Genetic control of flower morphogenesis in Arabidopsis thaliana: a logical analysis

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Abstract

**Motivation:** A large number of molecular mechanisms at the basis of gene regulation have been described during the last few decades. It is now becoming possible to address questions dealing with both the structure and the dynamics of genetic regulatory networks, at least in the case of some of the best-characterized organisms. Most recent attempts to address these questions deal with microbial or animal model systems. In contrast, we analyze here a gene network involved in the control of the morphogenesis of flowers in a model plant, Arabidopsis thaliana.

**Results:** The genetic control of flower morphogenesis in Arabidopsis involves a large number of genes, of which 10 are considered here. The network topology has been derived from published genetic and molecular data, mainly relying on mRNA expression patterns under wild-type and mutant backgrounds. Using a ‘generalized logical formalism’, we provide a qualitative model and derive the parameter constraints accounting for the different patterns of gene expression found in the four floral organs of Arabidopsis (sepals, petals, stamens and carpels), plus a ‘non-floral’ state. This model also allows the simulation of the prediction of various mutant phenotypes. On the basis of our model analysis, we predict the existence of a sixth stable pattern of gene expression, yet to be characterized experimentally. Moreover, our dynamical analysis leads to the prediction of at least one more regulator of the gene LFY, likely to be involved in the transition from the non-flowering state to the flowering pathways. Finally, this work, together with other theoretical and experimental considerations, leads us to propose some general conclusions about the structure of gene networks controlling development.

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Introduction

The study of the molecular basis underlying development and morphogenesis is an active area of biological research. Most studies use animals as model systems, mainly *Drosophila melanogaster*, *Caenorhabditis elegans*, *Xenopus laevis* and *Mus musculus*. More recently, plants have also become the focus of molecular geneticists, and new insights into the genetic and molecular mechanisms underlying plant development have been gained through the study of vegetal model systems, especially *Arabidopsis thaliana*. Flower development has received special attention, and many genes involved in this process have now been characterized at the genetic and molecular levels (for a review, see Weigel, 1995). Though still fragmentary, published data already support the existence of a complex regulatory network controlling flower morphogenesis in *Arabidopsis*.

A qualitative model for flower morphogenesis in *A. thaliana* was recently proposed (the ‘NET model’; Mendoza and Alvarez-Buylla, 1998), together with a preliminary analysis of its dynamical behavior. Here, we develop an extensive analysis of the relationship between the architecture of this gene network (in terms of feedback circuits) and its dynamical behavior. Basic formal prerequisites are introduced hereafter, whereas the experimental background supporting the model is summarized in the last part of this introductory section. A second section is devoted to the presentation of the NET model and its feedback circuit analysis, whereas a third section contains a thorough discussion of the biological relevance of the results, followed by some conclusions and further prospects.

Feedback circuits and logical formalization

Experimental and theoretical biologists have long been aware of the important biological and dynamical roles of genetic ‘feedback circuits’ or ‘feedback loops’ (see, for example, Monod and Jacob, 1961; Rosen, 1968). Feedback circuits are defined as circular chains of interactions, such that each element of a circuit influences its own future level. Whenever specific signs can be associated to each interaction, a given circuit can be unambiguously classified in one of the two following classes: either each element of the circuit exerts a positive direct or indirect effect on itself and the circuit is said to be ‘positive’, or each element of the circuit exerts a negative effect on itself and the circuit is said to be ‘negative’. The sign of a circuit is easily determined by
checking the parity of the number of negative interactions involved. If this number is even or zero, the circuit is positive, but if the number is odd the circuit is negative. Positive- and negative-feedback circuits have different dynamical and biological properties (for a review, see Thomas et al., 1995). From a dynamical point of view, positive circuits yield multistationarity, whereas negative circuits generate (damped or sustained) oscillatory behavior. Biologically, positive circuits are necessary to accomplish differentiation (i.e. alternative patterns of gene expression), whereas negative circuits are necessary to generate homeostasis. Formulated by Thomas about 20 years ago, these conjectures have recently been demonstrated in a general formal context (Plahte et al., 1995; Gouzé, 1998; Snoussi, 1998).

Formally, a gene network can be represented by a set of differential equations. Most of the time, however, little is known about the precise shapes of regulatory interactions, or about the values of the various parameters. This situation led several authors to propose a qualitative or ‘logical’ formalization of sets of interacting genes (e.g. Kauffman, 1969; Glass and Kauffman, 1973). In this paper, we use the formalism introduced by Thomas, which has the advantage of being particularly suited to evaluating the roles of the various feedback circuits present in a given network.

Following Thomas, we associate a logical variable (noted by a lowercase letter, e.g. ‘x’) and a logical function (noted by an uppercase letter, e.g. ‘X’) to each gene of a network. The logical variable represents the actual expression state of a gene, while its logical function indicates the gene’s future state as determined by the action of the genes (activators and inhibitors) that regulate it. The expression states are characterized by a limited number of integer values (0, 1, 2, …), but ‘threshold’ values \([s^{(1)}, s^{(2)}, …]\) that separate the expression states are also considered. Finally, logical parameters (noted by \(Ks\) and appropriate indices) are introduced to qualify the weight of each interaction, or combination of interactions, on the expression of a regulated gene (Snoussi, 1989).

For example, \(K_a\) represents the lowest expression state of a gene \(a\), \(K_{ab}\) indicates the effect of an activation of gene \(b\) over \(a\), \(K_{abc}\) the combined activatory effect of genes \(b\) and \(c\) over the state of \(a\), and so forth. In the simplest cases, our logical variables, functions and parameters take one of the three values \(\{0, s, 1\}\), where \(s\) stands for the threshold between ‘0’ and ‘1’ (for a review, see Thomas, 1991). A simple case of two interacting genes and the dynamical consequences of different values of \(Ks\) is shown in Figure 1.

In the context of this logical formalism, an \(n\)-element gene network can be described by two matrices: the first \((n \times n)\) matrix contains the signs (and eventually the thresholds) of all interactions, the second \((n \times 2^n)\) matrix contains the values of the logical parameters. The state of the system is represented by a vector of dimension \(n\). Whenever this state vector and its image (i.e. the vector formed by the values of the corresponding functions) are equal, there is a steady state in the system. Because we explicitly take into account threshold values, we will distinguish between ‘regular’ and ‘singular’ logical states. Regular states are those including only zeros and ones, while singular states may include one or more threshold values. Finally, the discrete approach used here guarantees the explicit calculation of numerical values of all parameters and functions. Therefore, it is possible to identify all the steady states of the system.

A much more interesting approach, however, consists of using the notion of feedback circuit. Clearly, the effect of a circuit will not depend only on the mere existence of the relevant interactions, but also on their relative strengths. Within appropriate parametric ranges, positive circuits generate multistationarity and negative circuits generate homeostasis. Therefore, if a circuit is found to generate its associated dynamical behavior, we will say that the circuit is ‘functional’.

That is, a positive circuit is functional if it actually produces different stable steady states separated by unstable steady states. Conversely, a negative circuit is functional when it generates damped or sustained oscillatory behavior. Figure 1 presents a simple example of how circuit functionality depends on the parametric values (\(Ks\)). In this example, the circuit is functional only for the first set of values.

In the context of Thomas’ logical formalism, it is possible to compute the parameter constraints making any single feedback circuit functional. Moreover, it was found that whenever such a circuit is functional, it generates a singular (‘characteristic’) steady state located on the thresholds of the interactions forming the circuit. More precisely, when the circuit is positive, its characteristic state is always unstable (typically a saddle point in two dimensions) and stands on a separatrix. When the circuit is negative, its characteristic state can be either stable or unstable (typically a focus in two dimensions). Snoussi and Thomas (1993) have demonstrated that only those singular states which are circuit characteristic can be steady, thus making superfluous the scanning of all ‘non-characteristic’ singular steady states.

The logical formalism outlined above enables us to dissociate a complex network into a well-defined set of feedback circuits and check their dynamical roles individually, yet keeping complete control of the ways in which these circuits are interconnected. The scope of this paper consists precisely in applying this approach to the analysis of the genetic network controlling flower morphogenesis in Arabidopsis. Before that, however, we still need to summarize the basic genetic and molecular data supporting the ‘NET model’.

**Flower development in Arabidopsis**

Mature flowers of Arabidopsis display a stereotyped architecture that consists of four concentric whorls of organs. These are from the external to the internal whorls: four sepals...
Fig. 1. Schematic representation of the parameter constraints on circuit functionality for a two-element cross-inhibitory positive circuit. The step curves give the value of the logical function $X$ (respectively $Y$) as a function of the value of the logical variable $y$ (respectively $x$). In biological terms, the step curve $X = F(y)$ represents the level of expression of gene $X$ in response to increasing concentrations of the regulatory product $y$ [similarly for $Y = F(x)$]. These step curves correspond to the ‘nullclines’ in the differential formalism (ODEs) and, consequently, intersections (circled) between the step curves give the steady states of the system. The number of steady states depends on the parameter values. The four diagrams correspond to four different combinations of parameter values. As we account only for two states corresponding to maximal and minimal transcriptional activities, the variables, functions and parameters can take only one of the two regular values 0 and 1, or, in special cases, the threshold value $s$. The logical parameters ($K$s) determine the amplitude of each function $F$, accordingly with the value of the relevant variable. For example, $K_x$ stands for the level of expression of gene $X$ (function $X$) in the presence of high concentrations of its repressor, the regulatory product $y$ (variable $y$); its value is set to 0 in (A) and (C), whereas it is set to 1 in (B) and (D) [leftmost vertical segment of $X = F(y)$]. $K_{x,y}$ stands for the level of expression of gene $X$ in the absence of the repressor $y$; it has the value 1 in all four illustrated cases [rightmost vertical segment of $X = F(y)$]. $K_y$ stands for the level of expression of gene $Y$ in the presence of its repressor $x$; its value is set to 0 in all four examples [rightmost horizontal segment of $Y = F(x)$]. Finally, $K_{y,x}$ stands for the level of expression of gene $Y$ in the absence of its repressor $x$; it has the value 1 in cases (A) and (B), and the value 0 in case (C) and (D) [leftmost horizontal segment of $X = F(y)$]. Note that multistationarity is found only in case (A), where $K_x = K_y = 0$ and $K_{x,y} = K_{y,x} = 1$.

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widely used to describe the morphology of wild-type and mutant Arabidopsis flowers. This model (Coen and Meyerowitz, 1991) postulates the existence of three different genetic functions or activities (A, B and C), each of them present in two adjacent whorls. According to this model, each whorl requires a specific combination of genetic activities: activity A in the first whorl determines sepal identity; activities A and B combined in the second whorl determine petal identity; activities B and C combined in the third whorl determine stamen identity; finally, activity C alone in the fourth whorl determines carpel identity. Furthermore, the ABC model postulates a mutual inhibition between activities A and C.

The ABC model also accounts for the phenotype of homeotic floral mutants (see Figure 2). Nevertheless, we still lack a thorough understanding of the molecular mechanisms underlying the ABC model. Recent genetic and molecular studies have already uncovered over a dozen genes involved in the control of flower morphogenesis. Some of these are clearly associated with each of the ABC functions described above. The gene APETALA1 (AP1) is associated with function A (Mandel et al., 1992; Bowman et al., 1993), APETALA3 (AP3) and PISTILLATA (PI) are B genes (Krizek and Meyerowitz, 1996), and AGAMOUS (AG) is the only gene associated with the C function (Yanofsky et al., 1990). Moreover, these genes have been found to cross-regulate with many others and thus form a complex regulatory network summarized in Figure 3. The molecular and morphological data underlying this net model are shown in Table 1.

### Feedback circuit analysis of the NET model

A quick look at the interaction matrix in Figure 3 indicates that EMF1, LUG, UFO and SUP do not receive any regulatory input, although each of them regulates at least one gene of the network. Closer inspection leads to the identification of eight feedback circuits involving from one to four of the remaining six genes. Moreover, considering the variables involved, we can split these circuits into two groups: TFL1-LFY, LFY-AP1, AP1-AG, LFY-AG-AP1 and TFL1-AG-AP1-LFY, on the one hand; AP3, PI and AP3-PI, on the other hand. Our 10-variable network can thus be considered as the combination of two smaller subnetworks, one formed by AP3 and PI, and the other one formed by TFL1, LFY, AP1 and AG. The other four genes (EMF1, LUG, UFO and SUP) can be represented as input variables. Note, however, that the two subnetworks are not isolated, because the smallest receives two inputs from LFY, which is part of the larger subnetwork. In the next two sections, we consider the dynamics of each of these two subnetworks and then reconstitute the dynamics of the whole network.

### Table 1. Experimental data that support the gene interactions included in the NET model.

<table>
<thead>
<tr>
<th>Interactions</th>
<th>Main evidence</th>
<th>Main references</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG —&gt; AP1</td>
<td>AP1 mRNA accumulates uniformly in ag-1 mutant flowers</td>
<td>Gustafsson-Brown et al. (1994)</td>
</tr>
<tr>
<td>AP1 —&gt; AG</td>
<td>Sepals are replaced by carpels, and petals by stamens in ap1 mutants. AG mRNA found in all the flower primordium of ap1-1 plants</td>
<td>Bowman et al. (1993)</td>
</tr>
<tr>
<td>AP3/Pi —&gt; AP3/Pi</td>
<td>AP3 and Pi mRNA levels are not maintained in ap3-3, pi-1 or double mutants. Co-immunoprecipitation of AP3 and Pi proteins</td>
<td>Bowman et al. (1993)</td>
</tr>
<tr>
<td>EMF1 —&gt; AP1,LFY</td>
<td>Inferred by morphological evidence that EMF1 inhibits the flowering promoting genes</td>
<td>Mendoza and Alvarez-Buylla (1998)</td>
</tr>
<tr>
<td>EMF1 —&gt; TFL1</td>
<td>Inferred by morphological evidence that EMF1 activates the late late-flowering genes</td>
<td>Mendoza and Alvarez-Buylla (1998)</td>
</tr>
<tr>
<td>LFY —&gt; AG</td>
<td>Early expression of AG is abnormally low in ify-6 flowers</td>
<td>Weigel and Nilsson (1995)</td>
</tr>
<tr>
<td>LFY —&gt; AP1</td>
<td>AP1 mRNA delayed in ify mutants. Earlier AP1 promoter induction in plants overexpressing LFY</td>
<td>Parcy et al. (1998)</td>
</tr>
<tr>
<td>LFY —&gt; AP3</td>
<td>Amount and domain of AP3 expression reduced in ify-6 mutants</td>
<td>Weigel and Meyerowitz (1993)</td>
</tr>
<tr>
<td>LFY —&gt; PI</td>
<td>Amount and domain of PI expression reduced in ify-6 mutants</td>
<td>Weigel and Meyerowitz (1993)</td>
</tr>
<tr>
<td>LFY —&gt; TFL1</td>
<td>Plants overexpressing LFY are very similar to tfl1 mutants</td>
<td>Weigel and Nilsson (1995)</td>
</tr>
<tr>
<td>SUP —&gt; AP3</td>
<td>Ectopic expression of AP3 in sup-1 mutants</td>
<td>Sakai et al. (1995)</td>
</tr>
<tr>
<td>SUP —&gt; PI</td>
<td>Contrary to wild type, PI expression is not reduced in the center of sup-1 flowers</td>
<td>Goto and Meyerowitz (1994)</td>
</tr>
<tr>
<td>TFL1 —&gt; AG</td>
<td>Inferred from morphological evidence. Double mutants ap1-1 ap2-2 have a disrupted C activity, which is rescued with the addition of tfl1 mutation</td>
<td>Mendoza and Alvarez-Buylla (1998)</td>
</tr>
<tr>
<td>TFL1 —&gt; LFY</td>
<td>Precocious appearance of floral buds expressing LFY in tfl1-2 plants</td>
<td>Weigel et al. (1992)</td>
</tr>
<tr>
<td>UFO —&gt; AP3</td>
<td>AP3 protein and messenger levels reduced in ufo-2 plants</td>
<td>Levin and Meyerowitz (1995)</td>
</tr>
<tr>
<td>UFO —&gt; PI</td>
<td>PI mRNA reduced in early stages of flower development in ufo-2 plants</td>
<td>Levin and Meyerowitz (1995)</td>
</tr>
</tbody>
</table>
The analysis of this subsystem is straightforward as it is made of only two elements. The general state table is given in Table 2.

Depending on the value of the logical parameters, this table covers various dynamics. Note that the system involves three positive feedback circuits. The parameter constraints that make each of these circuits functional are given in Table 3.

Remember that AP3 and PI genes are both associated with the B function. Both genes are expressed in petals and stamens, but not in sepals and carpels. Moreover, it is known that AP3 and PI regulate their own, as well as each other’s, transcription through the formation of a heterodimer of their protein products (Goto and Meyerowitz, 1994). In logical terms, these regulatory interactions can be represented by \( K_p = K_{p,p} = K_{p,i} = 0, K_{p,pi} = 1 \) for the parameters associated with the regulation of the expression of gene AP3 (indices \( p \)), and by \( K_i = K_{i,i} = K_{i,p} = 0, K_{i,pi} = 1 \), for the parameters associated with PI (indices \( i \)). Once included in Table 2, these parameter values give two stable states, [00] and [11], corresponding to the absence and presence of B function, respectively. For the parameter values selected, inspection of Table 3 reveals that the two-variable positive circuit is fully functional, whereas the one-variable positive circuits are functional only in some limited regions of the variable space. Additionally, the system comprises a third unstable steady state, namely [ss], which is the characteristic state of the two-element positive circuit.

### Table 2. General state table for the subnetwork AP3-PI. Variables \( p \) and \( i \) stand for the levels of activity of the regulatory products of genes AP3 and PI, respectively, whereas functions \( P \) and \( I \) stand for the levels of expression of the corresponding genes. Each row corresponds to a specific combination of the possible values (0 or 1) for the variables \( p \) and \( i \). In biological terms, the rows thus correspond to different combinations between the two levels of activity for the two regulatory products. For example, ‘10’ in the penultimate row means that the first regulatory product (AP3) is present at a high level, whereas the second product (PI) is absent (or present at a negligible level). Under the columns \( P \) and \( I \) are given the corresponding levels of gene expression in terms of the logical parameters (\( K_i \)). \( K_p \) stands for the basal expression of gene AP3, \( K_{pi} \) for its expression in the presence of the sole product of gene AP3 (autoregulation), \( K_{pi} \) for its expression in the presence of the sole product of gene PI and \( K_{pi} \) for its expression in the presence of both regulatory products. The meaning of parameters \( K_{pi} \), \( K_{pi} \), \( K_{pi} \) and \( K_{pi} \) is analogous to that associated with AP3. Clearly, different values of the logical parameters (\( K_i \)) may give rise to different dynamics.

<table>
<thead>
<tr>
<th>( p )</th>
<th>( i )</th>
<th>( P )</th>
<th>( I )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>( K_p )</td>
<td>( K_i )</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>( K_{p,i} )</td>
<td>( K_{i} )</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>( K_{p,pi} )</td>
<td>( K_{p} )</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>( K_{pi} )</td>
<td>( K_{pi} )</td>
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</table>

### Table 3. Feedback circuits of the AP3-PI subnetwork and the corresponding functionality constraints. This subnetwork formed by AP3 and PI (variables \( p \) and \( i \)) contains three circuits that are indicated in the leftmost column. The \( K \) columns give the parameter constraints to be fulfilled for each feedback circuit to be functional in a particular region of the phase space (‘Domain’ column). A ‘-’ means that there are no constraints on the corresponding parameter. The values selected on the basis of experimental data (see the text) are indicated in the lowermost row. Note that for these values, the two autorregulatory circuits are functional only in a limited region of the phase space, whereas the two-element circuit is functional in the entire state space. Notation of the parameters is as in Table 2.

<table>
<thead>
<tr>
<th>Loop</th>
<th>Domain</th>
<th>( K_p )</th>
<th>( K_{p,p} )</th>
<th>( K_{p,i} )</th>
<th>( K_{p,pi} )</th>
<th>( K_i )</th>
<th>( K_{p} )</th>
<th>( K_{pi} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )</td>
<td>S[1]</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
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<tr>
<td></td>
<td>S[0]</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( i )</td>
<td>[1]S</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>[0]S</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( pi )</td>
<td>SS</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>0</td>
<td>–</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

### AP3-PI subnetwork

The analysis of this subsystem is straightforward as it is made of only two elements. The general state table is given in Table 2.

For the four-variable subnetwork, we have also derived some of the parameter values from gene expression data in the outer and inner whorls of the flower. First, we need to account for a default ‘non-flowering state’. Considered as a flower suppressor, TFL1 is expressed in plant apices before flower development (Ratcliffe et al., 1998). In contrast, LFY expression has been shown to be very low before flowering, reaching its highest level upon flower induction (Nilsson et al., 1998). Finally, AP1 and AG genes are known to be specifically expressed during flowering. This leads us to associate the state 1000 (vector notation for TFL1 = 1, LFY = 0, AP1 = 0, and AG = 0) with the non-flowering state. Looking at the corresponding (tenth) row in Table 4, we find that state 1000 will be steady if \( K_{i,t} = 1 \), and \( K_i = K_{a,g} = K_{g,a} = 0 \), where indices \( t \), \( a \), and \( g \) stand for TFL1, LFY, AP1, and AG, respectively.

In contrast with the default state mentioned above, the flower suppressor gene TFL1 should be ‘off’ during flower morphogenesis and in each of the developing flower organs. In addition, as LFY is known to be only transiently expressed in most of the developing flower (Weigel et al., 1992), we consider that its final steady value is ‘0’ in the flower organs. Finally, taking into account the association of AP1 with A function, that of AG with C function, as well as their probable mutual inhibition, we are led to associate the states 0010 and 0001 with A and C functions, respectively. On the basis of Table 4, the first of these states will be steady if \( K_{i,t} = K_{i,ta} = K_{p,t} = 0 \) and \( K_{p,ta} = 1 \), whereas the second will be steady for \( K_{i,t} = K_{i,ta} = K_{p} = 0 \) and \( K_{p,ta} = 1 \).

### TFL1-LFY-AP1-AG subnetwork

Analysis of the four-variable subnetwork is somewhat more complex, but still straightforward. By hand or using a computer program (Thieffry et al., 1993), we obtain Table 4 (general state table) and 5 (functionality constraints for the five feedback circuits of the system).

For the four-variable subnetwork, we also derived some of the parameter values from gene expression data in the outer and inner whorls of the flower. First, we need to account for a default ‘non-flowering state’. Considered as a flower suppressor, TFL1 is expressed in plant apices before flower development (Ratcliffe et al., 1998). In contrast, LFY expression has been shown to be very low before flowering, reaching its highest level upon flower induction (Nilsson et al., 1998). Finally, AP1 and AG genes are known to be specifically expressed during flowering. This leads us to associate the state 1000 (vector notation for TFL1 = 1, LFY = 0, AP1 = 0, and AG = 0) with the non-flowering state. Looking at the corresponding (tenth) row in Table 4, we find that state 1000 will be steady if \( K_{i,t} = 1 \), and \( K_i = K_{a,g} = K_{g,a} = 0 \), where indices \( t \), \( a \), and \( g \) stand for TFL1, LFY, AP1, and AG, respectively.

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Fig. 2. Schematic representation of the wild-type Arabidopsis flower and the ABC model. The mutant types are in the leftmost column. The domains of expression of the ABC genetic activities in the four floral whorls are shown in the central column of the diagram. Longitudinal sections of wild-type (upper part) and mutant (lower part) Arabidopsis flowers are schematized in the rightmost column. For example, mutations in the *AP1* gene eliminate activity A, and activity C expands to the first two whorls; this causes the *ap1* mutant flowers to have carpels, stamens, stamens and carpels. se, sepals; pe, petals; st, stamens; ca, carpels.
Genetic control of flower morphogenesis in *Arabidopsis*. Activatory and repressory relationships are indicated by arrows and blunt lines, respectively. In the matrix, the rows give the sign of the transcriptional response of each gene (italics) to the gene products indicated in the column headings. Positive and negative transcriptional interactions are represented by plus (+) and minus (−) signs, respectively. Zeros indicate the absence of a transcriptional interaction. Focusing on the genes involved in feedback circuits, two subnetworks can be readily distinguished: one involving the genes *AP3* and *PI*, and the other involving the genes *TFL1*, *LFY*, *AP1* and *AG*. These subnetworks are highlighted in the matrix.

Note that the consideration of these different steady states leads to conflicting values for $K_{t.l}$ and $K_{a.g}$. In fact, this apparent contradiction disappears when we include the input variables in our description. Specifically, *EMF1* is a key flowering repressing element (Yang et al., 1995), that activates *TFL1* and represses *LFY* and *AP1* (see Table 1). Rather than directly introducing an additional index in the parameter notation, we hereafter consider two sets of parameter values, corresponding to the presence and the absence of *EMF1*, respectively.

In the first case, where the flower inhibitor is present (*EMF1* = 1), we have $K_{t.l} = 1$ and $K_{a.g} = 0$, giving rise to a unique, regular steady state, namely [1000] (all other $K$s = 0, except $K_{a.lg} = K_{g.tl} = K_{g.ta} = K_{g.la} = 1$). This state corresponds to the default state. Looking back to Table 5, we find that all three two-element circuits are functional, but only in a limited region of the variable space. Therefore, they do not generate any complete separatrix, while the system includes a single attractor representing the non-flowering stage.

In the second case, with the flower inhibitor absent (*EMF1* = 0), we have $K_{t.l} = 0$ and $K_{a.g} = 1$ (all other parameters identical to the preceding case). This leads to the two regular stable states [0010] and [0001], representing functions A and C, respectively. In this case, only one circuit is functional (see Table 5), corresponding to the mutual exclusion of *AP1* and *AG*. This circuit generates a separatrix across the variable space, defining two basins of attraction, each draining towards one of the stable states [0010] or [0001]. An additional, singular, steady state [00ss] is found on this separatrix. This singular state is unstable because it is generated by the positive circuit *AP1*-*AG*.
Table 4. General state table for the TFL1, LFY, AP1 and AG subnetwork. Variables \( t, l, a \) and \( g \) stand for the levels of activity of the regulatory products of genes TFL1, LFY, AP1 and AG, respectively, whereas functions \( T, L, A \) and \( G \) stand for the levels of expression of the corresponding genes. Notation of the parameters is as in Table 2.

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<th>( t )</th>
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Table 5. Feedback circuits of TFL1, LFY, AP1 and AG subnetwork and the corresponding functionality constraints. This subnetwork contains five circuits that are indicated in the leftmost column. The \( K \) columns give the parameter constraints to be fulfilled for each feedback circuit to be functional in a particular region of the phase space (‘Domain’ column). For the parameter notation, see Table 2.

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Dynamics of the whole network

On the basis of the results obtained for the two subnetworks, it is now possible to derive some key features of the dynamics of the whole system. The steady states of the whole network can be constructed by combining the steady states of the two subnetworks and relevant values of the four input variables. Since EMF1 is a floral repressor gene, it is considered active only in non-floral attractors. In addition, it is known that UFO, LUG and SUP regulate the fine spatial expression patterns of the organ identity genes (Levin and Meyerowitz, 1995; Liu and Meyerowitz, 1995; Sakai et al., 1995). Consequently, these three genes play a role only in the transient activation patterns of the organ identity genes AG.
AP3 and PI, but not in the establishment of the floral organs themselves. Therefore, the combination of the stable states found for the two subnetworks leads to the following six attractors:

\[
\begin{align*}
[00] + [0010] & \rightarrow [0001000000] \text{ (A = sepals)} \\
[11] + [0010] & \rightarrow [0001000110] \text{ (AB = petals)} \\
[11] + [0001] & \rightarrow [0001000110] \text{ (BC = stamen)} \\
[00] + [0001] & \rightarrow [0001000000] \text{ (C = carpels)} \\
[00] + [1000] & \rightarrow [1100000000] \text{ (flower inhibition)} \\
[11] + [1000] & \rightarrow [1100000110] \text{ (6th attractor)}
\end{align*}
\]

using the vector notation \([pi] + [itag] \rightarrow [etlagufpis]\), where \(e, t, l, a, g, u, f, p, i\) and \(s\) stand for EMF1, TFL1, LFY, AP1, AG, LUG, UFO, AP3, PI and SUP, respectively.

This set of attractors can be obtained for several combinations of parameter values, e.g., all values equal to zero, except \(K_{a,el}, K_{a,eg}, K_{g,tat}, K_{g,pis}, K_{g,pis,}\), and the parameters necessarily greater or equal to these. Note that these parameters take into account the input variables \(e, u\) and \(s\). As the two modules behave largely independently, we obtain all possible combinations of their respective steady states, including the sixth global attractor that does not correspond to any experimentally characterized cell type.

The methodology used here allows for the location of all the steady states of gene activation implied by the network connectivity, but in contrast to dynamic systems, this methodology does not identify the transitory activation pathways that lead to each attractor. The sequence of activation and repression of the input variables EMF1, UFO, SUP and LUG, as well as the timing of response of the genes in the circuit, determine which attractor is attained. In order to incorporate this information in the model, we need biological data such as the time needed for synthesis and degradation of regulatory factors. This information would enable us to obtain a flux diagram similar to that in Figure 4, which shows possible routes of gene activation starting at the vegetative cells of the fourth whorl which give rise to carpels. To simulate a null mutant in our model, it suffices to eliminate the variable \(l\). As a result, the LFY-dependent AG activation pathway is lost, whereas the LFY-independent pathway remains unaltered.

Simulation of mutations

Our model also allows the simulation or the prediction of mutant phenotypes. As an example, let us analyze the effect of LFY null mutation. Recall that LFY specifies the floral fate and activates the four organ identity genes represented in this model, i.e., AP1, AG, AP3 and PI. Null \(lfy\) mutants are practically devoid of flowers, and those that eventually appear possess genes with characteristics of wild-type sepals and carpels. To simulate a null \(lfy\) mutant in our model, it suffices to eliminate the variable \(l\). As a result, the LFY-dependent AG activation pathway is lost, whereas the LFY-independent pathway remains unaltered.

Similarly, we can simulate a mutation in AG, the only reported gene for the C activity. As AG is involved in a functional positive circuit, its inactivation altogether eliminates the corresponding multistable behavior. Indeed, eliminating variable \(g\) in the four-variable subnetwork leads to the single stable state \([001(0)]\), draining the entire variable space. Combining this steady state with those of the small subnetwork, we find that only the states corresponding to the A function alone and the combination AB are reached. This result accounts for the phenotype of strong \(ag\) mutants, whose flowers contain only sepals and petals (Gustafson-Brown et al., 1994).
Vegetative growth

[1100000000]

Floral induction

1100000000

0100000000 1110000000

0110000000

0010000000

Flower development

0011000000

0011010000 0010101000

0011010000 0011001110 0010101110 0011100000

0011000000 0011001110 0010100110 0010100000

0010100110

0000100111

[A,B,C]

A: sepals
B: petals
C: carpels

[0000100000] [0001000110] [0000100110] [0000100000]

Our model can also be used to predict floral phenotypes of mutations in the non-ABC genes included in it. For example, a simulation of a null mutation in TFL1 results in the elimination of the non-floral steady state. However, the stable ac-
activation of the A, B and C functions remains unaltered. The biological interpretation of such a result is that a mutation in the TFL1 gene gives rise to plants with severely reduced vegetative growth and flowers with wild-type phenotype. Plants with the tfl1 mutation have very short primary shoots terminated prematurely by a group of flowers with normal or slightly altered organs (Shannon and Meeks-Wagner, 1991). Therefore, the biological effect of inactivating TFL1 is described accurately in the model by the loss of stability of the non-floral state, converting it into a transient state. In contrast with the regulatory network presented here, the ABC model does not take into account a non-floral state, and hence cannot explain mutations that accelerate or retard the onset of flowering. Within flowers, we can also simulate the dynamical consequences of cis-regulatory mutations by adjusting the values of the relevant logical parameters. Moreover, it is possible to assign a phenotype to each simulation. Our model cannot, however, be used to describe mutant phenotypes of TFL1, or other genes, outside the flower. A comprehensive analysis of various types of loss and gain-of-function mutations will be presented elsewhere (Mendoza et al., in preparation).

Discussion

We previously presented the topology of the genetic regulatory network shown here and a system of difference equations to model the stable gene activation patterns observed in the flowers of Arabidopsis (Mendoza and Alvarez-Buylla, 1998). Here, we focused on the analysis of the dynamical roles of the feedback circuits contained in the same regulatory network. Despite the difference in the formalism used, both models predict the same stable states, but how much of these results depend on the precise genes involved in the network? New developmental genes are rapidly being discovered and characterized in Arabidopsis. Will the incorporation of these new genes soon invalidate our results? We think that this will not be the case, because the dynamic properties of feedback circuits depend on their sign (positive or negative) and not on their size (Thomas et al., 1995). Therefore, changes in the interaction matrix accounting for new intermediate elements should not invalidate our results regarding the number and nature of stable gene activation states. For example, if an intermediate gene between TFL1 and LFY were to be discovered, the interaction matrix of Figure 3 would be modified, but the positive circuit involving TFL1 and LFY would still operate and fulfill the same dynamical role. The only difference is that it would become a three-element instead of a two-element circuit. In short, the qualitative analysis presented here does not depend on the precise molecular nature of the regulatory pathways.

We found eight circuits in the regulatory graph and matrix (Figure 2), of which only two are functional in the whole space of gene expression states. These are the positive circuits AP1-AG and AP3-PI. These circuits, together with the input variable EMFI, account for the generation of several separatrices across the phase space, that define six basins of attraction, each containing one stable state. These attractors correspond to the patterns of gene expression found in the four floral organs of Arabidopsis (sepals, petals, stamens and carpels), plus a non-floral state and a new state awaiting experimental characterization. Although the regulatory matrix contains several other circuits, none of these are found to play any crucial dynamical role. Once again, we may be concerned by the discovery of new genes and interactions that define additional feedback circuits in the network. We predict that if such a hypothetical loop includes any of the ABC genes, this circuit should not be functional. Indeed, the experimental evidence available up to now suggests that there are only two functional feedback loops among the ABC genes, which correspond to the two functional circuits identified in this analysis. Messenger RNA in situ hybridization experiments in mutant backgrounds have shown that the A and C genes inhibit each other (Bowman et al., 1993). This creates the stable exclusive choice between A and C functions. There is also experimental data that support the view that the B function genes only have two possible stable states of activation. They are either both active or both inactive (Krizek and Meyerowitz, 1996). We exclude the possibility of additional functional circuits involving ABC genes because this would change the well-established combinations of activities described by the ABC model. However, we do not exclude the possibility of additional functional feedback circuits involving some of the non-ABC genes included in our model.

Our model accounts only for extreme levels of gene activation, either active (or more properly, with an activity above its threshold of functionality) or inactive (below this threshold). We used this simple binary representation because there is a scarcity of quantitative data. As a result, we reached a consistent but somewhat schematic picture of the differentiation pathways leading to the alternative states of gene expression in the developing flower of Arabidopsis. It would be more realistic to think in terms of a gradient of activation between these extreme values. As more data on different patterns of gene expression become available, we will be able to take advantage of the more sophisticated aspects of the generalized logical formalism (e.g. multilevel variables, functions and parameters, analysis of state transitions, etc.) to refine our model. These refined logical models could lead to more precise and detailed accounts of the kinetics of gene expression during flower development. This logical model and its potential further refinements could also help the development of a quantitative differential description. In this respect, note that previous theoretical analyses have shown that analogous continuous and discrete models yield com-
parable results in terms of steady states (Thomas et al., 1995).

The last issue regarding the accuracy of our model concerns the particular values of the parameters ($K$s) that we used. Most of these were based on existing data about the patterns of gene expression observed in Arabidopsis. However, not all of the parameters could be inferred from experimental data. Indeed, the values for $K_{al}$, $K_{e1}$, $K_{e4}$ and $K_{e6}$ were adjusted to obtain plausible transient gene activation patterns that did not affect the configuration of steady states. Nevertheless, an important prediction arises from the corresponding constraints on the values of some of the logical parameters. More precisely, our analysis predicts that LFY has at least one additional regulator yet to be discovered. Indeed, in order to obtain the three stable states experimentally found in wild-type Arabidopsis (non-flowering, activation only of AG or of AP1), $K_{e4}$ (representing the maximum expression state of LFY, i.e. when the products of TFL1 and EMF1 are below their respective inhibitory thresholds, and when the product of AP1 is over its activatory threshold) needs to be set equal to zero, but the experimental evidence available shows that LFY is expressed at high levels at the onset of flowering (Blázquez et al., 1997), and at low levels during later stages of flower development. This discrepancy strongly suggests that there is at least one additional regulator of LFY still to be discovered. We further predict that this regulator should play a key role in the transition from the non-floral attractors to the flowering pathways, implying both temporal and functional specific features.

The analysis presented here includes all the possible activation states of the network. However, cells in an organism likely only access a limited subset of these theoretical states during development. Each cell probably follows a limited set of states until it attains its final differentiated fate. In the particular case of Arabidopsis, some plausible pathways of gene activation states are shown in Figure 4. At the onset of flower development, flower inhibitors (EMF1 and TFL1) are active and cells are trapped in the stable non-flowering state [1100000000]. An external signal, which alters the activation of the flower inhibitors EMF1 and TFL1, is thus needed to move the regulatory network towards one of the floral steady states. From this point onwards, a choice between four main pathways that reflect alternative gene activation routes in different parts of the flower arises. AP1 mRNA is found throughout floral primordia during the very first stages of development, but by the time the sepals arise, its expression is restricted to the first two whorls. LFY follows a similar expression pattern, except that its mRNA becomes undetectable in the outer whorls at late stages of flower development. This temporal expression leads to the long-term activation pattern of the first and second whorls. On the other hand, LFY and AP1 expression are found in the inner whorls of the flower until sepals arise, when AG expression sets in (Weigel and Meyerowitz, 1993).

The dynamics explained above account for the differentiation of those regions of the flower characterized by either A or C activity. To complete this picture, it is necessary to take into account B activity, as well. In fact, the transient expression of LFY mentioned above might be important for the activation of AP3 and PI, ultimately leading to the stable states [001000110] (AB combination) and [000100110] (BC combination). This role of LFY in the establishment of B activity is supported by the observation that LFY mutant plants bear flowers that often lack petals and stamens, and that the expression of AP3 and PI messengers is reduced. Recent data on transgenic plants bearing a super activator form of the LFY product (LFY protein fused to the activation domain VP16) also suggest that LFY is an important activator of B genes, particularly AP3. In addition, UFO might act as a co-regulator in the activation of AP3 and PI (Lee et al., 1997; Parcy et al., 1998), but the mechanism is not yet known. Once AP3 and PI are fully active, the activation of LFY becomes dispensable, because AP3 and PI cross-activation ensures their mutual maintenance. Additional data on the kinetics of expression of cadastral genes and on their upstream regulators are needed to refine this scheme.

In addition to the vegetative state and the four states corresponding to floral organs already accounted for by the ABC model, our analysis predicts a sixth attractor [110000110], which implies a stable activation of EMF1, TFL1, AP3 and PI. This state automatically results from the combination of the steady states [11] and [1100] generated by the two regulatory modules. Such relative independence of B and A/C functions is supported by the fact that B activity can be disrupted without affecting other aspects of flower morphogenesis (Day et al., 1995).

Conclusions and prospects

We have derived a network of 10 genes involved in the genetic control of flower morphogenesis in Arabidopsis from published molecular and genetic data. Using a logical formalism, we provided a qualitative dynamical analysis of this regulatory network and derived the parameter constraints accounting for the different patterns of gene expression found in the four floral organs of Arabidopsis (sepals, petals, stamens and carpels), as well as for a non-floral state. In addition, our model leads to the prediction of a new stable state characterized by a simultaneous expression of the floral repressor and B function genes. Finally, we predict the discovery of at least a new regulator of the gene LFY, likely to be involved in the transition from the non-flowering state to the flowering pathways.

Our analysis also explains how specific genes cooperate to generate the alternative patterns of gene expression through two functional positive circuits. From a general perspective, this work further supports the idea that positive regulatory cir-
cuits play a central role in development. Such a role was postulated by several theoretical biologists in the late 1970s (see, for example Lewis et al., 1977; Meinhardt, 1978; Thomas, 1978). More recent studies have confirmed that key developmental genes are often involved in such positive circuits, thus positively affecting their own expression. Often, but not always, this is accomplished through a direct autoregulation, like in the case of the gene MyoD that codes for a muscle-cell specific transcription factor and is involved in both cell determination and differentiation [see Murre et al., 1989; more examples are found in Thieffry et al., 1995].

The present model also emphasizes another important feature of developmental gene networks, namely their modularity. Indeed, developmental biologists are now realizing that developmental genes can be grouped in relatively independent ‘regulatory modules’ or ‘syntagms’, acting in parallel or in temporal cascades (the best available example is the segmentation network of *D.melanogaster*; see, for example, Reinitz and Sharp, 1995; Sánchez et al., 1997). The present analysis points to a straightforward formal criterion to isolate regulatory modules in complex intertwined network, namely that a module can be simply defined as a set of feedback circuits sharing some element(s).

Our analysis accounts for various stable states and, to a lesser extent, for the kinetics of gene expression of the ABC and other interacting genes included in the network. It is clear, however, that pattern formation and cellular differentiation most often involve interplay between subcellular and supra-cellular levels. In addition, this interplay should be considered in the context of cellular growth and multiplication. Our model should thus be considered as a first step toward a more ambitious model of flower development, covering intercellular communication, cell growth and division, as well as three-dimensional morphogenesis. For example, our model could not account for flower phenotypes with the same four floral organs, but with a different spatial arrangement (e.g. sepal, petals, carpels and stamens), because it does not incorporate explicit spatial information. Future models will have to consider genes underlying cellular processes that are at the basis of aspects of floral morphology, such the shape, number or color of floral organs.

In spite of the limitations of our model, it can already be used to explore the role of regulatory genes in determining important aspects of floral morphological variation across angiosperms (plants with flowers). The stereotypical structure of flowers of >250 000 angiosperm species, and molecular and genetic data available for a few other model angiosperm systems, strongly suggest that the ABC model is widely conserved across flowering plants (Bowman, 1997). All flowering species, except one (*Lacandonia schismaticar*, Martínez and Ramos, 1989; Alvarez-Buylla et al., 1999), have concentric whorls of floral organs which, from the outside to the inside of the flower, are: sepal (or modified sterile organs), petals (or modified sterile organs), stamens and carpels. Furthermore, comparative molecular studies have shown that the ABC genes and their expression patterns are highly conserved across distantly related angiosperm species (Bradley et al., 1996; Mena et al., 1996; Purugganan, 1997). Therefore, the ABC model may be used to describe important aspects of the floral architecture of most angiosperms. In this context, the NET model is useful to elaborate hypotheses regarding variations in the network that underlie important aspects of flower morphological diversification during evolution. We refer to the morphological response that results from the variation of the patterns of gene expression involved in the control of the morphogenesis of floral organs (e.g. sepal, petal, stamens and carpels). This model also constitutes a formal framework susceptible to guiding future experiments aimed at identifying new regulatory genes or interactions. For example, our previous analysis of the NET model (Mendoza and Alvarez-Buylla, 1998) led us to propose that the gene AG was activated by *LFY*, a regulatory interaction which was soon confirmed experimentally by an independent group (Parcy et al., 1998).

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