IN THIS ISSUE

A Rose by Any Other Name?

"What's in a name? That which we call a rose, by any other word would smell as sweet." So declares Juliet as she laments the name of her beloved in Shakespeare's *Romeo and Juliet*. The fact is, today there are numerous varieties of ornamental rose that produce little or no fragrance. Garden roses of the 1500s (e.g., Damask, Alba, and Gallica varieties and Shakespeare's eglantine, *Rosa eglanteria*) were exceptionally fragrant, but the flowers tended to be less showy and shorter lived than modern roses. Conversely, many modern Hybrid Tea or Floribunda varieties that bear large, showy flowers and have long vase lives lack a strong fragrance. In this issue of *The Plant Cell*, **Guterman et al. (pages 2325–2338)** report on the identification of genes associated with floral fragrance in *Rosa hybrida* via a genomic approach that encompasses cDNA sequencing, microarray gene expression analysis, chemical analysis of the volatile composition of rose petals, and biochemical analysis of candidate proteins.

Floral scents are complex mixtures of chemicals. Although there are several main groups of compounds (e.g., monoterpenes and sesquiterpenes, aromatic alcohols, and esters), floral scent is highly species specific, and almost no two species produce identical mixtures of scent compounds. Even within species, often there is a great deal of variability in scent production. Rose is a prime example: many cultivars produce little or no scent, and among those that do, there is considerable variability in the type of scent produced.

Guterman et al. analyzed two cultivars of *R. hybrida*, Fragrant Cloud (FC), which produces large, strongly scented red flowers, and Golden Gate (GG), which yields smaller yellow flowers that have almost no fragrance (Figure 1). Gas chromatography–mass spectrometry analyses of the flower headspace showed that FC flowers

emit typical rose fragrance volatiles, including various esters, aromatic and aliphatic alcohols, monoterpenes, and sesquiterpenes. By contrast, 99% of the volatiles emitted by GG flowers were methoxylated phenols, which lack a distinct fragrance. Volatile emission in both cultivars was low early in flower development and increased to a peak as the flowers reached full bloom (developmental stages 4 to 6). The group created cDNA libraries using RNA isolated from stage-4 petals from each of the cultivars and sequenced 1834 and 1039 clones from the FC and GG libraries, respectively. A large percentage of cDNAs representing unique genes were found only in the FC database

(1288 genes) or only in the GG database (746 genes). This is probably attributable to the low redundancy of the library and the fact that the database is still far from saturation. A high percentage of unique genes showed homology with genes that encode proteins of unknown function (32%) or had no known homologs in plants or other species (17%). Therefore, these databases may represent a rich mine of novel genes, many of which are likely to have specific roles in petal development and/or secondary metabolism.

Guterman et al. conducted microarray gene expression analysis to compare the expression levels of a subset of 350 of the cloned genes in mature FC compared with

Figure 1. *Rosa hybrida* Cultivars Fragrant Cloud (Red) and Golden Gate (Yellow).

Fragrant Cloud flowers emit a strong fragrance comprising volatile esters, aromatic and aliphatic alcohols, monoterpenes, and sesquiterpenes. By contrast, Golden Gate flowers are nonscented, and volatiles emitted from these flowers are principally nonscented methoxylated phenols.

IN THIS ISSUE

GG petals and in stage-1 (young) compared with stage-4 (mature) FC petals. From this analysis, 40 genes were identified that showed upregulated expression during FC petal development and were expressed at a higher level in mature FC than in mature GG petals; thus, they were potential candidates for genes involved in scent production in FC petals.

One gene that showed strong upregulation in mature FC petals and no expression in GG petals was selected for detailed functional analysis because the DNA sequence was similar to those of known sesquiterpene synthase genes and FC petals produced relatively high levels of volatile sesquiterpenes. The gene product was shown to have germacrene D synthase activity in vitro when provided with farnesyl diphosphate as a substrate. Germacrene D was the major sesquiterpene emitted from FC petals and was not detected in the headspace of GG flowers, further suggesting that this gene product functions as germacrene D synthase in vivo.

Previous research on the genetic and biochemical pathways involved in the biosynthesis of floral scent compounds and the evolution of floral scent has focused largely on *Clarkia breweri* and *Antirrhinum majus*. These studies have confirmed that the ability to produce floral scent is easily acquired and easily altered or lost in natural populations as well as among cultivated species (Dudareva and Pichersky, 2000). For example, *C. breweri*, the flowers of which emit a strong, sweet scent, is believed to have evolved from the nonscented *C. concinna*. A major component of *C. breweri* fragrance is *S*-linalool, which is produced from geranyl pyrophosphate in a one-step reaction catalyzed by *S*-linalool synthase (LIS). *LIS* gene expression and protein activity is detected only in stigmata, petals, and other floral tissues in *C. breweri* and is concentrated in the secretory zone of the stigma and the epidermal layers of petals (Dudareva et al., 1996). Gene expression and enzyme activity appear first in the late bud stage and increase to a peak just after the flowers open, corresponding to peak scent emis-

sion. *LIS* gene expression and enzyme activity also was detected in *C. concinna*, but only in the stigma and at relatively low levels. Cseke et al. (1998) analyzed the coding and promoter regions of the *LIS* gene in *C. breweri* and *C. concinna* and found several insertions in the *C. concinna* promoter that do not occur in *C. breweri*. Thus, the ability of *C. breweri* flowers to produce relatively high amounts of *S*-linalool apparently is related to an alteration in the regulation of *LIS* gene expression and the corresponding enzyme activity.

The volatile phenylpropanoids methyleugenol and isomethyleugenol are two other major components of *C. breweri* fragrance. Wang and Pichersky (1999) showed that the ability of *C. breweri* to produce these compounds is the result of the recent evolution of a gene encoding *S*-adenosyl-L-Met:(iso)eugenol *O*-methyltransferase from a caffeic acid methyltransferase gene. Another scent-related gene that encodes acetyl-CoA:benzylalcohol acetyltransferase, which catalyzes the production of volatile benzylacetate, is highly expressed in *C. breweri*, but alternative transcript processing yields low gene expression and a gene product with altered enzyme activity in *C. concinna* (Dudareva and Pichersky, 2000). Thus, alterations in the coding and/or regulatory regions of a number of genes appear to be responsible for the evolution of a complex scent mixture in *C. breweri* compared with its nonscented progenitor *C. concinna*. It will be of interest to compare the germacrene D synthase gene coding and promoter regions in FC versus GG plants. Is the gene present in the GG cultivar but fails to produce an active gene product as a result of an alteration in the coding and/or noncoding regulatory region?

Although it is a major sesquiterpene emitted from FC flowers, germacrene D constituted <10% of the total volatiles measured in FC headspace. Also present, but not detected in the headspace of GG flowers, were various aromatic and aliphatic alcohols, monoterpenes, and esters. Guterman et al. identified several other genes whose expression increased

during FC petal development. Overexpression of these genes in bacteria and in vitro biochemical assays using bacterial lysate provided with various potential substrates identified protein products having enzyme activity consistent with a role in scent production, including an acetyltransferase and two *O*-methyltransferases (see also Lavid et al., 2002). Thus, *Rosa* spp promise to yield a wealth of additional information on the evolution, genetics, and biochemistry of floral scent production.

The sequence data presented by Guterman et al. represent a significant contribution to ESTs available for *Rosa* spp. Channelière et al. (2002) recently created another rose petal database, consisting of 1794 ESTs, representing ~900 unique genes, from a cDNA library from *Rosa chinensis* cv Old Blush. Similar to the FC and GG data collected by Guterman et al., ~36% of the genes were classified as having unknown function and/or no known homologs. The *R. chinensis* database is of particular interest because this species served as a progenitor for *R. hybrida* and contributed recurrent flowering and fragrance characters to many modern cultivars (Channelière et al., 2002). Thus, comparative analyses of scent-related genes, as well as genes associated with other petal characters, between *R. hybrida* and *R. chinensis* will be of considerable interest.

One of the major contributions of the work presented by Guterman et al. is the combination of sequencing and gene expression data with functional biochemical assays and metabolite analyses (in this case, volatile compounds emitted from petals) to test putative gene functions. Pichersky and Gang (2000) noted that assigning gene function based on sequence similarity may be particularly risky for scent-related genes—and for other genes involved in secondary metabolism—because of the variability within large gene families for numerous secondary metabolism genes and the observation that small changes in amino acid and/or promoter sequences can bring about large changes in enzyme activity and/or spatial and temporal expression. Of course, in vitro assays do not

prove the in vivo activity of an enzyme. For example, it is possible that the "germacrene D synthase" identified by Guterman et al. acts on a substrate other than farnesyl phosphate in vivo in FC flowers. However, the combination of in vitro biochemical assays with gene expression analysis and chemical analysis of floral headspace, indicating that germacrene D is a significant component of FC volatiles, is strong evidence that the enzyme acts as germacrene D synthase in vivo. An important test is to provide alternative substrates in vitro, and Guterman et al. noted that no monoterpenes were produced when geranyl diphosphate was presented as the substrate.

It also is important to note that forward genetics is not a particularly useful tool for isolating scent-related genes, because it is very difficult to screen for scent mutants. The search for scent-related genes is further complicated by the low level of volatile production from Arabidopsis flowers (and the corresponding lack of information related to scent production in the Arabidopsis genome). Guterman et al. provide an elegant demonstration of how genomics tools can overcome these limitations, in particular when used with cultivars that show contrasting phenotypes, and in combination with biochemical analyses to examine putative gene functions, to identify agriculturally important, cultivar-specific genes. Further studies of this nature can be expected to yield more valuable information about the genetics and biochemistry of floral scent production. In the meantime, we may be content to contemplate which fragrance-related genes inspired Shakespeare to pen some of his most exquisite lines:

I know a bank where the wild thyme blows,

Where oxlips and the nodding violet grows,

Quite over-canopied with luscious woodbine,

With sweet musk-roses and with eglantine:

There sleeps Titania sometime of the night,

Lull'd in these flowers with dances and delight....

Oberon to Puck in *A Midsummer Night's Dream*

Nancy A. Eckardt
News and Reviews Editor
neckardt@aspb.org

REFERENCES

Channelière, S., Rivière, S., Scalliet, G., Szecsi, J., Jullien, F., Dolle, C., Vergne, P., Dumas, C., Bendahmane, M., Hugueney, P., and Cock, M. (2002). Analysis of gene expression in rose petals using expressed sequence tags. FEBS Lett. **515,** 35–38.

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

Dudareva, N., Cseke, L., Blanc, V.M., and 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., and Pichersky, E. (2000). Biochemical and molecular genetic aspects of floral scents. Plant Physiol. **122,** 627–633.

Guterman, I., et al. (2002). Rose scent: Genomic approach to discovering novel floral fragrance-related genes. Plant Cell **14,** 2325–2338.

Lavid, N., et al. (2002). *O*-Methyltransferases involved in the biosynthesis of volatile phenolic derivatives in rose petals. Plant Physiol. **129,** 1899–1907.

Pichersky, E., and Gang, D. (2000). Genetics and biochemistry of secondary metabolites in plants: An evolutionary perspective. Trends Plant Sci. **5,** 439–445.

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

A Rose by Any Other Name?

Nancy A. Eckardt

This information is current as of October 19, 2019

References	This article cites 8 articles, 4 of which can be accessed free at: /content/14/10/2315.full.html#ref-list-1
Permissions	https://www.copyright.com/ccc/openurl.do?sid=pd_hw1532298X&issn=1532298X&WT.mc_id=pd_hw1532298X
eTOCs	Sign up for eTOCs at: http://www.plantcell.org/cgi/alerts/ctmain
CiteTrack Alerts	Sign up for CiteTrack Alerts at: http://www.plantcell.org/cgi/alerts/ctmain
Subscription Information	Subscription Information for *The Plant Cell* and *Plant Physiology* is available at: http://www.aspb.org/publications/subscriptions.cfm