Biogenesis of Floral Scents

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I. INTRODUCTION

Flowers of many plants emit scents, which are almost always a complex mixture of small (100–250 Da), volatile compounds (Fig. 2.1).The accumulated experience of research in the field and in controlled environment in the laboratory has shown that floral scents may function as both long- and short-distance attractants and nectar guides to a variety of animal pollinators (reviewed in Dobson 1993). 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 population structure and reproductive isolation (Galen 1985; Pellmyr 1986; Dodson et al. 1969).

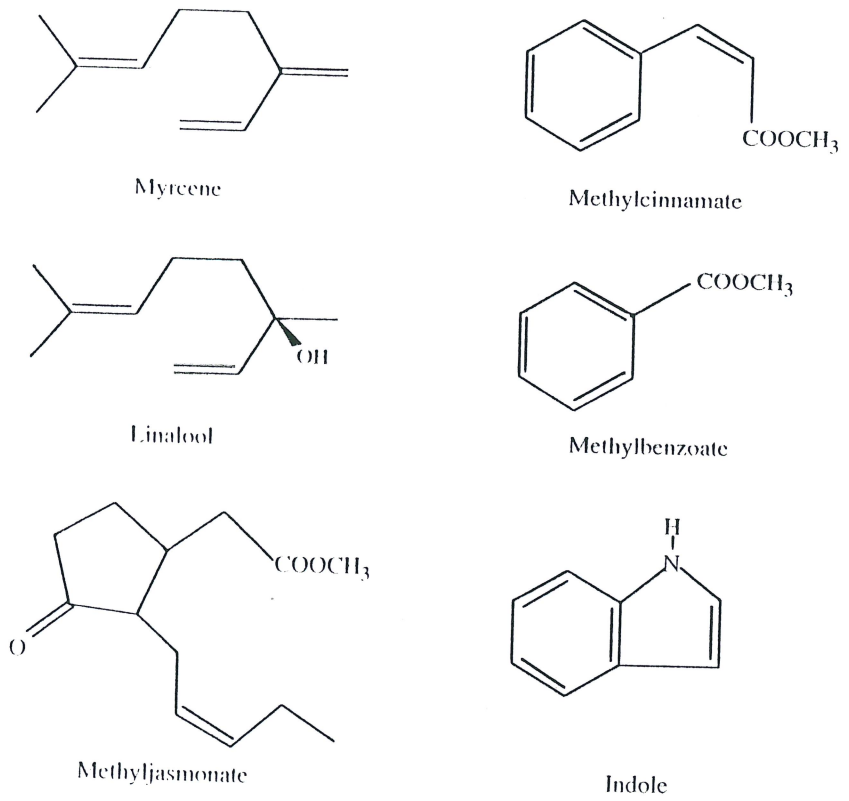

Fig. 2.1. Representative compounds of floral scent.

Floral scent is also an important character in some crop plants, since the presence or absent of a scent appropriate to the locally available insect pollinators may have a substantial impact on the level of pollination and therefore on seed (and fruit) set (Traub et al. 1942; Sugden 1986; Henning et al. 1992). Plants imported into a new environment by humans may be especially disadvantaged in this regard, as they have not coevolved with the local pollinators (DeGrandi-Hoffman 1987). 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 who may have the appropriate morphology (by chance) may not be successfully attracted to the plant.

The specific neurological effects of scent on humans are also little understood. However, it has been assumed since antiquity that at least some floral scents have beneficial or otherwise exploitable effects on humans and human behavior. This has in fact been the impetus for most of the research on floral scent done at the level of the plant. Over the last few hundred years, the perfume industry has amassed a vast catalogue of floral scent compounds. The desire to identify and purify such compounds, for commercial purposes, has been the driving force behind the development and/or refinement of several chemical techniques now widely used in many fields of chemistry and biochemistry, gas chromatography and oil-based extraction, to name just two.

Once a chemical from a floral scent has been identified, however, it could be synthetically made or obtained from sources other than flowers. Although some floral scent components are specific stereoisomers that cannot be synthetically made in pure form (others can be, by using chiral precursors), today the vast majority of perfumes (and scented food additives) are synthetically made. Perfumery has become an art that strives by chemical means to imitate life.

Plant scents are dominated by fatty acid derivatives, terpenoid, phenylpropanoid, and benzenoid compounds, with a smattering of other chemicals (see the excellent reviews by Knudsen et al. 1993; Knudsen and Tollsten 1993), all of which are regularly defined as "secondary metabolites." In contrast to the chemical emphasis in floral scent research, there have been few studies concerning the biosynthesis of floral scents. Most of the biochemical pathways and the enzymes involved in the synthesis of these compounds have not yet been elucidated. However, many of the chemicals found in scent are also occasionally found in vegetative tissue, e.g., limonene, farnesol, where more extensive biochemical investigations have been carried out. Only recently has attention been given to scent biogenesis. In this review, we describe what is known to date about the control of scent emission, the biosynthetic

pathways of floral scent compounds, the properties and regulation of the enzymes catalyzing these reactions, and the genes encoding these enzymes. It should be noted that most of the recent data concerning genes and enzymes involved in scent production come from one model organism, *Clarkia breweri*, which we have studied. It is hoped that similar investigations with other scented species will be forthcoming.

II. PATHWAYS AND SITE OF SYNTHESIS

While some plants emit scent from all, or a subset of, their floral parts, e.g. *Clarkia breweri* (Raguso and Pichersky 1995), other plants have been found to possess specialized "scent glands," e.g., orchids (Stern et al. 1986; Curry 1987). It is not yet clear how prevalent scent glands are among all scented flowers. Investigations of scent glands have so far been conducted mostly on the anatomical levels, and the question of whether such glands represents sites of emission only or also of synthesis of scent volatiles has not yet been addressed.

The determination of the location of synthesis of scent compounds has by necessity been tied to the elucidation of the pathways themselves. The first difficulty was trapping and identifying the actual volatile chemicals that are emitted under natural conditions, i.e., not by cut and/or dehydrated flowers. This was solved by the development of the "headspace" collection technique, in which flowers that are still attached to the rest of the plant are encased in a container from which the air is continuously purged. The volatiles in this "headspace" are collected onto a trapping sorbent, from which they are later eluted and analyzed (Kaiser 1991; Raguso and Pellmyr 1998).

While this approach has given us accurate descriptions of the emitted scent components, the specific pathways that operate in floral tissues or elsewhere to generate them have, in most cases, remained obscure. Historically, secondary metabolites in general were investigated by chemists who looked for intermediates (by pulse-chase experiments, for example) to piece together the pathways that lead to the final products. More recently, the emphasis has been on the discovery of enzymes catalyzing specific reactions as a proof that a postulated reaction indeed occurs in the cell (and is not an artifact of the experimental system). However, biochemical investigations of secondary metabolism have commonly been hampered by the lack of knowledge of the pathways, and, even when a substrate-product relationship is known or suspected, by the lack of pure substrates, especially radiolabelled ones, to perform the enzymatic assays.

Thus, the existence of scent biosynthetic enzymes in flower tissue has been demonstrated for the first time only recently.

Because it had proven difficult to find such biosynthetic enzymes, many investigations have focused instead on the possibility that some floral scent compounds are synthesized elsewhere in the plant and are then transported to the flowers. Several observations prompted such a hypothesis. First, many floral scent components are often found in glycosylated forms (as well as in free form) in fruits, and some of these may have been transported from the vegetative part of the plant (Gunata et al. 1985; Tang et al. 1990). Second, glycosylated compounds are also often found in buds (which are usually not scented), and later in flowers (Ackermann et al. 1989; Loughrin et al. 1992; Watanabe et al. 1993). And lastly, a report which appeared in Russian with an English abstract (Pogorel'skaya et al. 1980) claimed that such transport into buds occurs in roses (although this has not yet been repeated by other groups).

As is often the case in science, hypotheses that are relatively easy to test are tackled first. Thus, an important impetus for this line of investigation was simply the fact that it is relatively easy to measure the activity of the glycosidases, which were hypothesized to be the key enzymes releasing the aroma in the flower. In some cases it was reported that the levels of glycosidases increased during the development of the flower (Loughrin et al. 1992). It is important to note that the demonstration of glycosidase activity in flowers by itself says nothing about the site of synthesis of either the free scent component or its glycosylated form (both of which might of course occur in the flower itself). Moreover, when levels of glycosides present in the flowers at different stages are correlated with actual levels of emission, there is little to support the conclusion that glycosidically bound scent compounds are obligatory intermediates in scent biogenesis (Ackermann et al. 1989). This is because (1) the glycosides tend to accumulate more as the flower ages (i.e., they usually do not peak at or before the peak of scent emission), and (2) the pool of glycosides in the flower before or at peak emission time is only a small fraction of what the flower actually emits. In those studies neither the synthesis of the scent compounds themselves and their glycosylated forms in the vegetative tissues, nor the process of transporting them (or their glycosylated forms) into the flowers, have been examined. Thus, although it is possible that scent precursors are initially synthesized in vegetative tissue in some plant species, there is little empirical data to substantiate this scenario. This section will therefore review the growing evidence for flower-specific biogenesis of floral scent compounds. The published work deals with our model system *Clarkia breweri*, an annual flower from California. Recently, however,

similar results have been obtained with the cultivated ornamental species *Antirrhinum majus* (snapdragon) and *Narcissus pseudonarcissus* (daffodil) (N. Dudareva, J. D'Auria, and E. Pichersky, unpubl.).

A. Terpenes

Terpenes, especially monoterpenes but also some sesquiterpenes, are very common constituents of floral scent. Mettal and coworkers (Mettal et al. 1988) have shown that chromoplasts isolated from the petals of *Narcissus pseudonarcissus* flowers were capable of catalyzing the production of several monoterpenes, including limonene, myrcene, ocimene, and linalool. Interestingly, while the first three of these monoterpenes are regular constituents of the daffodil scent, linalool is not. The authors speculated that linalool is produced as an intermediate in the synthesis of other monoterpenes. Nevertheless, this work showed both that scent compounds could be synthesized in the flowers, and that the specific site of monoterpene synthesis is in the plastidic compartment. This is consistent with work on synthesis of monoterpenes in vegetative tissue, where it also occurs exclusively in plastids (McGarvey and Croteau 1995). It is now believed that in plants, monoterpenes (and diterpenes) are synthesized in the plastids via the Rohmer pathway, while sesquiterpenes are synthesized in the cytosol via the mevalonic acid pathway (Lichtenthaler et al. 1997).

We have looked in some detail at the synthesis of linalool, an acyclic monoterpene alcohol, in flowers of *Clarkia breweri*. Its flowers emit copious amounts of this monoterpene (~20μg/flower in 24 h), as well as a similar amount of two linalool oxide derivatives (Raguso and Pichersky 1995). Linalool synthase (LIS), the enzyme that catalyzes the formation of linalool from geranyl pyrophosphate (GPP) (Pichersky et al. 1995), is most abundant in the petals, stigma, and style, and is not found in the vegetative parts of the plant (Pichersky et al. 1994). The petals constitute the bulk of the flower, and most of the linalool emitted by the flower is synthesized in, and emitted from, the petals. In situ hybridization experiments have shown that the LIS gene is expressed in petals, mostly in the cells of the epidermis (from which linalool can easily escape into the atmosphere after being synthesized). In stigma and style, the transmitting tissue shows the highest concentration of LIS, but the linalool produced there is apparently mostly converted into linalool oxides by additional enzyme(s) not yet characterized. The purpose of the production of linalool and its oxides in the transmitting tissue is not clear, but may be related to defense, as linalool is relatively toxic to animals and micro-organisms (Brunetон 1995).

The gene encoding LIS has been isolated and characterized from several plant species (Cseke et al. 1998). The sequence of the deduced amino acid of the LIS protein shows that it is related to other terpene synthases, and LIS is therefore a member of a family of proteins that must have evolved from a common ancestor, although in a somewhat complicated manner (Bohlmann et al. 1998; Cseke et al. 1998).

B. Phenylpropanoids, Benzenoids, and Their Esters

The phenylpropanoids, derived from phenylalanine, 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 carboxy group at C9 is reduced (to either the aldehyde, alcohol, or alkane/alkene) and/or which contain alkane additions to the hydroxy groups of the benzyl ring or to the carboxy group (i.e., ethers and esters) are volatiles. Such compounds include eugenol, methyleugenol, and methylcinnamate.

The synthesis of methyleugenol and isomethyleugenol has been examined in *C. breweri* (Wang et al. 1997). An enzyme, S-adenosyl-L-methionine:(iso)eugenol methyltransferase (IEMT) has been isolated and characterized. The enzyme is most abundant in petal tissue, which, as in the case of linalool, accounts for the majority of the production and emission of (iso)methyleugenol. Again, in situ experiments show that the IEMT transcripts are most abundant in the epidermal cells of the petals, as well as in the epidermal layer of other floral tissues such as anthers (J. Wang, N. Dudareva, and E. Pichersky, unpubl.). IEMT activity is not found in vegetative tissue, nor in petals of a *C. breweri* variety that does not emit (iso)methyleugenol (Wang et al. 1997). The sequence of IEMT is very similar (84% identity) to the sequence of caffeic acid methyltransferase (COMT) from *C. breweri* (Wang and Pichersky 1997). COMT is an enzyme found universally in plants and it is involved in lignin biosynthesis. Thus, it appears that IEMT has evolved, perhaps recently, from a common enzyme by gene duplication and divergence.

Benzenoids, such as benzylalcohol (a component of petunia scent) are derived from phenylpropanoids by the loss of the C8-C9 carbons. Although the exact mechanism of their removal is still unclear, this process is probably *not* analogous to β-oxidation (Schnitzler et al. 1992). Many benzenoids are found in floral scent in an ester form (e.g., methylbenzoate, benzylbenzoate). The biosynthesis of two benzenoid esters, methylsalicylate and benzylacetate, have been examined in *C. breweri*. The formation of benzylacetate is catalyzed in flowers of *C. breweri* by

the enzyme acetyl-CoA:benzylalcohol acetyltransferase (BEAT). This enzyme is also most abundant in petal tissue, from where the bulk of benzylacetate emission occurs. The BEAT protein is a member of a newly defined family of acyltransferases. S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase (SAMT) is the enzyme that catalyzes the formation of methylsalicylate in petals of *C. breweri* (Dudareva et al. 1998a). IEMT, BEAT and SAMT, whose substrates are all phenylpropanoids/benzenoids, are most likely localized in the cytosol.

Recently, we have been able to demonstrate the activity of BEAT in petals of daffodils (J. D'Auria and E. Pichersky, unpublished). In addition, the activity of SAM:benzoic acid methyltransferase (BAMT), the enzyme that catalyzes the formation of methylbenzoate, a major component of the floral scent of *Antirrhinum majus*, has been demonstrated in their petals (N. Dudareva, unpublished).

C. Fatty Acids and Other Scent Compounds

Several floral scent components are derivatives of fatty acids. For example, the acetyl ester of cis-3-hexen-1-ol is common, as is methyljasmonate. Both cis-3-hexen-1-ol and jasmonate are breakdown products of linolenic acid (Creelman and Mullet 1997). Whereas it is well established that fatty acids themselves are synthesized in the plastids, the location of synthesis of their derivatives is still essentially uninvestigated. The jasmonates are important signaling compounds in vegetative tissues as well, and there it appears that at least the initial steps leading to their synthesis occur in the plastids. However, there are no reports that examine their synthesis in floral tissues.

In this category are mostly scent compounds that contain nitrogen, such as indole. Again, little is known about the biosynthesis of alkaloids in general (Kutchan 1995), and even less is known about volatile ones. In fact no information is available concerning the synthesis of any volatile alkaloids in floral tissues.

III. MOLECULAR GENETIC CONTROL

A. Terpenes

Although monoterpenes and sesquiterpenes are predominant in many floral fragrances, most of the work on terpene biosynthesis has been carried out on vegetative tissues, where these compounds also serve important functions such as defense (McGarvey and Groteau 1995). In the last few years, genes encoding the enzymes responsible for the synthesis of

many of these compounds have been identified and characterized (Bohlmann et al. 1998). However, to date only the gene encoding linalool synthase has been characterized specifically in respect to its role in floral scent biosynthesis (Dudareva et al. 1996). Linalool synthase was purified to homogeneity from stigmata of *Clarkia breweri* flowers and a protein-based cloning strategy was employed to obtain a cDNA encoding this protein. This enzyme produces exclusively S-linalool, a component of floral scent of many plant species (Knudsen et al. 1993; Gerlach and Schill 1991). It is a monomer with apparent molecular weight of 76 kDa (determined by gel permeation chromatography and polyacrylamide gel electrophoresis), and with a strict requirement for a divalent metal cofactor, preferentially Mn^{2+} (Pichersky et al. 1995). The LIS gene is unique in *C. breweri* genome and its coding region is interrupted by 11 introns (Dudareva et al. 1996; Cseke et al. 1998). The complete amino acid sequence of LIS, derived from the cDNA clone, showed it to be related to several enzymes involved in terpene synthesis (Dudareva et al. 1996). LIS was also isolated from a linalool-scented relative of *C. breweri*, *Oenothera arizonica*, and from non-scented *C. concinna* (Cseke et al. 1998), the proposed progenitor of *C. breweri*. Both sequence comparisons and comparisons of the location of introns in the genes suggest that LIS is actually a composite gene. It includes a portion of a copalyl pyrophosphate synthase-like sequence at its N-terminus coding region and a portion of another terpene synthase (a limonene synthase-like) in most of its second half (Cseke et al. 1998).

Expression of the LIS gene is temporally and spatially regulated during flower development. In scented *C. breweri*, LIS mRNA transcripts begin to accumulate in flower buds several days before opening the flower. One day before anthesis, LIS mRNA levels become approximately three and five times higher in petals than in pistil and stamens, respectively. There is a lag time of about a day between the peak levels of mRNA and the peak levels of LIS protein in petal and stigma tissues (Fig. 2.2), but not in style and anthers (Dudareva et al. 1996). During the lifespan of the flower, the protein levels in floral tissues show strong positive correlation with LIS activity (Fig. 2.2), indicating that the differences in LIS activity in different tissues and at different stages of flower development are due to changes in the amount of LIS protein and not to post-translational modifications.

Clarkia breweri has arisen from the non-scented species *C. concinna* (Raguso and Pichersky 1995), which nonetheless has been shown to express its LIS gene at a low level only in the stigma and to emit 1000-fold less linalool than *C. breweri* flowers (Pichersky et al. 1994; Dudareva et al. 1996). The most significant observation concerning the expression

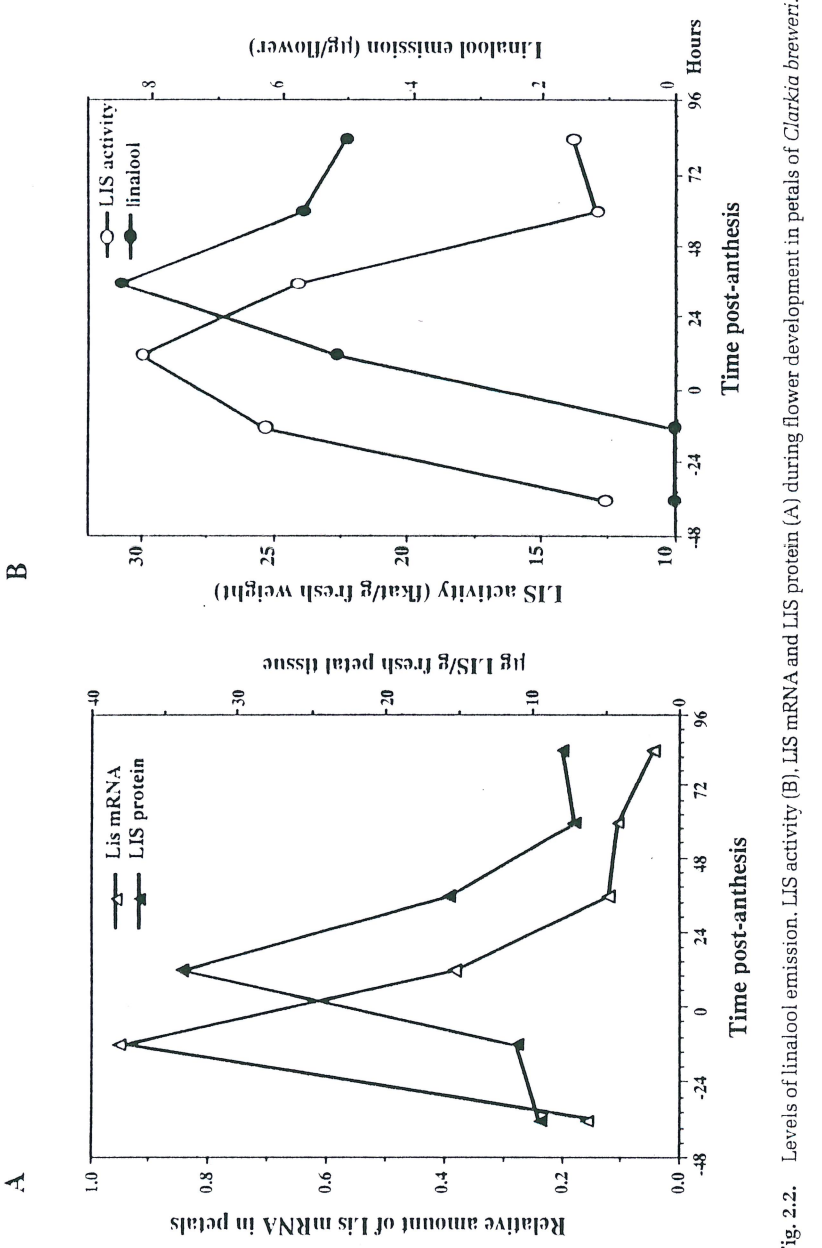

Fig. 2.2. Levels of linalool emission, LIS activity (B), LIS mRNA and LIS protein (A) during flower development in petals of *Clarkia breweri*.

of LIS in *C. breweri* is that in addition to being upregulated significantly in the stigma compared with its non-scented ancestor, *C. concinna*, the LIS gene is also highly expressed in other types of floral tissues such as styles, anthers, and petals. Up to 70% of the total LIS activity in the *C. breweri* flower was found in the petals, which are the major source of emitted S-linalool (Pichersky et al. 1994; Raguso and Pichersky 1995). In addition to high expression of the LIS gene in petals, a strict pattern of spatial regulation was also observed: LIS transcripts were found mainly in epidermal cells at the surface of the petals (Dudareva et al. 1996).

Overall, linalool production in *C. breweri* flowers involves a change in regulation of an existing gene. The expression of LIS gene was both upregulated and its range was expanded to include cells not expressing this gene in *C. concinna*, without the concomitant development of specialized "scent glands" as has been found in flowers of orchids (Stern et al. 1986).

B. Phenylpropanoids/Benzenoids

Two volatile phenylpropanoids, methyleugenol and isomethyleugenol, are important components of the floral scent of various species, including *C. breweri* (Raguso and Pichersky 1995; Wang et al. 1997). It has been shown that they are produced in *C. breweri* flowers by the action of a single enzyme, IEMT, which catalyzes the transfer of a methyl group to the 4-OH group of the benzyl ring of both eugenol and isoeugenol, using S-adenosyl-L-methionine as a donor of methyl group (Wang et al. 1997). IEMT has been purified to homogeneity from *C. breweri* petals. The corresponding cDNA has been isolated from *C. breweri* flower cDNA library, sequenced, and the catalytically active enzyme has been expressed in *Escherichia coli*. IEMT is active as a homodimer with a subunit molecular mass of 40 kDa and does not require any cofactors for enzymatic activity (Wang and Pichersky 1998).

IEMT is a single copy gene in the *C. breweri* genome and exhibits flower-specific and temporal expression patterns in (iso)methyleugenol emitters. The levels of IEMT activity and mRNA in different floral tissues strongly correlate with the production and emission of these two compounds by the same tissues, being highest in petals and absent in sepals and in leaf and stem tissue. Similar to LIS, the levels of IEMT mRNA in petals increased as the flower bud matured and peaked just before anthesis. Moreover, nonemitting plants did not have IEMT activity or IEMT mRNA in any floral tissues, but they do have the IEMT gene in their genome (Wang et al. 1997; J. Wang, unpubl.). These results suggest that similar to LIS, changes have occurred in the regulation of IEMT gene expression.

In many plant species, volatile esters contribute significantly to the total floral scent output and are important in attracting insect pollinators. Benzylacetate in particular is one of the most commonly found esters in moth-pollinated flowers (Knudsen and Tollsten 1993) and is often found in the aromas of other flowers as well (Knudsen et al. 1993; Van Dort et al. 1993; Watanabe et al. 1993). The enzyme BEAT, which catalyzes the formation of benzylacetate, has been purified from *C. breweri* petals, a cDNA encoding this enzyme has been isolated and characterized, and enzymatically active protein has been expressed in *Escherichia coli*. (Dudareva et al. 1998b). The sequence of the protein encoded by BEAT cDNA does not show extensive similarity to any other known protein sequences, but a short segment within it has significant similarity to short segments in other proteins known or hypothesized to use an acyl-CoA substrate.

There is a single copy of BEAT gene in the *C. breweri* genome. Its expression is developmentally and differentially regulated. Of the different parts of the *C. breweri* flower, petals contained the majority of BEAT transcripts, and no BEAT mRNA was detected in leaves. BEAT mRNA was first detected in petal cells just before the flower opened, and its level increased until it peaked on the day of anthesis (Dudareva et al. 1998b). These results are similar to those for LIS and IEMT, whose mRNA levels also peak at or around anthesis, suggesting a common regulatory mechanism.

Taken together, these results clearly indicate that, at least in *C. breweri* flowers, scent compounds are produced de novo in the tissues from which they are emitted and the levels of activity of enzymes involved in scent production are regulated mainly at the mRNA levels at the site of emission. Expression of genes encoding scent biosynthetic enzymes is relatively uniform, being highest in petals just before anthesis, and restricted to surface of the floral tissue (epidermal cells), without development of specialized "scent glands" as found in orchid flowers (Stern et al. 1986) and in vegetative tissue of some terpene-producing plants (Lewinsohn et al. 1991, 1998; McGarvey and Croteau, 1995).

IV. VARIATION IN BIOSYNTHESIS AND EMISSION OVER TIME

A. Variation During Development

The composition of the odor emitted determines the attractiveness of the flowers to specific insects, and is therefore responsible for pollinator

selectivity. For example, the intra- and inter-individual variation of odor composition of *Ophrys sphegodes* flowers and of flowers of other plants may influence the behavior of the male pollinator (Smith and Ayasse 1987). It is known that male bees can remember the floral bouquet of a visited flower and subsequently modify their response to it (Smith and Ayasse 1987; Ayasse et al. 1996, summarized in Schiestl et al. 1997). A decrease and/or an alteration of floral bouquets after pollination might lead to a lower attractiveness of these flowers. The advantage of these postpollination changes in scent emission is most likely that pollinators are directed to the unpollinated flowers of the plant.

Scent production and maintenance by flowers is expensive in terms of energy resources, therefore many flowers have evolved various mechanisms for energy conservation, such as cessation of scent production and fast wilting immediately after pollination (Arditti 1979) or decrease in scent production of unpollinated plants as a result of aging. The changes in scent during floral development involve not only a decrease of total amount of scent produced, but also an alteration of the odor bouquet. The effect of pollination on floral scent composition and production was studied in the moth-pollinated orchid *Platanthera bifolia*. A significant decrease (about 200 times) in scent production was detected five days after pollination, although some decrease (about 3 times) was already found two days after pollination. All scent compounds were affected by pollination, even though some compounds had a larger impact on the overall scent reduction. There was also a drop in scent production during the lifespan of the flower in unpollinated plants: the average amount of total volatiles collected per flower decreased about two times, from 69 µg at anthesis to 37 µg at day five (Tollsten 1993). In addition to quantitative changes, qualitative alterations of odor emission were found in the Mediterranean orchid genus *Ophrys* after pollination. Pollinated flowers produced significantly different odor bouquets due to the change in the relative amount of each constituent volatile, and the total amount of scent emitted two to four days after pollination was significantly lower compared with unpollinated flowers (Schiestl et al. 1997).

The comparative analysis of volatiles emitted at different stages of flower development in *Clarkia breweri* revealed variations in the quantitative contribution of the individual compounds to the floral fragrance (Pichersky et al. 1994; Wang et al. 1997; Dudareva et al. 1998a). Emission of volatiles began just before the flowers opened. Benzenoid esters (benzylacetate, benzylbenzoate, and methylsalicylate) were the first volatiles emitted from flower buds. The level of emission was 10–20% of the maximal level and remained relatively stable for the first 12 h after

anthesis. Unopened flowers (buds) emit no linalool, isoeugenol, and isomethyleugenol, but they do emit a small amount of linalool oxide, eugenol, and methyleugenol. Significant emission of volatile compounds begins at anthesis, peaks on day 1 or 2 (depending on compound) and declines thereafter. Developmentally, the activities of enzymes involved in the biosynthesis of these compounds of *C. breweri* scent follow two different patterns (Fig. 2.3). The activities of the first group of enzymes, such as LIS and SAMT (Fig. 2.3 A, D), increased in young flowers and declined in old flowers in parallel with emission of linalool and methylsalicylate, respectively. The activities of the second

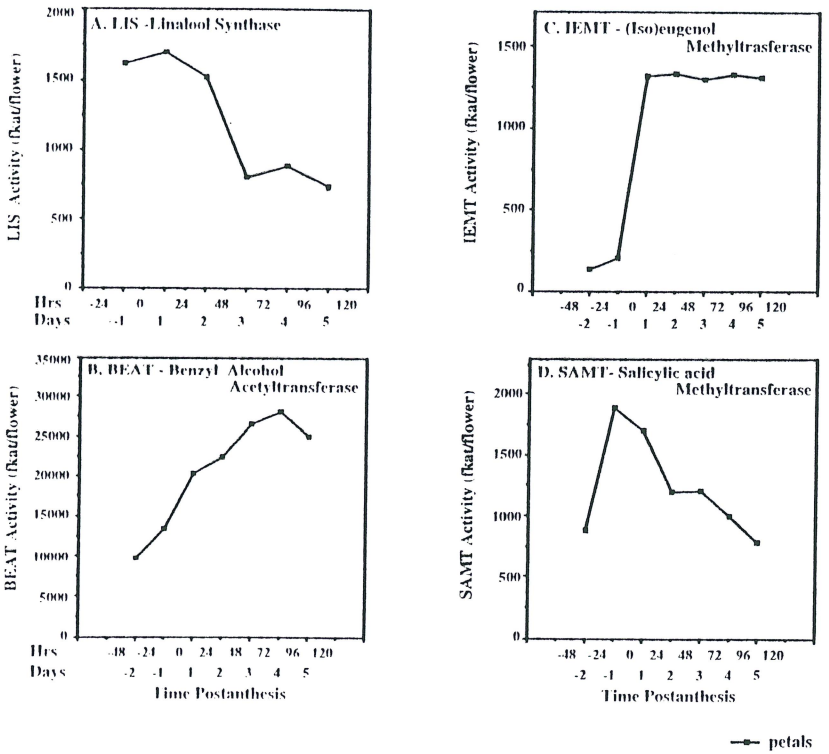

Fig. 2.3. Developmental regulation of activity of biosynthetic enzymes involved in floral scent production in *Clarkia breweri*. A—linalool synthase (LIS), B—acetyl-CoA: benzylalcohol acetyltransferase (BEAT), C—S-adenosyl-L-methionine:(iso)eugenol methyltransferase (IEMT), D—S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase (SAMT).

group of enzymes, such as IEMT and BEAT (Fig. 2.3 B, C), show little or no decline at the end of the lifespan of the flower, although emission of methyleugenol, isomethyleugenol, and benzylacetate do decline. The difference in developmental profiles of the latter two enzymes was that IEMT levels peaked on day 1 of anthesis and stayed stable afterward (Wang et al. 1997), whereas BEAT activity did not peak until the 4th day after anthesis (Dudareva et al. 1998a). The causes and consequences of high 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 produced in the reactions catalyzed by these enzymes are required for additional processes in the flowers other than scent emission. A third possibility is that as the flower ages, substrates may be diverted to other compartments and are not accessible to the scent biosynthetic enzymes.

The mRNAs for three isolated genes encoding scent biosynthetic enzymes in the *C. breweri* flower (LIS, IEMT and BEAT) were first detected in petal cells just before the flower opened and their levels increased until it peaked at or around anthesis, and afterwards began to decline (Dudareva et al. 1996, 1998b; Wang et al. 1997). For all three enzymes, the peak of mRNA was always 1–2 days ahead of enzyme activity and emission of corresponding component. In case of linalool, levels of emission, enzyme activity, and mRNA in the petals all rose and fell in parallel until the end of the lifespan of the flower (Fig. 2.2), whereas the situation with IEMT and BEAT was somewhat different. Isomethyleugenol and methyleugenol emission, IEMT activity, and mRNA levels in the petals all increased in parallel as the buds matured and the flowers opened. However, starting from the 3rd day post-anthesis (1 day after the stigma becomes receptive and most pollination occurs), emission began to decline but IEMT activity remained relatively stable. IEMT mRNA levels actually went up a little after declining 25% from their peak on the day before anthesis. In case of benzylacetate, the levels of BEAT mRNA in petals increased as the bud matured, and peaked at anthesis, paralleling changes in BEAT activity and emission. However, after the second day post-anthesis, mRNA levels declined sharply, whereas BEAT specific activity continued to increase and only began to decrease on day 5 after anthesis, suggesting that the BEAT protein is relatively stable. Overall, the data show that strong positive correlation exists among levels of enzyme activity, mRNA at the site of biosynthesis and emission of corresponding component in the *C. breweri* flower before and one day after anthesis, suggesting that the activity of scent

biosynthetic enzymes is regulated at a pretranslational level. These results also suggest a common regulatory mechanism for genes involved in scent production. Quantitative and/or qualitative changes of floral scent chemistry during flower development may be part of a mechanism to differentiate attraction cues between pollinated and unpollinated flowers.

B. Circadian Rhythm of Emission

While many plants continuously emit odor during flowering at a constant level, other flowers emit scent with the level of emission increasing or decreasing periodically. Yet other plants emit scent only at specific times during the day (diurnal emitters) or during the night (nocturnal emitters). In addition, some plants emit one set of compounds during the day and others during the night. Plant species that belong to one or the other category are listed in Table 2.1. The diurnal and nocturnal emission of odor correlates well (1) with the type of insect that pollinates the flower, e.g., the day-active insect (e.g. bees) and the night-active moths, and (2) with the time of flower opening either at a particular time of day and/or during a particular developmental phase. Odor is usually only emitted during a short period of flowering (a few days); thereafter no volatile compound can be detected, not even at constant

Table 2.1. Fragrance emission pattern.

Type	Species	Reference
Diurnal	*Citrus medica*	Matile and Altenburger 1988
	Odontoglossum constrictum	Matile and Altenburger 1988
	Ophrys sphegodes	Schiestl et al. 1997
	Platanthera chlorantha	Nilsson 1983
	Rosa hybrida (Hybrid tea)	Kaiser 1991
Nocturnal	*Cestrum nocturnum*	Overland 1960
	Hoya carnosa	Matile and Altenburger 1988
	Hyacynthus orientalis	Kaiser 1991
	Nicotiana sylvestris	Loughrin et al. 1991
	Nicotiana suaveolens	Loughrin et al. 1991
	Ophrys sphegodes	Schiestl et al. 1997
	Platanthera chlorantha	Nilsson 1983
	Stephanotis floribunda	Matile and Altenburger 1988
Constant[z]	*Malus × domestica*	Loughrin et al. 1990
	Nicotiana otophora	Loughrin et al. 1990

[z]Aromatic and benzyl alcohol compounds are present at constant levels throughout the day.

levels. For example, in *Nicotiana sylvestris*, *Hoya carnosa*, and *Stephanotis floribunda* the diurnal/nocturnal emission of flower volatiles was observed for four to seven days after anthesis (Matile and Altenburger 1988; Loughrin et al. 1991; Altenburger and Matile 1988).

The appearance of flower odor at specific times during the day is a prerequisite for pollination by diurnal or nocturnal insects. The regulation of the cycling of fragrance emission can be induced by either illumination or darkness, or alternatively be controlled by an endogenous clock. To find out whether diurnal or nocturnal odor emission is regulated by an internal mechanism, it is necessary to exclude possible external stimuli from the experiment. This is done by analyzing scent emission from plants grown under constant conditions, e.g. constant temperature and continuous illumination (LL) or constant temperature and continuous darkness (DD). If oscillations in scent emission are still observed under such conditions and have a periodic length of approximately 24 h, this means that such oscillations are controlled by an endogenous circadian clock. Results from such experiments are summarized in Table 2.2. The available data indicate that a circadian clock is likely to be a control mechanism involved in diurnal alterations of odor emission of several plant species. In the plants that show circadian rhythms, a peak fragrance emission occurs under free-running conditions (continuous light or continuous darkness) approximately every 25–28 h in flowers of intact plants and cut flowers of *N. suaveolens*, *N. sylvestris*, *H. carnosa*, *O. constrictum*, usually with a reduced or continuously dampening amplitude (Overland 1960; Loughrin et al. 1991; Altenburger and Matile 1988).

Table 2.2. Fragrance emission under constant conditions.

	Rhythmic appearance of fragrance			
Plant species	Continuous illumination	Continuous darkness	Period length[y] (h)	Reference
Cestrum nocturnum	+		25	Overland 1960
		+	28	
Hoya carnosa	+	n.d.[v]	29[x]	Altenburger and Matile 1988
Nicotiana suaveolens	+	n.d.	26–27[x]	Loughrin et al. 1991
Nicotiana sylvestris	+	n.d.	26–27	Loughrin et al. 1991

[y]Time between a maximum and next maximum of fragrance emission.

[v]n.d. = not detected.

[x]Entrainment adjusted after 2 days.

The period lengths of emission oscillations under free-running conditions are larger than 24 h, indicating that the endogenous clock controlling odor emission in flowers runs slower and is usually synchronized by environmental cues. The determination of odor emission to a precise time point during the day or night phase is predominantly synchronized by the day/night or night/day transitions, while alterations of temperature between day and night are usually less strong Zeitgebers (= time givers). Circadian oscillations are usually maintained throughout a wide temperature range (temperature compensation), but at unphysiological conditions, either the rhythm completely disappears or a rhythm with an extremely long period length occurs. The latter is observed in *Cestrum nocturnum* where a period length three times as long usually appears at 4°C (Overland 1960).

The observation of daily alterations of volatile appearance begs the question of the molecular mechanisms. Issues as yet to be addressed are: From what cellular compartment is the fragrance ultimately derived, and is its release controlled by a mechanism that is able to measure time? Does the synthesis of odor compounds depend on the time of day, and if so, how? Do the enzymes of the biosynthetic pathway differentially accumulate at different time points during the day or is their amount constant but their activity regulated by processes controlled by a circadian clock?

C. Differences in Rhythms of Emission of Specific Odor Components

As mentioned earlier, different volatiles emanating from the same flower may show independent patterns of emission oscillations. It is therefore of interest to distinguish between different volatiles of an odor bouquet and their characteristic emission pattern, e.g. rhythmic or constant appearance. This aspect is summarized in Table 2.3. In *H. carnosa*, five volatiles—linalool, cineole, β-pinene, iso-pentanol, and methylsalicylate (listed in order of their relative quantity: 21:4:2:1.5:1)—were identified that are major components of the odor, while a wealth of minor components of unknown identity possibly influence the scent specification as well. The emission of these five volatiles occurs during night (with a peak at 3 A.M.) and is in marked synchrony in continuous light conditions and light/dark entrained conditions (Matile and Altenburger 1988; Altenburger and Matile 1988). The oscillation in emission of these volatiles, with dampening amplitudes, is detected for four days in attached flowers of *H. carnosa*. Similar results were obtained with *O. constrictum* with two unidentified volatiles, and in *C. medica*, a

Table 2.3. Diurnal/circadian appearance of different odor volatiles.

Daylength	Species	Floral scent compounds	Reference
Long day	*Citrus medica*	linalool, nerolidol	Matile and Altenburger 1988
	Malus × *domestica*	*cis*-3-hexenyl acetate, *cis*-3-hexen-1-ol	Loughrin et al. 1990
	Nicotiana othophora	sabinene	Loughrin et al. 1990
	Odontoglossum constrictum	two unknown compounds	Matile and Altenburger 1988
	Ophrys sphegodes“	nonanal, decanal, hexadecane, 2-nonanone	Schiestl et al. 1997
	Platanthera chlorants	β-caryophyllene	Nilsson 1978, 1983
	Rosa hybrida	*cis*-hexenyl acetate, n-hexyl acetate.	Kaiser 1991
		trans-2-hexenyl acetate	
	Stanhopea anfracta	myrcene, ipsdienol	Curry et al. 1987
	Stephanotis floribunda	1-nitro-2-phenylethane	Matile and Altenburger 1988
Short day	*Hoya carnosa*	linalool, 1.8 cineole, α-pinene, β-pinene, iso-pentanol	Matile and Altenburger 1988
	Nicotiana suaveolens	sabinene, methylsalicylate, methylbenzoate	Loughrin et al. 1991
	Nicotiana sylvestris	benzyl acetate, benzyl alcohol, benzaldehyde. methylbenzoate	Loughrin et al. 1990, 1991
	Ophrys sphegodes“	limonene, α-pinene, heptanal, 1.8-cineole, 2-nonanol. 6-methyl-5-hepten-2-one	Schiestl et al. 1997
	Platanthera bifolia	methylbenzoate, linalool	Nilsson 1983; Tollsten and Bergstrom 1989
	Platanthera chlorantа	methylbenzoate, lilac alcohols, monoterpenes	Nilsson 1978, 1983
	Platanthera stricta	linalool	Patt et al. 1988
	Stephanotis floribunda	linalool, methylbenzoate	Matile and Altenburger 1988
12 h day	*Malus* × *domestica*	linalool, benzyl acetate, benzyl alcohol, 2-phenethyl alcohol	Loughrin et al. 1990
	Nicotiana othophora	myrcene, *trans*-β-ocimene, benzaldehyde. benzyl acetate, benzyl alcohol	Loughrin et al. 1990
	Nicotiana sylvestris	caryophyllene, linalool, α-terpineol, *cis*-jasmone	Loughrin et al. 1990, 1991
	Platanthera stricta“	α-pinene, myrcene, limonene	Patt et al. 1988

(Original paper lists more than 20 compounds, but only a few that occur at relatively high levels are listed here.

major component with rhythmic appearance was linalool, while oscillations with very small amplitude were measured for nerolidol (Matile and Altenburger 1988). It is worth noting that the volatiles of the latter two plant species were measured from excised flowers. This aspect is important, because excision can result in a dramatic loss of fragrance, e.g. *H. carnosa* (Matile and Altenburger 1988) and/or in major differences in fragrance composition compared to emission by attached flowers, e.g. yellow tea rose (Mookherjee et al. 1989; Kaiser 1991).

Synchronized rhythmic emanation of 2,6-dimethyl-3(E),5(E),7-octatrien-2-ol and its 5(Z) isomer, with a maximum between 6 and 10 P.M. was observed in *H. orientalis* (Kaiser 1991). Flowers of *S. floribunda* exhibit remarkable rhythmicity of three volatiles, methylbenzoate, RT 10.72 (tentatively identified as 1-nitro-2-phenylethane), and linalool (listed in order of their relative quantity: 4–7:2:1). Interestingly, the emission peaks of methylbenzoate and linalool occur at midnight, while the RT 10.72 compound peaks at noon, indicating that the appearance of the volatiles in this plant species does not coincide. Differences in daily odor composition was also detected in the genus *Ophrys*, a group of well-studied Mediterranean orchids. Comparison of volatiles at night versus day in *O. sphegodes* revealed that nocturnal emission contained significantly lower amounts of most aldehydes and 6-methyl-5-hepten-2-one and significantly higher amounts of most hydrocarbons, α-pinene, limonene, 1,8-cineole and 2-nonanol when collected during night, although the total amount of scent emission increased during night (Schiestl et al. 1997). Nilsson (1983) also found great differences in scent production during day and night of *Platanthera chlorantha*.

The data summarized in this section indicate that, in many plants, the odor composition and total emission output vary between day and night. Although it is clear that odor variability is important in influencing the behavior of the pollinators, and therefore plant fitness, the mechanisms responsible for the circadian rhythm in scent emission and for the temporal changes in scent composition presently remain unknown.

V. CONCLUSIONS

Fruit set in many agricultural and horticultural crops, such as most fruit trees, berries, nuts, oilseeds, and vegetables, rely on insect pollinators that are attracted by floral scents. Floral scent is typically a complex mixture of low molecular weight compounds, which gives the flower its unique characteristic fragrance. Although several thousand compounds have been identified from various floral scents and the chemical structures of most are known today, only a few studies have focused upon the biosynthesis of these compounds in planta. Recent investigations of floral scent production in *Clarkia* are the first examples of the isolation of enzymes and genes responsible for the formation of scent volatiles. In these investigations, it has been shown that scent compounds are produced de novo in open flowers, and that their emission levels, corresponding enzyme activities, and mRNA levels are all spatially and temporally correlated. However, our understanding of floral scent biosynthesis and its regulation is limited and based on analysis of a single model system in *Clarkia*. Obviously more research is needed in this field.

An understanding of the molecular, genetic, and biochemical basis of scent formation in plants will provide the knowledge for engineering plants with improved scent quality. Bioengineering of the metabolic pathways responsible for the production of volatile compounds in plants can involve either the modification of existing pathways and/or the introduction of new enzymes to produce novel products not normally found in the plant. The availability of an increasing number of cloned genes encoding scent biosynthetic enzymes is the first step toward engineering transgenic plants with modified volatile composition.

Development of crops with modified composition of volatiles and new introduced aroma could benefit agriculture by increasing crop productivity, pest resistance, and the value of ornamentals. Modified floral scent composition can increase attraction of flowers to pollinators and thereby increase reproduction efficiency and the yield of important agricultural crops. In addition, floral scent modification could be manipulated as a means of attracting beneficial insects and predators, and perhaps deter harmful ones. The manipulation of floral scents would be of immediate value for the floricultural industry. A large number of commercial flower cultivars have lost their scent during the selection and breeding processes due to the initial focus on maximizing postharvest shelf life and shipping characteristics. The lack of scent has long been recognized as a major problem in floriculture and a transgenic plant approach may help to solve this problem.

LITERATURE CITED

Ackermann, I. E., D. V. Banthorpe, W. D. Fordham, J. P. Kinder, and I. Poots. 1989. β-glucosides of aroma components from petals of Rosa species: assay, occurrence, and biosynthetic implications. J. Plant Physiol. 134:567–572.

Altenburger, R., and P. Matile. 1988. Circadian rhythmicity of fragrance emission in flowers of *Hoya carnosa* R.Br. Planta 174:248–252.

Arditti, J. 1979. Aspects of the physiology of orchids. In H. W. Woolhouse (ed.), Advances in botanical research, Vol. 7. p. 422–697. Academic Press, London.

Ayasse, M., F. P. Schiestl, H. F. Paulus, D. Erdmann, and W. Francke. 1996. Does an Ophrys plant cheat a pollinating male bee more than once? Proc., XX Int. Congr. on Entomology, Firenze. p. 225.

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

Bruneton, J. 1995. Parmacognosy, phytochemistry, medicinal plants. Elsevier Publ., New York.

Creelman, R. A., and J. A. Mullet. 1997. Biosynthesis and action of jasmonate in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:355–381.

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

Curry, K. J. 1987. Initiation of terpenoid synthesis in osmophores of *Stanhopea anfracta* (Orichadaceae): a cytochemic study. Am. J. Bot. 74:1332–1338.

DeGrandi-Hoffman, G. 1987. The honey bee pollination component of horticultural crop production system. Hort. Rev. 9:237–272.

Dobson, H. E. M. 1993. Floral volatiles in insect biology. p. 47–81. In: E. Bernays (ed.), Insect-plant interactions, Vol. V. CRC Press, Boca Raton, FL.

Dodson, C., R. Dressler, H. Hills, R. Adams, and N. Williams. 1969. Biologically active compounds in orchid fragrances. Science 164:1243–1249.

Dudareva, N., L. Cseke, V. M. Blanc, and E. Pichersky. 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., J. C. D'Auria, K. H. Nam, R. A. Raguso, and E. Pichersky. 1998b. Acetyl CoA:benzylalcohol acetyltransferase—an enzyme involved in floral scent production in *Clarkia breweri*. Plant J. 14:297–304.

Dudareva, N., R. A. Raguso, J. Wang, J. R. Ross, and E. Pichersky. 1998a. Floral scent production in *Clarkia breweri*. III. Biosynthesis and emission of benzenoid esters. Plant Physiol. 116:599–604.

Henning, J. A., Y-S. Peng, M. A. Montague, and L. R. Teuber. 1992. Honey bee (*Hymenoptera: Apidae*) behavioral response to primary alfalfa (*Rosales: Fabaceae*) floral volatiles. J. Econ. Entomol. 85:233–239.

Galen, C. 1985. Regulation of seed set in *Polemonium viscosum*: floral scents, pollination and resources. Ecology 66:792–797.

Gerlach, G., and R. Schill. 1991. Composition of orchid scents attracting euglossine bees. Bot. Acta 104:379–391.

Gunata, Y. Z., C. L. Bayonove, R. L. Baumes, and R. E. Cordonnier. 1985. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. J. Chromatography 331:83–90.

Kaiser, R. 1991. Trapping, investigation, and reconstitution of floral scent. p. 213–248. In: P. Muller and D. Lamparsky (eds.), Perfume: Art, science, and technology. Elsevier Publ., New York.

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

Knudsen, J. T., L. Tollsten, and G. Bergstrom. 1993. Floral scents—a check-list of volatile compounds isolated by head-space techniques. Phytochemistry 33:253–280.

Kutchan, T. M. 1995. Alkaloid biosynthesis—the basis for metabolic engineering of medicinal plants. Plant Cell 7:1059–1070.

Lewinsohn, E., N. Dudai, Y. Tadmor, I. Katzir, U. Ravid, E. Putievsky, and D. M. Joel. 1998. Histochemical localization of citral accumulation in lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf., Poaceae). Ann. Bot. 81:35–39.

Lewinsohn, E., M. Gijzen, T. J. Savage, and R. Croteau. 1991. Defense mechanisms in conifers. Relationship of monoterpene cyclase activity to anatomical specialization and oleoresin monoterpene content. Plant Physiol. 96:38–43.

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

Loughrin, J. H., T. R. Hamilton-Kemp, R. A. Anderson, and D. F. Hildebrand. 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.

Loughrin, J. H., T. R. Hamilton-Kemp, R. A. Anderson, D. F. Hildebrand. 1991. Circadian rhythm of volatile emission from flowers of *Nicotiana sylvestris* and *N. suaveolens*. Physiol. Plant. 83:492–496.

Loughrin, J., T. Hamilton-Kemp, H. R. Burton, R. A. Anderson, and D. F. Hildebrand. 1992. Glycosidically bound volatile components of *Nicotiana sylvestris* and *N. suaveolens* flowers. Phytochemistry 31:1537–1540.

Matile, P., and R. Altenburger. 1988. Rhythms of fragrance emission in flowers. Planta 174:242–247.

McGarvey, D. J., and R. Croteau. 1995. Terpenoid metabolism. Plant Cell 7:1015–1026.

Mettal, U., W. Boland, P. Beyer, and H. Kleinig. 1988. Biosynthesis of monoterpene hydrocarbons by isolated chromoplasts from daffodil flowers. Eur. J. Biochem. 170:613–616.

Mookherjee, B. D., R. W. Trenkle, and R. A. Wilson. 1989. Live vs. dead. Part II. A comparative analysis of the headspace volatiles of some important fragrance and flavor raw materials. J. Ess. Oil. Res. 2:85–90.

Nilsson, L. A. 1978. Pollination ecology and adaptation in *Platanthera chlorantha* (Orchidaceae). Botaniska Notiser 131:35–51.

Nilsson, L. A. 1983. Processes of isolation and introgressive interplay between *Platanthera bifolia* (L) Rich and *P. chlorantha* (Custer) Reichb. (Orchidaceae). Bot. J. Linn. Soc. 87:325–350.

Overland, L. 1960. Endogenous rhythm in opening and odor of flowers of *Cestrum nocturnum*. Am. J. Bot. 47:378–382.

Patt, J. M., D. F. Rhoades, and J. A. Corkill. 1988. Analysis of the floral fragrance of *Platanthera stricta*. Phytochemistry 27:91–95.

Pellmyr, O. 1986. Three pollination morphs in Cimicifuga simplex: incipient speciation due to inferiority in competition. Oecologia 8:304–307.

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

Pogorel'skaya, A. N., A. A. Prokof'ev, and S. A. Reznikova. 1980. Transport of monoterpenol and 2-phenylethanol alcohol glucosides from rose leaves to petals during flower maturation. Fiziol. Rast. (Moscow) 27:530–535.

Raguso, R. A., and O. Pellmyr. 1998. Dynamic headspace analysis of floral volatiles: a comparison of methods. OIKOS 81:238–254.

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

Schiestl, F. P., M. Ayasse, H. F. Paulus, D. Erdmann, and W. Francke. 1997. Variation of floral scent emission and post pollination changes in individual flowers of *Ophrys sphegodes* subsp. *Sphegodes*. J. Chem. Ecology 23:2881–2895.

Schnitzler, J. P., J. Madlung, A. Rose, and H. U. Seitz. 1992. Biosynthesis of β-hydroxybenzoic acid in elicitor-treated carrot cell cultures. Planta 188:594–600.

Smith, B. H., and M. Ayasse. 1987. Kin-based male mating preferences in two species of halictine bees. Behav. Ecol. Sociobiol. 20:313–318.

Stern, W., K. Curry, and W. M. Whitten. 1986. Staining fragrance glands in orchid flowers. Bul. Torrey Bot. Club 113:288–297.

Sugden, E. 1986. Anthecology and pollinator efficacy of *Styrax officinale* subsp. *redivivum* (Styracaceae). Am. J. Bot. 73:919–930.

Tang, J., Y. Zhang, T. G. Hartman, R. T. Rosen, and C. T. Ho. 1990. Free and glycosidically bound volatile compounds in fresh celery (*Apium graveolens* L.). J. Agr. Food Chem. 38:1937–1940.

Tollsten, L. 1993. A multivariate approach to post-pollination changes in the floral scent of *Platanthera bifolia* (Orchidaceae). Nord J. Bot 13:495–499.

Tollsten, L., and J. Bergstrom. 1989. Variation and post-pollination changes in floral odors released by *Platanthera bifolia* (Orchdaceae). Nord. J. Bot. 9:359–362.

Traub, H., T. Robinson, and H. Stevens. 1942. Papaya production in the United States. U.S. Dept. Agr. Cir. 633.

Van Dort, H., P. Jaegers, R. Ter Heide, and A. Van der Weerdt. 1993. *Narcissus trevithian* and *N. geranium*: analysis and synthesis of compounds J. Agr. Food Chem. 41:2063–2075.

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

Wang, J., and E. Pichersky. 1997. Nucleotide sequence of caffeic acid O-methyltransferase from *Clarkia breweri* (Accesssion No. AF006009) (PGR97-104). Plant Physiol. 114:1567.

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

Watanabe, N., S. Watanabe, R. Nakajima, J. H. Moon, K. Shimokihira, J. Inagaki, H. Etoh, T. Asai, K. Sakata, and K. Ina. 1993. Formation of flower fragrance compounds from their precursors by enzymic action during flower opening. Biosci. Biotech. Biochem. 57:1101–1106.