Integrative Hybrid Modelling of Plant Shoot Branching

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Introduction

The regulation of shoot branching in Arabidopsis involves a complex network of interacting genes, proteins, hormones, and environmental influences (Leyser, O. 2005). As our knowledge of the molecular biology of the individual processes increases, it becomes increasingly difficult to understand and visualise the system as a whole. We propose that techniques used in computer science for the development of complex software systems can be combined or integrated with existing computational biology techniques to produce new computer models of the shoot branching processes.

Shoot Branching

Shoot branching in Arabidopsis is thought to be controlled by two semi-discret regulatory systems. The first to be identified was the repression of branching by the hormone auxin which is produced by the primary shoot apex and transported down through the shoot stem. Auxin acts to down-regulate cytokinin which is a direct promoter of bud outgrowth (Nordstrom, A. 2004).

The second proposed regulatory system, known as the MAX pathway, is concerned with the control of the transport capacity for auxin in the stem, and consequently the number of branches (Bennett, T. et al 2006). The MAX pathway consists of 3 closely operating MAX genes, *MAX4*, *MAX3*, and *MAX1*, which are thought to be involved in the production of a yet be fully characterized hormone mds (max-derived-signal). The presence of mds is detected by MAX2 which then goes on to negatively regulate PIN1. PIN1 is a polar membrane bound auxin transport protein. The levels of PIN1 expressed in the membrane of vascular-associated cells determines the capacity of that cell to export auxin (PIN1 accumulates on the lower membrane of the vascular cell). Experiments have shown that the capacity of the shoot to transport auxin is positively associated with the number of branches on the shoot (Bennett, T., et al 2006). The production of PIN1 within the cell is regulated by the presence of auxin in a positive feedback loop, kept in check by *MAX2* down regulating PIN1. Therefore the removal of the *MAX2* activity by interruption of the MAX pathway causes a phenotype of runaway branching.

Taken alone, these systems are not trivial, but they should be considered even less so when thought of in in the larger context of a plant where phenotype must also be taken into account. Consequently it would be highly beneficial for any model of this system to capture not only the subtleties of the interacting genes, proteins, and hormones, but also their effect on the larger plant. We believe that combined modeling techniques from computer science and computational biology will assist in the production of models that are up to the challenge of answering such questions.

Software Engineering Plant Models

The Unified Modelling Language (UML, Object Management Group) is a mature visual modelling tool for the development of complex object oriented software systems, and is based on a series of diagrams. We use a UML based software engineering process to produce UML models of plants. The abstract concept of programming objects (from object oriented programming languages) within UML maps neatly onto the real physical objects that define plants and their cells. For example, programming objects can be used to describe the parts of the plant, such as cells, proteins, and genes. These objects can be modelled in UML and the different interactions captured. The UML model can then form the basis of an executable simulation. If later, after further research and experiments, the plant model needs to be modified, these changes can be made to the high level UML diagrams, which can then automatically update the simulation code structure appropriately. Fig 1 shows a high level UML diagram capturing some components of a plant cell. Other groups have started to apply UML to developing models of biological systems (Webb, K. White, T. 2005).

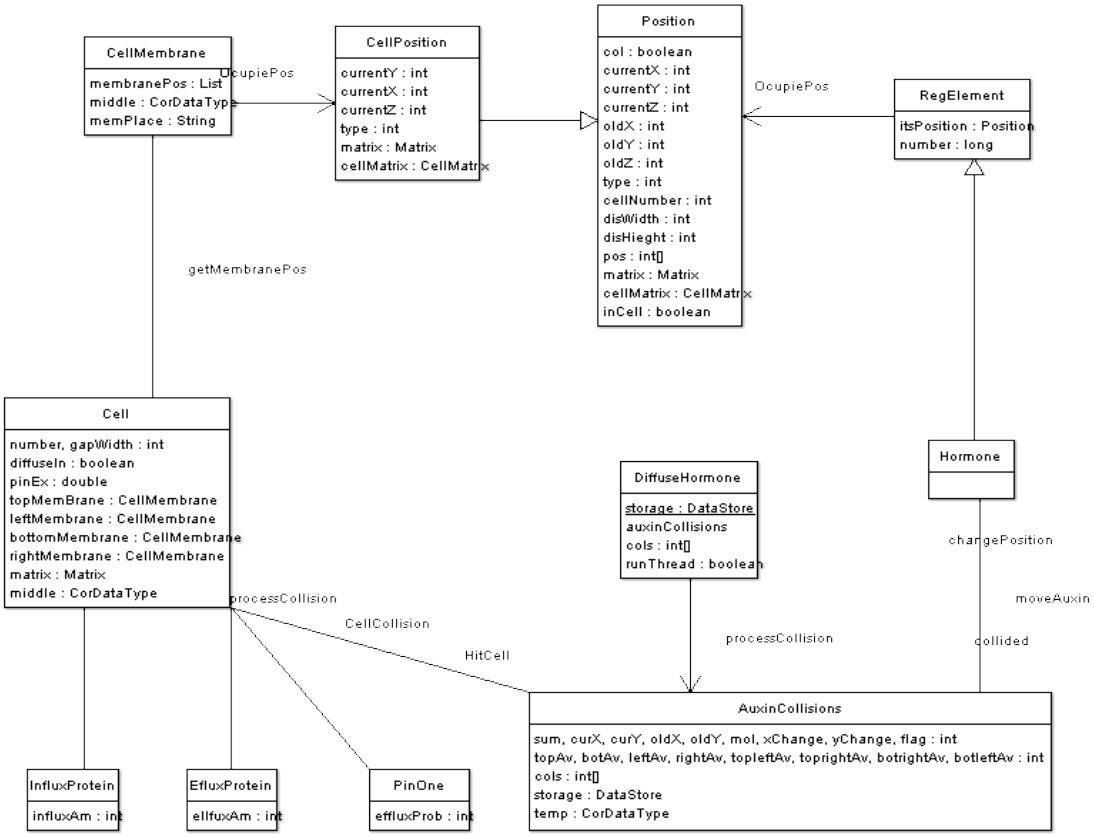


Fig1. The UML class diagram showing the interactions of the programming objects within the program. The boxes represent different classes within a program, each class defines the attributes of an object. The different classes represent parts of a real plant. The arrows show how the program interacts and is modelled on how those interactions are thought to be in real life. Objects are designed to be discrete and therefore it should be relatively easy to alter the interactions between them to alter the behaviour of the program (OMG).

We have applied this modelling approach to the problem of auxin transport canalisation, which is a small part of the greater question of shoot branching. The process of canalisation occurs during vascular tissue specification, and is thought to be an auxin regulated positive feedback loop, in which auxin increases its own transport by up-regulating proteins like PIN1. The differentiation of vascular tissue and the formation of canals of auxin flow has been hypothesised from a number of experiments by Tvsi Sachs (Sachs, T. 1981).

The formation of canals can easily be seen but the exact mechanism controlling this is not fully understood. There is a question mark over the mechanisms at work: are the canals produced at a higher or lower local auxin concentration relative to the surrounding tissue? Models developed to investigate canalisation originally suggested that the canals would be at a lower concentration relative to the surrounding tissue (Mitchison, G. 1980) but, experimental evidence now suggests otherwise. Newer models have been produced which agree with the experimental data, however they rely on the existence of transport proteins that have not yet been found by experimentation (Kramer, E. 2004).

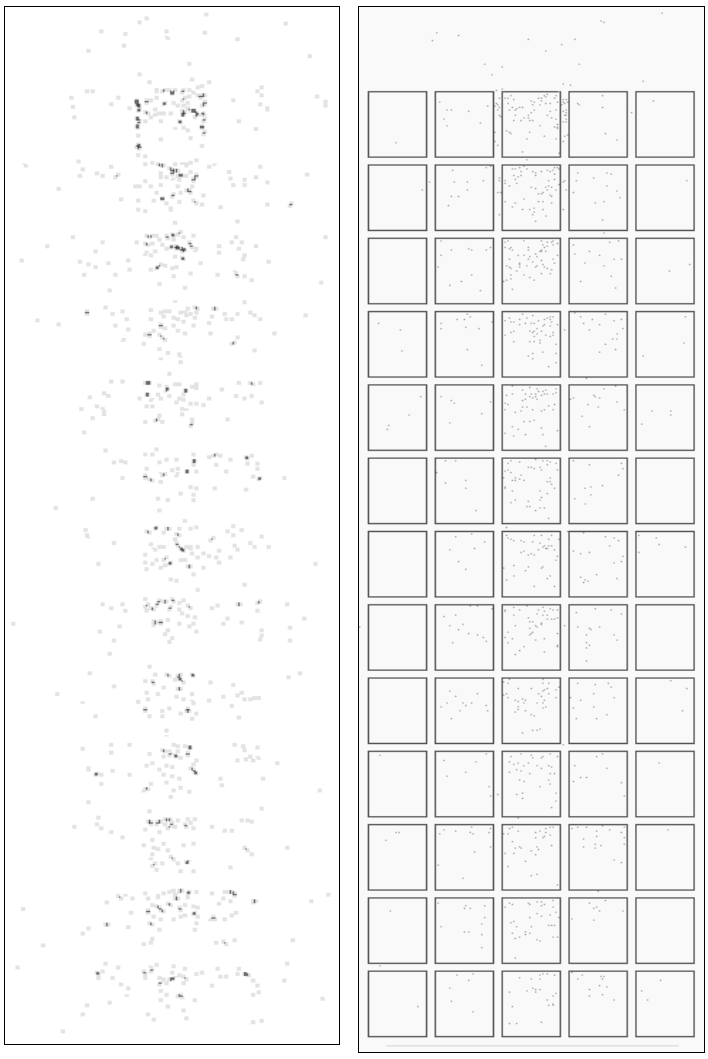
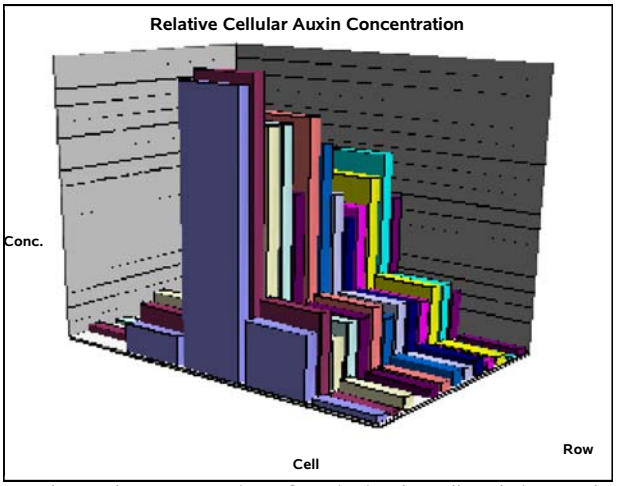


Fig 2: (A) Heatmap showing areas of high auxin concentration following the path of the canal. (B) The visual output of the 2D model, auxin enters the model in cell marked X and leaves via the sink at the bottom. The canal is formed by the diffusion of auxin into cells and the transport by proteins out of cells between an area of high concentration, the source X and an area of low concentration, the sink.

There are currently two models in development, one of which is 3D and the other 2D. Early data from both models indicate that the canals represent a local maximum in auxin concentration. The 2D model, shown in Fig. 2(A), shows the layout of the model and consists of a grid of cells with the auxin moving around, shown as dots. Auxin enters the model in the middle cell of the first row, a unit of auxin arrives here for every one lost at the sink (grey line at the bottom) so the system is closed. Currently when the model is started a set amount of auxin is released on top of the cells. The auxin moves around in the model by diffusion and transport. Diffusion into cells by auxin is permitted but diffusion out is not, as in reality the auxin would become deprotonated and thus unable to cross the membrane[Blakeslee, J. 2005].

The auxin can only leave the cells via an efflux protein. Efflux proteins are currently split into two types. Firstly, there are a set of efflux proteins which are randomly distributed around the cell membrane. Secondly, there are efflux proteins that represent the PIN1 family of proteins. These currently bind to the bottom membrane only and the amount in a cell at any one time is positively linked to the amount of auxin in the cell. This relationship can be altered within the program via a slidebar.



Graph 1: The concentration of auxin in the cells relative to the average across all cells. The cells forming part of the canal have a much higher relative concentration.

Graph 1 shows the relative concentration of auxin in the different cells in the model, and Fig 2(B) shows an auxin heatmap. Both show that a canal has formed between the cell that the auxin enters the model (as if it were coming from further up the plant) and the sink at the bottom where it leaves the plant. It is also clear that this canal is a local maxima of auxin concentration. The models are still undergoing testing and there are a few areas which require improvement. We would like to make the shape and layout of the cells more natural. This will be important for modelling how canals join up as seen in plants. Also, rather than releasing a set amount of auxin into the model, it would be better to have auxin producing cells that maintained a set cellular auxin concentration. Finally we would like to develop rules where the localisation and amount of all efflux proteins in the cells is influenced by the amount and position of auxin within them.

Future work: L-systems

L-systems are a well established method of computationally modelling the development of plants. They already have a facility whereby some of the modelling is captured in the form of and external computer program, and not as part of the L-system description itsself. Environmental models are a good example (Karwowski & Prusinkiewicz. 2003) as these models feed parameters into the L-system which affect is outcome. We are planning to produce more tightly linked models where an L-system that models branching and UML based models of auxin flow work closely together to model more of the developing plant.

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