Plant Physiology, March 2000, Vol. 122, pp. 627–633, www.plantphysiol.org © 2000 American Society of Plant Physiologists

Update on Biochemistry

Biochemical and Molecular Genetic Aspects of Floral Scents[1]

Natalia Dudareva* and Eran Pichersky

Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907 (N.D.); and Biology Department, University of Michigan, Ann Arbor, Michigan 48109 (E.P.)

The chemical composition of floral scents has been extensively investigated for hundreds of years because of the commercial value of floral volatiles in perfumery. More recently, several ecological studies have examined the roles of floral scent in the biology of the plant. However, in contrast to the chemical emphasis of the perfumers and the organismal emphasis of the ecologists, until recently, there have been few studies concerning the biochemical synthesis of floral scent compounds and the enzymes and genes that control these processes. In fact, our recent investigation into the biogenesis of floral scent production in *Clarkia breweri*, an annual plant native to California that served as our model organism, and our even more recent work on the scent of the cultivated snapdragon (*Antirrhinum majus*) represent the only examples to date (to our knowledge) in which isolation of enzymes and genes involved in the de novo synthesis of scent compounds in the flower have been reported. In this *Update* we review the research leading to our work, report our findings, and discuss implications for future directions of the field.

FLORAL SCENTS ARE IMPORTANT FOR PLANT FITNESS

Many plants emit floral scents, and such scents can attract a variety of animal pollinators, mostly insects. When present, scent is often the dominant means of long-distance attraction, particularly in moth-pollinated flowers, which are searched out and visited at night. Floral fragrances vary widely among species in terms of the number, identity, and relative amounts of constituent volatile compounds. Although little is known about how insects respond to individual components found in floral scents, it is clear that insects are able to distinguish between complex floral scent mixtures, and that discriminatory visitation based on floral scent has important implications for plant reproductive success (Pellmyr, 1986). Since floral scent can be crucial in ensuring fertilization, and therefore in determining seed or fruit set, the presence or absence of a scent attractive to the locally available insect pollinators may have a substantial impact on the yield of agronomically important crops (Free, 1970). Plants imported into a new environment by humans may be especially disadvantaged in this regard, as they have not co-evolved with the local pollinators (Traub et al., 1942). Even if a local pollinator is attracted to the flowers, it may not be physically suitable to be an effective pollinator. On the other hand, pollinators that may have the appropriate physique (by chance) may not be successfully attracted to the plant (Herrera, 1987).

Plants did not naturally evolve to produce their scent for the benefit of humans; nevertheless, it is clear that humans find an aesthetic value in certain types of floral scents, and the presence of floral scent may have contributed to the decision by humans to cultivate and propagate specific plant species. While there is certainly a wide variation in human taste, most people prefer the scents of bee-pollinated and, especially, moth-pollinated flowers, which they often describe as "sweet-smelling" (Knudsen and Tollsten, 1993). Unfortunately, very few plants are currently cultivated primarily for their scent. Moreover, a large number of commercial flower varieties have lost their scent during the selection and breeding processes due to, on the one hand, a focus on maximizing post-harvest shelf-life, shipping characteristics, and visual aesthetic values (i.e. color, shape), and on the other hand, to the lack of selection for the scent trait.

Some volatile compounds found in floral scent have important functions in vegetative processes as well. They may function as attractants for the natural predators of herbivores (Pare and Tumlinson, 1997) or as airborne signals that activate disease resistance via the expression of defense-related genes in neighboring plants and in the healthy tissues of infected plants (Shulaev et al., 1997). They may also serve as repellents against herbivores (Gershenzon and Croteau, 1991).

DETERMINATION OF FLORAL SCENT COMPOSITION

Until recently, investigations concerning floral scent have concentrated mainly on determining the chemical composition of floral fragrances. For this purpose, the "headspace" collection method was developed. In this procedure, a flower that is still connected to the rest of the plant is placed inside a glass chamber and its emitted volatiles are collected by continually purging the air inside the chamber through a polymer mesh that binds these volatiles. After a fixed period of time, the volatiles bound to the polymer are extracted with an organic solvent (a

[1] This work was supported by the National Science Foundation (grant nos. IBN–9904910 to N.D. and MCB–9974436 to E.P.) and The Fred C. Gloeckner Foundation (to N.D.).

* Corresponding author; e-mail dudareva@hort.purdue.edu; fax 765–494–0391.

variation of this procedure, the highly sensitive solid phase microextraction method, allows for "instant" sampling of headspace volatiles; Matich et al., 1996). The solution is then injected into a gas chromatograph, which separates the different volatiles, and each volatile is identified by mass spectrometry. These investigations have determined that floral scents are almost always a complex mixture of small (approximately 100–250 D) volatile molecules and are dominated by monoterpenoid and sesquiterpenoid, phenylpropanoid, and benzenoid compounds (Fig. 1). Fatty acid derivatives and a range of other chemicals, especially those containing nitrogen or sulfur, are also sometimes present (for review, see Knudsen et al., 1993).

LOCATION OF EMISSION OF FLORAL SCENT COMPOUNDS

The question of from which part of the flower the various scent components are emitted is a technically difficult one to answer. The identification of specific compounds inside floral organs is not sufficient to prove that these compounds are in fact emitted directly from such organs. The identification of anatomical features designated as "scent glands" by staining with stains that have affinity to aliphatic compounds (Curry, 1987) is also problematic, because this methodology again does not involve the actual measurement of scent emission from such anatomical features (nor from any other part of the flower). The best approach has been to conduct headspace analysis on flowers from which certain parts have been excised. These investigations have found that while the same floral scent components are often emitted from several parts of the flower (although not necessarily at the same amount or rate), specific compounds may sometimes be emitted from only a subset of the floral organs involved in total scent emission (Dobson et al., 1990; Pichersky et al., 1994). However, these results should also be viewed with caution, because the experiment involves injury to the flower (the removal of organs) and may therefore lead to changes in emission profiles. The use of appropriate controls and comparisons with whole-flower emission profiles should be employed to identify such changes should they occur (Dudareva et al., 1998b).

TEMPORAL AND PHYSIOLOGICAL VARIATIONS IN FLORAL SCENT EMISSION

There are many reasons why plants need to, and often do, vary the floral scent they emit during the lifespan of the flower, both in total output and in specific composition. It is to the advantage of the plant to have its scent output at maximal levels only when its potential pollinator is active. Thus, flowers that are pollinated by nocturnal insects such as moths tend to have maximal scent output in early evening (Loughrin et al., 1990), although this is not always the case (Pichersky et al., 1994). Aside from the obvious consideration of conservation of energy, it is also to the advantage of the plant not to attract more general pollinators, who might disperse its pollen non-productively. However, a certain amount of visitation by generalist pol-

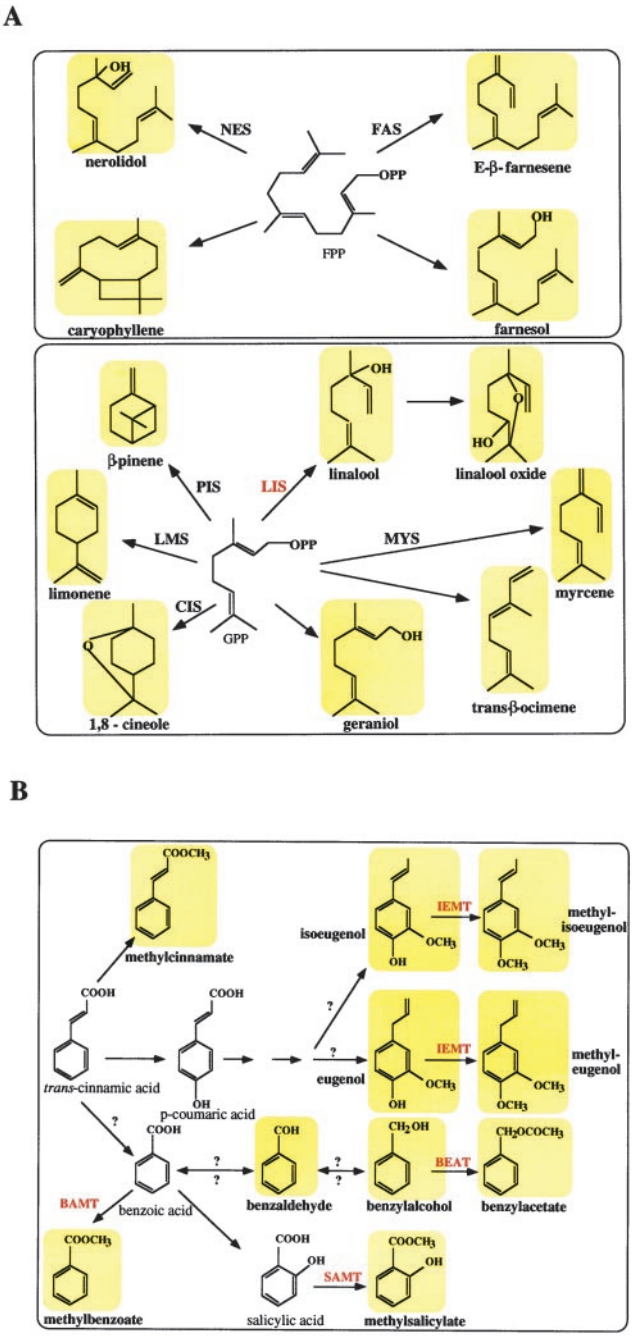

Figure 1. Pathways that lead to floral scent volatiles. Volatile compounds are shown with a yellow background; enzymes that have been identified in the synthesis of volatile compounds in vegetative tissues are shown in black; enzymes identified in floral tissues are shown in red. Not all reactions or enzymes have been identified. A, Sesquiterpenes (top) are synthesized in the cytosol. Biosynthesis of monoterpene (bottom) occurs in the plastids, although the location of the reactions leading to further modifications (e.g. linalool oxide) is not yet clear. B, The location of synthesis of most phenolpropanoids/benzenoids is not yet known, but is likely to be the cytosol and possibly the peroxisomes. IEMT, SAMT, and BAMT are methyltransferases that use SAM (not shown) as the methyl donor. BEAT is an acetyltransferase that uses acetyl-CoA (not shown) as the acetyl donor.

linators might have some benefit to the plant as "insurance," in case its specific pollinator is rare or absent.

It has been suggested that specific compounds in floral bouquets are designed to attract specific pollinators, and that some flowers can change their floral scent composition over time to attract more general pollinators if the flower has not yet been pollinated. In addition, it has been hypothesized that some flowers emit certain chemicals designed to repel insects that are non-beneficial to the plant (e.g. the so-called pollen or nectar "thieves," or generally destructive insects). Although specific changes in floral scents that follow diurnal, nocturnal, or circadian rhythms (and separate patterns may apply to different compounds in the same flower) or some other specific program over the lifespan of the flower have been well documented (Dudareva et al., 1999), our knowledge of how insect pollinators respond to specific floral volatiles is so rudimentary that it is not yet possible to assign specific adaptive values to such changes.

It should also be remembered that insects are capable of associative learning, and that the interests of the insect pollinators and those of the plants are not completely complementary, so there is a certain amount of "cheating" going on in these relationships. Sometimes the plants have the upper hand, as in the case of the *Ophrys* genus, where the insects are lured into visiting and pollinating the plants with the ruse of pheromone mimicry by floral scent (as well as morphological mimicry) (Borg-Karlson and Tengo, 1986), but often the plants are on the losing end. Nonetheless, it often benefits the flower to advertise truthfully when true rewards such as nectar or pollen are available (and their availability should coincide with the time of maturity of the male and female parts), and to stop advertising when the rewards are not available. This is because the insects will learn to associate the scent emanating from the flower with the rewards they get when they reach the flower, and will therefore continue to seek out and visit flowers with the same scent. On the other hand, even if the insect has been lured once by the scent to come and pollinate the flower when no rewards are available, it is less likely to come again to another flower of this kind.

In addition, once a flower has been sufficiently pollinated, post-pollination changes in scent emission could prevent additional, possibly destructive, visits to the pollinated flower, and could therefore also increase the chances of visitation to unpollinated flowers. Indeed, in most cases analyzed, the scent of flowers is markedly reduced soon after pollination, and in some cases it has been found to change qualitatively. The cessation of scent emission is often, but not always, accomplished simply because the petals (which usually constitute the bulk of the flower and the main source of scent emission), stigma, and style senesce and wilt.

Finally, the effect of temperature on fragrance emission has generally received even less attention, but it has been shown that temperature has a strong effect on the quantity of fragrance. For example, total emission of fragrance from *Trifolium repens* L. flowers was 58% higher at 20°C than at 10°C and all compounds of floral scent were affected by the change in temperature (Jakobsen and Olsen, 1994). It is not clear if the increase in emission is due solely to the greater volatility of these compounds at the higher temperature, or if it is also due to biological processes, including increased synthesis.

BIOSYNTHESIS OF FLORAL SCENT COMPOUNDS

As mentioned in the introduction, there have been few studies concerning the biochemical synthesis of floral scents, and our recent investigation into the biogenesis of floral scent production in our model organisms *C. breweri* and snapdragon represent, to our knowledge, the only examples to date in which isolation of enzymes and genes responsible for the formation of scent volatiles in the flower have been accomplished. These results are discussed below. Fortunately, too, many of the volatiles found in floral scents are also synthesized in vegetative tissues under specific conditions (mostly for defense purposes), and some information concerning their biosynthesis is also available. While it cannot be taken for granted that the synthesis of such compounds in vegetative tissue will in all cases be identical (i.e. same reactions, same enzymes) to their synthesis in flowers, and while there is currently no evidence that volatiles synthesized in vegetative tissues are transported into flowers, it is nevertheless instructive to review this information.

Terpenes, especially monoterpenes such as linalool, limonene, myrcene, and trans-β-ocimene, but also some sesquiterpenes such as farnesene, nerolidol, and caryophyllene, are common constituents of floral scent (Fig. 1A). They are also often found in vegetative tissues, where they serve mostly as defense compounds. In work done mostly with vegetative tissue, but also with daffodil petals, it was found that monoterpenes are synthesized in the plastidic compartment. In this cellular compartment, isopentenyl pyrophosphate (IPP) is derived from the mevalonate-independent "Rohmer" pathway (Lichtenthaler et al., 1997). IPP can be isomerized to dimethylallyl diphosphate (DMAPP), and one molecule of IPP is condensed with one molecule of DMAPP in a reaction catalyzed by the enzyme geranyl pyrophosphate synthase (GPPS) to form GPP, the universal precursor of all the monoterpenes. Similar work with vegetative tissue has revealed that in the cytosol, IPP is derived from the mevalonic acid pathway (McCaskill and Croteau, 1998), and two molecules of IPP and one molecule of DMAPP are condensed in a reaction catalyzed by the enzyme farnesyl pyrophosphate synthase (FPPS) to form FPP, the universal precursor of all the sesquiterpenes (McGarvey and Croteau, 1995).

In the last few years, genes encoding the enzymes responsible for the synthesis of many monoterpenes and sesquiterpenes have been identified and characterized (Bohlmann et al., 1998) (Fig. 1A). However, to date, only the enzyme that catalyzes the formation of the acyclic monoterpene linalool has been characterized in floral tissue. *C. breweri* flowers emit copious amounts of *S*-linalool from the petals, stigma, and style (the stigma and style also emit large amounts of linalool oxides), and we were able to demonstrate that linalool was synthesized from GPP in a one-step reaction (Fig. 1A) catalyzed by a monomeric en-

zyme linalool synthase (LIS) (Pichersky et al., 1994). We were also able to purify LIS from *C. breweri* stigmata by employing several chromatographic techniques (Pichersky et al., 1995) and to obtain peptide sequences that allowed us to isolate a LIS cDNA clone from a *C. breweri* flower cDNA library (Dudareva et al., 1996).

The phenylpropanoids, which are derived from Phe, constitute a large class of secondary metabolites in plants. Many are intermediates in the synthesis of structural cell components (e.g. lignin), pigments (e.g. anthocyanins), and defense compounds. These are not usually volatile. However, several phenylpropanoids whose carboxyl group at C9 is reduced (to either the aldehyde, alcohol, or alkane/alkene) and/or which contain alkyl additions to the hydroxyl groups of the benzyl ring or to the carboxyl group (i.e. ethers and esters) are volatiles (Fig. 1B). Our work with *C. breweri* flowers has now resulted in the identification and characterization of three enzymes that catalyze the formation of floral volatiles from this group: (iso)methyleugenol, benzylacetate, and methylsalicylate. The enzymes are, respectively, *S*-adenosyl-L-Met:(iso) eugenol *O*-methyltransferase (IEMT), acetyl-CoA:benzylalcohol acethyltransferase (BEAT), and *S*-adenosyl-L-Met:salicylic acid carboxyl methyltransferase (SAMT) (Wang et al., 1997; Dudareva et al., 1998a, 1998b; Wang and Pichersky, 1998; Ross et al., 1999). In addition, we have identified and characterized the enzyme *S*-adenosyl-L-Met:benzoic acid carboxyl methyltransferase (BAMT), which catalyzes the formation of methylbenzoate in snapdragon flowers (Bushue et al., 1999). cDNAs encoding all of these enzymes have also been characterized.

REGULATION OF SCENT BIOSYNTHESIS

In both *C. breweri* and snapdragon flowers, emission of the bulk of the volatiles occurs from the petals. Identification of enzymes responsible for the formation of these volatile compounds allowed us to determine how the levels of enzymatic activities are distributed in different floral parts and how they vary during flower development. When activity levels are calculated per total weight of each organ, the highest levels of activity of all these enzymes are found in the petals (Dudareva et al., 1999). Other parts of the flower, however, also contain detectable levels of activity, and the stigma actually contains higher levels of LIS specific activity (but because the mass of the stigma of *C. breweri* is so small compared with the mass of the petals, LIS in the petal still comprises the majority of activity present in the flower).

The specific types of cells expressing the genes encoding LIS and IEMT were determined by in situ hybridization. The results indicate that in *C. breweri* flowers, these scent genes are expressed uniformly and almost exclusively in cells of the epidermal layer of petals and other floral parts (Fig. 2, Dudareva et al., 1996). Volatile compounds produced in epidermal cells can apparently escape directly into the atmosphere after being synthesized.

C. breweri flowers, despite being moth-pollinated, do not show marked differences in emission between day and night. Snapdragon flowers, on the other hand, are bee-pollinated and have a marked peak of emission during the day. Both types of flowers follow a long-term pattern in which emission peaks within a few days of anthesis and then declines gradually. In *C. breweri*, the activities of scent enzymes follow two different patterns (Fig. 3). The activities of the first group of enzymes, represented by LIS and SAMT (Fig. 3, A and B), increase in maturing buds and young flowers, peaking about 12 to 24 h ahead of peak volatile emission. LIS and SAMT activities then decline in old (5-d) *C. breweri* flowers, but remain relatively high (40%–50% from the maximum level) even though emission of linalool and methylsalicylate has practically ceased. The activities of the second group of enzymes, represented by IEMT and BEAT (Fig. 3, C and D), show little or no decline at the end of the lifespan of the flower, although, again, emission of methyleugenol, isomethyleugenol, and benzylacetate virtually cease. A minor difference in developmental profiles of the latter two enzymes is that IEMT levels peak on d 1 of anthesis and stay stable afterward (Wang et al., 1997), whereas BEAT activity does not peak until the

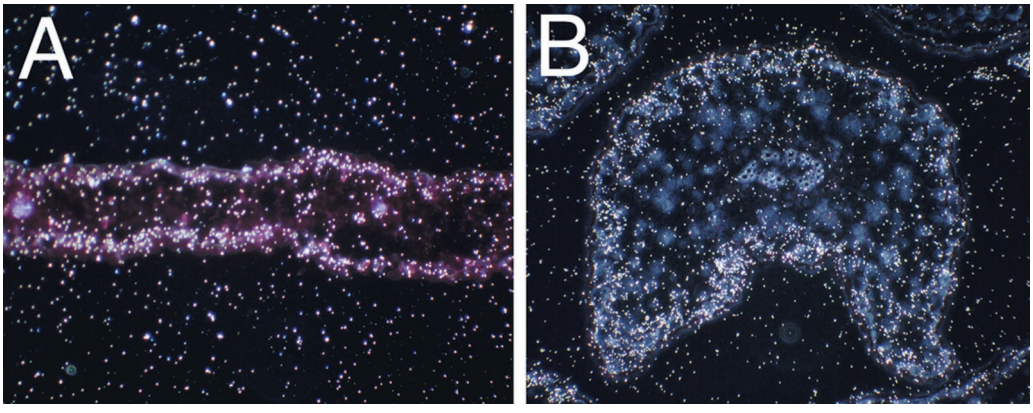

Figure 2. Localization of expression of the *IEMT* gene in *C. breweri* flowers by RNA in situ hybridization. A, Cross-section of a petal from a 1-d-old flower. B, Cross-section of an anther in a mature flower bud. Samples were hybridized with an IEMT antisense probe. White dots visualized by dark-field microscopy indicate location of hybridization. This figure was obtained by Dr. Jihong Wang (University of Michigan, Ann Arbor).

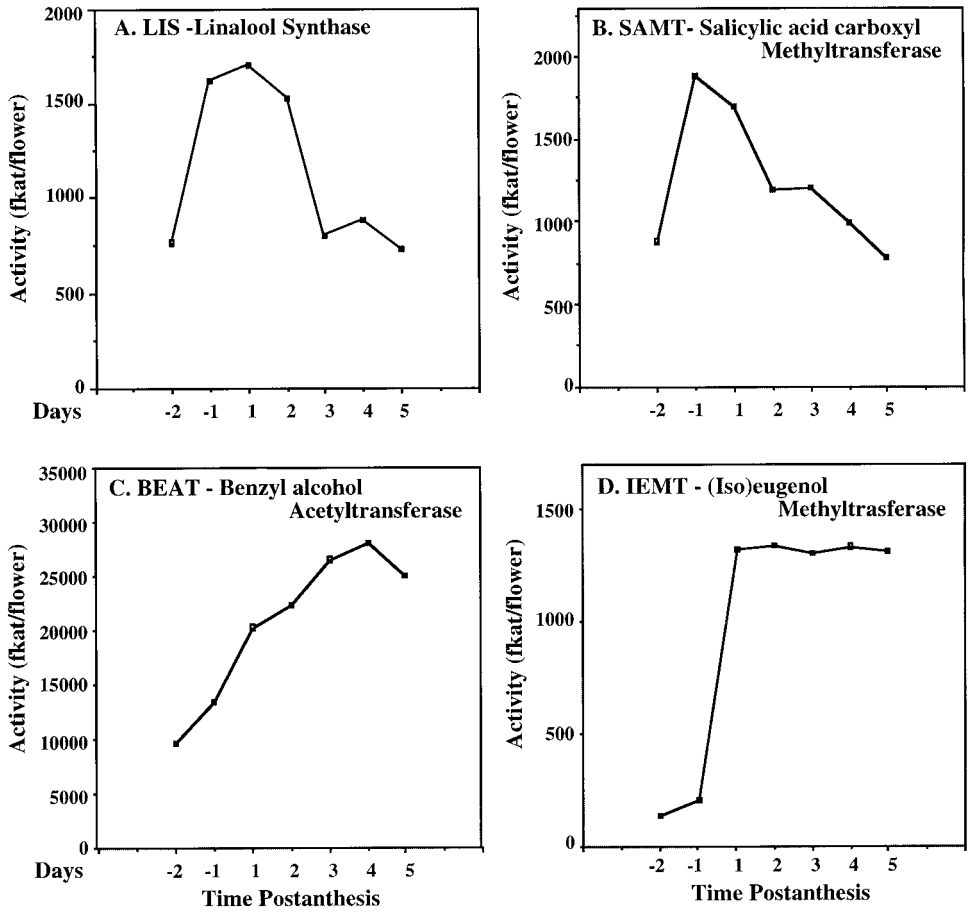

Figure 3. Levels of activities of four enzymes involved in floral scent production during flower development in petals of *C. breweri* flowers.

4th d after anthesis (Dudareva et al., 1998a). The BAMT enzyme from snapdragon flowers appears to belong to the first group, since its activity declines at the end of the lifespan of the flower (9–12 d after anthesis, snapdragon flowers are longer-lived than those of *C. breweri*).

The causes and consequences of appreciable levels of activity of biosynthetic enzymes in old flowers, without concomitant emission of the volatile products, are unknown. Although it is possible that the biosynthetic pathways in which these enzymes participate are blocked elsewhere, another possibility that remains to be investigated is that the products of the reactions catalyzed by these enzymes are required for processes other than scent emission in the flowers. Indeed, it has been found that the flowers of many species accumulate glycosides of scent compounds as they age (Oka et al., 1999). Such non-volatile glycosides are also sometimes found in buds, and were therefore originally hypothesized to be obligatory "scent precursors." However, closer examination has shown that, in most cases, an increase in emission of a particular volatile is not accompanied by a corresponding decrease in levels of the glycoside of this volatile, as would be predicted by this hypothesis (Oka et al., 1999). The increased synthesis of such glycosides as the flowers age may account for the cessation of scent emission, although the specific roles of such glycosides in the flower remain to be determined.

Expression of genes encoding scent-biosynthetic enzymes in the *C. breweri* flower is temporally and spatially regulated during flower development. The mRNAs encoding LIS, IEMT, and BEAT are first detected in petal cells just before the flower opens, and their levels increase until they peak at or around anthesis and then begin to decline (Dudareva et al., 1996, 1998a; Wang et al., 1997). For all of these three genes, peak levels of the mRNAs occur 1 to 2 d ahead of the peaks of enzyme activity and emission of the corresponding compound. Similar results were found for these mRNAs in other parts of the flower.

Overall, our data show that a good positive correlation exists between the amount of mRNA, the amount of protein and enzymatic activity for each of these enzymes, and emission of the corresponding component up to the second or third d post anthesis. But beyond that point, the levels of scent enzymes remain relatively high despite declining levels of the corresponding mRNA and also without the concomitant emission of volatiles (Dudareva et al., 1996, 1999). These results also indicate that in *C. breweri* flowers, scent compounds are synthesized de novo in the epidermal cells of organs from which they are emitted (primarily the petals). Thus, the levels of activity of enzymes involved in

scent production and, indirectly, scent emission are regulated mainly at the mRNA levels at the site of emission.

EVOLUTION OF FLORAL SCENT

An intriguing observation concerning floral scent is how variable this trait is. In many taxa, there are scented species that are closely related to non-scented ones, leading to the inescapable conclusion that the ability to produce and emit floral scent is an easily acquired, and easily lost, trait (Dudareva et al., 1996). Moreover, considering that floral scent is a complex mixture of chemicals, and practically no two closely related species emit identical mixtures of volatiles, it is clear that the ability to produce a specific floral scent volatile is an easily evolved trait. What is the basis for these evolutionary changes?

True to the complex nature of scent itself, the answer to the question posed above turns out to be complex as well. We have found that the genome of a close relative of *C. breweri* and its likely progenitor, the scentless *Clarkia concinna*, also contains the gene for linalool synthase (Cseke et al., 1998). Moreover, *C. concinna* flowers express *LIS*, but only in the stigma, and at such low levels that little linalool is produced and practically none is emitted (Raguso and Pichersky, 1995; Dudareva et al., 1996). Comparison of the promoter sequences of *LIS* from *C. breweri* and *C. concinna* revealed that although the promoter region in this gene from both species is almost identical, several insertions occur within the *C. concinna* promoter region relative to the *C. breweri LIS* promoter, two of them immediately upstream of the putative TATA box and a third inside the CAATT box (Cseke et al., 1998). It is possible that these differences in the *LIS* promoters are responsible for the vastly different expression characteristics of *LIS* in these two species, but this remains to be verified experimentally.

It appears that the difference between a linalool-emitting plant and a linalool non-emitter is not in the possession of a *LIS* gene per se, but in the mode of its regulation. In fact, the ability to synthesize linalool either in vegetative or floral parts is widespread in the plant kingdom, and has often been shown to be part of a defense response (as in maize, soybean, and possibly in the stigma of *C. concinna*), as well as being present in the scent of numerous flowers. It is therefore likely that virtually all plant genomes contain a *LIS* gene, and by a few changes in the regulation of expression of this gene, a plant may be able to easily recruit it for scent production.

A somewhat different scenario explains the ability of *C. breweri* flowers to produce methyleugenol and isomethyleugenol. We were able to show (Wang and Pichersky, 1999) that the *IEMT* gene, encoding the enzyme that methylates eugenol and isoeugenol, evolved recently from a gene encoding caffeic acid methyltransferase (COMT), which catalyzes the formation of ferulic acid and 5-hydroxyferulic acid, both non-volatile intermediates in the biosynthesis of lignin. The two enzymes are very similar, and changing only five to seven residues in one of them can convert the enzyme to preferentially act on the preferred substrates of the other (Wang and Pichersky, 1999). COMT is a ubiquitous gene throughout the plant kingdom, whereas IEMT most likely arose within Onagraceae, the family in which *C. breweri* is placed. Thus, unlike *LIS*, the ability of *C. breweri* to make the methyleugenol and isomethyleugenol components of its floral scent is due to the recent evolution of a new gene. However, like *LIS*, some *C. breweri* plants that do not emit (iso)methyleugenol have the *IEMT* gene in their genome but do not express it, indicating that the regulation of gene expression is also a factor in (iso)methyleugenol emission (Wang et al., 1997).

Non-scented *C. concinna* also contains genes with high sequence identify to *C. breweri BEAT*, but its flowers have little BEAT enzymatic activity. This is due first to the fact that the levels of properly processed transcripts of these genes in flowers are very low. In addition, the enzymes encoded by such transcripts have higher affinity with substrates other than benzylalcohol (e.g. heptanol; Nam et al., 1999). (Enzymes that can react with more than one substrate are not uncommon in secondary metabolism; see Wang and Pichersky, 1999.) It has recently been shown that BEAT is a member of a large family of plant acyltransferases (Dudareva et al., 1998; St.-Pierre et al., 1998). A few enzymes belonging to this family that catalyze the formation of various non-volatile (with the exception of benzylacetate) secondary compounds (e.g. anthocyanins and phytoalexins) have been identified. However, based on the presence in the Arabidopsis genome of perhaps 70 such related genes, it is clear that the functions of the majority of them remain to be determined. Such a large family represents a rich source of enzymes that may be involved in scent biosynthesis or serve as a pool for the evolution of new scent enzymes.

The methyltransferases SAMT and BAMT also belong to a newly identified family of enzymes. Although Arabidopsis has perhaps as many as 30 related genes of this family, and although several ESTs that are derived from genes of this family have been reported from Arabidopsis and other species, the reactions catalyzed by none of these proteins (with the exception of SAMT and BAMT) have been determined. This family is also likely to include scent enzymes such as the ones catalyzing the formation of methyljasmonate and methylcinnamate.

FUTURE PROSPECTS

It is clear from the above discussion that a major priority of scent research should be the continuation of the elucidation of biochemical pathways leading to scent biosynthesis and the identification and characterization of enzymes and genes controlling these pathways. In addition, the subcellular location of the synthesis of most of the scent compounds still needs to be determined, as well as the mechanisms controlling developmental changes and circadian rhythms of the pathways. On the evolutionary scale, it would be instructive to examine the molecular processes that bring about the variability in floral scent characteristics among different species, whether they are on the level of gene regulation, post-transcriptional regulation, or protein evolution. Finally, the contribution of specific scent compounds in attracting specific pollinators needs to be rigorously examined. The availability of scent genes should

allow us to create transgenic lines whose floral bouquets differ by a single component, thus simplifying this task.

Received October 15, 1999; accepted November 10, 1999.

LITERATURE CITED

Bohlmann J, Meyer-Gauen G, Croteau R (1998) Plant terpenoid synthases: molecular biology and phylogenetic analysis. Proc Natl Acad Sci USA **95:** 4126–4133

Borg-Karlson AK, Tengo J (1986) Odor mimetism? Key substances in *Ophrys lutea-Andrena* pollination relationship (Orchidaceae: Andrenidae). J Chem Ecol **12:** 1927–1941

Bushue L, Mann C, Gorenstein N, Dudareva N (1999) Floral scent production in *Antirrhinum majus*. *In* Plant Biology '99. American Society of Plant Physiologists, Rockville, MD, p 80

Cseke L, Dudareva N, Pichersky E (1998) Structure and evolution of the linalool synthase. Mol Biol Evol **15:** 1491–1498

Curry KJ (1987) Initiation of terpenoid synthesis in osmophores of *Stanhopea anfracta* (Orchidaceae): a cytochemical study. Am J Bot **74:** 1332–1338

Dobson HEM, Bergstrom G, Groth I (1990) Differences in fragrance chemistry between flower parts of *Rosa rugosa* Thunb. (Rosaceae). Isr J Bot **39:** 143–156

Dudareva N, Cseke L, Blanc VM, Pichersky E (1996) Evolution of floral scent in *Clarkia*: novel patterns of S-linalool synthase gene expression in the *C. breweri* flower. Plant Cell **8:** 1137–1148

Dudareva N, D'Auria JC, Nam KH, Raguso RA, Pichersky E (1998a) Acetyl CoA:benzylalcohol acetyltransferase: an enzyme involved in floral scent production in *Clarkia breweri*. Plant J **14:** 297–304

Dudareva N, Piechulla B, Pichersky E (1999) Biogenesis of floral scent. Hortic Rev **24:** 31–54

Dudareva N, Raguso RA, Wang J, Ross JR, Pichersky E (1998b) Floral scent production in *Clarkia breweri*: III. Enzymatic synthesis and emission of benzenoid esters. Plant Physiol **116:** 599–604

Free J (1970) Insect Pollination of Crops. Academic Press, London

Gershenzon J, Croteau R (1991) Terpenoids. *In* GA Rosenthal, M Berenbaum, eds, Herbivores: Their Interaction with Secondary Metabolites. Academic Press, New York, pp 165–219

Herrera C (1987) Components of pollinator "quality": comparative analysis of a diverse insect assemblage. Oikos **50:** 79–90

Jakobsen HB, Olsen CE (1994) Influence of climatic factors on rhythmic emission of volatiles from *Trifolium repens* L. flowers in situ. Planta **192:** 365–371

Knudsen JT, Tollsten L (1993) Trends in floral scent chemistry in pollination syndromes: floral scent composition in moth-pollinated taxa. Bot J Linn Soc **113:** 263–284

Knudsen JT, Tollsten L, Bergstrom G (1993) Floral scents: a checklist of volatile compounds isolated by head-space techniques. Phytochemistry **33:** 253–280

Lichtenthaler HK, Rohmer M, Schwender J (1997) Two independent biochemical pathways for isopentenyldiphosphate and isoprenoid biosynthesis in higher plants. Physiol Plant **101:** 643–652

Loughrin JH, Hamilton-Kemp TR, Andersen RA, Hildebrand DF (1990) Volatiles from flowers of *Nicotiana sylvestris, N. otophora* and *Malus* × *Domestica*: headspace components and day/night changes in their relative concentrations. Phytochemistry **29:** 2473–2477

Matich AJ, Rowan DD, Banks NH (1996) Solid phase microextraction for quantitative headspace sampling of apple volatiles. Anal Chem **68:** 4114–4118

McCaskill D, Croteau R (1998) Some caveats for bioengineering terpenoid metabolism in plants. Trends Biotechnol **16:** 349–355

McGarvey DJ, Croteau R (1995) Terpenoid metabolism. Plant Cell **7:** 1015–1026

Nam KH, Dudareva N, Pichersky E (1999) Characterization of benzylalcohol acetyltransferases in scented and non-scented *Clarkia* species. Plant Cell Physiol **40:** 916–923

Oka N, Ohishi H, Hatano T, Hornberger M, Sakata K, Watanabe N (1999) Aroma evolution during flower opening in *Rosa damascena* Mill. Z Naturforsch **54C:** 889–895

Pare PW, Tumlinson JH (1997) *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol **114:** 1161–1167

Pellmyr O (1986) Three pollination morphs in *Cimicifuga simplex*: incipient speciation due to inferiority in competition. Oecologia **78:** 304–307

Pichersky E, Lewinsohn E, Croteau R (1995) Purification and characterization of S-linalool synthase, an enzyme involved in the production of floral scent in *Clarkia breweri.* Arch Biochem Biophys **316:** 803–807

Pichersky E, Raguso RA, Lewinsohn E, Croteau R (1994) Floral scent production in *Clarkia* (Onagraceae): I. Localization and developmental modulation of monoterpene emission and linalool synthase activity. Plant Physiol **106:** 1533–1540

Raguso RA, Pichersky E (1995) Floral volatiles from *Clarkia breweri* and *C. concinna* (Onagraceae): recent evolution of floral scent and moth pollination. Plant Syst Evol **194:** 55–67

Ross JR, Nam KH, D'Auria JC, Pichersky E (1999) *S*-Adenosyl-L-methionine: salicylic acid carboxyl methyltransferase, an enzyme involved in floral scent production and plant defense, represents a new class of plant methyltransferases. Arch Biochem Bioiphys **367:** 9–16

Shulaev V, Silverman P, Raskin I (1997) Airborne signalling by methyl salicylate in plant pathogen resistance. Nature **385:** 718–721

St-Pierre B, Laflamme P, Alarco AM, De Luca V (1998) The terminal *O*-acetyltransferase involved in vindoline biosynthesis defines a new class of proteins responsible for coenzyme A-dependent acyl transfer. Plant J **14:** 703–713

Traub H, Robinson T, Stevens H (1942) Papaya Production in the United States. U.S. Department of Agriculture Circular No. 633. U.S. Department of Agriculture, Washington, DC

Wang J, Dudareva N, Bhakta S, Raguso RA, Pichersky E (1997) Floral scent production in *Clarkia breweri* (Onagraceae): II. Localization and developmental modulation of the enzyme *S*-adenosyl-L-methionine:(iso) eugenol *O*-methyltransferase and phenylpropanoid emission. Plant Physiol **114:** 213–221

Wang J, Pichersky E (1998) Characterization of *S*-adenosyl-L-methionine:(iso) eugenol *O*-methyltransferase involed in floral scent production in *Clarkia breweri*. Arch Biochem Biophys **349:** 153–160

Wang J, Pichersky E (1999) Identification of specific residues involved in substrate discrimination in two plant *O*-methyltransferases. Arch Biochem Biophys **368:** 172–180