

# Floral patterning in Sunflower – folding in a confined space

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## Introduction

Theories of organ patterning have a long history, from geometrical, to chemical, to mechanical bases. Recent research, both experimental and mathematical, indicates that the most complete patterning mechanisms likely encompasses all of the proposed mechanisms (1,2). The shoot apical meristem comprises a central zone of ‘stem cells’ and a peripheral zone where organs form and differentiate (Fig.1). The controlled accumulation of the phytohormone auxin, by transport proteins of the PIN family, triggers cell expansion and differentiation within the peripheral zone. The link between auxin and cell expansion has also been studied extensively. Most recently, it has been established that auxin triggers changes in cell wall mechanical properties that are necessary for organ formation (3,4). It also appears that the auxin transport machinery may be sensitive to tissue stresses, and possibly the mechanical state of the cell wall (4,5,6,7).

The sunflower capitulum has been a favoured system of study for chemical and mechanical phyllotaxis theories, but has been recalcitrant to genetic analysis. The most well know theories of phyllotactic patterning in sunflower are the geometrical (8) and that of mechanical buckling (9,10). Mechanical buckling has also been applied to the emergence of floral organ in *Anagallis* sp. (11); however, it has not been applied to sunflower floral patterning, only to initial floral meristem emergence. The development of sunflower flowers is a different case from *Anagallis*, and most other model systems, as it occurs in a laterally confined space (Fig.2AB). This imposes unique boundary conditions and creates a system of complex contact.

Mechanical buckling is easily described by considering a beam with a given bending stiffness, which is anchored by elastic springs along its lower edge (Fig.3A). When the beam is subjected to in-plane compressive stress, the material will buckle in characteristic wavelengths dependant on the material properties of the system (Fig.3B). This model was applied to annular rings and produced de novo ‘organ’ patterns; however, when extended to a disk undergoing expansion the mechanism could not create de novo the pattern seen in a sunflower capitulum (Fig.4). It is thus supposed that there is an annular ring, or generative zone (orange in Fig.1), where buckling occurs as opposed to the whole capitulum surface. This mechanism would give an enforced wavelength and positioning based upon the previous position of the annulus. This may be simply illustrated by examining the deformation of a metal sheet with successive movement of a heating bar (Fig.5); the position of the new hill/valley buckles is determined by the first row. In the case of the metal sheet, the heating causes material expansion to create in plane stress, in the sunflower this would be a result of growth from the centre of the capitulum towards the annular ring (12). As is, the model of Green can

explain the positioning of new floral primordia upon a growing capitulum (Fig.6); to explain the further patterning of floral primordia in sunflower, this model needs to be extended.

Floral meristems in the sunflower disk give rise to a paired bract (floral leaf) and bilaterally symmetrical flower. The first differentiation between the two occurs very early, when the floral primordia 'folds' midway (Fig.2C: drawn as an orange line); the physical presence of older primordia is strictly required for the initial folding event (Fig.7). The lower, floral primordia, then quickly folds across its middle, perpendicular to the first fold (Fig.2C, drawn as blue line). We can also see this geometrical evolution by examining the sample curvature (Fig.8A). We can also see that the mechanical properties of the tissue are not necessarily homogenous, adding a depth to previous buckling models (Fig.8B); additionally, we can track cell expansion and growth rates in relation to curvature (Fig.8C).

It is assumed that in order to explain these events via mechanical buckling, the existing model must be extended as follows (See Fig.9): 1) beginning with a linear elastic diamond of material with imposed lateral in-plane stress due to growth, 2) assuming that the outer boundary has a complex form, is fixed, and presents an immovable obstacle, and 3) that with time, lateral boundary conditions emerge to produce a new environment conducive to the secondary buckling event.

### **Overall Objective:**

It is of particular interest to determine whether a physical model encompassing tissue growth rate, mechanical properties, and restricted by contact and boundary conditions can explain the two folding/buckling events seen in the developing sunflower floral primordia.

### **Questions to be considered by Study Group participants:**

- Are simple boundary conditions enough to cause folding phenomenon?
- Will a model be sensitive to incoming growth rates (lateral forces) or can it be robust enough to withstand fluctuations (eg. Circadian growth rate)?
- Is a non-homogenous tissue in terms of mechanical properties stabilizing or troublesome? Does it matter if it occurs and drives the folding, or if occurs concurrent, or resultant from it?
- Is it possible to incorporate auxin transport as an instructive signal? Can the tissue mechanics be fed back onto the transport?

### **Available data (and data we can generate):**

- Curvature analysis and surface geometrical templates of discrete steps during floral formation.
- Growth rate parameters during floral formation.
- Tissue rigidity measurements during floral formation.
- (Possibly) PIN1 orientation data during floral formation.
- Physical manipulations of the surface and resultant imaging

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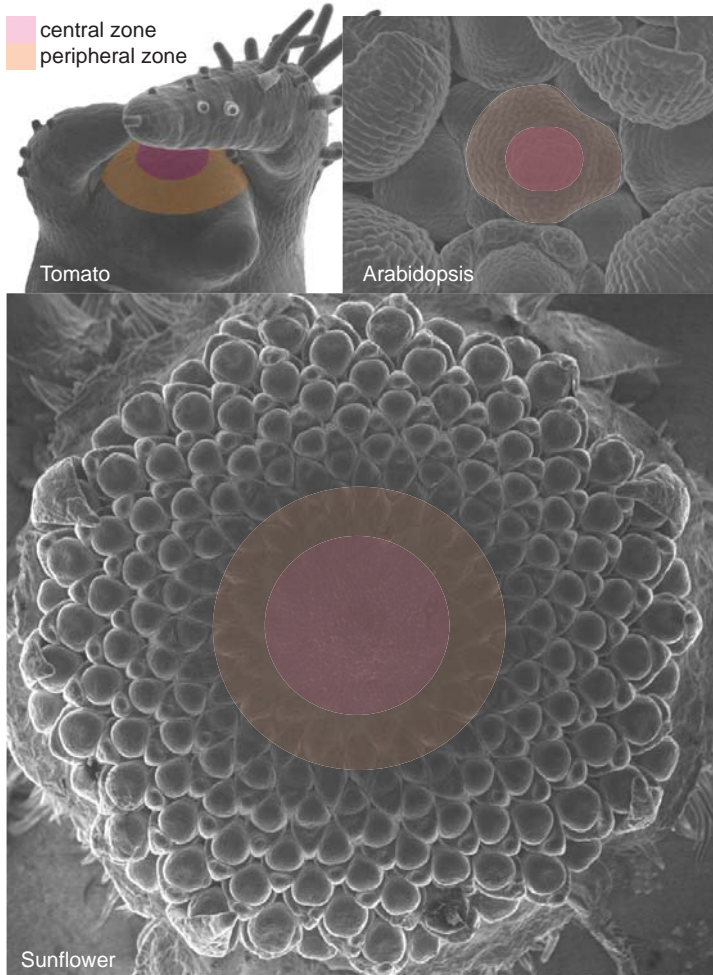


Fig.1: Basic shoot apical meristem organization

Shoot apical meristems are presented via scanning electron micrograph. The central zone is shaded in pink, being the area where the undifferentiated ‘stem cell’ population lies. The peripheral or generative zone is colored in orange, and is the area where new organs emerge.

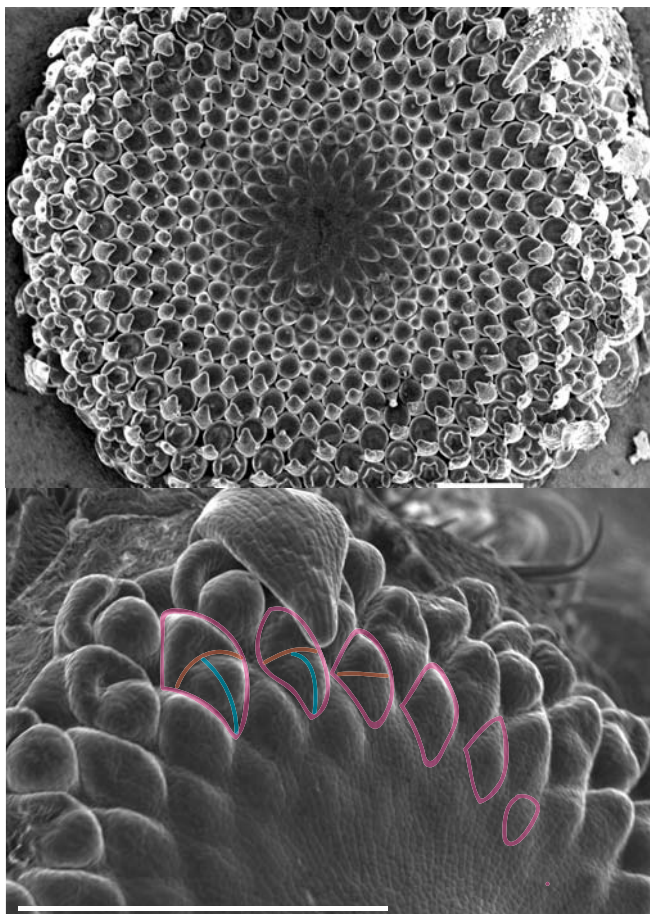


Fig.2: Patterning in the sunflower capitulum

An overview of a sunflower capitulum is presented, when floral meristem production is almost complete. Oldest flowers are at the edge, youngest towards the centre. Organs are presenting characteristic Fibonacci numbered paristiches. The lower image is a younger stage showing the first few rows of floral primordia forming. Characteristic geometrical transitions are outlined for a floral primordia: beginning as a single bulge, transitioning to a divided bract-floral pair (orange line), and lastly folding again radially (blue line).



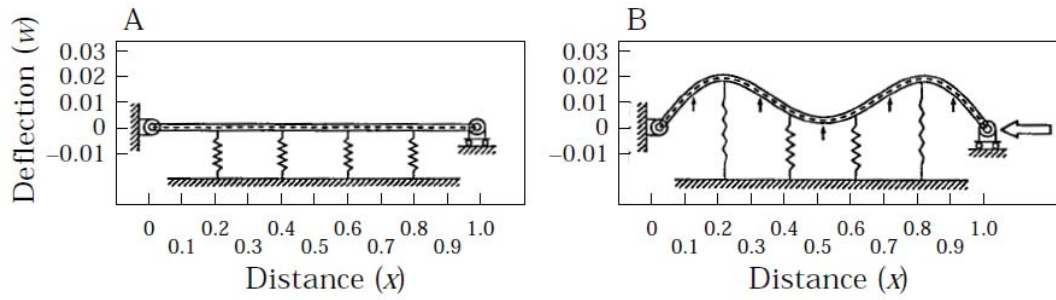


Fig.3: Mechanical buckling in a beam. *Taken from Green (1996).*

(A) a beam fixed to a surface by elastic springs, with simply supported boundaries (hinges). (B) When lateral in-plane stress is applied, the beam will buckle with a proscribed wavelength.

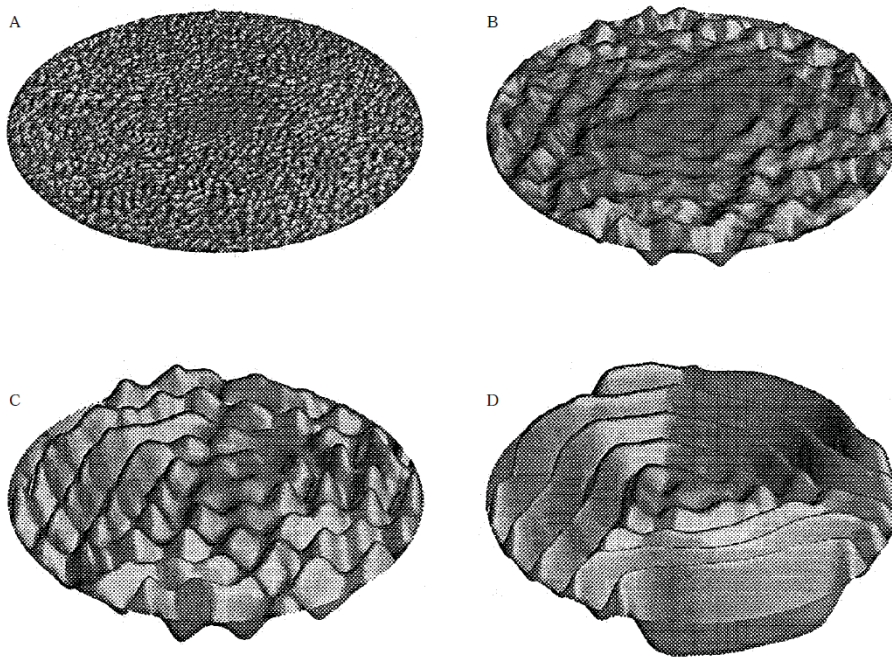


Fig.4: Mechanical buckling in a disk. *Taken from Green (1996).*

Evolution of buckling in an expanding disk. Initial noise (A) is swamped out as the deformation organizes into patterned ridges (D).

Fig.5: Mechanical buckling in a metal sheet upon heating. *Taken from Green (1992).*

Buckling in a sheet produced by heating (simulating localized growth). As the bar moves progressively, the pattern propagates.

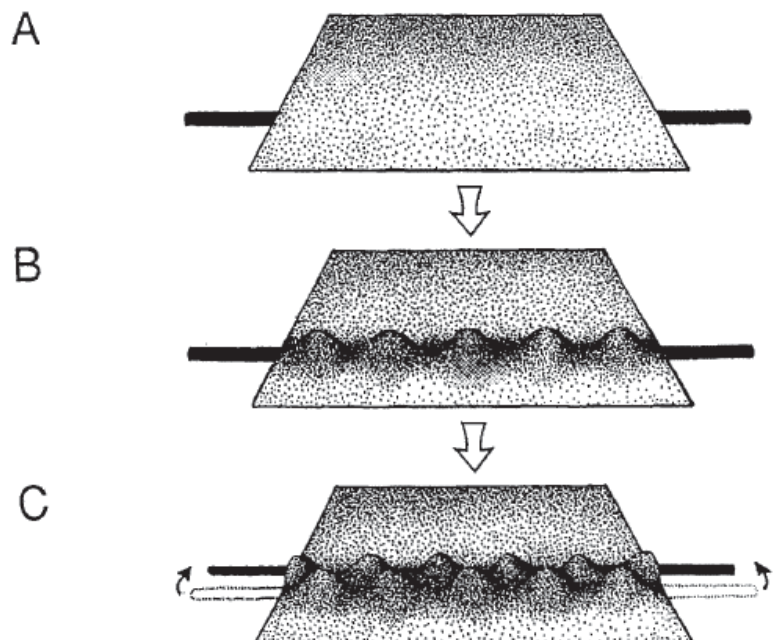


Fig.6: Minimal energy buckling in a sheet. Taken from Green (1992).

In a condition where two existing bumps are present (A-B), the application of in-plane stress results in a new bump positioned between the two existing ones (C-D). In this case as a bisection, with equally sized existing bumps. The system can be designed to produce new bumps at the golden section between two non-equal sized (simulating age) bumps.

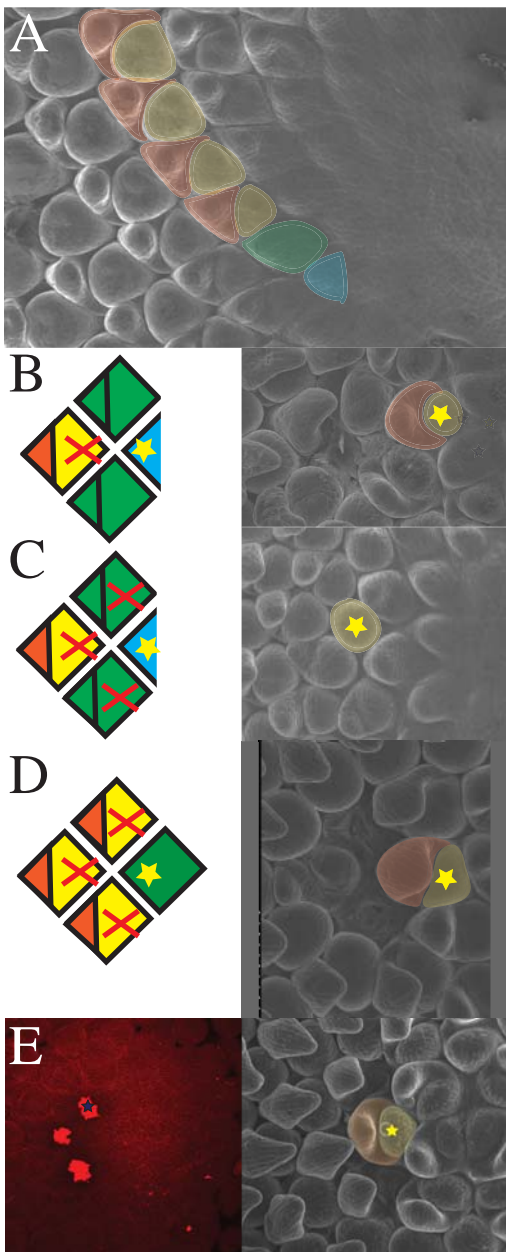
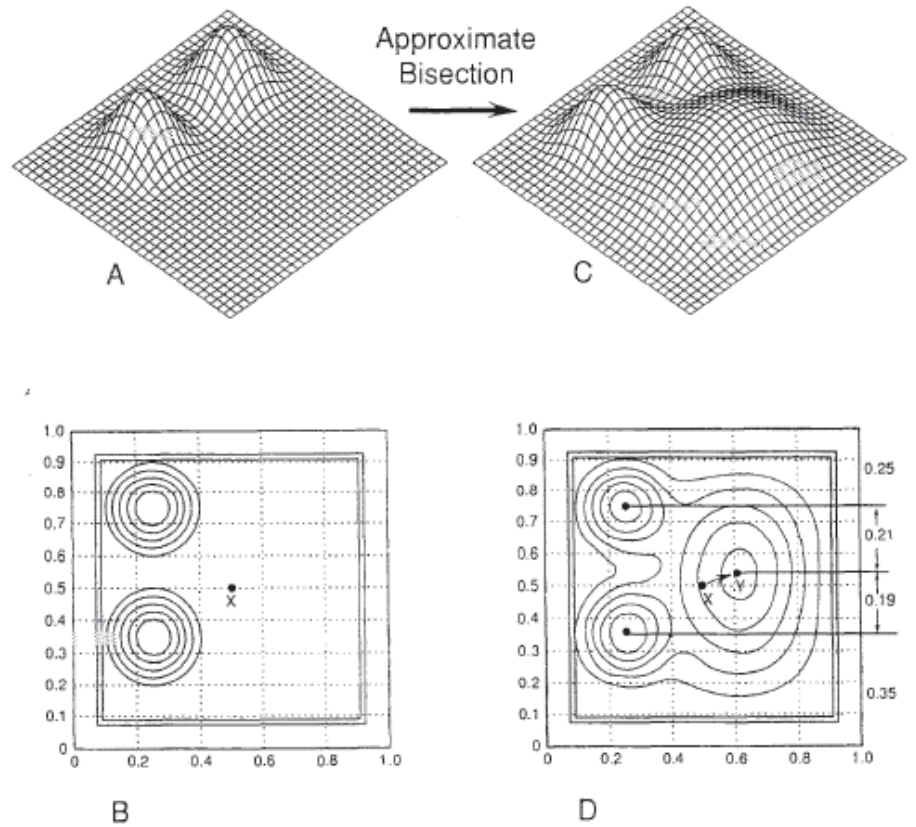


Figure 7: Physical disruption of older primordia effects identity of close contact neighbor

A) Normal floral development in *Helianthus*: floral primordia begin as asymmetric 'ramps' with a higher side presented to older primordia (blue). This shape grows more pronounced (green). Eventually the floral primordia splits in two presenting a flower primordia (yellow) and bract (orange). The bract always presents at the highest point of the young 'ramped' primordia, situated towards the outside of the capitulum. B) Physical ablation of a single proximal neighbor does not affect identity of a young ramp primordia. C) Physical ablation of all three proximal contact neighbours effects young ramp primordia, which no longer present bracts at later stages. D) Loss of bract is not seen when the young isolated primordia is past a certain maturation. E) Laser 'killing' of older contact neighbours does not cause loss of bract in the young ramped primordia.



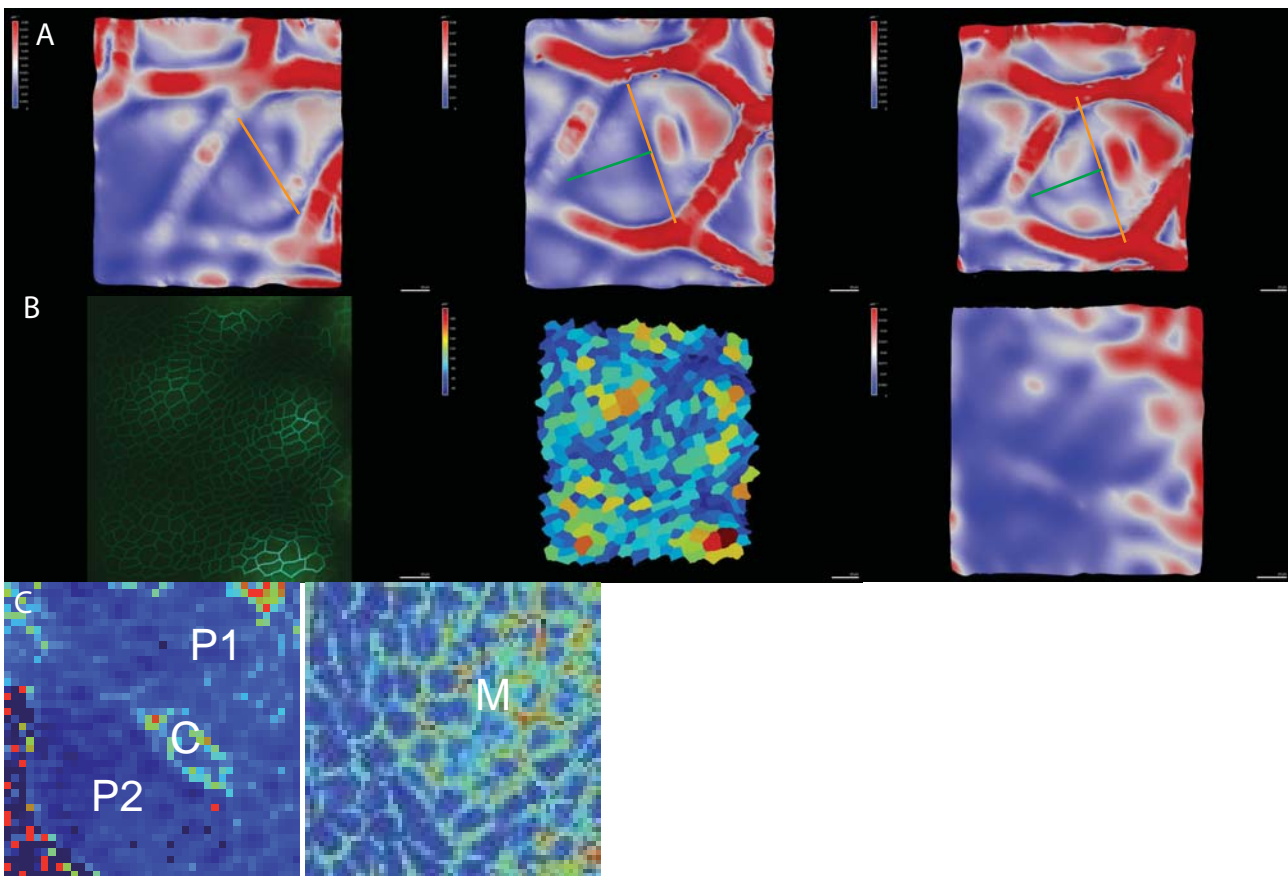


Fig.8: Characterizing parameters of floral development

(A) Gaussian curvature maps of developing floral primordia. The first fold is illustrated with an orange line, the second with a green line. These are based upon surfaces meshes obtained from confocal image stacks of cell wall staining. Meshes can be exported for modelling templates. (B) Meshes can be segmented as cells for surface area calculations (and growth analysis) to match with curvature development. (C) Atomic force microscopy based elasticity of epidermal cell walls within the developing capitulum. P for primordia, C for crease between two, and M for meristem or central zone.

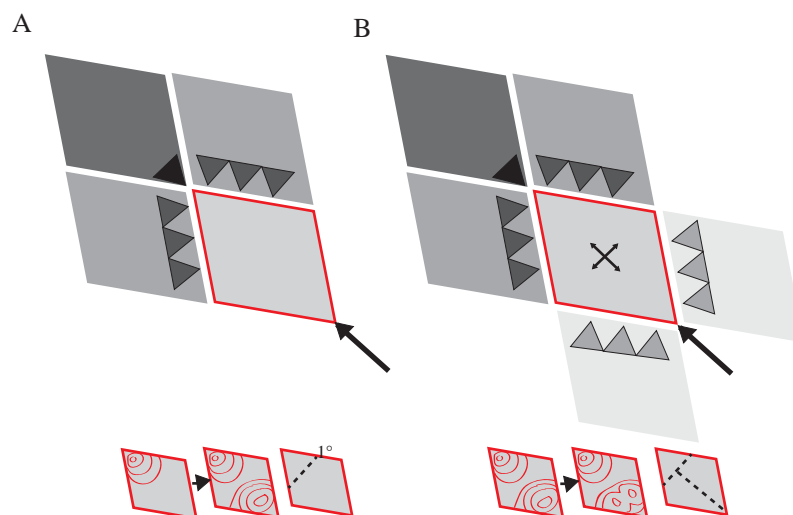


Fig.9: Proposed simplification of the system showing proposed boundaries and stress inputs

After the initial bump formation (Fig.6) we wish to simulate conditions whereby the floral primordia (diamond outlined in red) will undergo primary and secondary buckling events (A and B respectively). In the simplest case the floral primordia is a homogeneous linear elastic material. In the first instance (A) it is influenced by the three organs directly behind it (darker shades of grey, darkness representing age). In the second instance (B), it is also influenced by newly emerged organs below it (lighter grey). There is always an in-plane stress due to growth from the center of the meristem (black arrow), but also lateral constraints imposed by the surrounding organs (black arrow heads). 3D contours are shown below the diamond diagrams, illustrating the initial and resulting topographies and the positions of the primary and secondary buckling events.