A model for floral organ development in *Arabidopsis*

Problem presented by

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1 Introduction

1.1 Preliminaries

Arabidopsis thaliana is a member of the mustard family (Brassicaceae) and is used as a model organism for studying a variety of processes in plants, including (among others) flower development, light sensing, root growth and circadian rhythms. In this report we develop a mathematical model of floral organ development and use it to investigate the phenotype observed in *ask1* (*arabidopsis skp1-like1*) and *ufo* (*unusual floral organs*) mutants (these being described below). A key process the development of a flower is the formation of its organs, these being sepals, petals, stamens and carpels. This process is marked by twelve anatomically defined stages [1], during which the organs emerge to form a rather distinct spatial pattern, whereby each type of organ is arranged in a series of concentric circles (denoted whorls) about the centre of the flower (see Figure 1). These whorls are numbered one through four, with four in the centre and one at the periphery. Importantly, the four types of organ are initiated in a sequential

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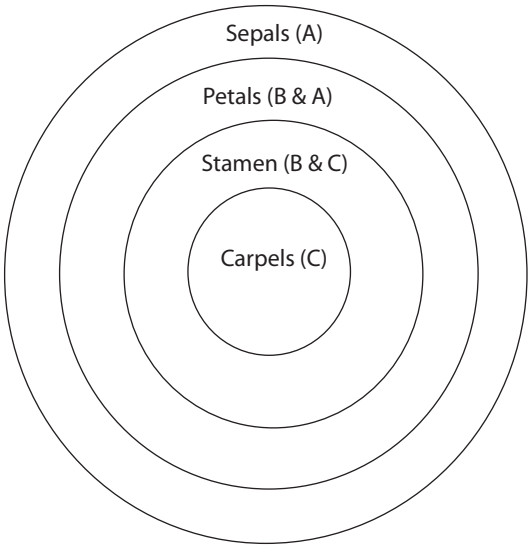

Figure 1: Schematic of floral organ arrangement in a developing flower. The spatial expression patterns of the A, B and C-class genes are indicated.

manner: first, sepals grow to overlie the flower primordia (whorl one); second, petal (whorl two) and stamen (whorl three) emerge to be surrounded by the sepals; third, carpels (whorl four) form in the centre of the flower. The resulting wild-type pattern (from the whorl one to four) is: sepals, petals, stamens, carpels. It is not yet fully understood how these patterns form. However, a number of the genes that direct flower pattern formation have now been identified through mutant studies. We discuss the relevant experimental observations below.

1.2 Experimental observations

1.2.1 The ABC model

By considering mutants of *A. thaliana* where the identity of the organ that occupy a particular whorl is affected, Meyerowitz *et. al.* [2, 3] proposed the so-called 'ABC' model. Here, the mutations were found to fall in three distinct classes. In the first class, whorls one and two are affected, so that carpels form in the former and stamens in the latter: the phenotype being carpel, stamen, stamen, carpel. In the second class, mutations affect whorls two and three, giving sepal in two and carpels in three. The resulting pattern is: sepal, sepal, carpel, carpel. In these first two classes, organ number is also altered. The third class of mutations has have petals in whorl three and sepals in four. The phenotype is: sepal,

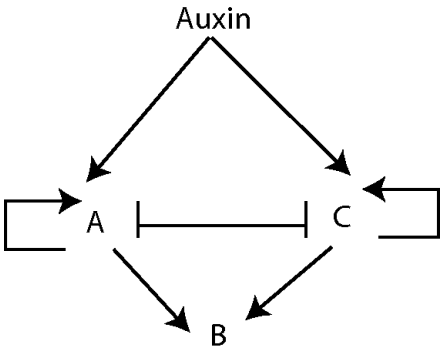

Figure 2: A simplified version of the ABC-gene network which summarises the key interactions considered in our mathematical model (2). We note that the activation of A and C class genes by auxin is largely hypothetical. See Section 1.3 for a discussion of the available data.

petal, petal, sepal. These observations lead the authors to postulate the existence of three categories of gene – A, B and C. In the model, the expression domains of these three genes is sufficient to determine the floral pattern. Thus, gene A is expressed in whorls one and two only, B in whorls two and three and C in whorls three and four. The proposed wild-type pattern being: A, AB, BC, C. Furthermore, sepals form if gene A is expressed in the absence of B and C; expression of both A and B lead to the development of petals; cells co-expressing B and C develop into stamens; expression of C alone specifies carpels [2, 3]. A number of candidates for A, B and C class gene have now been identified: representative examples of A class genes are *AP1* and *AP2*, B class ones are *AP3* and *PI* and C class ones are *AG* [2, 3, 4]. Members of the A and C class of genes are mutually anatagonistic, and both A and C class genes upregulate B-class genes (see [5] and references therein). A and C-class genes can also upregulate their own expression. This network is illustrated in Figure 2.

The above considerations indicate that the fate of an individual cell in a developing flower is to a large extent determined by the interactions between various ABC-type genes. By considering all the known interactions between both ABC-type and the relevant non-ABC type genes (this constituting a gene network), Espinosa-Soto *et al.* [5] developed a Boolean-network model of the genetic interaction in a single cell of a developing flower. The authors found that the gene network can have steady states that are consistent with gene expression profiles found in both wild-type and mutant plants. We note that, however, the particular steady state to which the system will evolve depends crucially on

the initial conditions. One hypothesis is that the gene expression patterns that appear in wild-type and mutant plants correspond to a particular spatial distribution of the steady-states of the single cell model. However, the model does not explain how this spatial pattern is attained. Typically, biological systems require some form of cell-cell communication to establish spatial patterns of a gene expression. One candidate to mediate this communication is the phytohormone auxin, which is thought to play an important role a variety of patterning processes in plants [6, 7], including floral development [8, 9]. We discuss the relevant experimental observations in Section 1.2.3.

1.2.2 *ufo* and *ask1* mutants

The ABC model is based on mutant studies where the plants have a very particular phenotype, namely, that whilst wild-type organs form on each of the whorls, the spatial distribution of these organs differs from that of the wild-type. Intrigingly, this model does not appear to account for another type of mutation, where some of the primordia appear to be a mixture of wild-type organs (and so that they appear to be a fusion of them). Two prominent examples of this are *ask1* (*arabidopsis skp1-like1*) and *ufo* (*unusual floral organs*), which are mutants in two components of the SCF (Skp1-Cullin-F-box protein) ubiquitin ligase complex. A striking feature of these mutants is the frequent occurrence of fused organs between adjacent stamens or between a stamen and a petal, which are also typically accompanied by a reduction in the size of the affected organs [10]. One possibility is that the mutation affects the cell proliferation rate. As we now explain, this hypothesis may account for why organs appear fused and reduced in these mutants. In a wild-type plant, the regions that form stamens and petals initially overlap [11, 12]. Cells within these domains undergo a number of divisions as their identity is established [13]. It is expected that, as a consequence of this cell proliferation, the two domains move farther apart until they separate. The outcome of this process is thus the creation of separate organs in normal development, or fused organs in mutants when the cell proliferation rate is reduced.

1.2.3 Auxin perception and transport in developing flowers

As a consequence of its patterning role in plant development, auxin has been compared to animal morphogens [14]. For example, auxin is known to play a crucial role in positioning of new leaves and

flowers in a developing plant shoot [7, 6]. In individual flowers (as well as other aerial tissues), the position of new organs is associated with auxin maxima [7, 6]. Unlike classical animal morphogens, which are typically thought to passively diffuse between cells [15], auxin is predominently transported through tissues of the plant. This 'pumping' of auxin is mediated by members of the PIN and AUX families, with the former being auxin efflux carriers and the latter influx ones (we recommend the reader consult [16] for further details). PIN (and AUX) proteins are typically localised to a particular face of a cell. Thus, the direction of auxin flux, in contrast to that of a classical morphogen, is highly regulated. Furthermore, auxin has been shown to regulate the polarity of PIN proteins [17], although the mechanism that controls this remains to be characterised. However, computational models of auxin transport that incorporate a phenomelogical version of this feedback loop can generate the types of whorl-like patterns found in developing shoots [18]. In this model, the auxin maxima are estabished and maintained through the polarity of the PIN proteins: in cells close to the apex of an auxin maxima, PINs predomimently pump auxin toward it. In more distal cells, PIN polarity is distributed so that auxin is transported away from the maxima. A similar patterning of PIN polarity can been observed in developing floral primordia [19], suggesting that this mechanism is operating in developing flowers.

Auxin perception is mediated by members of the ARF (Auxin Response Factor) family of transcription factors [20]. Disruption of either auxin transport (corresponding to a PIN mutant) [8] or its perception (corresponding to an ARF mutant) [9] can cause flowers to form abnormal structures.

1.3 Coupling auxin transport to the ABC gene network

The precise details on how auxin might regulate the ABC-type genes is currently unknown and we propose one possible mechanism by which it could occur. First, the transcrition factor *LEAFY* can up-regulate *AP1* (A class) and *AG* (C class) genes (see [5] and references therein). Second, *LEAFY* expression dynamics are highly correlated with the polarity of PIN proteins and the expression dynamics of an auxin-reporter gene, *DR5*. Together, these data suggest that auxin may up-regulate A and C class genes by mediating the activation of *LEAFY*. For the sake of simplicity, we will henceforth assume that auxin activates A and C class genes directly.

2 Model development

In this section we develop a mathematical model of the patterning of the ABC network in response to auxin a line of cells. We assume that cells periodically divide, reflecting the growth of the floral primordium.

2.1 The ABC network

Our mathematical model of the ABC gene network illustrated in Figure 2. We assume that the proteins encoded by A and C class are both required to activate a B class gene (this corresponding to an 'AND' gate). Henceforth, we denote the concentration of the protein encoded by A, B and C class genes by A_i, B_i and C_i respectively (in each cell i). The concentration of auxin is denoted by U_i. For each protein X in the model, we take its rate of production to be proportional to the probability that its gene (**X**) is activated (with proportionality constant λ_X). We model this probability using a Hill function. For example, if protein Y (with concentration Y) activates the gene that encodes protein X, then the probability its gene is active is taken to be

$$f(Y) = \frac{Y^m}{Y^m + \theta_X^m}. \tag{1}$$

Similarly, if Y represses **X**, then the probability that it is active taken to be $1 - f(Y)$. Here, θ_X is the concentration of Y at which $f = 1/2$ and m is the number of Y binding sites. Furthermore, we assume that each protein X in the model is degraded at a rate that is proportional to its concentration (with proportionality constant μ_X). The governing equations for the gene network are thus

$$\frac{dA_i}{dt} = \left(\lambda_{UA}\frac{U_i^2}{U_i^2 + \theta_{UA}^2} + \lambda_{AA}\frac{A_i}{A_i + \theta_{AA}}\right)\frac{\theta_{CA}^2}{\theta_{CA}^2 + C_i^2} - \mu_A A_i, \tag{2a}$$

$$\frac{dB_i}{dt} = \lambda_B \frac{A_i C_i}{A_i C_i + \theta_{AB}\theta_{CB}} - \mu_B B_i, \tag{2b}$$

$$\frac{dC_i}{dt} = \left(\lambda_{XC}\frac{U_i^2}{U_i^2 + \theta_{UC}^2} + \lambda_{CC}\frac{C_i}{C_i + \theta_{CC}}\right)\frac{\theta_{AC}^2}{\theta_{AC}^2 + A_i^2} - \mu_C C_i. \tag{2c}$$

We note that (2a) and (2c) decouple from (2b). Equations (2a) and (2c) have been studied extensively in the literature for a variety of processes in biology (see, for example [21, 22]). Such systems can be bistable (depending on parameter values) with stable steady-states corresponding to either dominant expression of A (and low C expression) or dominant C expression (and low A expression). Choosing

initial conditions $A_i(0) = 0$, $B_i(0) = 0$ and $C_i(0) = 0$ (so that none of the ABC genes are initially expressed in the developing primordia), there can exist a critical concentration of auxin (U^{c}) at which for $U_i > U^{\mathrm{c}}$ the system will evolve to one steady state, but for $U_i < U^{\mathrm{c}}$ the system evolves to the other steady-state [21]. Thus, (2) provide a mechanism through which a cell can determine its fate (i.e. whether it expresses A or C class genes) depending on the concentration of signal (in this case, auxin) it is exposed to. We choose the model parameters so that the system predominently expresses A (i.e. is a sepal) at low concentrations of auxin, and at high concentrations a cell predominently expresses C (i.e. is a carpel). This concludes our discussion of the ABC gene network. In the next section we describe a model for the transport of auxin.

2.2 Auxin transport

We adopt the model of auxin transport recently developed by Jonsson *et al.* [18]. As noted in Section 1.2.3, this incorporates auxin regulation of PIN polarity. We take the equations that govern the concentration of auxin in cell i to be

$$\frac{dU_i}{dt} = D(U_{i-1} - 2U_i + U_{i+1}) + c_i - \mu_U U_i + TP\left(\frac{U_{i-1}U_i}{\kappa + U_{i-2} + U_i} - \frac{U_{i-1}U_i}{\kappa + U_{i-1} + U_{i+1}} + \frac{U_{i+1}U_i}{\kappa + U_i + U_{i+2}} - \frac{U_{i+1}U_i}{\kappa + U_{i-1} + U_{i+1}}\right), \qquad (3)$$

where μ_U is the rate of auxin decay, c_i is the rate of auxin synthesis, D is its diffusion rate, T its rate of transport (mediated by PIN proteins), P is the total level of PIN protein in an individual cell and κ is the ratio between the rate of pin exocytocis to its rate of endocytosis. Jonsson *et al.* [18] analysed these equations (for $c_i = c$) and found that the system patterns, provided that PT/D is sufficiently large. Here, we assume that $c_i = c$ (a constant) for $i > 1$, and that $c_1 > c$ (corresponding to a source of auxin coming from the stem of the flower). We note that (3) decouples from (2).

2.3 Cell division

We cells within the flower synchronously divide evey τ time units. To incorporate cell division in the model, we solve (2)-(3) for the between times $[k\tau, (k+1)\tau]$ for $k = 0, \ldots, K-1$ (where k is the generation number and K is the total number of divisions that occur), doubling the number of cells every generation.

We assume that when the cell divides, its volume and contents are divided equally between its progeny and thus we choose our initial conditions for every generation accordingly. Thus, for example, at the start of each generation the initial level of auxin within a cell is half that of the parent cell.

3 Model results

We numerically solve 2-3 using Matlab routinue ode15s for stiff ODEs subject to the initial conditions described in Section 2.3. Default parameter values are provided in Table 1. A representative solution to the model is provided in Figure 3. As expected, auxin levels evolve to form an undulating pattern, with the amplitude of the waves decreasing from left to right. Consider for example generation four in Figure 3. Starting from the left, where auxin levels are highest, cells prodominently express C (corresponding to carpels). Moving to the right, the system passes through a critical concentration of auxin and the cell predominently expresses A and B (petals). Moving further to the right, the system passes through a second critical level of auxin, whereby cells only express A (sepals). Thus, the model is able to capture aspects of the wild-type pattern as described in Section 1.2. It is notable, however, that cells do not co-express C and B class genes (these corresponding to stamen). The gene regulation model we introduced in Section 2.1 is missing a number of key interactions (see [5]) that could explain this inconsistency, and this merits further investigation. The two mutants, *ask1* and *ufo* (see Section 1.2.2) can be captured in the model by varying the proliferation rate of cells within the model. This will effect the size of the domain (i.e. the number of cells within the system), and therefore the underlying auxin pattern. This in turn effects the patterning of the ABC network and hence the morphology of the plant.

4 Discussion

We have developed a mathematical model of the spatial patterning of the ABC-type genes in order provide into the phenotypes of mutants *ufo* and *ask1* (see Section 1.2.2). Based on our model results and the available experimental data, it is reasonable to expect that the patterning of these genes is determined by the distribution of auxin in developing flower. The spatial patterns generated by the mathematical model presented here (see (3)) are dependent on domain size. Thus, based on our model

Parameter	Value	Units	Parameter	Value	Units	Parameter	Value	Units
κ	10^{-7}	[C]	μ_U	10^{-3}	[T^{-1}]	P	1	[C]
c_1	10	[C]	c	1	[C]	T	100	[$(CT)^{-1}$]
D	10	[T^{-1}]	λ_{UA}	30	[CT^{-1}]	λ_{AA}	30	[CT^{-1}]
λ_{CC}	30	[CT^{-1}]	λ_B	0.1	[CT^{-1}]	λ_{XC}	40	[CT^{-1}]
μ_A	1	[T^{-1}]	μ_B	100	[T^{-1}]	μ_C	1	[T^{-1}]
θ_{CA}	0.01	[C]	θ_{AB}	1	[C]	θ_{CB}	1	[C]
θ_{AC}	1	[C]	θ_{XA}	1	[C]	θ_{AA}	2	[C]
θ_{CC}	2	[C]						

Table 1: Default parameter values (with their dimensions) in the floral organ model (2)-(3). Here, c_i is the rate of auxin synthesis, D is the rate of auxin diffusion, T is its rate of transport (mediated by PIN proteins), P is the total level of PIN protein in an individual cell and κ is the ratio between the maximal rate of PIN exocytocis to its rate of endocytosis. λ's represent maximum rates of gene transcription, θ's are disociation constants for protein-DNA binding and μ's are rates of protein decay.

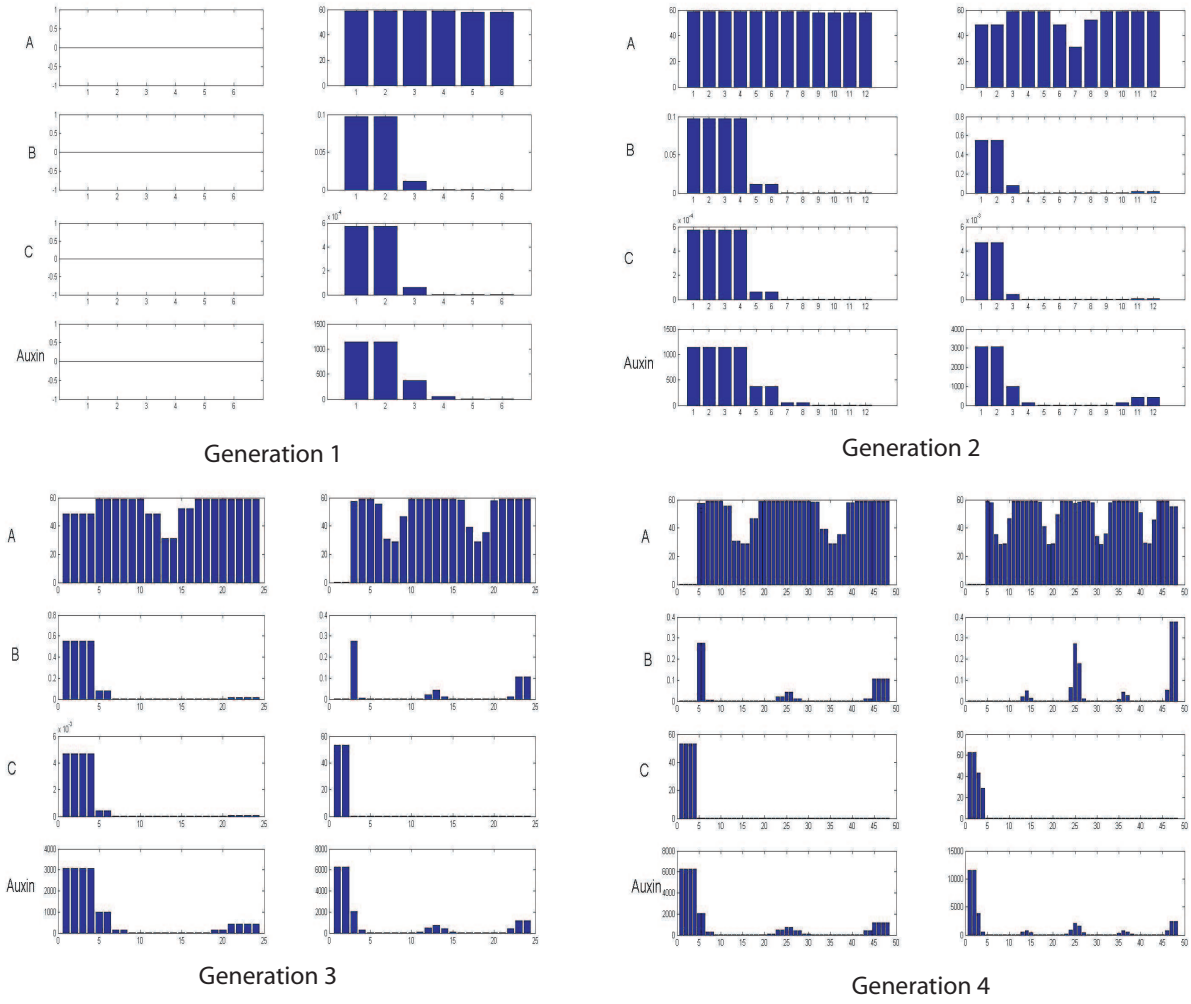

Figure 3: Representative solutions to the floral organ model (2)-(3) for four generations (corresponding to $k = 0, \ldots, 3$. For each generation initial conditions are chosen according to the scheme described in Section 2.3). The levels of the key components of the model in each cell are provided just after (right panel) and just before (left panal) a round of cell division.

results, it seems feasable that the spatial patterning of the ABC-type genes could be severly affected by a reduction in the rate of cell division (which could correspond to *ufo* or *ask1* mutants). In particular, if the number of cells was sufficiently small, then the system would contain one auxin maximum that contains both A and C-expressing cells (this corresponding to 'fusion' of the organs). The model that we have developed here provides a solid framework with which to explore these issue further. First, however, a more detailed analysis of the ABC gene network would have to be performed so that the wild-type pattern is reproduced in full by the model.

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