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Inflorescence development in petunia: through the maze of botanical terminology

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Abstract

Flowering plants have developed many ways to arrange their flowers. A flower-bearing branch or system of branches is called an inflorescence. The number of flowers that an inflorescence contains ranges from a single flower to endless flower-clusters. Over the past centuries, botanists have classified inflorescences based on their morphology, which has led to an unfortunate maze of complex botanical terminology. With the rise of molecular developmental biology, research has become increasingly focused on how inflorescences develop, rather than on their morphology. It is the decisions taken by groups of stem cells at the growing tips of shoots, called meristems, on when and where to produce a flower or a shoot that specify the course of inflorescence development. Modelling is a helpful aid to follow the consequences of these decisions for inflorescence development. The so-called transient model can produce the broad inflorescence types: cyme, raceme, and panicle, into which most inflorescences found in nature can be classified. The analysis of several inflorescence branching mutants has led to a solid understanding of cymose inflorescence development in petunia (Petunia hybrida). The cyme of petunia is a distinct body plan compared with the well-studied racemes of Arabidopsis and Antirrhinum, which provides an excellent opportunity to study evolutionary developmental biology (evo-devo) related questions. However, thus far, limited use has been made of this opportunity, which may, at least in part, be due to researchers getting lost in the terminology. Some general issues are discussed here, while focusing on inflorescence development in petunia.

Key words: Cyme, flowering plants, inflorescence architecture, meristem, monopodial, panicle, petunia inflorescence, raceme, sympodial.

Introduction

Flowering plants (angiosperms) are a widespread, diverse, and large group of approximately 260,000 described species that have long been admired for the endless variation in the shape, size, and colour of their flowers (Bell et al., 2005; Krizek and Fletcher, 2005). Besides being appreciated for their beauty, they provide us with numerous resources, of which food is arguably the most important one. A major determinant of crop yield is the way that a plant arranges its flowers (Wyatt, 1982). The flower-bearing branches of a plant are called the inflorescence, and the shape of an inflorescence is referred to as the inflorescence architecture (Rickett, 1944, 1955; Weberling, 1989). Figure 1 shows four basic inflorescence architectures: a single flower (A), raceme (B), cyme (C), and panicle (D). Perhaps surprisingly, most inflorescences found in nature can be classified into one of these broad categories (Prusinkiewicz et al., 2007). Rather elaborate sets of descriptive terms are used to characterize subordinate architectural types. As straightforward as this classification system may seem, in reality there is no generally used, adequate system for the characterization and classification of the immensely diverse range of inflorescence architectures. Over 60 years ago, Rickett pointed out that ‘the names applied to inflorescences were confused from the beginning’ (Rickett, 1944). Unfortunately, the puzzling maze of inflorescence terminologies has still not been solved; it is arguably even more complex now than it ever was. For example, if one is to look up in literature the inflorescence type of the well-known and extensively studied
tomato plant (Solanum lycopersicum), one is sure to be left in total confusion, wondering whether the tomato inflorescence is correctly called a cyme, raceme, indeterminate cyme, racemose cyme, symposium, or whether yet another new label should be introduced to define its inflorescence (Cooper, 1927; Allen and Sussex, 1996; Molinero-Rosales et al., 1999, 2004; Quinet et al., 2006; Welty et al., 2007; Lippman et al., 2008). Clearly, no science can afford to have its descriptive terms, which are supposed to clarify, be such major sources of confusion.

Like tomato, petunia (Petunia hybrida) is a versatile model plant that belongs to the diverse Solanaeace family, which includes 3000–4000 species including pepper (Capsicum annuum), tobacco (Nicotiana tabacum), potato (Solanum tuberosum), and eggplant (Solanum melongena) (Knapp et al., 2004; Gerats and Vandenbussche, 2005). Based on combined data from in situ hybridization experiments, sequential replica series of developing meristems, and molecular analyses of mutants that are defective in inflorescence branching, there is now a detailed, solid understanding of inflorescence development in petunia at both morphological and molecular levels. The purpose of this review is to discuss and clear up some of the most relevant aspects of the persistent confusion and controversy concerning the classification of inflorescence architectures by means of a detailed description of inflorescence development in petunia.

What is an inflorescence?

One key source of confusion is the ambiguous meaning of the term inflorescence. Nowadays, the inflorescence of a plant is its reproductive part: the system of branches that bears the flowers, or other types of sexually reproductive structures (Rickett, 1944, 1955; Weberling, 1989; Tucker and Grimes, 1999; Benloch et al., 2007; Prenner et al., 2009). Thus, an inflorescence is a structure, the end-product of a course of development. The original meaning of inflorescence was different. Carl Linnaeus applied the term inflorescence to the mode of flowering (Linnaeus, 1751; Rickett, 1944), so to him an inflorescence was not a structure, but a mode of development. The change in meaning of the word ‘inflorescence’ is understandable, considering that past botanists were predominantly occupied with naming and classifying plants, not so much with understanding their development.

To understand the confusion that using the two different meanings of inflorescence creates, look again at Fig. 1. The single flower (Fig. 1A) and raceme (Fig. 1B) are obviously different under both interpretations of inflorescence. However, confusion sets in when the raceme and cyme are compared. From a developmental biologist’s point of view, a raceme and cyme develop quite differently. The raceme shown in Fig. 1B consists of a monopodial (continuous) main stem with lateral flowers. By contrast, the main stem of the cyme (Fig. 1C) terminates in a flower, and growth continues from lateral shoots that also end in flowers, thereby creating a so-called sympodial (composite) main axis that is actually built of separate shoots (Rickett, 1944, 1955). However, if the inflorescence is regarded as a flower-cluster, the only difference between the cyme and raceme is that the cyme has a zigzag shaped stem, whereas the raceme has a straight stem. In nature this may be less obvious; the sympodial axis might straighten, creating the false impression of a monopodial axis with lateral flowers. Or, alternatively, a monopodial axis might have a zigzag shape. Indeed, practice shows that the flower clusters of cymes and racemes cannot be properly distinguished. At least in part, this has possibly contributed to the ambiguous classification of tomato, and other species, as cymes or racemes. Therefore, it is reasonable that Troll and Weberling (Troll, 1964–1969; Weberling, 1989), who both define the inflorescence as a structure, state in their influential works that the ‘distinction between cymose and racemose inflorescences is of an artificial nature’, and that ‘although a cymose form of branching exists ..., there is no such thing as a cymose inflorescence’. After all, under their definition of inflorescence, cymes cannot be properly distinguished from racemes. This creates the confusing situation in which a plant’s inflorescence may develop by cymose branching, but is not classified as a cyme. Regarding the inflorescence as a mode of development makes the classification of inflorescences much more straightforward.

According to Rickett (1944) ‘it would be foolish to attempt to change’ the definition of inflorescence back to its original meaning. However, in present-day developmental research it is usually just used in this original sense: a mode of development. A prime example is the so-called transient model proposed by Prusinkiewicz et al. (2007). Since there seems to be a tendency to misinterpret and underestimate the value of the transient model (Alvarez-Buylla et al., 2007; Prusinkiewicz and Coen, 2007; Prenner et al., 2009), its virtues will be discussed in the next section.

The transient model: a unifying inflorescence model

Separate developmental models for different inflorescence types have been proposed in the past; however, assuming that different inflorescence types are evolutionarily related and have diverged due to genetic changes, a model is required that can produce cymes and racemes, as well as panicles. The transient model by Prusinkiewicz et al. captures all three architectural types (Prusinkiewicz and Coen, 2007; Prusinkiewicz et al., 2007; Koes, 2008). In this model the level of a single continuous variable, called
vegetativeness, determines whether meristems, which are the groups of stem cells at the growing tips of shoots, produce flowers or shoots. Vegetativeness is not a substance, but a quantitative measure of meristem identity; a simplifying abstraction of the many factors that may influence the identity of meristems, such as plant age or complex molecular mechanisms. If, during a set time interval (a plastochron), the level of vegetativeness in a meristem remains high, it will produce a shoot that consists of an internode and a lateral meristem that is subtended by a leaf. However, if vegetativeness falls below a certain floral threshold the meristem will transform into a flower. Meristems that lose vegetativeness and acquire a floral fate are called determinate, as they are destined to terminate after forming the last floral organs (carpels, in most cases), whereas meristems that maintain a high level of vegetativeness and proceed by forming the next plastochron are called indeterminate.

If vegetativeness is uniformly decreased in all meristems, then after a period of branching all meristems will simultaneously terminate in flowers and a panicle will be formed (Fig. 1D). To generate racemes, the level of vegetativeness in lateral meristems is transiently decreased; therefore they reach the floral threshold before apical meristems do (Fig. 1B). If vegetativeness is transiently increased in lateral meristems, apical meristems will terminate in flowers before the lateral meristems do; a cymose inflorescence is formed (Fig. 1C). How the levels of vegetativeness specify the basic architectures of racemes, cymes, and panicles is an easily grasped concept, but it is difficult to comprehend the precise course of inflorescence development over a number of plastochrons without the aid of modelling. Therefore, we recommend experimenting with the model by using the available software, which is available on request (Prusinkiewicz et al., 2007), in order to get a full understanding of how the model works and to appreciate its full potential.

Clearly, inflorescence architectures exist that require further elaboration of the transient model. For instance, besides the shoot or flower subtending leaves, meristems might produce additional leaves, as is the case in petunia, which will be shown later. Also, other characteristics are not included in the model, such as the three-dimensional organization, branch lengths, and the number of lateral meristems. However, including those aspects would probably only distract from the main focus of the model (Prusinkiewicz and Coen, 2007). As it is, the transient model helps to comprehend the meaning and logic of differently regulated molecular mechanisms in the development of distinct inflorescence architectures in current model plant species. With research focused on inflorescence development still in its infancy, the model serves as a solid reference point based on which predictions can be made, hypotheses can be tested, and more elaborate models can be built. The transient model also breaks with the past habit of labelling inflorescence architectures based on subjective criteria, due to loosely defined inflorescence types; an issue which will be discussed next.

The divergent definitions of cyme, raceme, and panicle

Whenever the terms cyme, raceme, and panicle are used without further description, it is important to be aware that dictionaries and scientific literature contain divergent definitions of these terms (Rickett, 1944, 1955; Benlloch et al., 2007; Prusinkiewicz et al., 2007; Prenner et al., 2009). Particularly the term panicle should be treated with caution, as its definitions have always been vague and ambiguous (Rickett, 1944, 1955). Panicles produced by the transient model are similar to those in the descriptions of Rickett and Weberling (Rickett, 1955; Weberling, 1989).

Definitions of inflorescence architectures often include whether an inflorescence is ‘open’ (indeterminate; axes do not terminate in flowers), or ‘closed’ (determinate; all axes terminate in flowers). Racemes are usually defined as ‘open’, which fits for example Arabidopsis (Arabidopsis thaliana) and Antirrhinum (Antirrhinum majus). However, in many (non-model plant) racemes the main axis does end in a flower, for instance, blackberry and raspberry plants (Rickett, 1955). The transient model is in line with the existence of open and closed racemes; it depends on the decrease of vegetativeness in apical meristems. If during a plant’s lifetime vegetativeness in primary apical meristems reaches the floral threshold, it will be a closed raceme, if it does not, it will be an open raceme. Thus, a raceme is a monopodial inflorescence that can be either determinate or indeterminate. By contrast, cymes are always determinate in the transient model; any given stem is terminated by its first flower, and is sympodially continued via a series of lateral branches (sympodial shoots). These lateral branches are subtended by leaves, which are called bracts. Bracts are useful markers that help to distinguish between monopodial and sympodial development. For example, the bracts of Antirrhinum sub tend the flowers, revealing its monopodial nature; the bracts in the cyme of petunia do not subtend the flowers, but the lateral shoots. However, bracts can be suppressed; in some species their growth aborts early in development. For instance, judged by the naked eye, Arabidopsis plants may seem bractless, but by different techniques it has been shown that floral meristems actually arise in the axes of rudimentary bracts (Long and Barton, 2000; Hepworth et al., 2006; Kwiatkowska, 2006, 2008). Similarly, no obvious bracts are present in the tomato inflorescence, but the presence of rudimentary bracts that subtend inflorescence meristems has been shown by scanning electron microscopy (Reinhardt and Kuhlemeier, 2002). Despite these reports, both Arabidopsis and tomato are still inaccurately described as bractless (Benlloch et al., 2007; Prenner et al., 2009).

We will continue with a detailed description of inflorescence development in petunia. Besides the general issues related to inflorescences, some cyme-specific controversies will be dealt with, and extent to which the transient model holds up to our experimental data will be discussed.
Inflorescence development in petunia

A short history

The first petunia species was collected in the 18th century by the French naturalist Philibert Commerson on the north bank of the River Plate in what is now Uruguay. It was described for the first time by Jean-Baptiste Lamarck as Nicotiana axillaris (Lamarck, 1793). Seen through contemporary eyes, this name is a misnomer (Ferguson and Ottley, 1932). First of all, Nicotiana and Petunia are no longer considered to be sufficiently closely related that they belong to the same genus. In that sense, Antoine Laurent de Jussieu was more accurate in 1803, when he unwittingly described Lamarck’s Nicotiana axillaris under the name of Petunia nyctaginiflora, thereby founding the genus Petunia, a name he had derived from a Native American word for tobacco. In 1888, after a period during which at least three different names for the same species had existed (Sims, 1825), the final name became Petunia axillaris, which is still in use today. However, settling on Petunia nyctaginiflora, though perhaps a bit of a tongue twister, would have been more appropriate, since axillary refers to the position of the flowers, which is not axillary, but rather apical. Apparently, neither Lamarck, nor Jussieu appreciated the cymose nature of the petunia inflorescence in which the primary axis is terminated by the first flower and is continued sympodially by a series of axillary two-leafed shoots, which each terminate in a flower (Ferguson and Ottley, 1932; Napoli and Ruhle, 1996). Figure 2A shows a petunia inflorescence. From Fig. 2A it is difficult to judge whether the main axis is built of separate shoots (Fig. 2B), or is continuous (Fig. 2C). Figure 2D shows the detail of a branching point. The axis that leads directly to the flower is continuous; the lateral shoot that leads to the next pair of bracts is an autonomous separate shoot that originates in the axil of a bract. This implies that the inflorescence of petunia is sympodial, thus a cyme. The petunia flowers are in an apical, and the shoots are in a lateral position, which is in contrast to the racemose (monopodial) inflorescence of for instance Arabidopsis, which has lateral flowers arranged around a continuous primary axis, as illustrated in Fig. 2C. In all fairness to Lamarck and Jussieu, both undisputed botanical heavyweights, the concepts of sympodial and monopodial would not be introduced for decades (Rickett, 1944). It is not our intention to criticize their judgment, but rather to illustrate the long history of confusing botanical terminology.

The petunia inflorescence is a monochasium

In petunia, after a given shoot has terminated in a flower, only one sympodial shoot develops (Napoli and Ruhle, 1996; Souer et al., 2008) (Fig. 2A); this type of cyme is called a monochasial cyme, or monochasium. A dichasium has two, and a pleiochasium more than two sympodial shoots. There are four types of monochasial cymes: the drepanium, rhipidium, helicoid cyme (bostryx), and scorpioid cyme (cincinnus), which differ in the positions of the successive lateral shoots (Fig. 3). If the successive lateral shoots always appear on the same side of the sympodial axis, and the flowers in the resulting inflorescence are thus in one plane, with the oldest flower on one side and the youngest on the other, this is called a drepanium (Fig. 3A). If, however, the next shoot appears on the opposite side of the previous one, the flowers in the resulting inflorescence are also in one plane, but in this case new flowers appear in between the older flowers. This type of monochasial cyme is a rhipidium (Fig. 3B). In the two other types, the successive sympodial shoots appear at an angle between 0° and 180° to the previous one. If this angle is always in one direction, the result is a helicoid cyme, or bostryx (Fig. 3C), in which the flowers are ultimately arranged in a helix, and the youngest flower is in the centre of the inflorescence. In the scorpioid cyme, or cincinnus (Fig. 3D), the new lateral shoots appear alternately on one and the other side of the axis. In this case the flowers are in a zigzag pattern, and the youngest flower is not in the centre of the inflorescence, but on the outer edge of the inflorescence. This is why scorpioid cymes are usually (but not always) coiled like a scorpion’s tail.
These botanical terms have been, and still are, misused, mixed up, and inadequately explained by many (Rickett, 1944, 1955; Welty et al., 2007; Prenner et al., 2009). This may hardly be surprising, considering that even Asa Gray, the most prominent American botanist of the 19th century, failed to grasp the essential distinctions, as he reckoned that a single name for all four kinds of monochasial cymes would be sufficient, based on the argument that scorpioid and helicoid cymes ‘are not always readily discriminated’ (Gray, 1879). It will not only be shown that this distinction is easily made, but also that it is an important aid to interpreting molecular data correctly, such as RNA in situ hybridizations.

The petunia inflorescence is a scorpioid cyme

Using the sequential replica method (Williams and Green, 1988), the late Paul Green (Silk, 2000) made a day-by-day scanning electron micrograph series of a single developing petunia inflorescence apex (Fig. 4). A hard copy picture of the series was digitally processed by scanning, colouring, and removing the labels added by Green. The uncoloured, scanned image of the original picture by Paul Green is available as Supplementary Fig. S1 at JXB online. The first picture (Fig. 4A) shows a well-developed flower on the right (F1), of which all floral organ primordia are visible: five sepals, five petals, five stamens, and in the centre the two fused carpels. To the left of this flower, a floral meristem (fm2), of which at this stage only the sepal whorl can be discerned, terminates a lateral shoot. The bracts labelled ‘b2’ belong to that same shoot, and in the axil of one of them the next shoot appears. The floral meristem at the tip of this shoot has yet to produce the first floral organ primordia (sepals), and in the axil of one of its bracts a sympodial meristem emerges that will again produce a shoot that consists of two bracts and a terminal flower. After a sympodial meristem has produced the two bracts, it is labelled as a floral meristem. Following the development of the sympodial meristem and the youngest floral meristem (fm3) from Fig. 4A to Fig. 4E, a number of things are

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Fig. 3. The four types of monochasial cymes: (A) drepanium; (B) rhipidium; note that the drepanium and rhipidium are flattened in the plane of the paper; (C) helicoid cyme; note that the flowers are arranged in a helical pattern; (D) scorpioid cyme; note the twist that occurs in the scorpioid cyme as it turns away from the observer. Flowers are numbered in order of development. The four flowers in the lower right corners show top views of the inflorescences. Nomenclature after Gray (1879) and Rickett (1955).

Fig. 4. Day-by-day scanning electron micrographs of casts of a developing petunia inflorescence apex (Paul Green: Silk, 2000). The apex shown in (J) is the same apex as shown (A), but 9 d later. F, flower; sm, sympodial meristem; fm, floral meristem; b, bract, bracts that have the same number belong to the same shoot; s, sepal; p, petal; st, stamen; c, carpel. Flowers and sepals are numbered in order of development. Scale bar, 100 μm.
noticeable. They are best seen in Fig. 4B, and are indicated in Fig. 5. Firstly, the bracts are not exactly opposite, but have a divergence angle of about 137.5°: the ‘golden angle’ (Kuhlemeier, 2007) (Fig. 5A). Secondly, one bract is slightly ahead in development: there is an upper and a lower bract. Sympodial meristems always develop in the upper bracts (Fig. 5A). Thirdly, the sepals and bracts appear in a continuous spiral pattern (Fig. 5B).

From Fig. 4A alone it is immediately clear that petunia is a scorpioid cyme. The flowers are not in one plane, which they would be if the inflorescence were a drepanium or rhipidium. If it were a helicoid cyme, then the sympodial meristem would emerge in the axil of the bract between fm3 and F1, in the middle of the image. However, the sympodial meristem arises in the axil of the other bract; at the edge rather than in the middle. Another, perhaps more comprehensible way to determine the scorpioid nature is to imagine that you are standing on the mature flower (F1), facing the next pair of bracts (b2 in this case); then the next flower (fm2) would appear to your left. Standing on fm2, the next flower would appear to your right. Thus, the position of subsequent flowers would alternate between your left- and right-hand sides. Knowing this, it can be predicted that, standing on the third flower, the fourth flower (fm4) will appear to the left, which indeed it does (Fig. 4F). If petunia were a helicoid cyme, subsequent flowers would always appear on the same side; either right or left. Knowing that petunia is a scorpioid cyme makes it easy to distinguish young floral meristems from sympodial meristems, which are morphologically similar through a scanning electron microscope; see Fig. 4B, C, G. Also in transverse and longitudinal sections used for in situ hybridization experiments it is difficult to determine whether a gene is expressed in sympodial or young floral meristems. This was experienced while determining the expression pattern of EVERGREEN (EVG) (Rebocho et al., 2008). Since evg mutants lack flowers, it is intuitively expected that EVG would be expressed in floral meristems. However, knowing that petunia is a scorpioid cyme, it was immediately clear from RNA in situ hybridization experiments that the meristems that express EVG actually are not floral meristems, but sympodial meristems (Fig. 5C).

ABERRANT LEAF AND FLOWER and DOUBLE TOP as marker genes

Most of the artificial colouring was straightforward, as structures such as bracts and floral organs are easily recognized by their shapes and positions. However, how we determined the sizes of the sympodial meristems in Fig. 4A,
F, and J may seem rather arbitrary; we could have coloured a larger or smaller part blue, since at that stage there is no visible separation between floral and sympodial meristems. However, our judgment was based upon meristems from other plants of which longitudinal sections had been made and RNA in situ hybridization carried out of two key genes that specify the floral identity of meristems: ABERRANT LEAF AND FLOWER (ALF) and DOUBLE TOP (DOT). ALF is the orthologue of LEAFY (LFY), and DOT is the orthologue of UNUSUAL FLORAL ORGANS (UFO) from Arabidopsis. In both strong alf and dot mutants, flowers are homeotically transformed into shoots that continue to produce bract-subtended sympodial meristems, thereby creating very similar flowerless inflorescences (Fig. 6A) (Souer et al., 1998, 2008).

Figure 6 illustrates how the expression patterns of ALF and DOT help to map a petunia inflorescence apex. Figure 6B shows an apparently uniform meristematic dome that is enclosed by two bracts. At this stage, it would be impossible accurately to draw a line that separates the sympodial meristem from the floral meristem. Figure 6C shows the same inflorescence apex section as in Fig. 6B, but now it has been hybridized with a digoxigenin-labelled antisense RNA probe of ALF. This makes the sympodial meristem visible at its earliest stages of development as a small area between bract and floral meristem that lacks ALF mRNA.

Another challenge is morphologically to distinguish a meristem with associated bract primordia from a floral meristem that has just started to produce sepal. However, they can be easily distinguished by molecular criteria, because DOT is expressed in the axils of sepal, not in the axis of bracts (Fig. 6D). If DOT is not functional, which it is in both dot and alf loss-of-function mutants (Souer et al., 2008), sepal will develop as bracts, from the axis of which sympodial meristems will arise, thus creating a flowerless inflorescence. Besides their specific expression patterns, ALF and DOT mRNA are, probably due to relatively high expression levels, easy to detect and are expressed during all stages of inflorescence development. These properties make ALF and DOT very suitable marker and control genes relative to which the expression of other genes can be mapped.

Since ALF and DOT are such major determinants of flower formation, and therefore inflorescence architecture, it is expected that their reduced activity would lead to increased branching. This is obvious in strong transposon-induced alf and dot knockout mutants: the flowerless inflorescences (Fig. 6A). Weak alf and dot mutants that contain flowers with petal defects have been described, but those plants have normal monochasial branching inflorescences (Souer et al., 1998, 2008). Another phenotype was recently observed among wild-type petunia plants that had been transformed with a construct that contains the genomic region of ALF (ALFg) (Fig. 6E). This phenotype seems due to a slightly stronger loss-of-function defect that is possibly triggered by unintended RNA interference, rather than a gain-of-function defect, as gain-of-function mutants that express ALF constitutively from the viral 35S promoter develop normally (Souer et al., 2008). Of those plants, the internodes between bracts of the same shoot are typically much larger than they are in the wild type, which makes it easier to see that the lateral shoots, not the flowers, are subtended by bracts. Most strikingly, although shoots do end with flowers, they generate new lateral shoots from the axils of the leaf-like organs that wild-type plants would develop as the first sepal. Presumably, the first sepal is homeotically transformed into a bract with an associated sympodial meristem, because floral commitment is delayed due to reduced ALF activity. As a result, instead of ending with a single flower, shoots end with cymose clusters of flowers (Fig. 6E).
In the terms of the transient model (Prusinkiewicz et al., 2007), both ALF and DOT are clear examples of genes required for the reduction of the levels of vegetativeness in meristems, so that it reaches the floral threshold, thereby making meristems terminate in flowers. Furthermore, the delayed expression of ALF and DOT in lateral meristems corresponds to a transient state of high vegetativeness in lateral meristems. We discuss whether the transient model could indeed produce the observed mutant phenotypes in the next section.

Petunia inflorescence branching mutants and the transient model

The transient model can readily simulate cymose, monochasial inflorescence development. However, the transient model produces only one type of monochasium: the drepanium. Incorporating the other types would go beyond the scope of the model, and really not add any value to it, because in terms of vegetativeness each type of monochasium develops similarly.

Without the activity of DOT or ALF, vegetativeness would remain high in all meristems and a flowerless, infinite inflorescence would be the result, which indeed is what alf and dot mutants look like (Fig. 6A). Following this logic, constitutive expression of ALF or DOT would make the level of vegetativeness reach the floral threshold prematurely; before bracts with associated new lateral shoots are produced. This predicts the production of an inflorescence that contains few flowers (Fig. 1A). Indeed, constitutive expression of DOT reduces the multi-flower petunia cyme to a single flowered inflorescence (Souver et al., 2008). By contrast, constitutive expression of ALF has no effect on inflorescence development whatsoever. However, this is because ALF activity depends on the post-translational activation by DOT, which therefore is the limiting factor. Constitutive expression of DOT in the absence of ALF (in alf mutants) has no effect on inflorescence architecture either; however, when ALF and DOT are simultaneously constitutively expressed, flowering commences at the seedling-stage (Souver et al., 2008).

Constitutive expression of DOT, or ALF and DOT together, and alf and dot knockout mutants represent two extremes of the level of vegetativeness; a very fast decline and a very slow decline, respectively. In these cases, the outcome may seem obvious, but predicting the inflorescence architecture when levels of vegetativeness are intermediate is difficult without modelling. Using the software of Prusinkiewicz et al. (2007), the intermediate alf phenotype (Fig. 6E) could be reproduced by reducing the decline of vegetativeness. Thus, the transient model can account for most empirical data on ALF and DOT.

Other inflorescence branching mutants are shown in Fig. 7. In the late-flowering mutant veggie, termination of a sympodial meristem in a flower is much delayed; instead of two bracts, a series of leaves precedes each flower, but the monochasial nature of the inflorescence is unaffected (Fig. 7A, B) (Koes et al., 2009; I Roobeek and R Koes, unpublished data). If the level of vegetativeness is increased in sympodial meristems of veggie, increased branching would be expected, but this is not observed. One explanation is that lateral meristems have such high levels of vegetativeness that they have a vegetative rather than an inflorescence identity and generate leaves rather than lateral meristems. Even though the transient model cannot produce a veggie inflorescence, which is because it does not comprise fully vegetative meristems that produce vegetative leaves, the veggie phenotype can be explained in terms of the concept of vegetativeness underpinning the model.

Both extrapetals (exp) and hermit (her) mutants have a single flower inflorescence, similar to transgenic plants that constitutively express DOT (Souver et al., 1996; Rebocho et al., 2008) (Fig. 7C, D); after the main axis has terminated in a flower, no sympodial shoots develop. This makes EXP and HER candidate genes that control the repression of floral identity in sympodial meristems, or in other words, promoters of vegetativeness. Indeed, it has now been shown that EXP transiently represses floral identity in sympodial meristems (Castel, 2009) (R Castel, E Kusters, A Procissi, M Bliek, E Souer, R Koes, unpublished data). Cymose branching is inhibited in exp mutants, because sympodial meristems acquire floral identity before they have produced bracts, and become incorporated in the first flower. However, HER does not control the level of vegetativeness in sympodial meristems, but rather is required for their initiation and/or maintenance (Castel, 2009) (R Castel, E Kusters, M Vandenbussche, R Koes,
unpublished data). The exp and her mutants are examples of how similar inflorescence phenotypes can be caused by distinct mutations in apparently unrelated pathways. Either sympodial meristems acquire floral identity prematurely (exp), or they fail to be initiated and/or maintained (her); both defects effectively abort cymose branching. As the transient model predicts, branching can be restored by increasing the level of vegetativeness in sympodial meristems in both exp and transgenic plants that constitutively express DOT. In exp this is accomplished by mutating DOT, and constitutive expression of DOT can be antagonized by constitutive expression of EXP: both exp dot double mutants and plants that constitutively express both DOT and EXP simultaneously, produce monochasial cymes. As expected, cymose branching in her mutants is not restored in a dot mutant background (Castel, 2009).

Thus, our experimental data illustrate that the transient model is simplistic, but they do not undermine the underlying core principles of the model.

Inflorescence development in petunia compared to other Solanaceae

The series of the petunia inflorescence apex shown in Fig. 4 is morphologically very similar to scanning electron micrographs of inflorescence apices of tomato, potato, and tobacco, which are all closely related to petunia (Green and Linstead, 1990; Allen and Sussex, 1996; Hart and Hannapel, 2002; Reinhardt and Kuhlemeier, 2002; Welty et al., 2007; Lippman et al., 2008). Yet there are two schools of thought on the ontogeny of these inflorescences. One is that after the main stem (or the previous sympodial shoot), has ended in a flower, sympodial meristems arise in the axils of bracts (Pnueli et al., 1998; Reinhardt and Kuhlemeier, 2002; Lifschitz and Eshed, 2006; Lippman et al., 2008; Elitzur et al., 2009). The other is that lateral shoots are produced by bifurcations of the main apex, thereby maintaining an indeterminate apical meristem (Allen and Sussex, 1996; Hart and Hannapel, 2002; Quinet et al., 2006; Welty et al., 2007). Such contrasting views on a basic issue are undesirable, as they add confusion to a field that already is bothered by troublesome terminology.

The splitting of an inflorescence meristem into two halves, of which one differentiates into a flower, and the other half will divide again into two parts, is a process known as dichotomy (Rickett, 1944; Nolan, 1969). The concept that cymose inflorescences are produced by dichotomy may have been invoked to explain the existence of putatively bractless cymose inflorescences. It was accepted during a period of the second half of the 19th century, but had largely been discredited and ignored, until it received renewed attention after Allen and Sussex (1996), based on scanning electron micrographs, had argued that the inflorescence meristems of tomato produce floral meristems by a series of unequal divisions. Their view is shared by others (Quinet et al., 2006; Welty et al., 2007). However, these authors ignored the fact that the inflorescence meristems in tomato are subtended by rudimentary bracts, in the axils of which they might have been initiated. This raises the question whether truly bractless Solanaceous inflorescences exist at all. A careful reinvestigation into the possible development of bracts in putatively bractless species, for instance potato (Hart and Hannapel, 2002), may lead to the conclusion that truly bractless inflorescence branching, at least among the Solanaceae, is a botanical myth. Therefore, until convincingly shown, perhaps it would be best to avoid using the term bractless in the description of inflorescence development in a given species.

At first sight, the cleft that is formed in between sympodial and floral meristems, as seen through a scanning electron microscope, is suggestive of a bifurcating meristem. However, because of the nature of scanning electron microscopy the images are entirely in focus, hence sympodial and floral meristem domes appear flatter and therefore smaller than they really are. This makes sympodial meristems visible only when they are fairly well developed, and it also makes sympodial and floral meristems seem of approximately equal size, while from in situ hybridized sections (Fig. 6) it is clear that sympodial meristems initiate in petunia as small groups of cells in the axils of bracts, and that floral meristems are much larger than sympodial meristems. The same appears to hold for tomato, as the expression patterns of ANANTHA (AN) and COMPOUND INFLORESCENCE (S) within the inflorescence apex are very similar to those of the petunia homologues DOT and Evg, respectively (Lippman et al., 2008).

Another factor that contributes to the appearance of a bifurcating meristem is the vigorous growth of sympodial meristems directly on the flanks of floral meristems, by which they push the flowers aside, which makes them seem to appear suddenly, especially in images that are not part of a chronological series. Furthermore, the fact that the bracts and sepals that are produced by a sympodial meristem form a continuous spiral supports the idea that the main axis is built of separate shoots. From an evolutionary point of view, branching from the axils of bracts is the most parsimonious scenario, as this would imply that the initiation of new sympodial inflorescence meristems in petunia and tomato is essentially similar to the formation of lateral (floral) meristems in racemes like Arabidopsis and Antirrhinum and that architectural differences primarily result from changes in the identity that these meristems assume, in line with the model of Prusinkiewisz et al. (2007). Taking all observations into account, we favour the view that monochasial branching in petunia occurs sympodially from the axils of bracts, not monopodially by the division of meristems. Since petunia, tomato, potato, and tobacco are such closely related species, it might be expected that their mode of development will be similar and that none of the inflorescences would develop by dichotomy.

Tomato differs in one important aspect from petunia, in that concomitant with the development of the first inflorescence (or truss), a lateral meristem emerges in the axil
of the last formed leaf that generates several leaves and then the next truss (Lippman et al., 2008; Shalit et al., 2009). Because this latter meristem, which is also called a sympodial meristem, grows more vigorously it tends to push the truss to a more lateral position creating the false impression of a straight shoot with many lateral trusses. Even though the inflorescence of tomato (the truss) and petunia develop in very similar ways, it is unclear whether both structures are truly homologous in the evolutionary sense of the word.

At least two scenarios can be envisaged by which the tomato and petunia architecture diverged (Fig. 8). The first scenario involves two steps: (i) the conversion of the cymose tomato inflorescence into a solitary flower and (ii) an accelerated development of the vegetative sympodial shoot and reduction of vegetativeness, for example, by alterations in the expression of floral meristem identity genes, so that it generates only two leaves before the next flower. In the second scenario, the vegetative sympodial shoot of tomato acquires a dormancy similar to other meristems in leaf axils, because of which it does not sprout precociously. In a petunia mutant, floozy, with a defect in local auxin synthesis, meristems in bract axils sprout precociously resulting in an architecture that resembles to some extent that of tomato (Tobena-Santamaria et al., 2002). Although both scenarios are conceivable and fully compatible with the existing data, they imply different homology relations between tomato and petunia structures. Scenario 1 implies that the tomato truss is homologous to a single petunia flower, whereas scenario 2 implies that it is homologous to the entire petunia inflorescence. The two scenarios share the same start and endpoint and differ only with regard to the hypothetical intermediate. Hence, they cannot be distinguished by comparing petunia and tomato alone and require analyses of additional species in order to identify (or obtain indirect information on) evolutionary intermediates.

Concluding remarks

In this review, we hope to have illustrated that the current botanical jargon is confusing and understandably discouraging to scientists who might consider joining the field. It is natural for the meaning of terms to change over time as science advances, but if terms are applied too loosely, they risk becoming meaningless and confusing. We recommend that the terms cyme, raceme, and panicle are applied to the main broad types of inflorescence architectures with distinct modes of development, in agreement with the transient model of Prusinkiewicz et al. (2007). Although cymes and racemes may occasionally produce morphologically similar structures, they do develop quite differently and represent different body plans. Clearly the mode of development is the most important factor to consider in evolutionary developmental biology as well as taxonomy. Since advanced molecular tools are available for a variety of (relatively closely related) species that have different body plans, plants are promising models for evolutionary developmental biology (evo-devo), but, unfortunately, have been relatively neglected in that field (Jenner and Wills, 2007). Possibly this has to do with the existing maze of botanical terminology in which one is easily lost.

Supplementary data

Supplementary data are available at JXB online.

Supplementary Fig. S1. The uncoloured day-by-day scanning electron micrographs showing casts of a developing petunia inflorescence apex: made by the late Paul Green (Silk, 2000). See main Fig. 4.

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