Morphogenesis of Flowers--Our Evolving View

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HISTORICAL PERSPECTIVE ESSAY

Morphogenesis of Flowers—Our Evolving View

In this essay, the time course of our understanding of the structure and function of flowers will first be outlined from prehistoric times to the mid-twentieth century. Information is taken mostly from Sachs (1875), Arber (1950), and especially Morton (1981). More recent studies on the genetic basis of flower development will then be reviewed, focusing on floral organs rather than ovules, seeds, or fruits. Finally, major gaps in our present understanding and possible future advances will be discussed.

EARLY IDEAS ABOUT FLORAL STRUCTURE AND FUNCTION

Until ~12,000 years ago, humankind survived by exploiting wild plants and animals for food and shelter. Since then, domestication of many species led to major population increases, urbanization, and the availability of time for creative pursuits. These changes have been associated with the rise of mechanical invention, literacy, and ultimately modern civilization.

The domestication of plants required knowledge of how to cultivate them successfully. However, the initial choice of species and the improvement of strains were rather haphazard and empirical processes. Improvement mostly relied on early farmers selecting seeds from the best performing plants of the current crop to grow in the next season. Occasionally, rare genetic variants or hybrids would arise that were seized on as offering major benefits in quality and yield. For example, domesticated maize differs from its wild Mexican ancestor teosinte by several major genetic variants that result in reduced branching and larger, nonshattering ears (Doebley, 2004). Also, the domestication of bread wheat in the Middle East included the selection of two sequential interspecies hybrids that apparently arose after spontaneous hybridization events between cultivated and weedy species (Feldman et al., 1995). Ancient civilizations did not know about plant sexual reproduction. The closest they got was to realize that there are two forms of date palm and that fruit set could be promoted if dust of a flowering shoot of a sterile tree was shaken over the flowering shoots of potentially fertile trees.

The first historical records of attempts to comprehend the general properties of plants are the writings of the Greek philosopher Theophrastus (~370–285 BCE). The father of botanical science, Theophrastus was a colleague of Aristotle, whom he succeeded as leader of the Lyceum. Theophrastus considered plants to be made up of persistent parts (roots, stems, branches, and twigs) and ephemeral parts (leaves, flowers, fruits, including seeds, and the stalks of these organs). In his view, flowers were basically defined by the petals, although they did include stamens and the styles of carpels. Sepals were considered small leaves, and the ovary seemed to be viewed not as a floral part but as the future fruit case. Thus, the flower represented only those organs that abscised from the developing fruit. Sexual reproduction was not understood or distinguished from vegetative reproduction. The role of stamens, the fertilization of ovules, and the universal occurrence of seeds as a stage in the life cycle were not comprehended.

For the next 1800 years, interest in the study of plants was mostly limited to their role in medicine, with descriptions of useful species being reproduced down the generations in compendia called herbals. In Oriental cultures, plant descriptions were particularly accurate, and they also included species of aesthetic attraction such as peonies, lilies, and chrysanthemums in addition to medicinal plants.

EUROPEAN AWAKENING: ANALYTICAL AND EXPERIMENTAL APPROACHES

In Europe from the sixteenth century on, attempts were renewed to understand the basic principles of plant structure, function, and classification. An Italian physician, Andrea Cesalpino (1519–1603), proposed the first natural system of plant classification (i.e., one in which plants are grouped by their degree of relationship) using their fructification properties. These included the position of the abscising floral organs on the seed case (i.e., whether the ovary is superior with the outer organs at its base or inferior [organs at its apex]), the number of seeds in a fruit, and the number of cavities (locules) per fruit. Joachim Jung (1587–1657), a professor of Natural Sciences in Hamburg, clarified the distinction between petals and the organs that surround the flower (which Jung called the perianthium and which includes what we now call floral bracts as well as sepals). He also noted that stamens are often divisible into a pediculus (filament) and a capitulus (head or anther).

The invention of the microscope allowed two major contributions to plant anatomy soon after. Italian Marcello Malpighi (1628–1694) and Englishman Nehemiah Grew (1641–1712) observed not only plant morphology, but they linked mature structures with their development for the first time. They recognized the generality of properties of different floral organs across species. For example, they observed that what we now call perianth organs (sepals and petals) are usually essentially leaf like and that stamens, almost universal components of flowers, always released dust from their upper regions. The Englishman John Ray (1623–1705) subsequently named this dust pollen and also distinguished between the calyx (now called sepals) and the internal corolla made up of showy "petals," a name he popularized and which came into common use. Thus, the components of the flower were now defined almost as in current usage except that the ovary was still not recognized as a floral component, only the style.

Surprising as it now seems, it wasn't until the end of the seventeenth century that the

role of pollen in fertilization was established and that sexual reproduction was confirmed in plants. Rudolph Jacob Camerarius (1665–1721), director of the Botanic Garden in Tübingen, Germany, observed that most flowers had stamens positioned close to the style that topped the future seed case. He proposed that such flowers were hermaphrodite and that pollen released from the anthers landed on the style and ensured that seeds subsequently developed. He concluded that petals were not involved because many flowers lack petals but set seeds (e.g., vines and cereals), and also some garden plant variants had extra petals at the expense of stamens (double flowers), and even though these may have styles, fruit was not usually set. Camerarius then performed experiments to test the role of pollen. He used plant species in which flowers of two types occurred: male (stamens only) and female (styles only). These were present either on separate plants (dioecious, including the mulberry Morus and dog's mercury Mercurialis) or on the same plant (monoecious, castor oil plant Ricinus and maize Zea). By preventing any pollen from landing on styles in each case, in 1694, he confirmed the necessary role of pollen in fruit and seed set. Again, it is surprising in hindsight how long it took for this conclusion to be extended to understanding the role of insects in transfer of pollen between anthers and styles (the 1760s by Joseph Gottlieb Koelreuter [1733–1806] in Karlsruhe) and the adaptation of many flowers to insect-driven cross-pollination rather than self-fertilization (in 1793 by Christian Konrad Sprengel [1750–1816] in Berlin).

The mechanics of fertilization were not fully defined until even later. In 1833, British botanist Robert Brown (1773–1858) confirmed that pollen tubes emerge from pollen grains and grow down the style, and he showed for the first time that a pollen tube enters the micropyle of an ovule immediately before the embryo starts to develop. But it wasn't until the 1840s that Wilhelm Friedrich Hofmeister (1824–1877), working in Hamburg, showed that the embryo was derived from the egg cell within the embryo sac, and the actual process of nuclear fusion of the sperm and egg nuclei was not recorded until 1878 by Eduard Strasburger of Bonn (1844–1912). More generally, it was Hofmeister who established in 1851 that alternating generations involving sexual reproduction (now called the sporophyte-gametophyte cycle) was a property common to bryophytes, ferns, lycopods, and gymnosperms as well as angiosperms. This was a major unifying theory that linked all land plants and was dependent on the key finding that ferns contained cells obviously equivalent to the male and female gametes of animals (motile sperm and sessile eggs). Interestingly, the chromosomal basis of this alternation of generations was not deduced until the end of the nineteenth century, another major contribution by Strasburger.

Returning to the eighteenth century, Swedish botanist Carolus Linnaeus (Carl von Linné, 1708–1778) had a major influence on plant science. Linnaeus' use of floral and fruit properties as the basis of classification of plants helped emphasize these structural features, although floral function was not involved. His hierarchical scheme was based firstly on stamen number and arrangement within the flower (Figure 1). He proposed 24 primary groups that he called classes, with the first 10 corresponding to plants with 1, 2, and so on up to 10 free stamens per flower all of the same length and the next 10 also including other stamen properties, such as different lengths and degrees of fusion to each other and to other floral organs. The last four groups included monoecious, dioecious, mixed dioecious-hermaphrodite, and apparently flowerless plants. Within the first 13 classes, Linnaeus then defined subgroups (orders) based on the number of styles per flower, and in the other classes he used fruit characters. As this scheme was based on stamens and styles/fruits, Linnaeus called it the "sexual system." He did not claim that this was a natural scheme, although related plants often clearly grouped together, but its simplicity and ease of use meant that it was widely followed until displaced by more natural schemes.

Linnaeus' second contribution, after classification, was the invention of the binomial system of nomenclature. Within his orders, he followed earlier authors in grouping plants into named genera with shared properties, in his case involving six parts: the calyx, corolla, stamens, pistil, pericarp, and seeds. But his innovation was to provide just one word to specify each plant type within a genus, rather than a wordy descriptive phrase. This word is now known as the species name, and his binomial system of nomenclature is followed to this day.

DEVELOPMENT OF IDEAS ABOUT FLOWER MORPHOLOGY AND MORPHOGENESIS

The study of plant development was not neglected. In 1790, the German poet Johann Wolfgang von Goethe (1749–1832) published an influential extended essay entitled "Versuch die Metamorphose der Pflanzen zu erklären" (An attempt to interpret the metamorphosis of plants) in 1790. The idea of metamorphosis had been proposed much earlier by Cesalpino and refined by Linnaeus. In Linnaeus' words "the flower [can be regarded] as the interior portions of the plant which emerge from the bursting rind; the calyx as a thicker portion of the shoot; the corolla as an inner and thinner rind; the stamens as the interior fibers of the wood, and the pistil as the pith of the plant" (Sachs, 1875). Based on his observations of variations in normal plant growth and on abnormalities that sometimes occur, Goethe (1790) provided an alternative view of metamorphosis: "The same organ which on the stem expands itself as a leaf, and assumes a great variety of forms, then contracts in a calyx—expands again in the corolla—contracts in the reproductive organs—and for the last time expands in the fruit." Goethe named the generalized organ "Blatt," and thought of it as a generalized plant organ rather than as a leaf, leaves being just one of the forms it could adopt. This simple and attractive proposal has influenced the thinking of plant scientists until the present day (see below).

In line with this scheme, sepals and petals are obviously similar in form to leaves, but stamens and carpels are less so. In this regard, Robert Brown independently obtained evidence that pointed to all floral organs sharing leaf-like properties.

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Figure 1. The Basis of Linnaeus' Sexual System for the Classification of Flowering Plants.

Linnaeus defined 24 classes based on the number of stamens per flower (A to N), their variable length (O to P), their degree of fusion (Q to U), and the presence of some flowers without stamens (V to Y) and of plants apparently without flowers (Z). He then subdivided classes into orders mostly based on the number of styles (carpels) in each flower. Each order was then again divided into genera based on six other floral and fruit characteristics. Finally, similar plants within a genus were given a single Latin descriptor, now know as the species name. The genus and species names are the binomial system we use today. (Watercolor by Georg Dionysius Ehret drawn in 1736. Original held in the Natural History Museum, London, UK, and reproduced with permission.)

For carpels, his interpretation was that the units of multilocular gynoecia can be thought of as leaf-like carpels joined along their edges. For both carpels and stamens, he proposed that the reproductive tissues (ovules and pollen) arose along the edges of the foliar-like organ. This interpretation of the gynoecium and its relationship to later developing fruit and seeds provided the basis for our modern view of the flower as including the ovary as well as the styles (and stigmas), rather than the former being looked on solely as the future fruit. In passing, in 1827, Brown clarified the difference between the naked seeds produced by conifers and cycads and the single-seeded indehiscent fruits that include a pericarp derived from the ovary (such as those that occur in grasses and daisies), leading to the establishment of the fundamental difference between gymnosperms and angiosperms.

A new developmental approach to the natural classification of plants was introduced in 1813 by the Swiss botanist Augustin-Pyrame de Candolle (1778–1841). de Candolle highlighted the symmetry of flowers, the number and relative placement of organs set up early in development, as being of key importance in classification. Furthermore, he identified three processes by which this symmetry could apparently be modified later in development: organ abortion, organ modification, and organ adherence, modifications that should be taken into account when grouping species with the same overall symmetry. Such similarities and differences were apparent in the increasingly accurate and detailed descriptions of early flower development highlighted by Jean-Baptiste Payer's (1818–1860) masterpiece ''Traité d'Organogénie Comparée de la Fleur'' (Treatise on the comparative organogenesis of flowers) published in Paris in 1857.

During the nineteenth century, details of the cellular basis of plant development were described. The universal presence of one nucleus in each cell had been established by Robert Brown, and the cell as the unit of all plant tissues generalized by Matthias Jakob Schleiden (1804–1881) of Jena in 1839. The fact that cells only arise

from preexisting cells by a process of binary separation was established in plants by the Swiss botanist Karl Wilhelm von Nägeli (1817–1891) in Munich in 1846, well before the same conclusion was reached by Virchow in animals (1859). The chromosomal events that underlie cell division awaited Eduard Strasburger's contribution at the end of the nineteenth century.

THE TWENTIETH CENTURY—MERISTEMS, COMPARATIVE MORPHOLOGY, AND EVOLUTION

The first half of the last century saw progress in our understanding of the properties of meristems (Wardlaw, 1965; Steeves and Sussex, 1989). The structure of shoot apical meristems and flower meristems was defined, and their functional partition into stem cells in the central zone, and organogenic cells in the peripheral zone, deduced. The origin and maintenance of epidermal (L1) and subepidermal (L2) cell layers was also followed through the use of genetically marked lineages. In addition, some understanding of the basis of the phyllotactic pattern of leaf initiation was obtained by manipulating the developing meristem, although these studies were not extended to flowers.

Study of the comparative development of flowers reached its zenith later in the twentieth century. The invention of the scanning electron microscope in 1952 allowed simple and detailed examination of early developmental events. These studies provided new characters, such as the order of initiation of floral organs, to help distinguish between closely related species. More generally, such comparative studies provided insights into the relative rates and possible directions of evolution of floral form. For example, Endress (1994) highlights three aspects of flower structure with different underlying rates of evolutionary change. He calls these organization, construction, and mode. Essentially, these correspond with, first, the floral blueprint (bauplan) that defines the number and position of organs (including their degree of fusion with each other), second, the basic three-dimensional structure of the

flower (gestalt), and third, the later elaboration of specialized characteristics (style). The blueprint is relatively stable in evolutionary terms, growth patterns that define the overall size and shape of the flower less so, and elaborations such as pollinator-specific colors and perfumes are relatively fluid.

The origin of flowers has been the subject of much speculation for the last 100 years (Arber, 1937; Cronquist, 1988; Doyle, 1994), although resolution is still not in sight. Two alternative schemes were proposed early: the euanthial theory, in which flowers arose de novo, and the pseudanthial theory, in which they arose through the combination of originally separate female and male inflorescence shoots. The main difficulty in distinguishing between these (in their many versions) is the lack of obviously ancestral fossils or of closely related extant groups. A recent euanthial scheme, the anthophyte hypothesis, proposed that the flower-like structures of living Gnetophytes and fossils, including the Bennettitales and the true flowers of Angiosperms, are evolutionarily related (Doyle, 1994). However, this particular scheme is now discredited because molecular phylogenetic study has revealed that the Gnetophytes are most closely related to conifers among the Gymnosperms and are not the sister group of the Angiosperms, and so their reproductive structures are presumably similar to flowers by convergent evolution (Crepet, 2000). The evolutionary origin of each type of floral organ also was not resolved (Arber, 1937; Cronquist, 1988; Endress, 1994).

On the other hand, firm deductions about the directions of floral evolution within the angiosperms have recently become possible. Accurate phylogenies of most orders and many families have been assembled from multiple data sets: morphology, the sequences of translated genes from nuclei, plastids and mitochondria, and the sequences of nuclear rRNA genes (Soltis et al., 1999; Angiosperm Phylogeny Group, 2003). These definite lineages have, for example, allowed the structure of ancestral flowers to be deduced as probably being small and primarily bisexual, with few to moderate numbers of organs often

with spiral phyllotaxy, sepals and petals often not distinguished, stamens with short filaments, and free carpels lacking styles and sealed by secretion (Endress, 2001a).

GENES, GENES, GENES

For many years, it had been clear that the development of flowers must be under genetic control. Mutants that disrupt the normal processes were well known. From the early 1980s, new technology allowed the responsible genes to be cloned, their molecular nature to be deduced, their expression patterns to be mapped, consequences of their loss or gain of function to be assessed, and their interactions with other genes, including related genes, to be determined. Access to the molecular and cellular functions of flower developmental genes was available at last (Lohmann and Weigel, 2002; Leyser and Day, 2003; Jack, 2004).

Organ Identity Genes

One question investigated in detail was how the individual developmental path followed by a newly arising organ—either sepal, petal, stamen, or carpel—was determined. The answers came from the study of mutants. Plant biologists have long been fascinated with the abnormal, the monstrous, and the defective (Meyerowitz et al., 1989). Goethe (1790) had drawn attention to observations of what he called abnormal metamorphosis. This refers to the situation where the organs that are normally formed on a plant in a time series (cotyledons, leaves, bracts, sepals, petals, stamens, and styles) "are sometimes transformed, so that they assume—either wholly or in some lesser degree—the form of the nearest in the series." Bateson (1894) invented a new name for abnormal metamorphosis, homeosis, arguing that "the essential phenomenon is not that there has been a change, but that something has been changed into the likeness of something else." Homeotic changes were recognized as providing clues about the normal organogenetic process (Meyerowitz et al., 1989), although some have erroneously argued

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that they represent atavistic reversions to more primitive forms.

It was argued that the normal function of such homeotic mutant genes was to define the identity of the organ. Their cloning and characterization would test this idea. Such an approach had proved spectacularly successful in insects in gaining an understanding of how body segment identity is determined through the action of homeobox genes (Bender et al., 1983). For plants, two model species were mainly involved, the mouse ear cress *Arabidopsis thaliana* and the snapdragon *Antirrhinum majus*. For Arabidopsis, its convenient genetics (Koornneef et al., 1983) and small genome allowed genes to be cloned based on their map position. For Antirrhinum, active transposable elements had already been cloned, and this, coupled with a large series of characterized flower mutants (Stubbe, 1966), was the basis of cloning of genes with unstable, transposon-induced mutants.

Based on single and multiple mutant phenotypes, it had already been proposed that organ identity genes ''allow cells to determine their place in the developing flower,'' and that they acted combinatorially by ''setting up or responding to concentric, overlapping fields within the flower primordium'' (Bowman et al., 1989). When homeotic genes were cloned, these predictions were borne out. The first to be cloned was the *DEFICIENS* gene of Antirrhinum (Sommer et al., 1990), soon followed by the *AGAMOUS* gene of Arabidopsis (Yanofsky et al., 1990). In each case, the genes encoded transcription factors related to several already known in humans and yeast and together were called the MADS family. The action of organ identity genes in Arabidopsis and Antirrhinum was soon summarized in the ABC model (Coen and Meyerowitz, 1991), now well known. It can be summarized as follows: A function defines sepals, A+B petals, B+C stamens, and C carpels, with A and C antagonizing each other's function. Subsequent studies have modified and refined the ABC model and extended it to many other species (Lohmann and Weigel, 2002; Jack, 2004).

An important recent discovery was that another set of MADS genes, *SEPALLATA1*,

2, and 3, is redundantly involved in defining the petal, stamen, and carpel domain of the flower primordium in Arabidopsis (Pelaz et al., 2000). SEPALLATA function, sometimes called E function, is sufficient to transform leaves into petal-like structures in combination with A and B function and into stamen-like organs with B and C function (Honma and Goto, 2001). This was soon nicely integrated with the finding that MADS polypeptides associate as multimers (Egea-Cortines et al., 1999), when pairs of known ABC MADS proteins were shown to also associate with SEPALLATA proteins (Honma and Goto, 2001).

Of the proposed organ identity functions, A function has not been confidently defined beyond Arabidopsis. The main MADS gene associated with A function, *APETALA1*, has an earlier flower meristem identity function that is present in many other species, but a role in sepal and petal identity specification elsewhere is not apparent. The other known A function gene from Arabidopsis, *APETALA2*, is a member of a different family of plant-specific transcription factors (Jofuku et al., 1994). Its ortholog from Antirrhinum occurs as recently duplicated genes, *LIPLESS1* and *2*, and these do not repress expression of C function genes, although they do influence sepal and petal development to some extent (Keck et al., 2003). *APETALA2* is expressed throughout the flower, even in the stamen and carpel regions where C function inhibits its action. Recently, it has been discovered that APETALA2 function is regulated posttrascriptionally by a specific microRNA miR172, with evidence that this includes inhibition of translation (Aukerman and Sakai, 2003; Chen, 2004). Thus, it seems that C function is inhibiting APETALA2 A function through its sharing of a common expression domain in the flower primordium with this microRNA. How this domain is jointly defined is not yet known.

Flower Meristem Genes

Just as floral organs have a genetically defined fate, so do floral meristems. Genes involved here were first identified from mutants in which flowers were replaced by

shoots with inflorescence-like properties. The *FLORICAULA* gene of Antirrhinum (Coen et al., 1990) and *LEAFY*, its ortholog from Arabidopsis (Weigel et al., 1992), encode transcription factors that impose a floral identity on primordia that arise from the flank of shoot apical meristems after their floral induction. Floral meristem identity is also promoted by a MADS box transcription factor gene in these species, the orthologs *SQUAMOSA* and *APETALA1* in Antirrhinum and Arabidopsis, respectively. Interestingly, in other species, the functions of all these genes do not always include specification of flower meristem identity. In passing, study of *LEAFY* gene function in Arabidopsis has revealed the basis of the unusual flower initiation property of this species—flowers arise naked from the flank of the inflorescence meristem. In many species (including Antirrhinum), they arise from the axil of a leaf-like bract generated by the meristem. It seems this potential remains in Arabidopsis, but bract development is normally inhibited by LEAFY function.

A later role of these genes is to establish the expression of floral organ identity genes, at least in Arabidopsis (Lohmann and Weigel, 2002; Jack, 2004). For example, expression of B function genes is lost in *leafy apetala1* double mutants, and expression of the C function gene *AGAMOUS* is directly activated by LEAFY.

In flower meristems, the maintenance of the central stem cell zone is at first regulated by the same genes that maintain the shoot apical meristem (Leyser and Day, 2003). In Arabidopsis, this apparently involves a self-sustaining feedback loop. The homeobox transcription factor WUSCHEL promotes stem cell properties immediately above the organizing center where it is expressed. The size of this center is constrained by CLAVATA signaling proteins. WUSCHEL promotes production of the CLAVATA3 ligand in the overlying stem cells, and this ligand then moves downward and sideways and likely interacts with the CLAVATA1 receptor. This receptor is present in a wider zone than WUSCHEL, and it apparently prevents CLAVATA3 from moving further in and inhibiting WUSCHEL expression. A constant number of stem cells is thus maintained. However, unlike shoots, flower

meristems eventually terminate growth (they are determinate). The C function gene *AGAMOUS* is involved here with another function in addition to its role in organ identity. It also suppresses the transcription of *WUSCHEL*, thus terminating cell proliferation in the central zone (Lenhard et al., 2001; Lohmann et al., 2001).

Organ Boundary Genes

The genetic mechanisms by which organ spacing is set up and maintained constitute a different category of genetic function within the flower, and our understanding of this area is also growing. Genes involved in organ spacing can be conveniently divided into two categories: those that regulate boundaries between whorls of different organs and those that keep organs of the same type separate within whorls. In the former category, the *SUPERMAN* (*SUP*) gene of Arabidopsis was characterized early, and its function is best understood (Sakai et al., 1995, 2000). In *sup* mutants, additional stamens develop at the expense of carpels. Cloning of the gene revealed that it encodes a C2H2 zinc finger transcription factor and that the gene is expressed early in the third whorl where stamens subsequently arise, and later only in the region adjacent to the fourth whorl. Genetic and molecular evidence indicates that the SUP protein inhibits proliferation of cells at the inner boundary of the third whorl.

The paradigm for genes that control organ boundaries within whorls is the *CUP-SHAPED COTYLEDON* (*CUC*) family of genes of Arabidopsis, first identified in Petunia as the *NO APICAL MERISTEM* gene (Souer et al., 1996). *cuc* mutants are characterized by lateral fusion of radially adjacent organs, including sepals and stamens (Aida et al., 1997). At least three *CUC* genes, all members of the multimember NAC family of transcription factors, are generally expressed in boundaries between organs, where they also may act individually to suppress intercalary growth. Recently, it has been shown that the boundary-specific location of CUC1 and CUC2 function is regulated posttranscriptionally by microRNA miR164 (Laufs et al., 2004; Mallory et al., 2004).

Organ Polarity Genes

Another aspect of spatial divergence involves specification of the polarity of individual organs. Genes that control which side of an organ is which (i.e., whether adaxial or abaxial) were discovered in floral organs and leaves, and the same functions seem to apply in each case. Generally, flattened organs arising from the flanks of both shoot and flower meristems are now called lateral organs, although I suggest that an Anglicized version of the term Blatt in the sense that Goethe used it (see above) is more appropriate. Genes from three families of transcription factors are involved. Two, first discovered through floral mutants of Arabidopsis, were the *YABBY* family (Sawa et al., 1999; Siegfried et al., 1999) and the *KANADI* family (Eshed et al., 2001). These are expressed in outer (abaxial) regions of newly growing primordia. Conversely, the third family, comprising the type III HD-Zip transcription factors *PHABULOSA*, *PHAVOLUTA*, and *REVOLUTA*, is expressed in the inner (adaxial) domain (McConnell et al., 2001). It seems that the functions of the adaxial-promoting PHABULOSA-like family and the abaxial-promoting KANADI family members are mutually antagonistic, based on complementary phenotypes following either their loss or gain of function. The YABBY family seems to be involved in sideways outgrowth of organs following earlier establishment of their polarity by members of the other two families. Again, it has recently been found that regulation of the three *PHABULOSA*-like genes includes a posttranscriptional step, this time by microRNA miR165/166 (e.g., Emery et al., 2003).

Lateral organs (blatts) also have polarity in a second dimension, lateral/medial, and this is influenced by another gene, the *PRESSED FLOWER* homeodomain gene of Arabidopsis (Matsumoto and Okada, 2001). *PRESSED FLOWER* is expressed specifically in lateral domains of leaves, sepals, petals, and stamens and, at least in the sepals, seems to promote lateral growth.

Genes Controlling Bilateral Symmetry

It has long been recognized that flowers occur in two basic designs, those with radial symmetry (with two, three, four, five, or more axes of symmetry) and those with bilateral symmetry (with only one axis, so-called mirror image flowers) (Figure 2A). Biologically, the latter are associated with insect and vertebrate pollination where bilateral visual cues direct the pollinator to a reward within the flower. Bilaterality in flowers seems to be the derived form, having arisen independently in many lineages, especially advanced ones (Donoghue et al., 1998; Endress, 2001b). The symmetry difference is apparently genetically controlled, as botanists interested in deformed flowers had noticed a special category called ''peloric,'' in which large radial flowers sometimes occurred in species that are normally bilateral. The cloning of two related genes associated with peloric mutant phenotypes in the bilateral model species Antirrhinum revealed how bilaterality may be imposed (Luo et al., 1995, 1999). These genes, *CYCLOIDEA* and *DICHOTOMA*, encode transcription factors of the TCP class that apparently act to suppress growth in the upper (adaxial) part of the developing flower primordium. This creates an abaxial–adaxial polarity that results in diversity in the form that floral organs, especially petals and stamens, adopt in upper and lower parts of the flower. Without the function of the two genes, all petals and all stamens are very similar in each case.

WHAT DON'T WE KNOW?

How Is the Floral Ground Plan Established?

Despite much progress, we still don't understand how spatial information is generated to set up the blueprint (bauplan) of the flower (Figure 2). The number of organs of each type is highly conserved. For instance, most monocots have trimerous flowers, whereas many eudicots are tetramerous or pentamerous (Figure 2A) (Cronquist, 1988; Endress, 1994; Judd et al., 2002). Loss of function of the bZIP transcription factor gene *PERIANTHIA* often makes Arabidopsis flowers pentamerous (with five sepals, petals, and stamens), although how this occurs is not clear (Chuang et al., 1999).
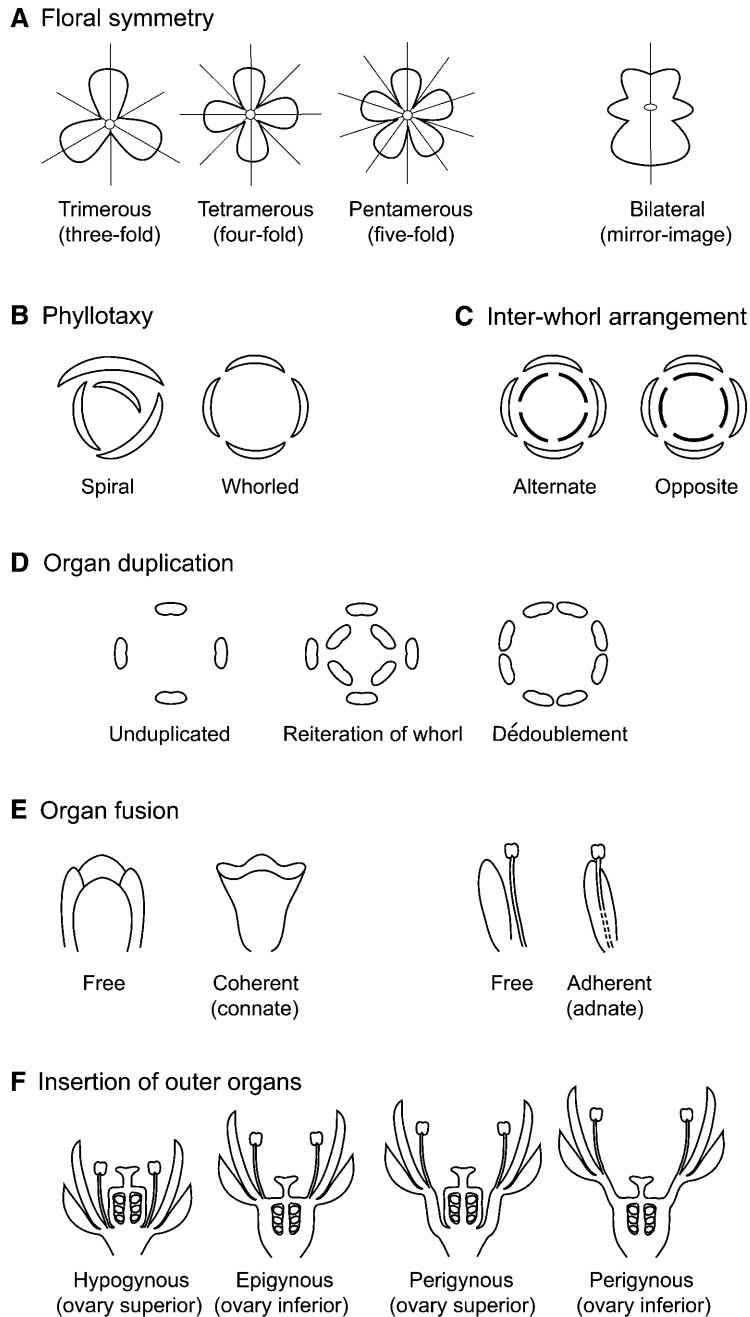
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A Floral symmetry

Trimerous
(three-fold)   Tetramerous
(four-fold)   Pentamerous
(five-fold)   Bilateral
(mirror-image)

B Phyllotaxy

Spiral   Whorled

C Inter-whorl arrangement

Alternate   Opposite

D Organ duplication

Unduplicated   Reiteration of whorl   Dédoublement

E Organ fusion

Free   Coherent
(connate)   Free   Adherent
(adnate)

F Insertion of outer organs

Hypogynous
(ovary superior)   Epigynous
(ovary inferior)   Perigynous
(ovary superior)   Perigynous
(ovary inferior)

Figure 2. Variations in Floral Structure.

(A) The symmetry of flowers is defined by the number of similar axes that can be drawn through its plan. Radially symmetric flowers (also called regular or actinomorphic) have two or more axes and include many monocots (3), Arabidopsis (2, derived from 4), tomato, and Petunia (5). Bilaterally symmetric flowers (also called irregular, zygomorphic, or mirror-imaged) have only one and include Antirrhinum and the pea family (Papilionaceae).

(B) Phyllotaxy of organs may be spiral (especially in the basal angiosperms) or whorled.

(C) Organs may arise alternate with, or opposite to, those in the adjacent whorl.

Loss of function of *CLAVATA* genes also leads to extra organs, but this seems to be a consequence of an increase in size of the flower meristem (Clark et al., 1993).

Recently, the spacing and timing of leaf primordium initiation has been shown to be regulated by auxin and cytokinin gradients (Reinhardt et al., 2003; Guilini et al., 2004), and similar processes may well be involved for floral organs. Certainly, disruption of auxin transport results in major upsets to floral organ number (Okada et al., 1991). However, the situation in flowers is much more complex than in the vegetative shoot: with up to four different organ types arising in defined succession, either in a whorled or spiral phyllotaxy (Figure 2B); with the new primordia arising either opposite or alternate to organs that have already arisen (Figure 2C); with whole whorls sometimes reiterated, and in some cases two organs arising in place of one (dédoublement), or vice versa (Figure 2D) (Endress, 1992). It is challenging to believe that such complexity could be set up as a self-organizing system, but at least we have a simpler precedent in the shoot apical meristem.

Other aspects of the floral ground plan are also conserved. These include whether

(D) Duplication of organs may occur by the reiteration of a whorl (as in poppies) or by two organs arising in place of one or vice versa (dédoublement, as in Lepidium [Brassicaceae]).

(E) Organs may either be free or show different patterns of fusion with each other. Those within a whorl may be coherent in a tube (e.g., the corolla of Antirrhinum). Different types of organ may be adherent (e.g., the stamens and corolla of Antirrhinum). These forms are usually congenital. Post-genital fusions occur after organs have formed (e.g., anthers in the daisy family, Asteraceae).

(F) The place of attachment of the perianth organs and stamens varies. They may be attached to the receptacle near the base of the ovary (hypogynous) or at the top of the ovary (epigynous). They are sometimes attached on the rim of a floral tube (perigynous), which itself may be basal or apical to the ovary. The ovary is either superior if the other organs (or a floral tube) are attached to its base or inferior if they are attached to its apex. The floral tube is sometimes called a hypanthium.

organs are attached to each other in a pattern that is either connate (cohesion within a whorl) or adnate (adhesion between whorls) (Figure 2E). In evolutionary terms, lack of fusion seems to be ancestral (Endress, 2001a). Modifications to this pattern can be envisioned as arising from differential changes in growth. For example, fusion of organs within a whorl, such as fused petals within the corolla, mostly seems to result from intercalary growth between initially distinguishable primordia. Such fusion is congenital, compared with post-genital fusions between already formed structures that presumably occur by different mechanisms (Raven and Weyers, 2001).

Another aspect of the ground plan of phylogenetic importance is the site of insertion of the outer organs relative to the ovary (Cronquist, 1988; Judd et al., 2002). They may be either attached close to its base (hypogyny, with the ovary superior) or to its apex (epigyny, with the ovary inferior) (Figure 2F). In some cases, the peripheral organs all arise at the top of a floral tube (perigyny). Perigyny can occur with the tube attached either at the base or the apex of the ovary, although intermediate attachment also occurs. Differential early growth can account for these different patterns (Soltis et al., 2003). Compared with the hypogynous ancestral condition (Endress, 2001a), additional growth of the flower primordium outside the ovary could result in epigyny if it remains coherent (apparently fused) with the ovary. If such growth is not attached, or if it continues above the ovary, then perigyny would result. To me, arguments about whether such growth represents receptacle tissue or floral organ tissue seem semantic because the receptacle is defined by its location rather than being a specific tissue type (Raven and Weyers, 2001).

Understanding how the complex topographical interplay between growth promotion and suppression is set up and maintained within the flower primordium is a big challenge, although roles for hormones and other unknown morphogens, microRNAs, and growth-suppressing boundary genes can be predicted. Attempts are now being made to define complete cell lineages within developing Arabidopsis flowers (Reddy et al., 2004) and the allometry of growth in developing Antirrhinum petals (Rolland-Lagan et al., 2003), with the ultimate aim of understanding their regulatory basis.

Most flowers are hermaphrodite, but a derived form of floral architecture is found in unisexual flowers of dioecious and monoecious species. It seems that in many unisexual flowers, the male and female organ primordia arise, but those of one sex or other stop growing early in development. Despite some clues from mutants of monoecious maize, the nature of the molecular switch that generates unisexual flowers is still not known in any species (Tanurdzic and Banks, 2004). On the other hand, the molecular basis of self-incompatibility not associated with physical differences between plants is relatively well understood (Kachroo et al., 2002; Franklin-Tong and Franklin, 2003). Surprisingly, this does not yet extend to the molecular genetic basis of heterostyly, a form of genetically controlled self-incompatibility associated with reciprocal differences in stamen and style length (Barrett et al., 2000).

Transcription Factors—What Are Their Regulators, Interactors, and Targets?

It is apparent that most flower development genes known to date encode transcription factors, but the function of a transcription factor is to regulate the expression of other genes, and until we know what these genes are, we won't know exactly how the transcription factor regulates morphogenesis. Very few direct downstream target genes are known so far (Jack, 2004). We do know that LEAFY directly activates *APETALA3* and *AGAMOUS* expression and that AGAMOUS activates *SPOROCYTELESS*, but there are few other examples. This may soon change, however, as genomic approaches are adopted. Targets may be identified by comparing expression patterns of all genes in plants that differ by only one specific transcription factor function (e.g., Schmid et al., 2003; Zik and Irish, 2003). The resolution of these methods can be greatly improved by confining the comparison to short time intervals through using a conditionally expressed version of the gene (William et al., 2004) or by assessing expression in limited amounts of tissue achievable by laser capture microdissection (Meyers et al., 2004).

The context of action of transcription factors is also now being better understood. It is clear that the condition of the chromatin in which target genes are embedded is important (e.g., histone modification status and DNA methylation and the maintenance of these states) (Goodrich and Tweedie, 2002; Reyes et al., 2002). These may lead to stable epigenetic changes within extensive and long-lived cell lineages, such as those that occur in plants. Also, the combinatorial presence of other transcription factors, and coactivator or corepressor proteins, helps generate diversity of outcomes based on a limited number of factors and targets. In yeast, global approaches to identifying all possible interactions between proteins (the interactome) have been performed (Uetz et al., 2000), and similar mapping of plant proteins is possible in yeast and in plant cells (Immink et al., 2002). Genetic approaches are also possible. For example, mutant screens to identify enhancers of A and C organ identity function have uncovered the AGAMOUS corepressor proteins LEUNIG and SEUSS (Franks et al., 2002) and a gene (*HUA ENHANCER1*) involved in processing microRNA 172 that targets APETALA2 mRNA for inactivation (Chen, 2004). It is interesting that a relatively large number of transcription factor genes involved in plant morphogenesis are susceptible to microRNA regulation. Within flowers, these include members of the *APETALA2*, *CUC*, and *PHABULOSA* families. It may be that this form of mRNA degradation or blockage occurs rapidly, avoiding any effect of long-lived transcripts in fast changing cellular environments (Rhoades et al., 2002).

Although not transcription factors, roles in flower development are known for at least one of the 700 or so F-box genes found in the Arabidopsis genome, *UNUSUAL FLORAL ORGANS*, and its

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Antirrhinum ortholog *FIMBRIATA* (Lohmann and Weigel, 2002; Jack, 2004). F-box proteins target specific proteins for degradation via the proteasome. It seems likely that many additional F-box proteins will be uncovered when the targets of flower development transcription factors are defined. Perhaps extensive redundancy, or vital pleiotropic actions earlier in development, have kept them hidden so far during mutant screens of flower development.

One outcome of all this knowledge about developmental cascades of transcription factor action will be the ability to model flower development so that gaps will be revealed and predictions made about unknown outcomes. Indeed, such model building using known organ identity genes of Arabidopsis has already begun (Espinosa-Soto et al., 2004).

How Is Spatial and Dimensional Information Signaled?

Perhaps the largest gap in our present understanding is how morphogenetic signals are generated, transmitted, perceived, and acted on in the developing flower (Golz and Hudson, 2002). We know such signals exist because, for example, floral organs retain fixed relative locations and orientations within the flower meristem (e.g., Griffith et al., 1999). Within organs, feedback must somehow be signaled so that the organ adopts the appropriate size and shape. This is apparently not related to cell number because size and cell number can be uncoupled (Foard and Haber, 1961; Hemerly et al., 1995). Whether or not physical forces are involved (Green, 1999) has not been established, although they alone cannot be responsible. It seems likely that morphogens, by triggering specific actions in a concentration-dependent manner, are central to developmental processes.

The nature of intercellular signaling molecules, and their pathways of movement, are beginning to be established, both between adjacent cells (either by direct secretion or through plasmodesmata) and between organs (through the epidermis, cortex, or phloem) (Haywood et al., 2002; Wu et al., 2002). We know that several

transcription factor proteins are capable of limited movement between cell layers. Also, the small CLAVATA3 peptide, a ligand for the CLAVATA1 receptor-like kinase, is mobile within the central zone of the shoot and flower meristem (Lenhard and Laux, 2003). We also know that those RNAs involved in RNA silencing are capable of acting at a distance within a plant, although how far microRNAs move needs to be examined, especially those that target developmentally regulated transcripts. A role for lipid-like molecules, including sterols, is not established, although they are implicated by the presence of putative sterol binding sites in, for example, the PHABULOSA-family of transcription factors (McConnell et al., 2001). Tantalizingly, these sites are also targets of microRNA binding (Emery et al., 2003). Also, among the hormones, auxin is known to move in specific directions associated with early developmental decisions (e.g., Reinhardt et al., 2003). Overall, it is an open question how far these scattered observations will be generalized and whether unknown signaling mechanisms, especially any that sense internal physical forces, await discovery.

How Do Flowers Evolve?

Evolution involves genetic change. We are already approaching an understanding of the underlying genetic basis for rapidly evolving aspects of the flower. Genes controlling traits such as the pollination syndrome can be identified in segregating populations from crosses between different interfertile species (i.e., quantitative trait mapping). Recent successful examples of this approach are the mapping of loci corresponding to 12 floral traits, mostly involving petal color and shape, in the monkey flower (Mimulus) (Bradshaw et al., 1998), and of petal color, corolla and stamen length, nectar production, and fragrance in Petunia (Stuurman et al., 2004). Eventually, the molecular function of these genes may be identified through a candidate gene approach using the growing knowledge base in model species.

The basis of evolution of more conserved properties of the flower is less accessible. Recently a floral genome project was

established to extend knowledge of developmental genes known from model species more broadly across a selection of angiosperms (Soltis et al., 2002). The aim is to identify cDNAs of orthologous genes and to map their expression patterns as an indication of their function. This will help test the generality of function of already known genes, although genes not yet identified, or those that do not function in the model species, are unapproachable by this strategy (Baum et al., 2002). Baum et al. (2002) argue that a more informative approach would be to develop a wider range of model species in which function is examined in depth. Already, functional and genomic information is accumulating in other model species, with rice (Shimamoto and Kyozuka, 2002) and maize (Lawrence et al., 2004) providing divergent monocot information that is intrinsically important as well as allowing comparisons with data from the established core eudicots. Baum et al. (2002) also argue that hypotheses about function will be readily testable in species closely related to established models. For example, comparative study of the role of the LEAFY transcription factor in controlling the generation of single rosette flowers in two different relatives of Arabidopsis has indicated that it has occurred by parallelism, with the same morphological outcome resulting from independent modifications to *LEAFY* gene function (Yoon and Baum, 2004).

Generally, our understanding of the mechanisms of evolution of morphology (evo-devo) is at an exciting stage. In flowering plants, the rapid explosion in diversity that followed their origin in the early Cretaceous (~130 million years ago) may be linked to modularity within their new structure, the flower (Carroll, 2001). Synergies resulting from interactions between floral organ modules with different functions may have promoted the relatively fast rate of evolution because floral structure is itself strongly associated with reproductive success. The origin of these modules is still an open question, however, and the discovery of new fossils of the immediate ancestors of angiosperms would be a major advance (Crepet, 2000; Stuessy, 2004).

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Just as modular structures have proliferated, so have the controlling genes. There are more than 1500 transcription factor genes in Arabidopsis (Riechmann, 2002), many occurring in large families. Duplication of a transcription factor gene immediately opens the possibility of divergence in function as either gene can maintain the existing function. Two routes are available: changes in the translated sequence that may alter target genes and influence interacting proteins or changes to the regulatory sequences that may result in transference of function in developmental time or space. For example, recent evidence reveals that the B function gene *APETALA3* duplicated and the C-terminal end diversified in ancestors of core eudicots such that B function now controls the identity of petals as well as stamens (Lamb and Irish, 2003).

Extending phylogenetic assays of known floral regulatory genes into more primitive plants, including the gymnosperms, is helping generate new speculative schemes about the origin of flowers (e.g., Theißen and Becker, 2004). More generally, sequencing of the genomes of representatives of even more primitive orders of green plants, including green algae (Chlamydomonas), bryophytes (Physcomitrella), and lycopods (Selaginella), will allow gene phylogenies to be traced and, ultimately, the origin and diversification of their morphogenetic functions to be deduced (http://www.jgi.doe.gov/sequencing/index.html).

In conclusion, we are beginning to understand aspects of the genetic basis of flower development. However, we still don't know how the underlying design of flowers is established, which genes are targets of the cascades of regulatory gene action, the nature of signals defining the location, size, shape, and differentiation of floral organs, or the pathways and mechanisms of evolution of floral morphology. Better understanding will depend upon integrating findings from functional studies with those that provide global information about genes and their action. Established model species will be the focus at first, but increasingly a comparative approach will be informative. There is no doubt that our views and perspectives of floral morphogenesis will continue their own rapid evolution.

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REFERENCES

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997). Genes involved in organ separation in Arabidopsis: An analysis of the *cup-shaped cotyledon* mutant. Plant Cell **9,** 841–857.

Angiosperm Phylogeny Group (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc. **141,** 399–436.

Arber, A. (1937). The interpretation of the flower: A study of some aspects of morphological thought. Biol. Rev. **12,** 157–184.

Arber, A. (1950). The Natural Philosophy of Plant Form. (Cambridge, UK: Cambridge University Press).

Aukerman, M.L., and Sakai, H. (2003). Regulation of flowering time and floral organ identity by a microRNA and its *APETALA2*-like target genes. Plant Cell **15,** 2730–2741.

Barrett, S.C.H., Jesson, L.K., and Baker, A.M. (2000). The evolution and function of stylar polymorphisms in flowering plants. Ann. Bot. **85** (suppl. A), 253–265.

Bateson, W. (1894). Materials for the Study of Variation. (London: Macmillan).

Baum, D.A., Doebley, J., Irish, V.F., and Kramer, E.M. (2002). Response: Missing links: The genetic architecture of flowers and floral diversification. Trends Plant Sci. **7,** 31–34.

Bender, W., Akam, M., Karch, F., Beachy, P.A., Peifer, M., Spierer, P., Lewis, E.B., and Hogness, D.S. (1983). Molecular genetics of the bithorax complex in *Drosophila melanogaster*. Science **221,** 23–29.

Bowman, J.L., Smyth, D.R., and Meyerowitz, E.M. (1989). Genes directing flower development in Arabidopsis. Plant Cell **1,** 37–52.

Bradshaw, H.D., Jr., Otto, K.G., Frewen, B.E., McKay, J.K., and Schemske, D.W. (1998). Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). Genetics **149,** 367–382.

Carroll, S.B. (2001). Chance and necessity: The evolution of morphological complexity and diversity. Science **409,** 1102–1109.

Chen, X. (2004). A microRNA as a translational repressor of *APETALA2* in *Arabidopsis* flower development. Science **303,** 2022–2025.

Chuang, C.-F., Running, M.P., Williams, R.W., and Meyerowitz, E.M. (1999). The *PERIANTHIA* gene encodes a bZIP protein involved in the determination of floral organ number in *Arabidopsis thaliana*. Genes Dev. **13,** 334–344.

Clark, S.E., Running, M.P., and Meyerowitz, E.M. (1993). *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. Development **119,** 397–418.

Coen, E.S., and Meyerowitz, E.M. (1991). The war of the whorls: Genetic interactions controlling flower development. Nature **353,** 31–37.

Coen, E.S., Romero, J.M., Doyle, S., Elliott, R., Murphy, G., and Carpenter, R. (1990). *floricaula*: A homeotic gene required for flower development in *Antirrhinum majus*. Cell **63,** 1311–1322.

Crepet, W.L. (2000). Progress in understanding angiosperm history, success and relationships: Darwin's abominably ''perplexing phenomenon.'' Proc. Natl. Acad. Sci. USA **97,** 12939–12941.

Cronquist, A. (1988). The Evolution and Classification of Flowering Plants, 2nd Ed. (Bronx, NY: New York Botanical Garden).

Doebley, J. (2004). The genetics of maize evolution. Annu. Rev. Genet. **38,** 37–59.

Donoghue, M.J., Ree, R.H., and Baum, D.A. (1998). Phylogeny and the evolution of flower asymmetry in the Asteridae. Trends Plant Sci. **3,** 311–317.

Doyle, J.A. (1994). Origin of the angiosperm flower: A phylogenetic perspective. Plant Sys. Evol. Suppl. **8,** 7–29.

Egea-Cortines, M., Saedler, H., and Sommer, H. (1999). Ternary complex formation between MADS-box proteins *SQUAMOSA*, *DEFICIENS* and *GLOBOSA* is involved in the control of floral architecture in *Antirrhinum majus*. EMBO J. **18,** 5370–5379.

Emery, J.F., Floyd, S.K., Alvarez, J., Eshed, Y., Hawker, N.P., Izhaki, A., Baum, S.F., and

HISTORICAL PERSPECTIVE ESSAY

Bowman, J.L. (2003). Radial patterning of *Arabidopsis* shoots by class III HD-ZIP and KANADI genes. Curr. Biol. **13,** 1768–1774.

Endress, P.K. (1992). Evolution and floral diversity: The phylogenetic surroundings of *Arabidopsis* and *Antirrhinum*. Int. J. Plant Sci. **153** (suppl.), S106–S122.

Endress, P.K. (1994). Diversity and Evolutionary Biology of Tropical Flowers. (Cambridge, UK: Cambridge University Press).

Endress, P.K. (2001a). The flowers of extant basal angiosperms and inferences on ancestral flowers. Int. J. Plant Sci. **162,** 1111–1140.

Endress, P.K. (2001b). Evolution of floral symmetry. Curr. Opin. Plant Biol. **4,** 86–91.

Eshed, Y., Baum, S.F., Perea, J.V., and Bowman, J.L. (2001). Establishment of polarity of lateral organs. Curr. Biol. **11,** 1251–1260.

Espinosa-Soto, C., Padilla-Longoria, P., and Alvarez-Buylla, E.R. (2004). A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles. Plant Cell **16,** 2923–2939.

Feldman, M., Lupton, F.G.H., and Miller, T.E. (1995). Wheats. In Evolution of Crop Plants, 2nd Ed., J. Smart and N.W. Simmonds, eds (London: Longman Scientific), pp. 184–192.

Foard, D.F., and Haber, A.H. (1961). Anatomic studies of gamma-irradiated wheat growing without cell division. Am. J. Bot. **48,** 438–446.

Franklin-Tong, V.E., and Franklin, F.C.H. (2003). The different mechanisms of gametophytic self-incompatibility. Phil. Trans. R. Soc. Lond. B Biol. Sci. **358,** 1025–1032.

Franks, R.G., Wang, C., Levin, J.Z., and Liu, Z. (2002). SEUSS, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with LEUNIG. Development **129,** 253–263.

Goethe, J.W. von (1790). Versuch die Metamorphose der Pflanzen zu erklären. (Gotha: Carl Wilhelm Ettinger). [English translation: Arber, A. (1946). Goethe's botany. Chronica Botanica **10,** 63–126.]

Golz, J.F., and Hudson, A. (2002). Signalling in plant lateral organ development. Plant Cell **14** (suppl.), S277–S288.

Goodrich, J., and Tweedie, S. (2002). Remembrance of things past: Chromatin remodeling in plant development. Annu. Rev. Cell Dev. Biol. **18,** 707–746.

Green, P.B. (1999). Expression of pattern in plants: Combining molecular and calculus-based biophysical programs. Am. J. Bot. **86,** 1059–1076.

Griffith, M.E., da Silva Conceição, A., and Smyth, D.R. (1999). *PETAL LOSS* gene regulates initiation and orientation of second whorl organs in the *Arabidopsis* flower. Development **126,** 5635–5644.

Guilini, A., Wang, J., and Jackson, D. (2004). Control of phyllotaxy by the cytokinin-inducible response regulator *ABPHYL1*. Nature **430,** 1031–1034.

Haywood, V., Kragler, F., and Lucas, W.J. (2002). Plasmodesmata: Pathways for protein and ribonucleoprotein signaling. Plant Cell **14** (suppl.), S303–S325.

Hemerly, A., de Engler, A.J., Bergounioux, C., Van Montagu, M., Engler, G., Inze, D., and Ferreira, P. (1995). Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. EMBO J. **14,** 3925–3936.

Honma, T., and Goto, K. (2001). Complex of MADS-box proteins are sufficient to convert leaves into floral organs. Nature **409,** 525–529.

Immink, R.G.H., Gadella, T.W.J., Ferrario, S., Busscher, M., and Angenent, G.C. (2002). Analysis of MADS box protein-protein interactions in living plant cells. Proc. Natl. Acad. Sci. USA **99,** 2416–2421.

Jack, T. (2004). Molecular and genetic mechanisms of floral control. Plant Cell **16** (suppl.), S1–S17.

Jofuku, K.D., den Boer, B., Van Montagu, M., and Okamuro, J. (1994). Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. Plant Cell **6,** 1211–1225.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F., and Donoghue, M.J. (2002). Plant Systematics: A Phylogenetic Approach, 2nd Ed. (Sunderland, MA: Sinauer Associates).

Kachroo, A., Nasrallah, M.E., and Nasrallah, J.B. (2002). Self-incompatibility in the Brassicaceae: Receptor-ligand signaling and cell-to-cell communication. Plant Cell **14** (suppl.), S227–S238.

Keck, E., McSteen, P., Carpenter, R., and Coen, E.S. (2003). Separation of genetic functions controlling organ identity in flowers. EMBO J. **22,** 1058–1066.

Koornneef, M., Van Eden, J., Hanhart, C.J., Stamp, P., Braaksma, F.J., and Feenstra, W.J. (1983). Linkage map of *Arabidopsis thaliana*. J. Hered. **74,** 265–273.

Lamb, R.S., and Irish, V.F. (2003). Functional divergence within the *APETALA3/PISTILLATA* floral homeotic gene lineages. Proc. Natl. Acad. Sci. USA **100,** 6558–6563.

Laufs, P., Peaucelle, A., and Traas, J. (2004). MicroRNA regulation of the CUC genes is required for boundary size control in *Arabidopsis* meristems. Development **131,** 4311–4322.

Lawrence, C.J., Dong, Q., Polacco, M.L., Seigfried, T.E., and Brendel, V. (2004). MaizeGDB, the community database for maize genetics and genomics. Nucleic Acids Res. **32,** D393–D397.

Lenhard, M., Bohnert, A., Jürgens, G., and Laux, T. (2001). Termination of stem cell maintenance in Arabidopsis floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. Cell **105,** 805–814.

Lenhard, M., and Laux, T. (2003). Stem cell homeostasis in the *Arabidopsis* shoot apical meristem is regulated by intercellular movement of CLAVATA3 and its sequestration by CLAVATA1. Development **130,** 3163–3173.

Leyser, O., and Day, S. (2003). Mechanisms of Plant Development. (Oxford: Blackwell Science).

Lohmann, J.U., Hong, R.L., Hobe, M., Busch, M.A., Parcy, F., Simon, R., and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in Arabidopsis. Cell **105,** 793–803.

Lohmann, J.U., and Weigel, D. (2002). Building beauty: The genetic control of floral patterning. Dev. Cell **2,** 135–142.

Luo, D., Carpenter, R., Copsey, L., Vincent, C., Clark, J., and Coen, E.S. (1999). Control of organ asymmetry in flowers of *Antirrhinum*. Cell **99,** 367–376.

Luo, D., Carpenter, R., Vincent, C., Copsey, L., and Coen, E.S. (1995). Origin of floral asymmetry in *Antirrhinum*. Nature **383,** 794–799.

Mallory, A.C., Dugas, D.V., Bartel, D.P., and Bartel, B. (2004). MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. Curr. Biol. **14,** 1035–1046.

Matsumoto, N., and Okada, K. (2001). A homeobox gene, *PRESSED FLOWER*, regulates lateral axis-dependent development of *Arabidopsis* flowers. Genes Dev. **15,** 3355–3364.

McConnell, J.R., Emery, J., Eshed, Y., Bao, N., Bowman, J., and Barton, M.K. (2001). Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. Nature **411,** 709–713.

Meyerowitz, E.M., Smyth, D.R., and Bowman, J.L. (1989). Abnormal flowers and pattern formation in floral development. Development **106,** 209–217.

Meyers, B.C., Galbraith, D.W., Nelson, T., and Agrawal, V. (2004). Methods for transcriptional profiling in plants. Be fruitful and replicate. Plant Physiol. **135,** 637–652.

Morton, A.G. (1981). History of Botanical Science. (London: Academic Press).

Okada, K., Ueda, J., Komaki, M.K., Bell, C.J., and Shimura, Y. (1991). Requirement of the polar auxin transport system in early stages of *Arabidopsis* floral bud formation. Plant Cell **3,** 677–684.

Payer, J.-B. (1857). Traité d'Organogénie Comparée de la Fleur. (Paris: Librairie de Victor Masson).

Pelaz, S., Ditta, G.S., Baumann, E., Wisman, E., and Yanofsky, M.F. (2000). B and C floral organ identity functions require *SEPALLATA* MADS-box genes. Nature **405,** 200–203.

Raven, J.A., and Weyers, J.D.B. (2001). Significance of epidermal fusion and intercalary growth for angiosperm evolution. Trends Plant Sci. **6,** 111–113.

Reddy, G.P., Heisler, M.G., Ehrhardt, D.W., and Meyerowitz, E.M. (2004). Real-time lineage analysis reveals oriented cell divisions associated with morphogenesis in the shoot apex of *Arabidopsis thaliana*. Development **131,** 4225–4237.

Reinhardt, D., Pesce, E.-R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J., and Kuhlemeier, C. (2003). Regulation of phyllotaxis by polar auxin transport. Nature **426,** 255–260.

Reyes, J.C., Hennig, L., and Gruissem, W. (2002). Chromatin-remodeling and memory factors. New regulators of plant development. Plant Physiol. **130,** 1090–1101.

Rhoades, M.W., Reinhart, B.J., Lim, L.P., Burge, C.B., Bartel, B., and Bartel, D.B. (2002). Prediction of plant microRNA targets. Cell **110,** 513–520.

Riechmann, J.-L. (2002). Transcriptional regulation: A genomic overview. In The Arabidopsis Book, C.R. Somerville and E.M. Meyerowitz, eds (Rockville, MD: American Society of Plant Biologists), doi/10.1199/tab.0085, http://www.aspb.org/publications/arabidopsis/.

Rolland-Lagan, A.-G., Bangham, J.A., and Coen, E.S. (2003). Growth dynamics underlying petal shape and asymmetry. Nature **422,** 161–163.

Sachs, J. von (1875). Geschichte der Botanik vom 16. Jahrhundert bis 1860. (Munich: R. Oldenburg). [English translation: Garnsey, H.E.F., and Balfour, I.B. (1890). Sachs' History of Botany (1530–1860). (Oxford: Clarendon Press).]

Sakai, H., Krizek, B.A., Jacobsen, S.E., and Meyerowitz, E.M. (2000). Regulation of *SUP* expression identifies multiple regulators involved in Arabidopsis floral meristem development. Plant Cell **12,** 1607–1618.

Sakai, H., Medrano, L.J., and Meyerowitz, E.M. (1995). Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. Nature **378,** 199–203.

Sawa, S., Watanabe, K., Goto, K., Kanaya, E., Morita, E.H., and Okada, K. (1999). *FILAMENTOUS FLOWER*, a meristem and organ identity gene of *Arabidopsis*, encodes a protein with zinc finger and HMG-related domains. Genes Dev. **13,** 1079–1088.

Schmid, M., Uhlenhaut, N.H., Godard, F., Demar, M., Bressan, R., Weigel, D., and Lohmann, J.U. (2003). Dissection of floral induction pathways using global expression analysis. Development **130,** 6001–6012.

Shimamoto, K., and Kyozuka, J. (2002). Rice as a model for the comparative genomics of plants. Annu. Rev. Plant Biol. **53,** 339–419.

Siegfried, K.R., Eshed, Y., Baum, S.F., Otsuga, D., Drews, G.N., and Bowman, J.L. (1999). Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis*. Development **126,** 4117–4128.

Soltis, D.E., Fishbein, M., and Kuzoff, R.K. (2003). Reevaluating the evolution of epigyny: Data from phylogenetics and floral ontogeny. Int. J. Plant Sci. **164** (suppl.), S251–S264.

Soltis, D.E., Soltis, P.S., Albert, V.A., Oppenheimer, D.G., dePamphilis, C.W., Ma, H., Frohlich, M.W., and Theißen, G. (2002). Missing links: The genetic architecture of flowers and floral diversification. Trends Plant Sci. **7,** 22–31.

Soltis, P.S., Soltis, D.E., and Chase, M.W. (1999). Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature **402,** 402–404.

Sommer, H., Beltrán, J.-P., Huijser, P., Pape, H., Lönnig, W.-E., Saedler, H., and Schwarz-Sommer, Z. (1990). *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: The protein shows homology to transcription factors. EMBO J. **9,** 605–613.

Souer, E., van Houwelingen, A., Kloos, D., Mol, J., and Koes, R. (1996). The *No Apical Meristem* gene of Petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. Cell **85,** 159–170.

Steeves, T.A., and Sussex, I.M. (1989). Patterns in Plant Development, 2nd Ed. (Cambridge, UK: Cambridge University Press).

Stubbe, H. (1966). Genetik und Zytologie von *Antirrhinum* L. sect. *Antirrhinum*. (Jena, Germany: Fischer Verlag).

Stuessy, T.F. (2004). A transitional-combinatorial theory for the origin of the angiosperms. Taxon **53,** 3–16.

Stuurman, J., Hoballah, M.E., Broger, L., Moore, J., Basten, C., and Kuhlemeier, C. (2004). Dissection of floral pollination syndromes in Petunia. Genetics **168,** 1585–1599.

Tanurdzic, M., and Banks, J.A. (2004). Sex-determining mechanisms in land plants. Plant Cell **16** (suppl.), S61–S71.

Theißen, G., and Becker, A. (2004). Gymnosperm orthologues of class B floral homeotic genes and their impact on understanding flower origin. Crit. Rev. Plant Sci. **23,** 129–148.

Uetz, P., et al. (2000). A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. Nature **403,** 623–627.

Wardlaw, C.W. (1965). Organization and Evolution in Plants. (London: Longmans Green).

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F., and Meyerowitz, E.M. (1992). *LEAFY* controls floral meristem identity in *Arabidopsis*. Cell **69,** 843–859.

William, D.A., Su, Y., Smith, M.R., Lu, M., Baldwin, D.A., and Wagner, D. (2004). Genomic identification of direct target genes of LEAFY. Proc. Natl. Acad. Sci. USA **101,** 1775–1780.

Wu, X., Weigel, D., and Wigge, P.A. (2002). Signaling in plants by intercellular RNA and protein movement. Genes Dev. **16,** 151–158.

Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., and Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. Nature **346,** 35–39.

Yoon, H.-S., and Baum, D.A. (2004). Transgenic study of parallelism in plant morphological evolution. Proc. Natl. Acad. Sci. USA **101,** 6524–6529.

Zik, M., and Irish, V.F. (2003). Global identification of target genes regulated by *APETALA3* and *PISTILLATA* floral homeotic gene action. Plant Cell **15,** 207–222.