

Collaborative Research: New Methods in Phyllotaxis

Introduction

Many patterns in Nature are made of units repeated regularly in space (Fig. 1). The symmetries found in these patterns reflect two simple geometrical rules:

- i) Equivalent or nearly equivalent units are added in succession.
- ii) The position of new units is determined by interactions with the units already in place.

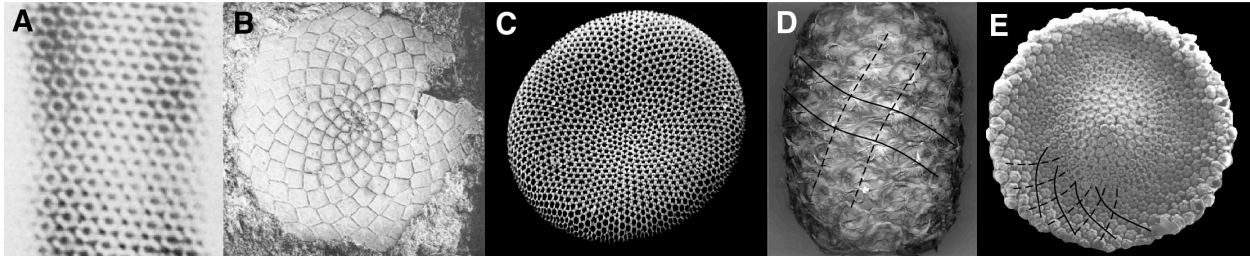


Figure 1. Lattice-like patterns in Nature. A) Hexamers in a polyhead bacteriophage T4 (Erickson, 1973). B) Plates of a fossil receptaculitids (Gould and Katz, 1975). C) The mineralized silica shell of a centric diatom (Bart, 2000). D) The pineapple fruit, with two parastichies of each family drawn. Parastichy numbers are (8, 13) in this case. E) A young artichoke head, with parastichy numbers (34, 55).

That proteins can assemble to create patterns that emulate the beautiful regularity found in crystals may not come as a great surprise. When the same regularity is found at the level of an entire living organism, one may justly be astonished. This is, however, a common occurrence in plants where the arrangement of leaves and flowers around the stem, known as phyllotaxis, can be very regular (Fig. 1D and E).

To understand the origin of phyllotactic configurations in plants one must focus on the shoot apex where these configurations are established. The shoot apex is composed of a group of slowly dividing cells, the meristem, whose activity generates leaves, flowers, and other lateral organs of the shoot as bulges of cells called primordia (Fig. 2). The angle between two successive primordia is called the divergence angle. The most common configuration is one where the elements are arranged in two families of spirals called parastichies (Figs. 1D and 1E). Moreover, the numbers of parastichies in these two families are most often successors in the Fibonacci sequence (1, 2, 3, 5, 8, 13, ...) and the divergence angle between primordia is close to the golden angle $\alpha = 360(2 - \tau)^\circ \approx 137.5^\circ$ where $\tau \approx 1.608$ is the golden mean (Figs. 1 and 2).

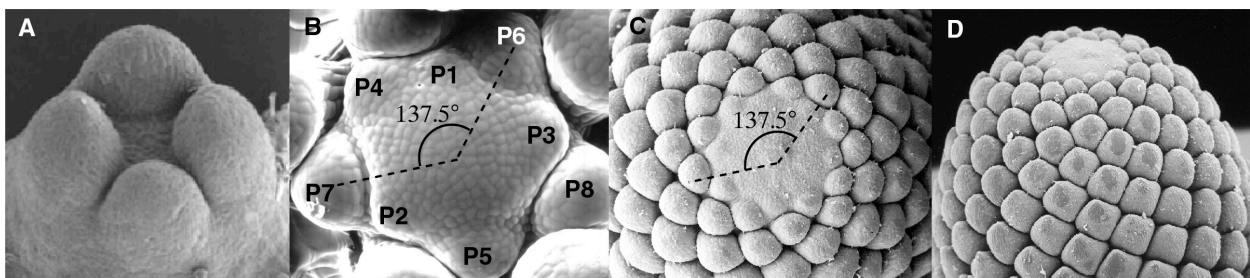


Figure 2. Whorled and spiral configurations at the shoot apical meristem. A) Whorl of four in the larch embryo (Harrison and von Aderkas, 2004). B) Inflorescence meristem of Arabidopsis

thaliana with primordia numbered according to their age. C) Spruce meristem showing a (8, 13) pattern (Rutishauser 1998). In B) and C), the divergence angle is close to the golden angle. D) Side view of the meristem in C).

Our overall goal is to understand the universe of all possible phyllotactic configurations and to determine why, in plants, some configurations are more common than others. To fulfill this goal we take a multidisciplinary approach. On the mathematical side, it has been necessary to develop a geometric framework that encompasses all the phyllotactic configurations found in Nature. We propose a drastic extension of the classical framework of parastichies and lattices to the new paradigm of *primordia front* and *multilattices*. A subset of multilattices is an attractor in our dynamical systems model, and contains the classical phyllotactic spirals and whorls, and continua of configurations close to these. On the biology side, we developed techniques that allow us to monitor and manipulate pattern formation at the meristem. By perturbing phyllotactic development, it will be possible to explore the set of stable configurations that are accessible to plants. The proposal includes four objectives:

Objective 1: Develop the geometric framework of primordia fronts and multilattices.

Objective 2: Generate a comprehensive dataset of time-resolved phyllotactic configurations and calibrate the dynamical models according to these data.

Objective 3: Study the topology of the attractor in various systems and its implications on the frequency of certain configurations (e.g., Fibonacci configurations) and on the transitions of patterns in plants.

Objective 4: Provide biologists with conceptual and computational tools to study the dynamics of meristem development, concentrating on *Arabidopsis thaliana*.

Background

Biological Control of Phyllotaxis

One central question for phyllotaxis is the nature of the interactions that determine the position of new primordia. Recent observations indicate that these interactions could involve molecular and mechanical signals. At the molecular level, polar transport of the plant hormone auxin is now emerging as one of the key players in determining the position of new primordia at the meristem (Reinhardt et al., 2000 and 2003a). Auxin is believed to be transported in and out of cells by influx and efflux carriers located in the cell membrane. Putative influx and efflux carriers, AUX1 and PIN1 respectively, in *Arabidopsis* are present in cells of young primordia and at the site where the next primordium forms. At least one of these membrane proteins, PIN1, shows a clear subcellular localization that is believed to be the basis for polar transport of auxin. One putative mechanism for the propagation of phyllotactic configurations posits that auxin is actively transported up the stem to the apex where a high local level of auxin favors differentiation of a new primordium. On the other hand, young primordia act as auxin sinks thus lowering the level of auxin and preventing the differentiation of new primordia in their vicinity. The interaction between the apical flow of auxin and the activity of primordia as auxin sinks offers a possible molecular basis for the geometrical rules stated in the introduction. Tissue mechanics has also been implicated in the control of phyllotaxis. For example, it is possible to alter phyllotaxis by constraining the meristem mechanically during development (Hernández and

Green, 1993; Green, 1999). Another intriguing observation is the possibility of initiating primordia by local application or expression of the wall loosening molecule expansin (Fleming et al., 1997, Reinhardt et al., 1998; Pien et al., 2001). Based on these observations, it has been argued that “mechanical communication”, perhaps in the form of buckling of the meristem surface, offers another putative mechanism for the placement of new primordia at the meristem.

Biologists have also attempted to identify the set of primordia directly involved in determining the location of new primordia. Through a series of ablation experiments, it was shown that only the set of most recently formed primordia around the circumference of the meristem plays a role in determining the position of the next primordium (Snow and Snow, 1932; Reinhardt et al., 2003b). Moreover, within this set some primordia seem to be playing a more important role although no quantitative analysis is currently available.

From the results presented above it is clear that phyllotaxis is a dynamic process that evolves in space and time. This conclusion served as the impetus for the development of experimental approaches to observe meristem growth and the evolution of phyllotactic configurations over time (Hernández et al., 1991; Dumais and Kwiatkowska, 2002; Grandjean et al., 2004; Reddy et al., 2004). These experimental tools have achieved a high degree of sophistication but additional tools are needed to analyze quantitatively the massive amount of data that is being generated. Moreover, biologists are increasingly challenged by the complexity of processes such as phyllotaxis that evolve in space and time. There is therefore a need for a modeling environment that will help researchers test alternative hypotheses for the control of phyllotaxis.

Classical Geometric Models of Common Phyllotactic Patterns

The geometry of the surface on which the primordia configuration is formed can range anywhere from a cylinder to a dome or a flat disk. For simplicity, in this text we will concentrate on a cylinder of unit circumference. Since Bravais and Bravais (1837) it has been assumed that the regular configurations of cylindrical plant phyllotaxis are *cylindrical lattices*. A cylindrical lattice is obtained by placing primordia one at a time, at constant angular and height increments (x, y) along the cylinder (Figs. 3A and 3B). If one were to use the cylinder as a printing press and rolled it out on a plane, the successive primordia of a cylindrical lattice would appear as part of a straight line (which, rolled back on the cylinder, is called the “generative helix”). There are also other helices that are usually more visible in lattices: those going through nearest neighbors. These are called *parastichies*, and they often come in pairs of Fibonacci numbers in plants.

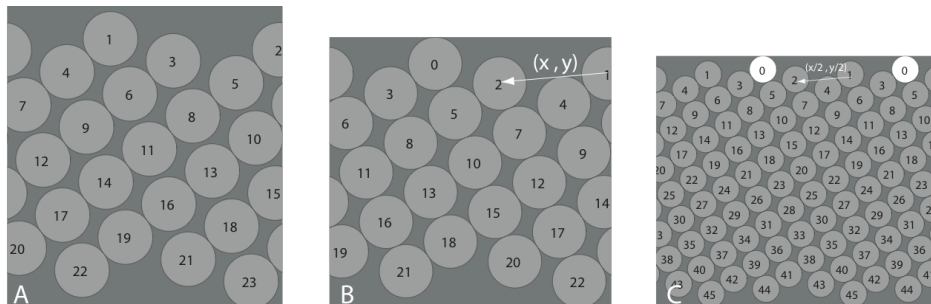


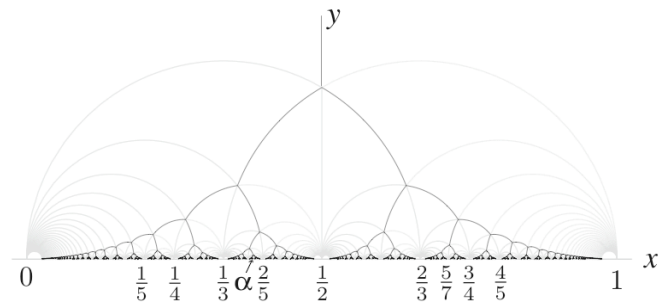
Figure 3. Cylindrical lattices and multijugate configurations. The cylinder is flattened out on the plane. A) A lattice that is not phyllotactic: primordia are not equidistant to their nearest neighbors. B) A phyllotactic lattice with its generating vector (x, y) . All other points can be obtained by adding integer multiples of this vector, and a multiple of the vector $(1, 0)$. This lattice

has parastichy numbers (3, 5): this is the number of distinct helices joining nearest neighbors, three parallel to (0, 3, 6, 9, ...) and five to (0, 5, 10, 15, ...). This is a common Fibonacci lattice. C) a multijugate (bijugate in this case) configuration that is comprised of two copies of the lattice in B) scaled by 1/2 and shifted by 1/2 turn from one another. This configuration has parastichy numbers (6,10).

A common assumption is that points of lattices in nature are equidistant to their two nearest neighbors. Lattices satisfying this equidistant condition are called *rhombic lattices* and can be represented by configurations of tangent disks of same diameter D (Fig. 3). The parameter D , which biologically represents the ratio of the primordia diameter over the circumference of the meristem, is central to our study.

Van Iterson (1907) charted out all possible values of (x,y) corresponding to rhombic lattices in a planar diagram (Fig. 4). This diagram is central in phyllotaxis, and an accepted measure of validity of a model of phyllotaxis is to be able to reproduce some of its qualitative and quantitative features.

Figure 4. The van Iterson diagram (in black) is the set of (x, y) corresponding to rhombic lattices. Each branch corresponds to lattices with a given pair of parastichy numbers. Zigzagging down along the branches starting from the top, one follows the Fibonacci sequence, limiting down to the golden angle α (here the interval $[0,1]$ corresponds to a full turn of 360°).



However, the phyllotactic configurations observed in Nature conform to rhombic lattices only to a first approximation. There are in fact many reasons why it is desirable to break free of such a rigid description. In particular, lattices do not include whorl and multijugate¹ configurations, so an entire class of patterns is unaccounted for. Moreover, the configurations seen in nature do not have time to stabilize to perfect lattices. We propose a new geometric framework, the primordia front and multilattices, to deal with these facts (see Proposed Work section).

Discrete Dynamical Systems Models

A large part of the literature offers geometric studies of models with no time evolution, involving only spiral configurations (Bravais 1837; van Iterson 1907; Levitov 1991; Adler 1998). Unfortunately, they cannot explain the convergence to (and hence the stability of) regular spiral configurations from general initial conditions. For this project, we choose to emphasize a finite dimensional geometric and dynamical systems approach, as initiated in Atela et. al. (2002) (see also Kunz 1997; d'Ovidio and Mosekilde 2000). We were inspired by the iterative models of the physicists Douady and Couder (1996) (see also the related systems proposed by Schwabe and Klewer 1984; Koch, Bernasconi and Rothen 1998): they are sufficiently simple to be cast as finite dimensional, discrete dynamical systems; yet, they are compatible with most of the biological mechanisms proposed such as buckling and auxin transport. Importantly, our models offer a bridge to the geometric framework of multilattices and primordia fronts.

¹ Spiral structures where several equally spaced primordia form at the same level (Fig. 3C).

These dynamical systems models are an implementation of the two rules stated in the introduction. More specifically:

- 1) A new primordium forms in “the least crowded spot.”
- 2) It then moves away from the center of the meristem (either vertically or radially depending on the meristem geometry).

If one assumes that primordia form periodically (more precisely, constant plastochrone ratio), then one obtains what Douady and Couder called *Hofmeister’s hypothesis*. If one assumes that new primordia form *when* and *where* there is enough space for them, then one obtains the so-called *Snow hypothesis*. The Snow hypothesis has the advantage to allow the simultaneous formation of several primordia (multijugate configurations). Douady and Couder numerically reproduced many features of the van Iterson diagram. They also showed that, for a given set of parameters, configurations starting with different initial conditions could converge to either spiral or whorl configurations. This result suggests that the initial configuration of primordia found in the meristem of the plant seedling could play an important role in determining the phyllotaxis later in development.

Mathematically, these models can be seen as discrete dynamical systems whose configuration space is the set of $N+1$ -tuplets of points $(x_0, y_0), \dots, (x_N, y_N)$ of the cylinder (each representing the center of a primordium) with a transformation of the form

$$F(x_0, y_0, \dots, x_N, y_N) = (f(x_0, y_0, \dots, x_N, y_N), x_0, y_0, \dots, x_{N-1}, y_{N-1}),$$

where the function f determines the location of the new primordium that minimizes the interaction between it and the existing ones. The Hofmeister type models have the internodal distance pre-established: $y_k - y_{k-1}$ is constant and the variables y need not be explicitly part of the definition of F . In the Snow-type models, f expresses the location *and* time at which the interaction W falls below a certain threshold. In either case, most authors consider an interaction of the form

$$W(x, y, x_0, y_0, \dots, x_N, y_N) = \sum_k u(x, y, x_k, y_k),$$

where (x, y) is the test location of the new primordium and u is a more or less explicit function of the distance between (x_k, y_k) and (x, y) . Douady and Couder used, among others,

$$u = \frac{c}{dis^a}$$

where dis means distance between (x_k, y_k) and (x, t) . The distance function may itself vary with the geometry considered (*e.g.*, Douady and Couder 1996 use the angle of conicity). It is not hard to show that the configurations that are fixed by F are, up to translation, cylindrical lattices.

Our group has proposed, in either the Hofmeister or Snow models (Atela et. al. 2002; Atela and Golé 2005) a radical simplification of the interaction as

$$W^*(x, y, x_0, y_0, \dots, x_N, y_N) = \max_k u(x, y, x_k, y_k).$$

In other words, we neglect all but the largest interaction – equivalently, *we assume that a new primordium only feels its closest neighbor*. It is easy to see that W^* attains its minima at locations equidistant to the two nearest primordia. The potential W^* can be seen as a limit of W as $a \rightarrow \infty$

with the interaction law $u = \frac{c}{dis^a}$. This limit is sometimes called the hard disk limit. In this

proposal we refer to the hard disk Snow model by Snow0. This assumption has the distinct advantage of making the connection to the classical van Iterson diagram clear. In both the Snow and Hofmeister models, we show that the fixed-point bifurcation diagrams are the same subset of the van Iterson diagram. To our knowledge, it has allowed the first entirely complete and rigorous mathematical analysis of a bifurcation diagram in phyllotaxis.

Proposed Work

Many physico-chemical models have been used to simulate phyllotactic patterns some of which are based on diametrically different biological mechanisms. This diversity suggests that some critical information is missing to constrain the models to a limited set of likely candidates. We believe that this information can come from precise quantitative measurements of phyllotactic patterns both in space and time. This leads us to study phyllotaxis both at the organ level and in terms of geometrical dynamical systems.

The first subsection (Objective 1) of the project's Proposed Work develops the framework of primordia front and multilattices. These concepts are central to this project and inform all of its parts. They address the serious limitations of the classical approach to phyllotaxis via lattices and parastichies.

The second subsection (Objective 2) addresses the biological aspects of the project and the connection between experiments and modeling. Here is where the intimate interaction (surprisingly rare in phyllotaxis) between biologist and mathematicians that this project supports will be most crucial. This part of the project will be a portal for the undergraduate students participating in this project.

The third subsection (Objective 3) deals with the dynamical study of different models, in light of the experimental data. The reference model Snow0 will serve as a bridge between the geometrical framework and the calibrated models.

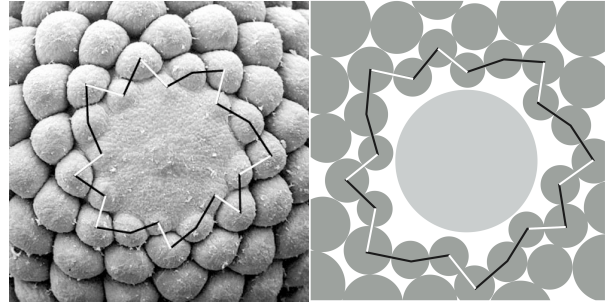
The final subsection (Objective 4) describes the development of research and educational computational tools and their dissemination. Here again, there will be an extensive collaboration between biologists, mathematicians and undergraduate students.

Objective 1: Develop the geometric framework of primordia fronts and multilattices

A key element in our project is the new concept of *primordia front*: in botanical terminology, a closed chain of adjacent primordia surrounding the meristem that were, at one point in the morphogenetic process, the innermost primordia of the meristem (Figs. 5 and 6), see Section Objective 3 for a precise mathematical definition. The primordia front is both a mathematically convenient and a biologically relevant concept. The primordia front embodies the experimental observation that only the “ring” of recently formed primordia contributes to the formation of new

primordia (Snow and Snow, 1932; Reinhardt et al., 2003). This observation provides experimental evidence that the concept of primordia front is more than just a mathematical tool.²

Figure 5. A) A primordia front around the meristem of *Picea*, formed by the newest layer of primordia. Note that there are 21 primordia in this front. Of the 21 line segments joining these primordia, 13 (in black) point inward as one travels along the front counterclockwise, and 8 (in white) point outward. This corresponds to the parastichy numbers.



B) Result of a simulation of a Snow model (centric representation). The primordia front has the same structure as in the *Picea* meristem.

Primordia fronts lead naturally to the new geometric structure of *cylindrical rhombic multilattice*. As Figure 6 shows, a variation of the primordia front of a rhombic lattice yields a rhombic multilattice, with undulating parastichies. These small variations of lattices are closer to configurations actually observed in nature. Moreover, the set of rhombic multilattices includes whorl and multijugate configurations. Primordia fronts, under iteration of our Snow model, converge to rhombic multilattices, which are periodic orbits for the system. We will use primordia fronts as a way to parameterize the set of rhombic multilattices and to chart out transitions between different configurations.

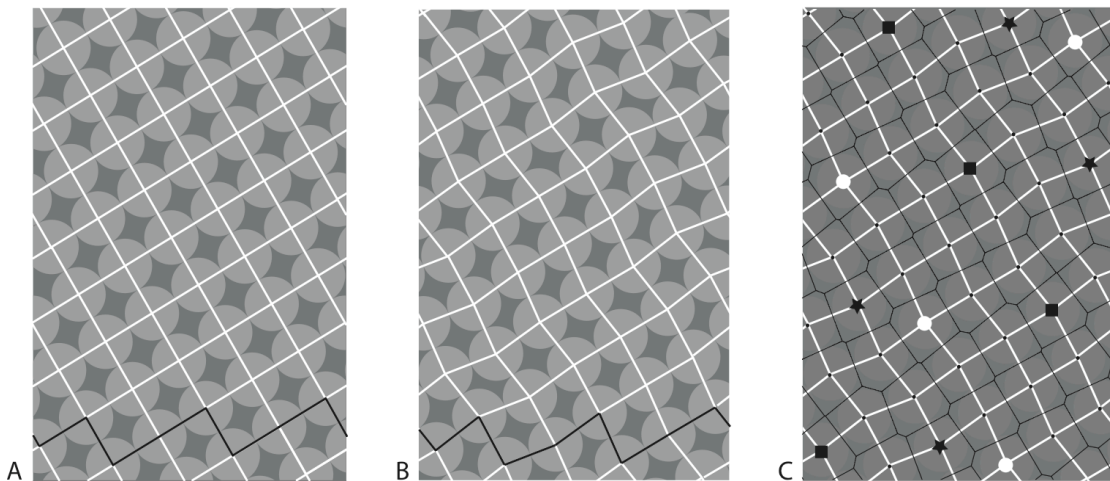


Figure 6. A) A rhombic cylindrical lattice with parastichy numbers (3,5). The white lines are the parastichies going through the points of the lattice. B) A rhombic multilattice. It was obtained by deforming a primordia front (black broken lines) of the lattice on the left. Notice that, although undulating, the multilattice still has distinctive parastichies, with same parastichy numbers (3,5). Note also that, in both cases, the front has 3 down segments and 5 up, in correspondence to the parastichy numbers. C) The same multilattice with its Voronoi cells added for a more biological

² As another possible evidence, the fact that the number of primordia in a front is the sum of the parastichy numbers may be related to the statistical predominance of Fibonacci numbers of ray florets in some plants.

look. Compare with Fig. 1D. The multilattice is formed of 15 translated copies of the lattice in white disks. We have shown 2 of these copies: one as stars, the other as squares.

Mathematically, we define a cylindrical multilattice L to be the union of a finite number of translated copies of a single cylindrical lattice Λ :

$$L = \bigcup_{k=1}^n (\Lambda + v_k)$$

where the vectors v_k gives the translations along the circumference and the axis of the cylinder. A lattice is trivially a multilattice, and multijugate configurations can be obtained by setting $v_k = (k/n, 0)$ for some n (See Fig. 3C for a bijugate example). In the same way that rhombic lattices play a special role in classical phyllotaxis (van Iterson diagram, Fig. 4) the set of *rhombic multilattices* is central to phyllotaxis in the new context of multilattices. We say that a cylindrical multilattice is rhombic if its points form a rhombic tiling of the cylinder (Figs. 6B and 6C). To our knowledge this set has not been studied before.

A primordia front of a multilattice contains all the information about the multilattice, including its parastichy numbers (given by the numbers of up and down segments in the front). Any deformation (in a certain range) of the front yields a multilattice with same parastichy numbers. This kind of deformation when performed on rhombic lattices yields high dimensional surfaces of multilattices around every rhombic lattice (of dimension $m+n-2$ for a lattice of parastichy numbers (m, n)).

Topology of the sets of primordia fronts and of rhombic multilattices – Fronts can be parameterized by a finite sequence of angles with certain restrictions. The length of the individual segments in a front is equal to the parameter D (ratio of the disk diameter over the circumference of the cylinder), . It is a crucial parameter that governs transitions of fronts in plants. The set of all fronts then comes together as a sort of branched manifold with branches of various dimensions. We will perform an exhaustive study of this set in the range of parameter D where the dimensions of the branches are less than or equal to 3. This contains configurations of parastichy numbers (1,1) on up to (2,3) and (3,3). (Interestingly, we have observed that, for a range of values of the parameter, the space of fronts is topologically the projective plane.)

As mentioned earlier, there is an intimate relation between the set of rhombic multilattices and the set of primordia fronts: fronts can be used to parameterize rhombic multilattices. The set of rhombic multilattices is thus itself a sort of branched manifold containing the van Iterson diagram and all its “multijugate copies” as well as continua of other rhombic multilattices. Extending the methods of hyperbolic geometry of Atela et. al. (2002) we will use renormalization methods to study its topology. (See Tuffley 2003 for a topological study of the related notion of finite subset spaces of a surface.)

Objective 2: Generation of Dataset of Time-Resolved Patterns and Calibration of the Models to Biology

Quantification of the evolution of phyllotactic configurations in time and space is important to set precisely the range over which putative models must be operating. Such data can set a benchmark to decide whether a model is fully compatible with the biological process. Our experimental approach is based on two important techniques: *i*) an *in vivo* replica method that

allows us to follow the evolution of phyllotactic configurations as they unfold; *ii*) microsurgical techniques to modulate pattern formation at the meristem.

In vivo analysis of developing phyllotactic configurations

Observation of meristems is based on a replica technique (Williams and Green, 1988) that allows us to follow precisely the development of phyllotactic configurations in time (Fig. 7). The non-invasive replica technique uses dental impression polymer to obtain accurate moulds of the meristem surface at different time points. These moulds are filled with epoxy resin and the resulting casts can then be imaged with light microscopy or scanning electron microscopy. The replica technique has now been applied to a wide range of meristems (Tiwari and Green, 1991; Hernández et al., 1991; Selker and Lyndon, 1996; Hill, 2001; Dumais and Kwiatkowska, 2002; Kwiatkowska, 2004). The flexibility and ease of use of the approach means that a large data bank of time-resolved phyllotactic configurations can be generated quickly. This is a clear improvement over previous experimental work that was either static or offered poor time and/or space resolution of the growing meristem.

Microsurgical manipulations of pattern formation

We have perfected microsurgical techniques to modify pattern formation at the shoot meristem. Thin flexible razor blades are used to perform superficial cuts on the meristem surface. The approach is particularly useful when applied to the sunflower meristem where it is possible to isolate large unpatterned regions and to follow how a new pattern is formed with or without the influence of older primordia (Fig. 7). The technique offers an unprecedented level of control over the morphogenetic process. In particular, the ability to alter the size and shape of the isolated region is a powerful tool to probe the role of geometry in pattern formation.

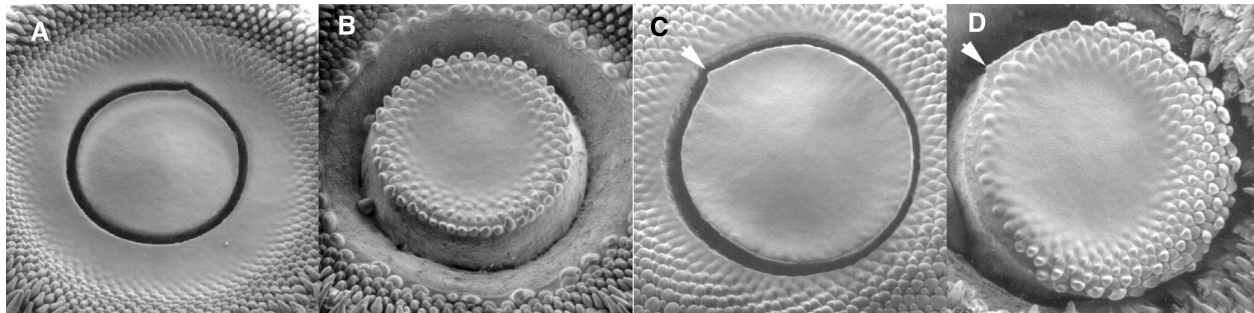


Figure 7: Sequential replicas of sunflower meristems after microsurgical manipulation. A) and B) The same meristem immediately after (A) and three days after (B) performing a centered cut. C) and D) another meristem but this time with an off-centered cut. Note in C) the inclusion of some patterned tissue in the lower right of the plug leading to unequal development of the plug in D). An arrowhead indicates the same tissue projection in C) and D).

Generation of dataset

Much of the work done on the quantification of phyllotactic patterns has focused on fully developed structures (notable exceptions include Meicenheimer 1998; Rutishauser 1998). Although one might argue that this approach is an expedient way to gather information about the development of phyllotaxis, there are also many reasons to look at such data with circumspection. Perhaps the greatest shortcoming is that growth of the meristem may have altered substantially the geometry of the structure and the quantitative relationship between

organs. We want to avoid such difficulties by proposing to quantify phyllotactic patterns as they unfold.

Plant material and data collection – To achieve a comprehensive understanding of the development of phyllotaxis, it is important to sample broadly among plant species with various phyllotactic configurations and meristem geometry. We will perform the primary selection during summer '05 starting with a wide range of asteraceae, conifer seedlings, and brassicaceae, including *Arabidopsis*. The sunflower capitulum offers many advantages among which are the simple, disk-like geometry of the meristem and the ability to alter phyllotaxis as discussed above.

The data collection is technically easy but labor intensive. Most of the data will be collected in the context of a work-study program at Smith College. Starting July 2005, two Smith College students will work for six weeks in the Dumais laboratory at Harvard University. For the duration of the grant, three students per year will spend up to two months in the Dumais laboratory. The students will be trained in the preparation of plant material, microsurgical techniques, and microscopic techniques. It is expected that they will gain full autonomy during the two-month training period and that they will continue their work after returning to Smith.

Extraction of phyllotactic parameters for primordia front – A wealth of data can be generated with the replica method but computational tools are required to extract the relevant phyllotactic parameters. First, three-dimensional reconstructions are required to characterize the geometry of the meristem surface and to localize precisely the position of primordia. Stereoscopic techniques and computational tools were already developed by our group to make 3-D reconstructions from scanning electron micrographs (Dumais and Kwiatkowska, 2002; Kwiatkowska and Dumais, 2003) from which the surface curvature can be quantified. As indicated above, prior experimental work indicates that only the most recent primordia affect the positioning of new primordia. Therefore our reconstruction efforts will focus primarily on the unpatterned central region of the meristem and the youngest primordia fronts. We will develop computer algorithms to locate the position of primordia on the meristem surface. Our long-standing collaborator Scott Hotton has developed computational tools to treat data of primordia configurations (Hotton, 2003). Our group, and especially the Postdoc, will develop new tools to extract primordia fronts and their time evolution. The important parameters that will be extracted are the distance of the primordia from the center of the phyllotactic pattern, the distance between adjacent primordia, and the set of angles that define the primordia front.

Experimental manipulation of phyllotactic configurations – In order to explore fully the range of configurations that are accessible to plants, we will follow phyllotactic development in meristems that have been microsurgically manipulated. By changing the size of the plugs and their geometry, we will attempt to cover systematically the space of configurations that will be studied formally under objectives 1 and 3. The transition from whorl to spiral, which occurs in most dicotyledons, will also be used to investigate how the evolution of phyllotaxis is affected by the initial configuration of the system.

Calibration of the dynamical models – The above data will be crucial in establishing the kind of interaction laws and motion of primordia, as well as the underlying geometry of the meristem in our models. We will experiment with different interaction potentials restricting the contributing primordia to a subset of the primordia front. We will also experiment with small deformations of the front as new primordia develop.

Objective 3: Dynamical Systems and Topology of the Attractors

The Snow0 model described in the Background section will serve as central reference for all the threshold models we will study. It has direct ties to the set of rhombic multilattices (its attractor is a subset of it); it is a mathematically fertile ground for the development of fundamental concepts of phyllotaxis (for instance, it lead us to the concepts of primordia fronts and multilattices); it allows rigorous mathematical treatment of these concepts; and features of its attractor will inform those of nearby threshold models.

The reference model: Snow0

Snow0 has a simple geometric interpretation in terms of configurations of disks on a cylinder of circumference normalized to 1. The ratio parameter D is then, simply, the diameter of the disks. It represents the threshold value. Given a configuration at a given time, the next one is obtained by placing a new disk on top of the existing ones at the lowest place possible without overlapping (Fig. 8). As a consequence, the new disk is tangent to at least two existing disks. For mathematical consistency, after a certain number N of disks have been placed, the oldest disk is discarded as the new one is added.

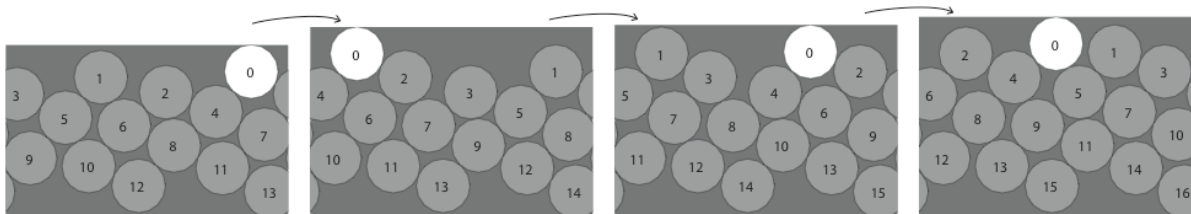
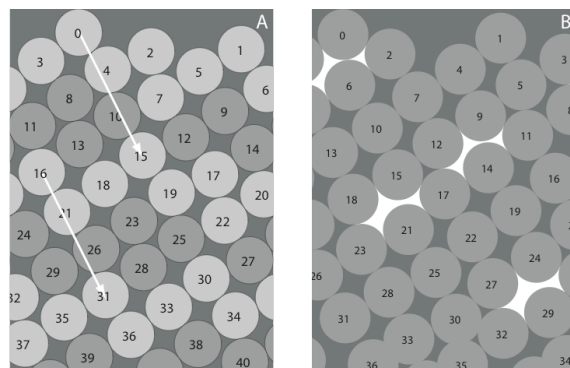


Figure 8. Three iterations of Snow0. At each iteration, the new disk (in white) goes on top of the existing ones at the lowest place possible without overlapping. Note that the internodal distance, which can be visualized as the increment of height in the successive pictures, is not constant.

Interestingly, we have observed that regardless of the initial condition, after a finite number of iterations (with fixed parameter D), only two types of configurations remain (Fig. 9):

- 1) Periodic configurations in which primordia fronts repeat periodically (up to rotation). These configurations are cylindrical rhombic multilattices. These include, among many others, the classic phyllotactic lattices (Fig. 6), which appear as fixed configurations of the system, and multijugate configurations (see Section Objective 1).
- 2) Asymptotically periodic configurations that feature sequences of pentagonal interspaces (in white) that become thinner and thinner.

Figure 9. Examples of the two types of configs formed after a finite number of iterations. A) A periodic configuration, of period 15. This is a multilattice with parastichy numbers (3,5) (hence the period equals 3×5). A front (light grey) repeats, translated. B) A configuration containing pentagonal interspaces (in white). In all the many cases that we have observed, the width of the pentagons tends to 0 exponentially while the configuration tends to a periodic one.



In this mathematical context, a primordia front is a closed chain of adjacent disks such that:

- The line segments joining the centers of adjacent disks form the graph of a piecewise linear function.
- The next primordium in the iteration will be higher than any primordia of the chain.

It is easy to see that, after a finite number of iterations, any configuration will yield a primordia front at the top boundary. Once a front is formed, the top boundary primordia of each of the subsequent iterates also constitute a front. In this sense, the dynamics is eventually confined to the set of fronts and this set can be viewed as an attractor for the system. Hence, it is crucial to understand the topology of this set and the dynamics induced on it, especially as the parameter D varies. The set of fronts of all diameters contains not only the fixed-point bifurcation diagram studied until now, but also the corresponding bifurcation diagrams for multijugate configurations. This project will show that the key to understand transitions between different such configurations lies in understanding the topology of the space of primordia fronts and its relation to the set of rhombic multilattices.

Dynamics in the space of primordia fronts – As stated above, after a finite number of iterates, all configurations obtain a primordia front. Based on the topological study (Objective 1) of the set of fronts restricted to the low dimensions, we will perform an exhaustive study of the dynamics of Snow0 in this case. We will then use renormalization to study the dynamics in the branches of higher dimensions.

Convergence of pentagons – We have seen that some fronts yield configurations that are asymptotically periodic and featuring pentagonal interspaces whose width tends exponentially to zero. We will study the set of fronts that yield these configurations and the set of multilattices that these pentagonal configurations converge to.

Generalization of the fundamental theorem of phyllotaxis to the multilattice case – The Fundamental Theorem of Phyllotaxis gives the range of possible divergence angles for rhombic lattices with given parastichy numbers. This theorem is an easy consequence of our study of the fixed-point bifurcation diagram using hyperbolic geometry (Atela et. al. 2002). We propose to find a relationship between parastichy numbers of rhombic multilattices and divergence angles.

Study of the attractor and stability of steady states in other Snow systems

The calibration process (Objective 2) will focus our attention on a subset of Snow models with different geometries (cone, domes, disk, ...) and different dynamical features. In turn, the mathematical and numerical study will inform the experiments.

Persistence of attractors – We will show that the attractor of Snow0 persists as sets of invariant manifolds in other Snow systems. (Using persistence of normally hyperbolic manifolds or numerical methods, Simó 1990). We will show that configurations similar to those in the attractor show up in plants in transient modes.

Transitions – Various studies (Atela et. al. 2002; d’Ovidio 2000; Shipman and Newell 2004; Kunz 1997; Douady & Couder 1996; Levitov 1991) have indicated that transitions of parastichy numbers following the Fibonacci sequence result from parameter changes. Other common transitions observed in plants, from multijugate or whorl to spiral (and vice-versa) also occur in simulations where parameters are varied. At the level of primordia fronts, transitions are simply detected as a change in the number of primordia in successive fronts. This has simple geometric

consequences on the configuration (Fig. 10), and corresponds to jumps from one branch to another in the branched manifold of primordia fronts. The topology of the branch manifold will inform our study of these transitions.

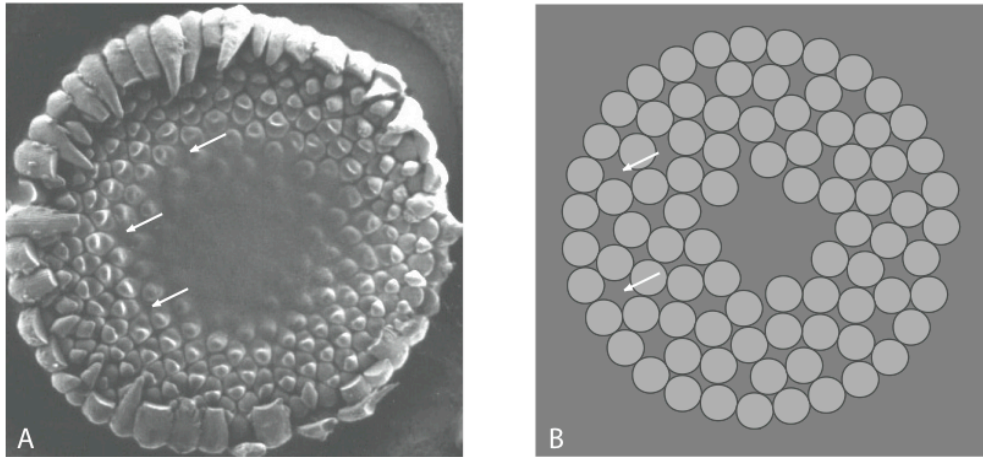


Figure 10. In the disk geometry, if the primordia are of constant size and the morphogenetic region decreases in size, the ratio parameter D increases. Eventually, the number of primordia in a growth front must decrease. It is easy to check that this implies the appearance of pentagonal interspaces (or even higher n -gons). This simple mathematical prediction is confirmed in the incised sunflower meristem in A). In B), a simulation of the Snow0 system in the disk representation (no attempt to match the sunflower's parameters was made here). Arrows point at some of the pentagons in each picture.

Stability and basins of attraction of periodic orbits and fixed points – In the reference system, fixed points, which are rhombic lattices, are surrounded by periodic orbits (rhombic multilattices obtained by perturbation of the front). Hence, these orbits are stable but not asymptotically stable. Numerical studies indicate that for many interaction laws and geometries, the fixed points exhibit stability in ranges of the parameter (Douady and Couder 1996). We will use perturbation methods as well as numerical studies to demonstrate the stability of fixed points and periodic orbits in certain ranges of the parameters. We will also study the basins of attraction of the stable orbits within the attractor.

Objective 4: Development and Dissemination of Tools for Biologists

Over the years, biologists have uncovered a number of methods to disrupt the phyllotactic development of plants. Historically, surgical interventions and drugs have been used to change phyllotaxis (Snow and Snow, 1932; Maksymowych and Erickson, 1977). Recently, mutants were identified where phyllotaxis is perturbed in a more or less systematic way (Byrne et al., 2003). However, because phyllotaxis is a process that evolves in space and time, it is particularly difficult to identify precisely how the patterning process is affected. We thus propose to develop computational tools to support the intuition of researchers in the field. In particular, we want to develop a simulation environment where it is possible to check the global effect of a local disruption of the patterning process. These tools could then be used to predict the possible states that the system can reach given the initial perturbation.

Development of a plant system – The predictive power of the model may depend to a large extent on the quality of the calibration to the biological system under study. Therefore, although our

modeling approach is quite general, it is important to select a specific plant for in-depth study of the phyllotactic process. Given the fundamental role played by *Arabidopsis thaliana* in plant science, we have selected this plant for our initial work. The *Columbia*, *Landsberg erecta*, and *Wassilewskija* ecotypes are among those most widely used in *Arabidopsis thaliana* and were therefore selected. The replica method has already been applied to the *Columbia* and *Landsberg erecta* ecotypes (Kwiatkowska, 2004; preliminary results from the Dumais lab) so it is clear that the proposed experiments are technically feasible. As background data for the modeling work, we will collect a large data set of time-resolved phyllotactic configurations in the inflorescence meristem of *Arabidopsis* grown under some of the standard conditions used in the literature. These data will be used to calibrate the dynamical system.

Development of the simulation environment – We will develop interactive software at two levels: research and education, which will be available on our web site www.math.smith.edu/phyll0. The research oriented software will implement families of Snow and Hofmeister type models. The user will be able to implement arbitrary initial conditions. Different choices of geometry (cylinder, cone, dome, disk,...) and parameters (primordia and meristem sizes, primordia shape and velocities, etc.) will be available. The user will also have the choice of implementing the calibrations from biological experiments performed by our group for *Arabidopsis thaliana*. The educational software will be a simplification of these tools, with didactic demonstrations and tutorials. The languages used will be MATLAB GUI, *Mathematica* and JAVA.

Development of a web database – in conjunction with the simulation tools, the project will develop a large database of digitized images as well as configuration data.

Undergraduate students working in this project will be an integral part of the development of the software, including coding, testing and data entry.

Organization of Human Resources and Broader Impact

Human Resources

Personnel involved in the project: two mathematics faculty (Pau Atela (Hispanic) and Chris Golé (Caucasian), Smith College), one biology faculty (Jacques Dumais (Caucasian), Harvard University), one postdoc, one graduate student (Enrique Rojas (Hispanic), Harvard University), three female undergraduate students (Smith College).

Mathematics faculty will continue developing the geometric model. With constant feedback from the experiments, they will refine the fit of the models to the diverse geometries and growth mechanisms. They will take part in some of the lab experiments at Harvard. They will provide support and guidance to the undergraduate students in the development of computational tools, outreach material and in mathematical research.

The biology faculty will direct the experimental work to collect the data sets of phyllotactic configurations and the computer work necessary to extract the phyllotactic parameters. He will provide guidance for undergraduate students from Smith during their summer internship.

The Postdoc, with training in mathematical biology, will embed him/herself in the lab environment at Harvard, where he/she will get direct experience in the biological experimental techniques. Working full time on the project, he/she will help provide continuity and cohesiveness to the project with constant interactions with the faculty and students, and

frequently traveling between the two institutions. He/she will actively participate in all aspects of the research, including mentoring of the students.

The graduate student will be in charge of the extraction of phyllotactic parameters from the data set. A Physics student, Enrique Rojas has already been selected. Enrique is a current member of the Dumais lab and will work half time on the project. He will be able to advise experimentalists on the collection of data and will, along with the biology faculty, assure continuity of the biological aspects of the project.

The three (female) Smith College students (mathematics and biology majors) per year will be supported for a ten week summer internship each, a large part at the Dumais lab at Harvard University training and doing research on plant surgery, electron microscopy, and data collecting. They will spend the remaining part of the internship at Smith working on data fitting and model calibration, development of software and on the phyllotaxis website. Continuity in their multidisciplinary experience will be assured during the academic year through seminars, independent studies, and independent research projects, with the support of the Postdoc and faculty. Continuity from one year to the next will be achieved by mixing prospective students and experienced students. This will start immediately since two Smith College students will train at Harvard University this July 2005 in a pilot project.

Broader Impact

Multidisciplinary experience – All the participating members will have multidisciplinary research experience. This will be especially important for the students and the Postdoc.

Publications – The project will produce publications for different audiences: mathematical biologists, biologists, general scientific audience and general audience.

Educational material – Our group has a strong track record in developing educational material for general audiences. The idea of this project was initiated as a result of a visit of Jacques Dumais to the exhibit “Plant Spirals: Beauty You Can Count On” designed by the two Smith faculty and staff of the Smith Botanic Garden. The mathematicians have included phyllotaxis material in numerous undergraduate courses at different levels (from “math for poets” courses to advanced seminars in dynamical systems and analysis). They also have taught visitor classes at elementary schools in the area. See the RUI statement for further educational impact.

Web site – Our group has a strong track record in Web-based dissemination of both research and educational material on phyllotaxis. Our extensive web site www.math.smith.edu/phyll appears first in a google search for the word “phyllotaxis” and receives constant positive feedback from the public at large, and fosters professional interactions. As an example, the botanical garden of Genoa, Italy, has asked to borrow our exhibit that will show as part of a city-wide science fair in Fall 2005. The web site will link to a new database, to the simulation tools and it will develop the presentation of the Snow models.