

# Chemical pattern formation and plant morphogenesis: controlling branching via reaction-diffusion dynamics

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[http://commons.bcit.ca/math/faculty/david\\_holloway.html](http://commons.bcit.ca/math/faculty/david_holloway.html)

# Spatial pattern formation:

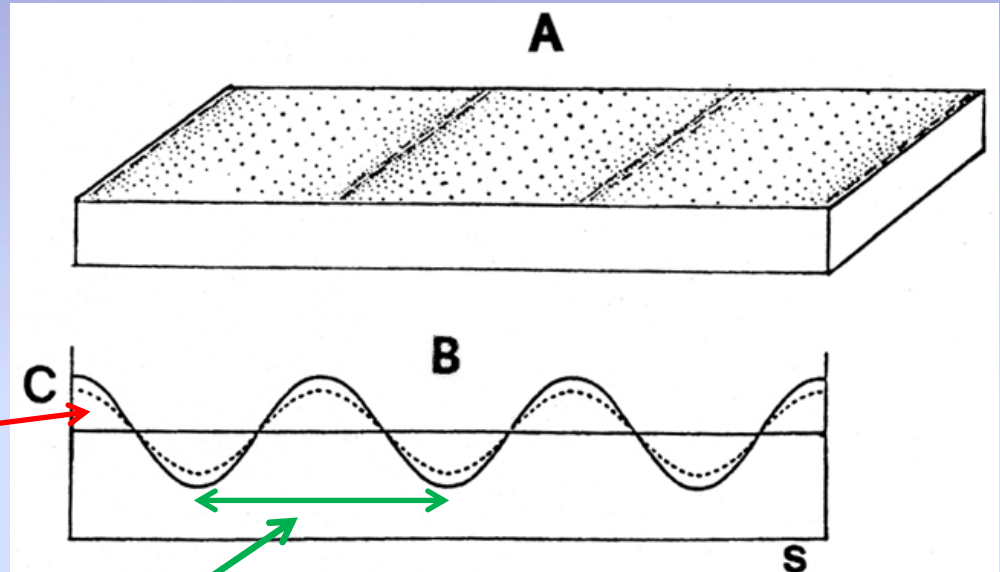
Turing's (1952) "Chemical basis of morphogenesis"

Reaction      Diffusion

$$\frac{\partial U}{\partial t} = k_1 U + k_2 V + D_X \Delta U$$

$$\frac{\partial V}{\partial t} = k_3 U + k_4 V + D_Y \Delta V$$

$$U = \cos(\omega s) e^{\sigma t}$$

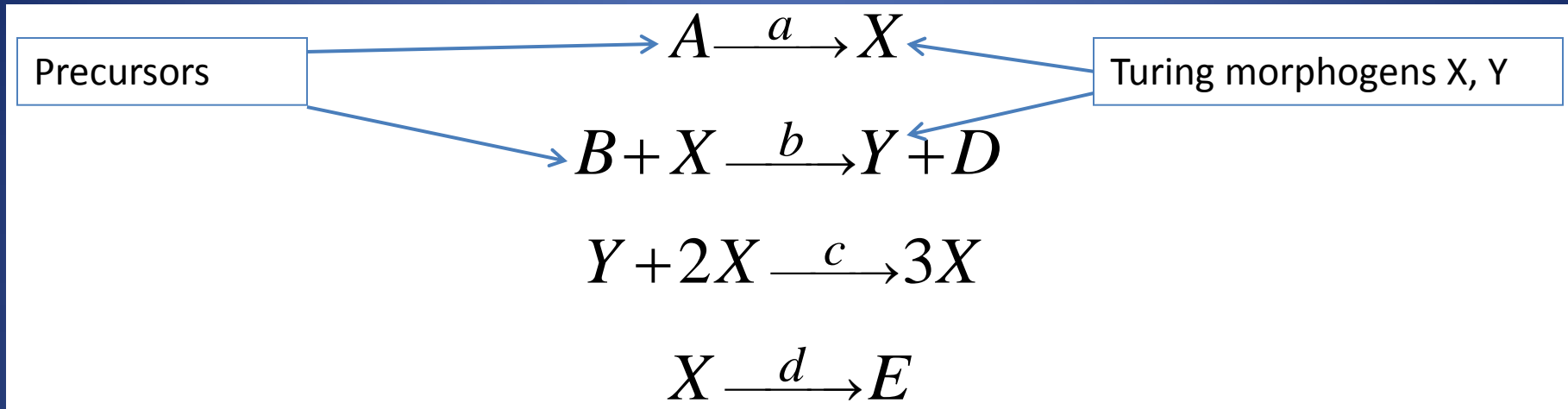


$$\frac{2\pi}{\omega} = \lambda \propto \sqrt{\frac{D}{k}}$$

← Diffusivity  
 ← Reaction rate

# The Brusselator

Prigogine and Lefever, 1968



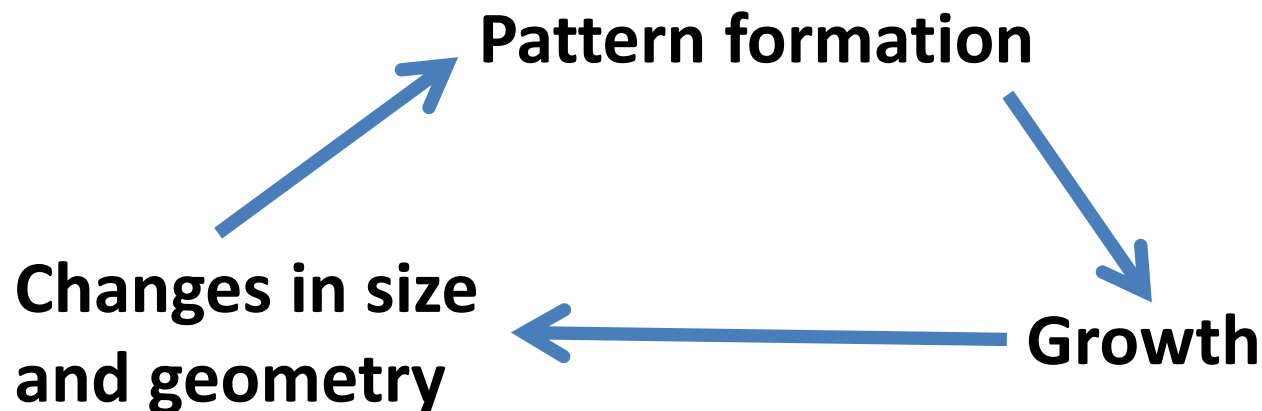
$$\frac{\partial X}{\partial t} = aA - bBX + cX^2Y - dX + D_X \nabla^2 X$$

$$\frac{\partial Y}{\partial t} = bBX - cX^2Y + D_Y \nabla^2 Y$$

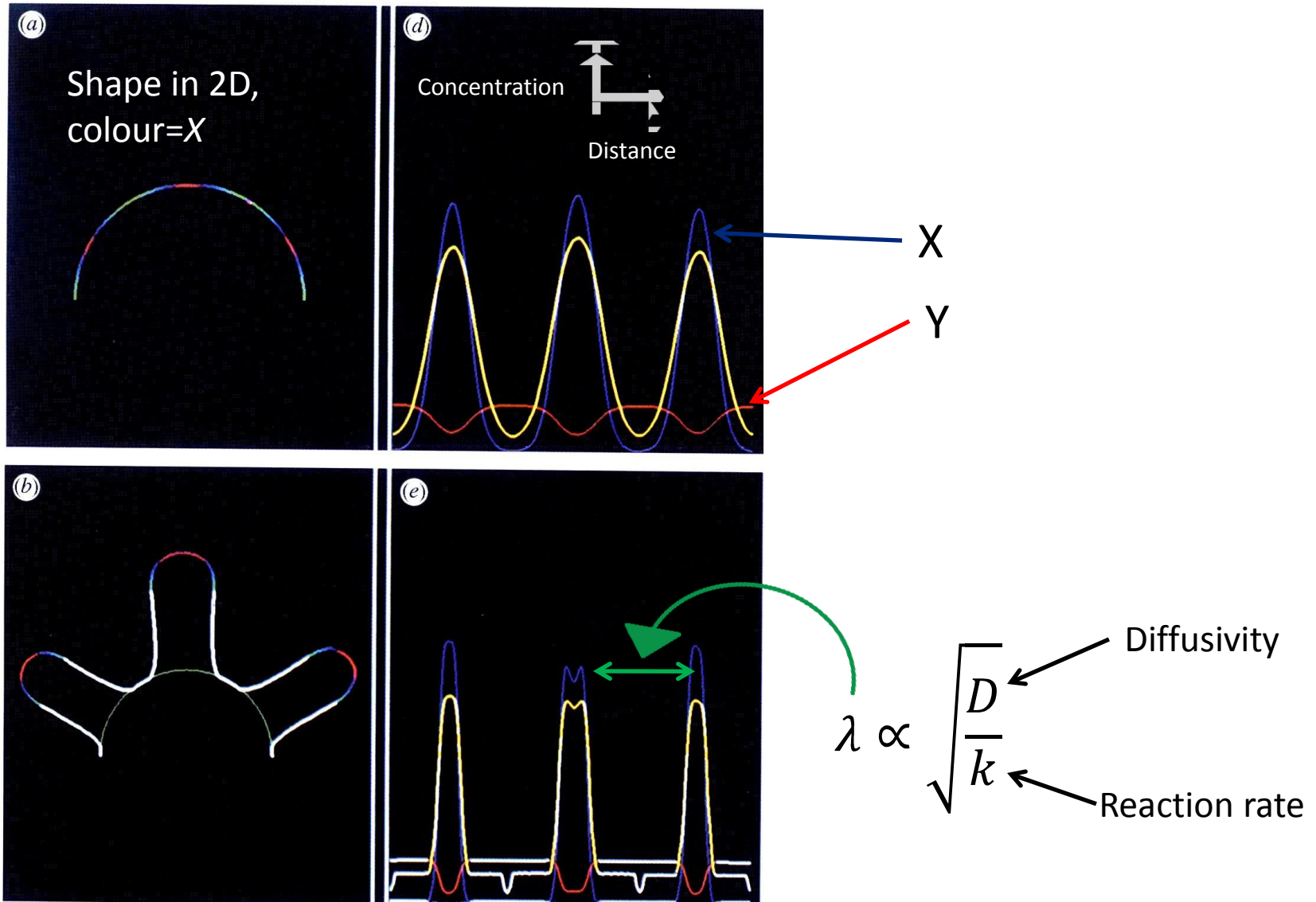
Good RD (reaction-diffusion) model for regular periodic pattern; unlike the Gierer-Meinhardt, which has 'spike' solutions (Lacalli, 1981; Holloway&Harrison, 1995; Iron et al., 2001)

# The Challenge of Plant Morphogenesis

- Plants are continually growing.
- A chemical explanation of shape generation, in which, e.g., a growth catalyst is patterned by Turing (RD) dynamics, must consider the full feedback cycle of:

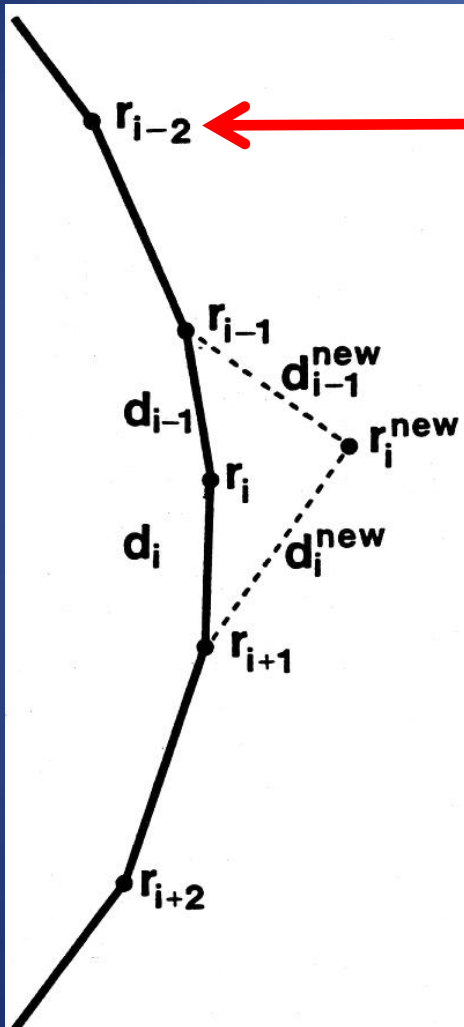


# Coupling chemical patterning and growth



# Can Pattern Drive Growth?

## Implementation of Growth in 2D



RD model solved (finite-difference) on set of nodes ( $r_i$ ) in 2D space;

-Segments ( $d_i$ ) grow in proportion to  $X$ ;

-Nodes ( $r_i$ ) moved to accommodate growth.

**Harrison and Kolář**  
***J. Theor. Biol.* 1988**

# RD making shapes:

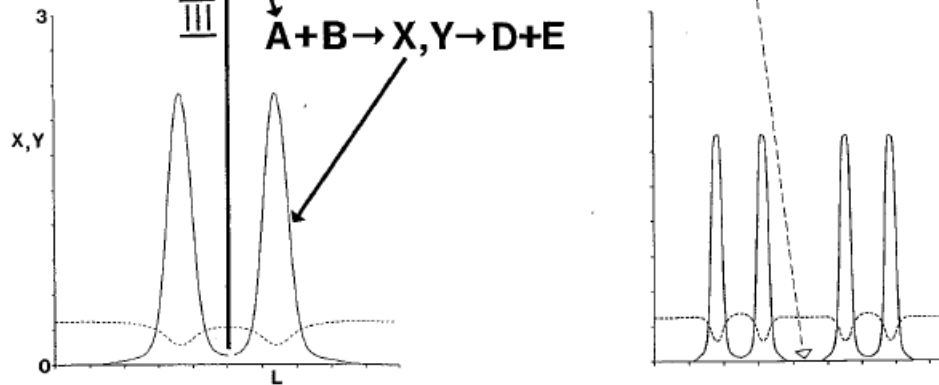
What is needed to make plant shapes?

2D Shape



- **X** catalyzes surface growth (determines local growth rate).

RD pattern on the shape

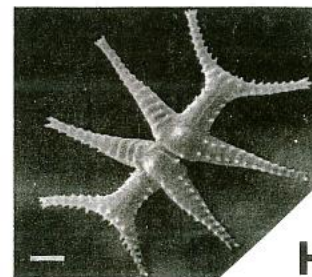
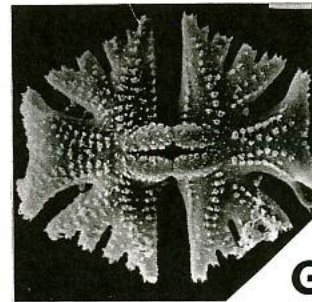
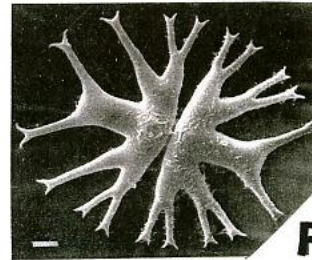
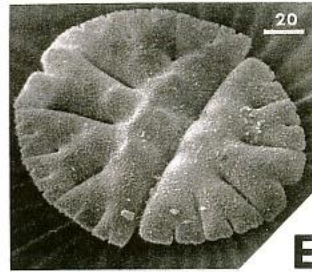
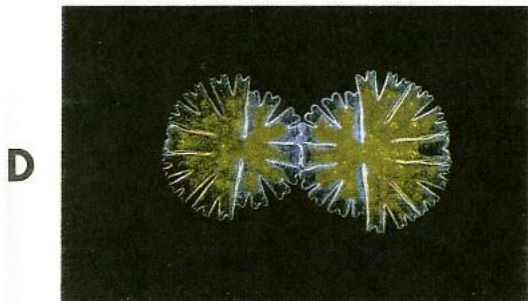
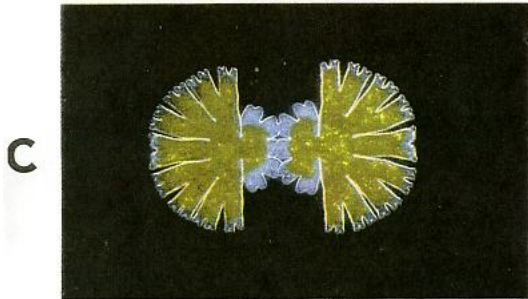
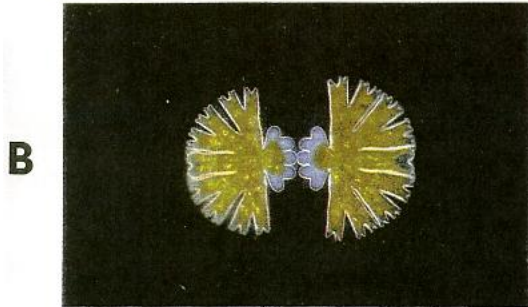
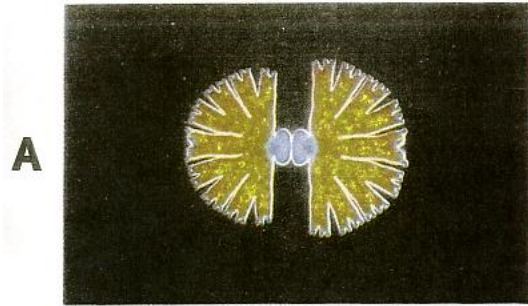


- Aging mechanism in **A** separates patterning regions.

Earlier

Later

In general, plant morphogenesis requires clear distinction (boundaries) between regions with fast growth (high X) and slow growth (low X) .



## *Micrasterias*:

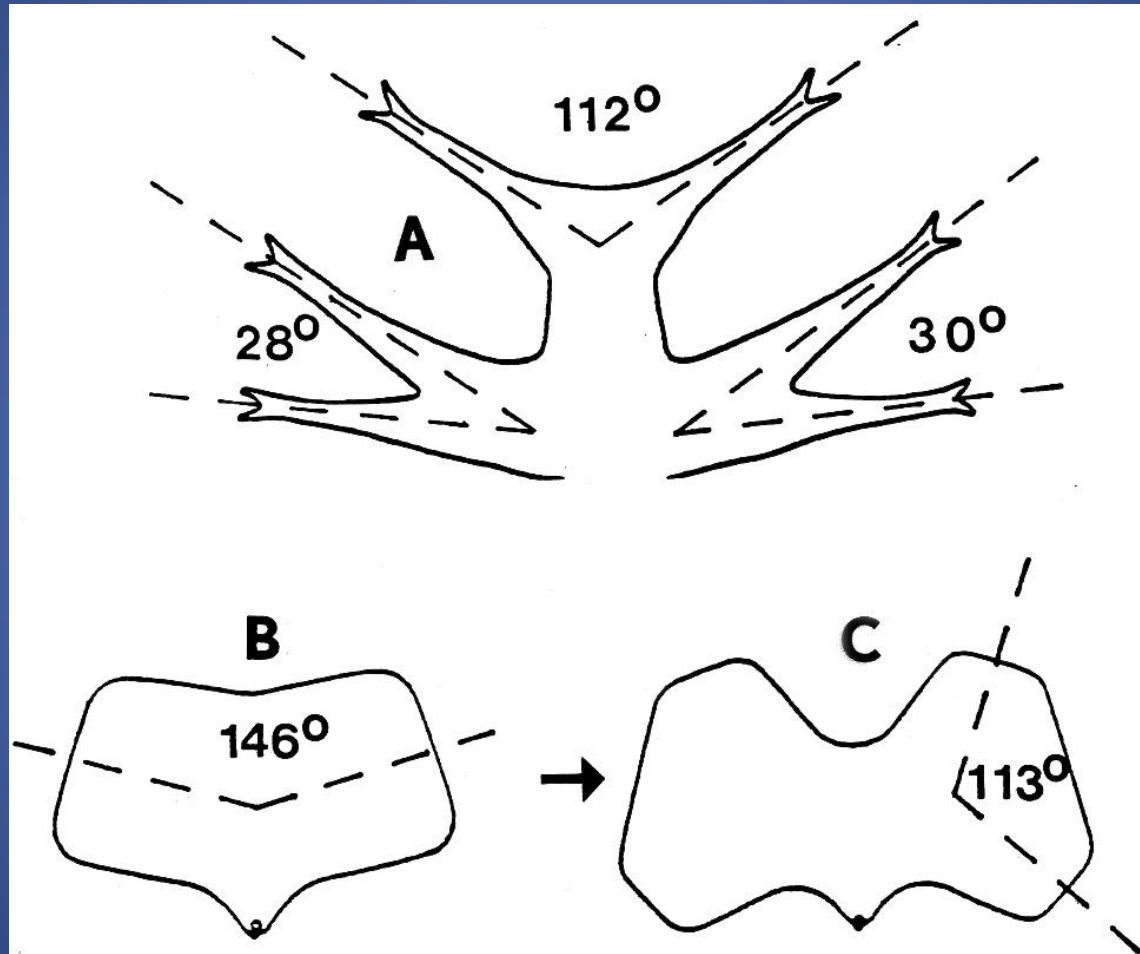
single-celled, freshwater algae

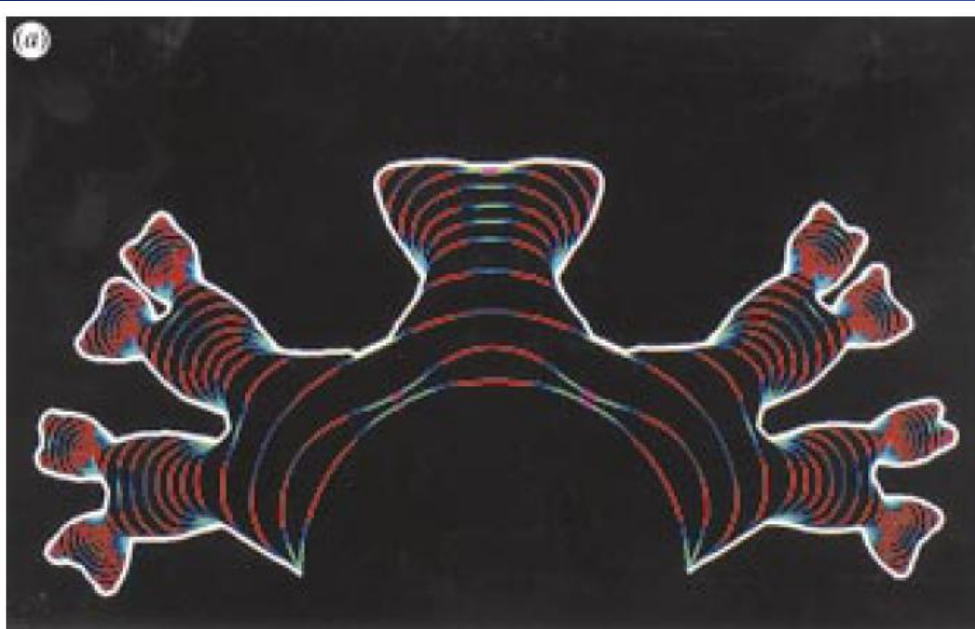
A-D: after cell division, repeated dichotomous branching regenerates the adult shape (~4 hrs).

E-H: large variety of shapes across species



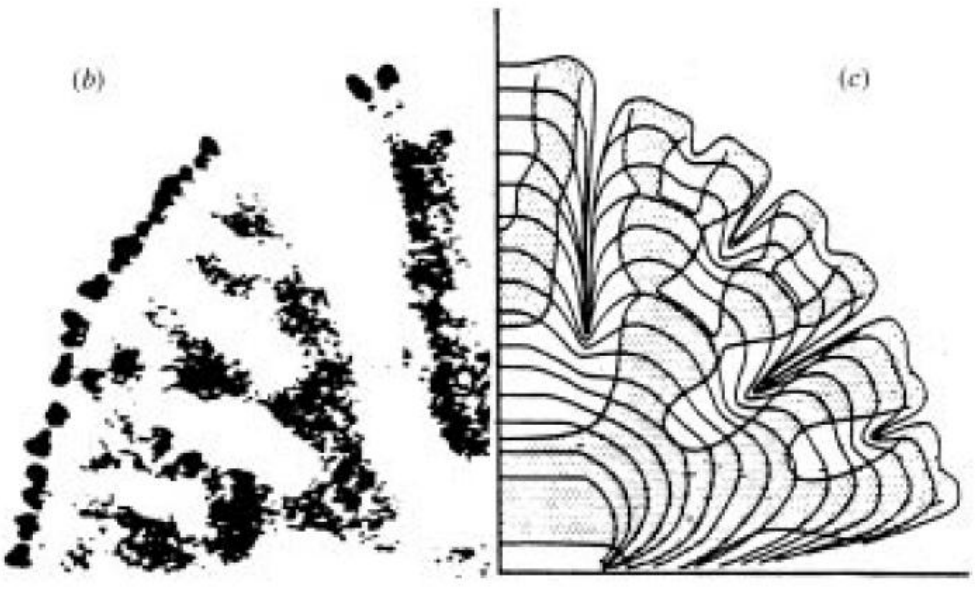
# Obtuse angles vs. *Micrasterias*





Acute branches: we switched from the aging mechanism, to a threshold mechanism, where patterning ceases for  $X < X_{th}$ .

Holloway and Harrison, 1999  
*Phil. Trans. R. Soc. Lond B*



Modelled growth rates match experimental rates of wall material incorporation.

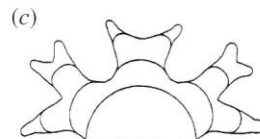
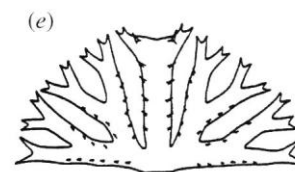
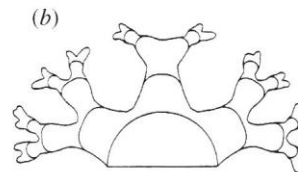
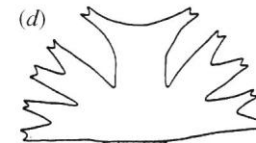
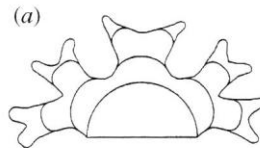
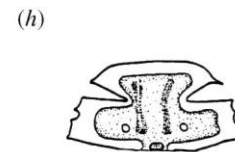
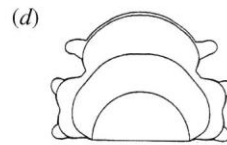
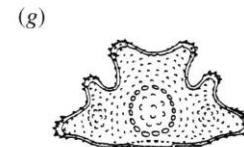
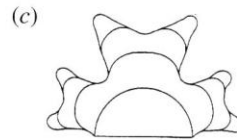
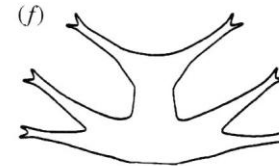
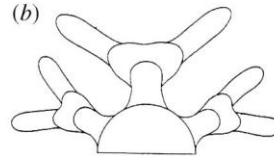
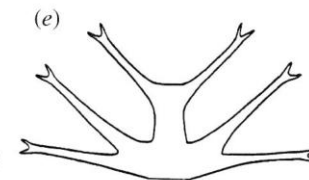
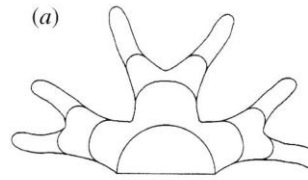
T.C. Lacalli, 1975, *J. Embryol. Exp. Morphol.*

Matching species diversity in *Micrasterias*.

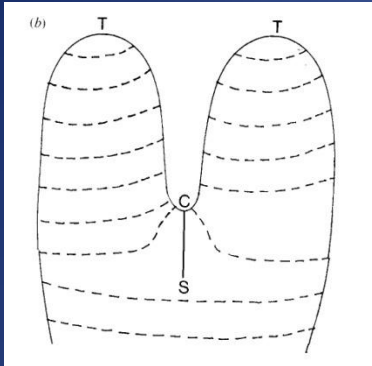
Variation of  $X_{th}$ : the coupling between patterning and growth shapes the cell.

Simulations

Algae



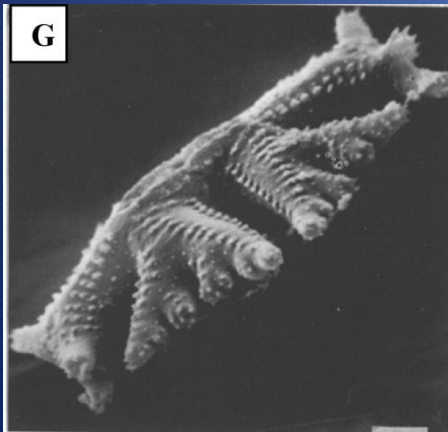
# Three Dimensions – why?



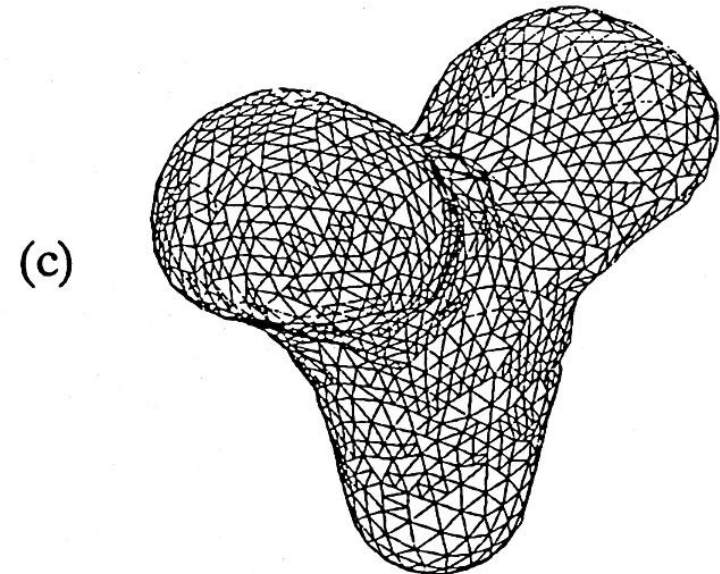
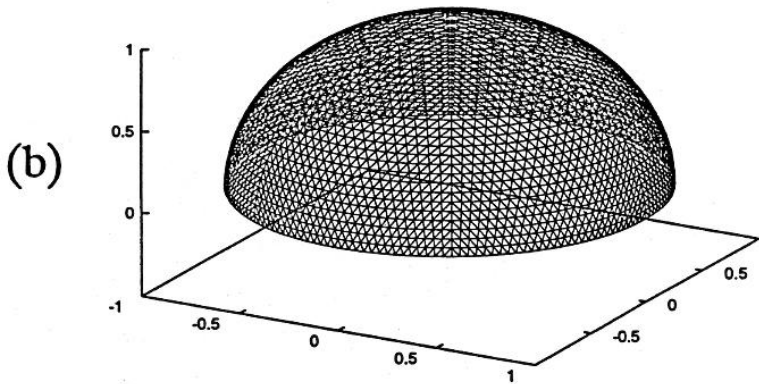
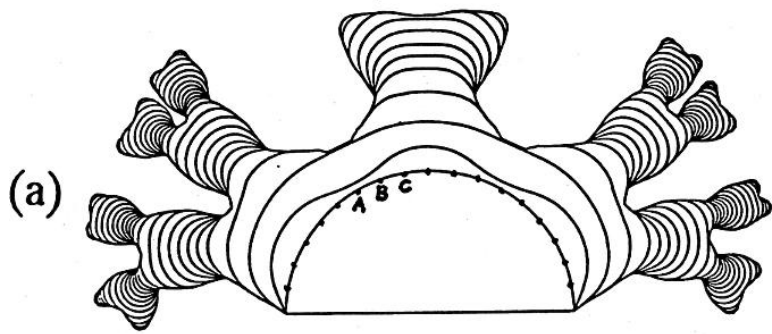
1. What constrains clefts? (Geometry, slow growth.)

Harrison et al., 2001

2. Pattern selection: there are many more possible patterns in 3D than 2D: i.e., what looks like a branch in 2D could be a branch in 3D, or an annular flattened tip, or a whorl of structures. What selects among these options?



3. What keeps *Micrasterias* planar, i.e. why are successive branches in the same plane? (Also critical in leaf planarity.)



Moving to 3D: requires a growing, proliferating mesh. We turned to a triangulated mesh, in order to be able to insert new triangles as growth occurs.

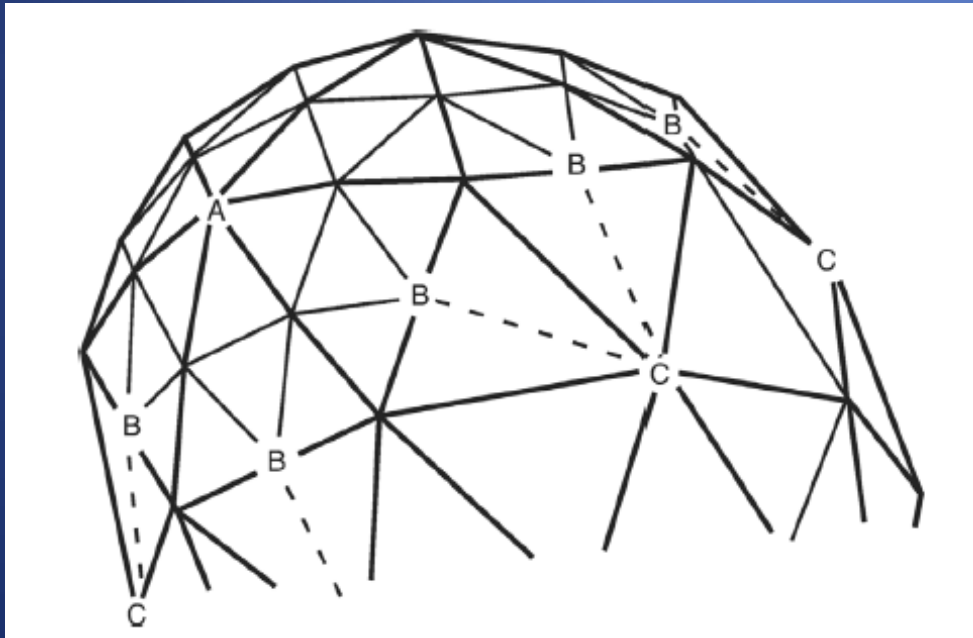
(Earlier work: Mirek Kolar tried hexagonal meshes - growth was followed by polynomial fitting and regular relatticing.)

Harrison, Wehner and Holloway  
(2001, *Faraday Discussions*)

# Full Growth $\leftrightarrow$ Patterning Feedback in 3D

**Growth:** nodes move normal to surface, proportional to the amount of X in an element.

**Proliferation:** when the side of a triangle has doubled, a new triangle is inserted.

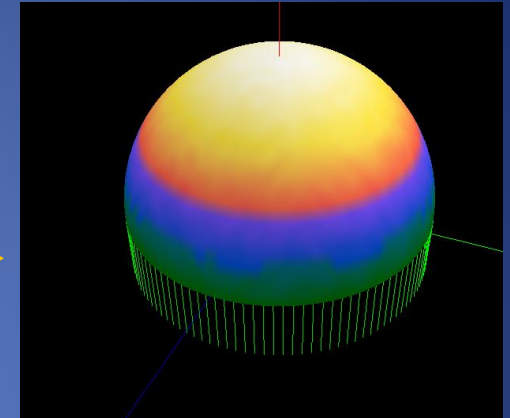


**Stability:** large deformations end up 'spiking' nodes out to infinity. We stabilized this somewhat by limiting the number of neighbours a node could have to 5 or 6. This limits the formation of 'long, skinny' triangles.

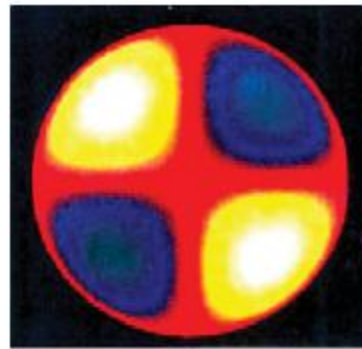
# Pattern Selection on Hemispheres

TABLE 1. *Harmonic wave patterns on a hemispherical shell*

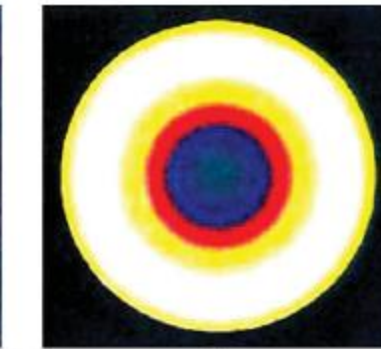
$l$	$l(l+1)$	Analogous atomic orbital	Spherical surface harmonic, $Y_l^m$	Plant tip pattern
1	2	$p_z$	$Y_1^0$ or $z$	Dome-shaped tip
2	6	$d_{xz,yz}$	$Y_2^{\pm 1}$ or $xz, yz$ *	Monocot embryo
3	12	$f_z^2$	$Y_3^0$ or $z^3$	Annulus (cup-shaped)
"	"	$f_{xyz}(x^2-y^2)$	$Y_3^{\pm 2}$ or $xyz, z(x^2-y^2)$	Dichotomous branches



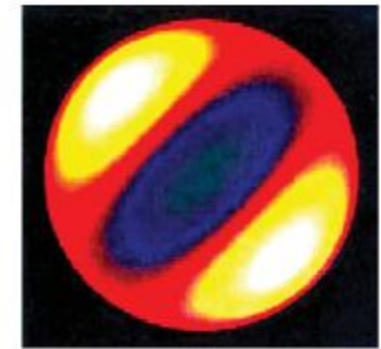
\* ' $\pm$ ' indicates a sum of the modes.



A



B

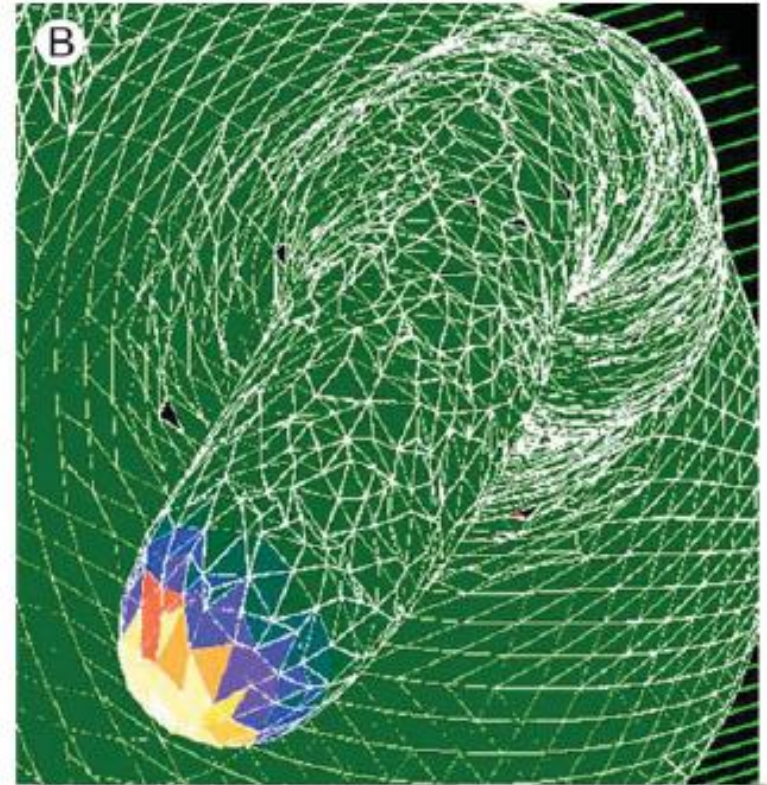
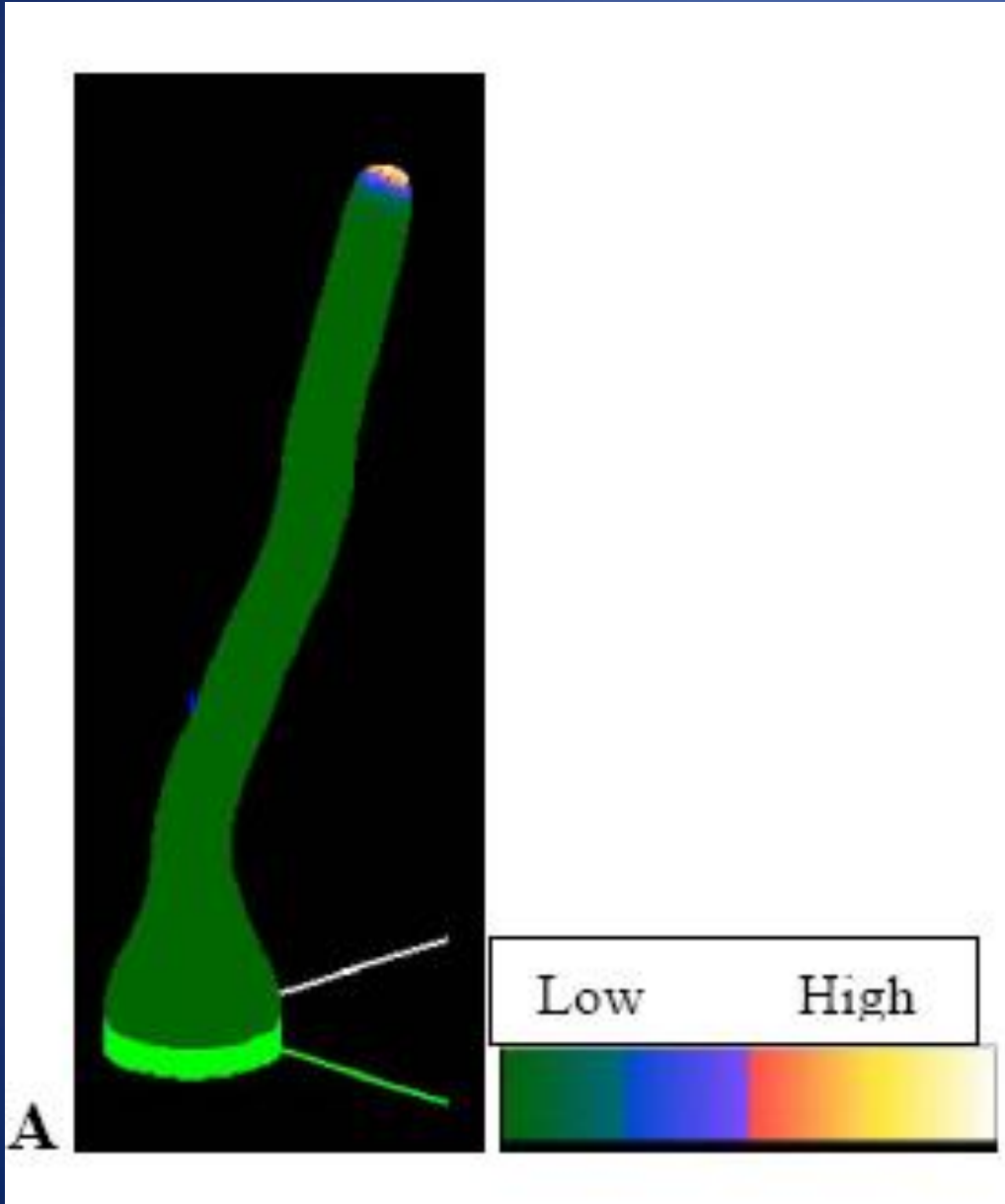


C

+

=

# Tip Growth $(\gamma_{1,0})$

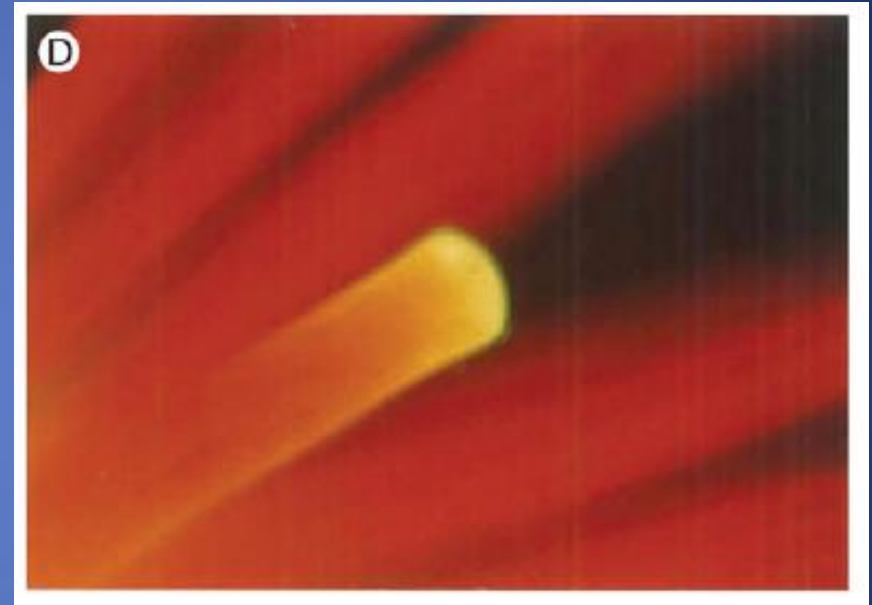
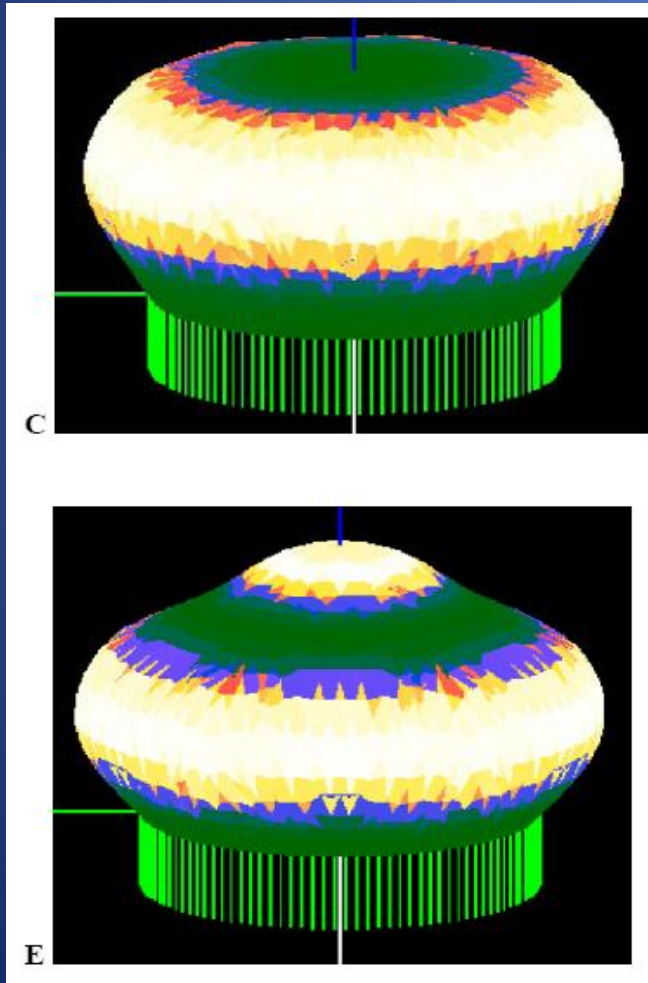


Holloway and Harrison,  
*Annals of Botany*, 2008



# Tip Flattening $(Y_{3,0})$

Decreased wavelength causes tip flattening (C), and tip re-initiation (E).

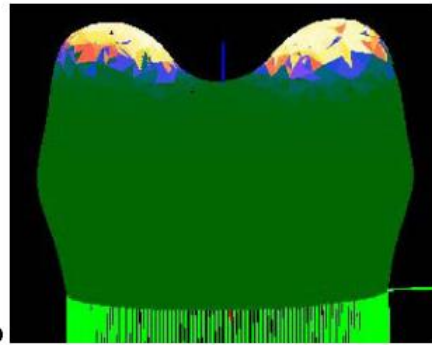
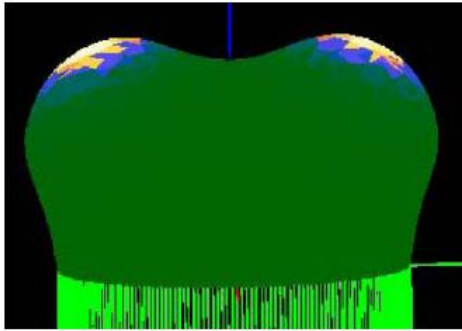
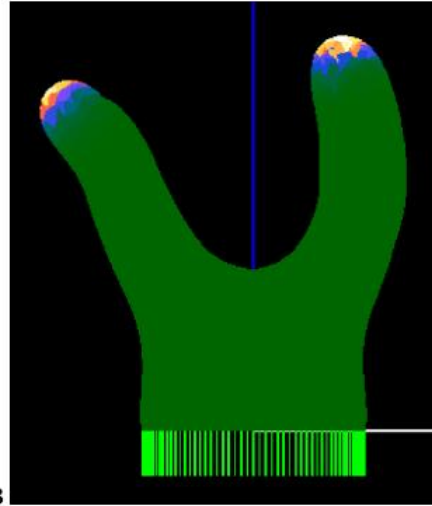
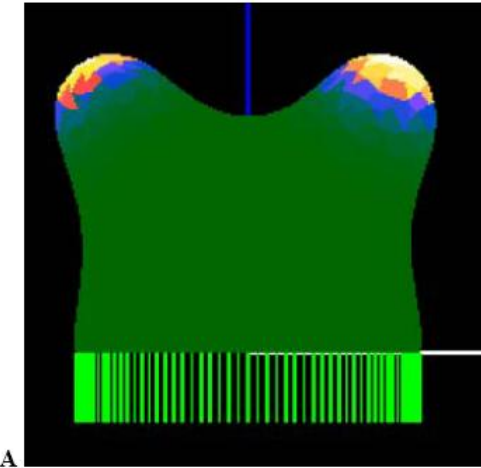


Annular pattern / flattened tip.  
Fluorescent labelling of surface-bound protein in *Acetabularia* (a single celled algae). Harrison et al., 1988, *Development*.

# Dichotomous Branching ( $Y_{3,0} + Y_{3,2}$ )

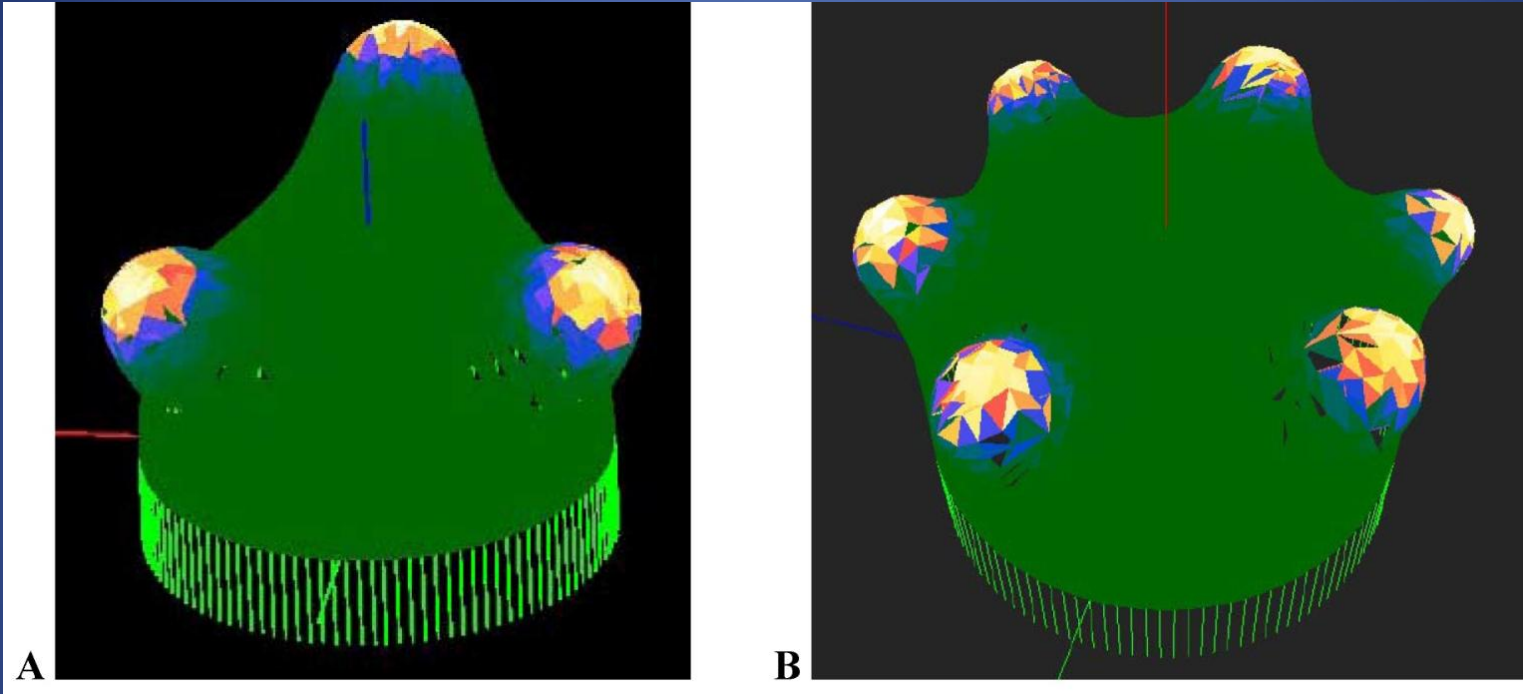
## Breaking Circular Symmetry

Start with tip growth,  
shorter wavelength causes  
branch.



3D geometry gives better  
control of branching  
angle: C,  $\sim 80^\circ$ ; D,  $\sim 20^\circ$

# Whorls



Patterns with up to about 6 structures can be created with decreased wavelength. We postulate that higher numbers (*Acetabularia*) may require a hierarchy of patterning events.

# Pattern Selection, Symmetry Breaking

Increasingly complex patterns (tips to dichotomous branches to multiple branches) are due to higher harmonics (shorter wavelength) within the active-patterning (fast-growing) regions. These result from:

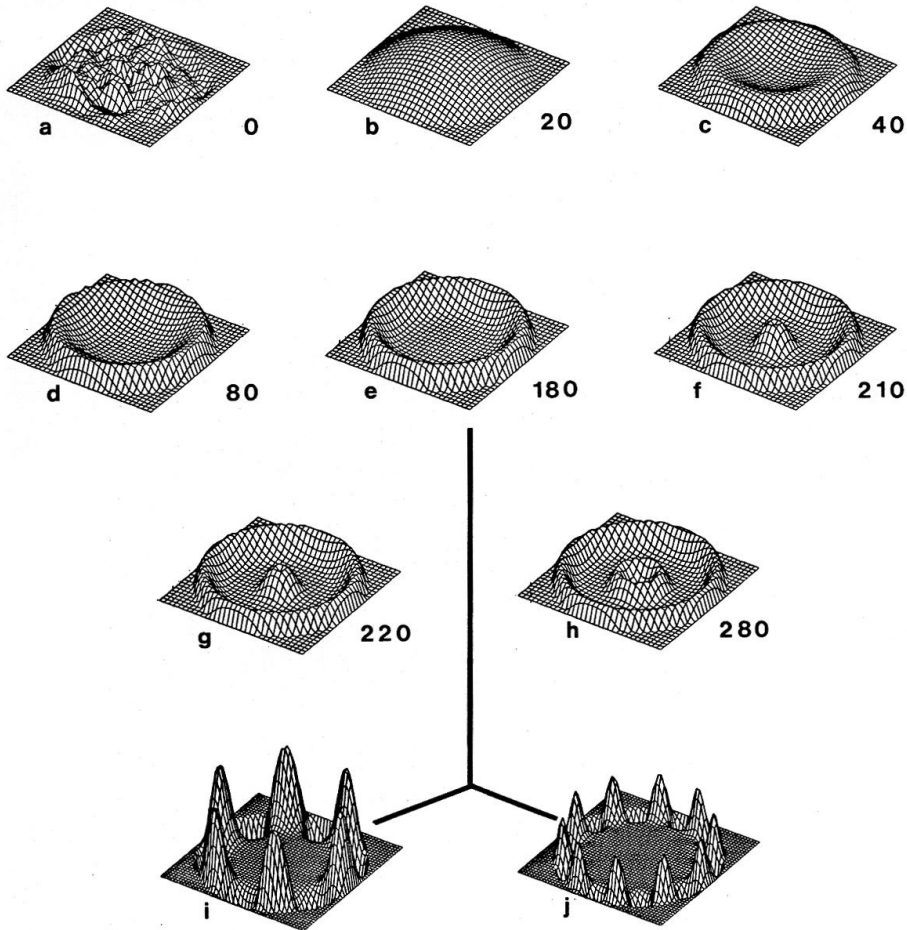
- Slower diffusivities

- Faster reactions

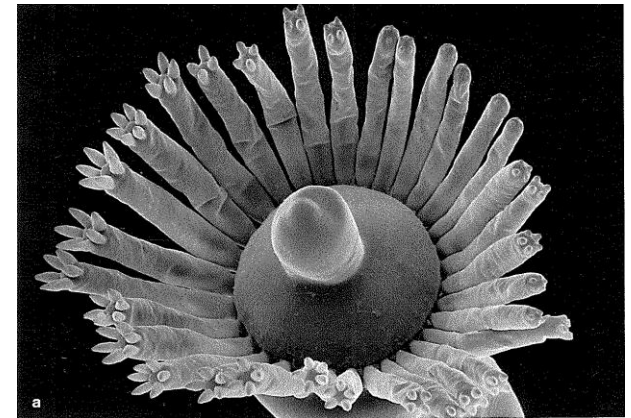
- Slower growth-patterning feedback (low  $X_{th}$ ).

# Early Modelling (Harrison et al., 1981):

Pattern selection on a uniformly growing hemisphere



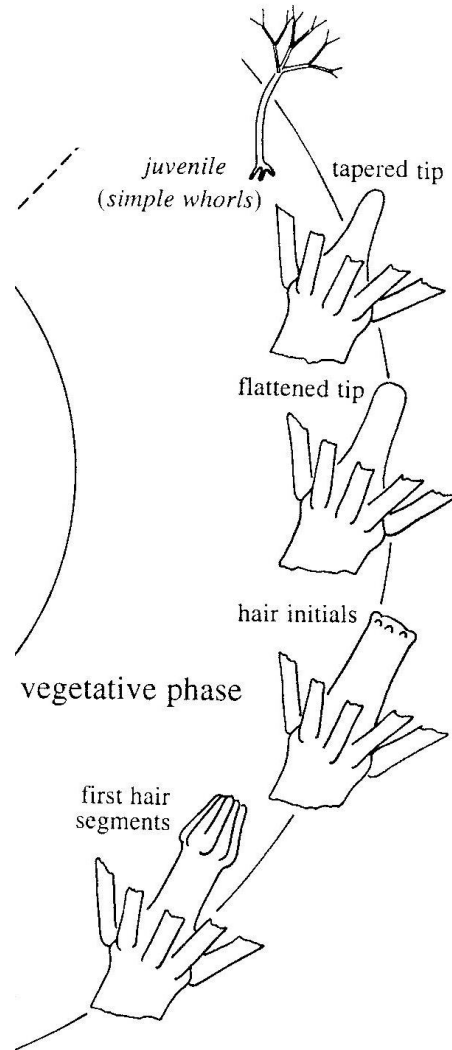
*Acetabularia*  
(a single cell)



**Growth->Patterning**

Two Brusselator hierarchical feed-forward

# Experimental evidence for reaction-diffusion in *Acetabularia*

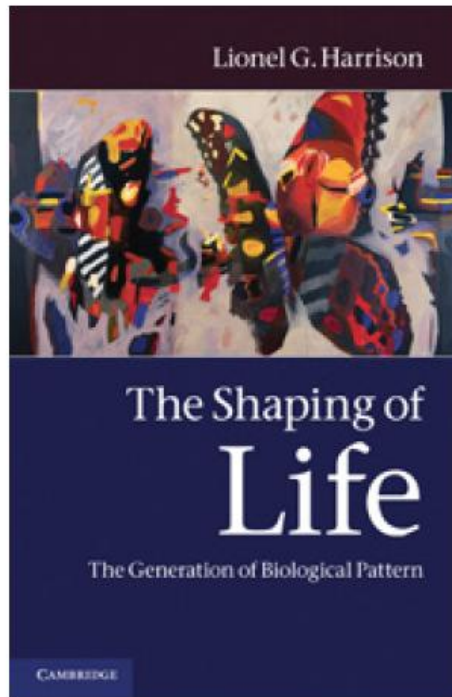


## Harrison et al., 1981 – 1997

- Hairs are constantly spaced,  $\lambda$
- Spacing can be controlled by:
  - Temperature
  - $[Ca^{2+}]$
  - $Ca^{2+}$  inhibition (EGTA)
- Prepatterns can be seen for membrane-bound  $Ca^{2+}$ , cytosolic  $Ca^{2+}$  is not patterned.
- Spacing is consistent with endothermic binding of  $Ca^{2+}$  by a membrane-bound protein.
- Model: reaction-diffusion patterning of membrane-bound proteins controls hair spacing.

# The Shaping of Life

*The Generation of Biological Pattern*



**New!**

Lionel G. Harrison, University of British Columbia, Vancouver

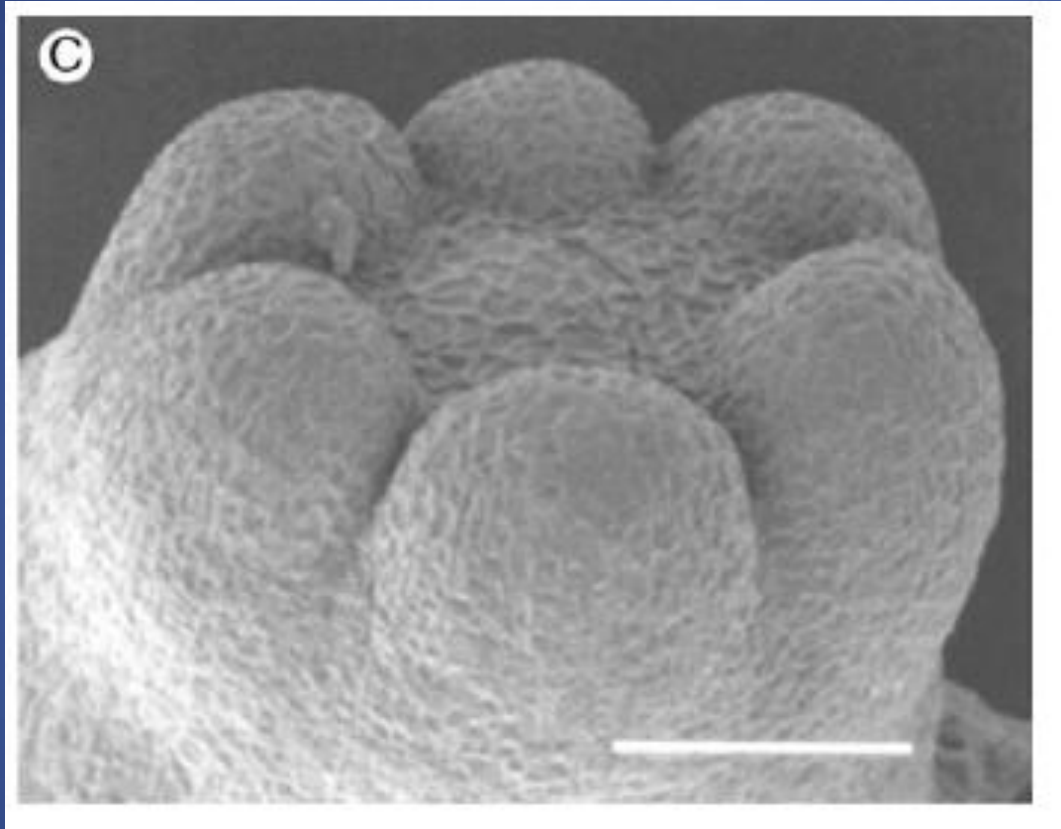


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Hardback / 9780521553506

# Conifer embryogenesis

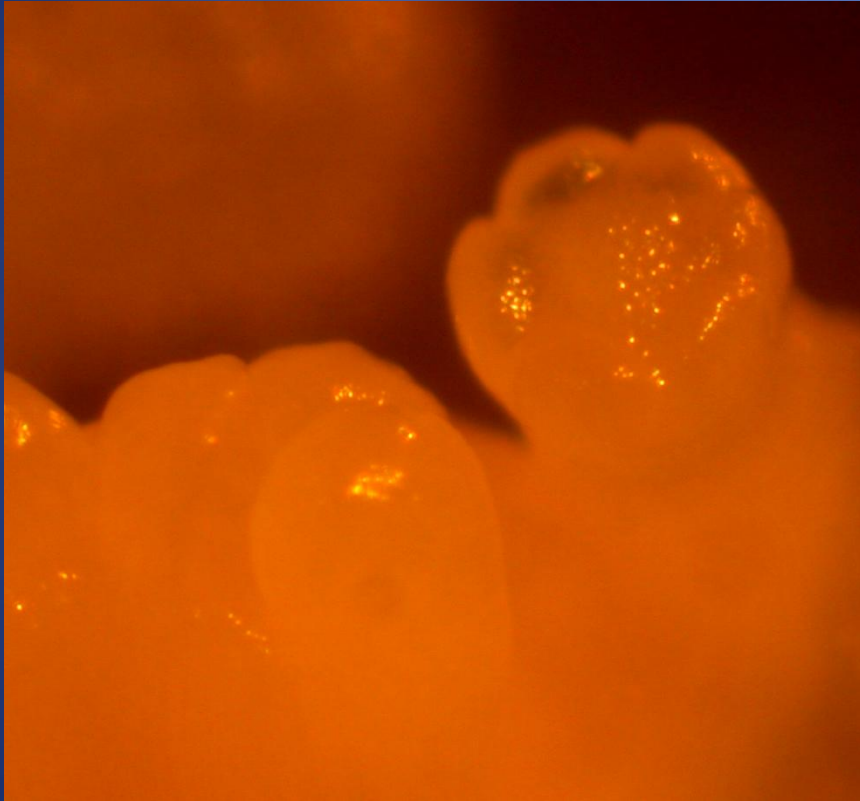
multicellular plants, treated as a continuum



The first structures (cotyledons) coming out of larch tree embryos fit well to Bessel functions, suggesting pattern formation on a flattened disk (Harrison and von Aderkas, *Annals of Botany*, 2004).



# Experiments



1) Do the flattened disk patterns fit as well for other conifer species?  
- At BCIT, we're seeing constant spacing with Douglas fir and spruce (collaboration with Patrick von Aderkas, UVic).

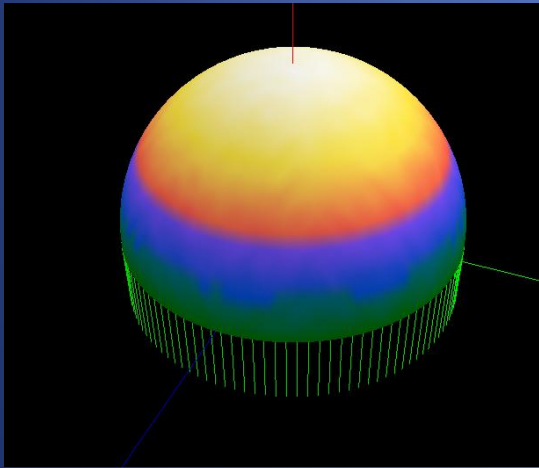
2) Can we control the cotyledon spacing chemically (NPA inhibition of auxin transport), or by temperature?

3) Can we quantify the dome to disk shape change, for use in mathematical analysis?

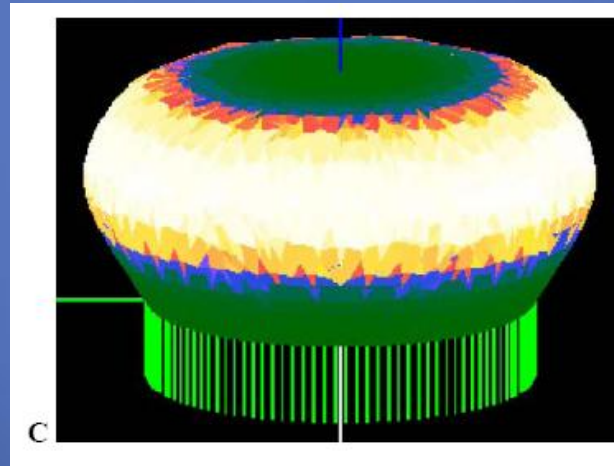
# Analysis

How does pattern selection proceed as tips flatten?

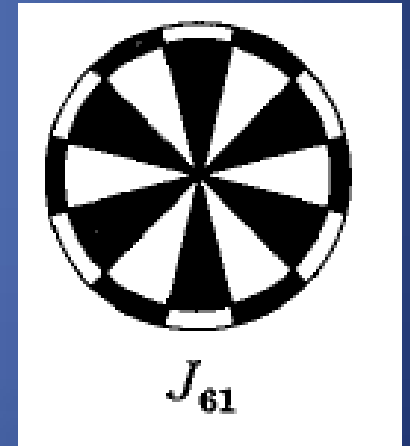
With Wayne Nagata and Hamid Zangeneh, UBC Mathematics.



$Y_{1,0}$



$Y_{3,0}$



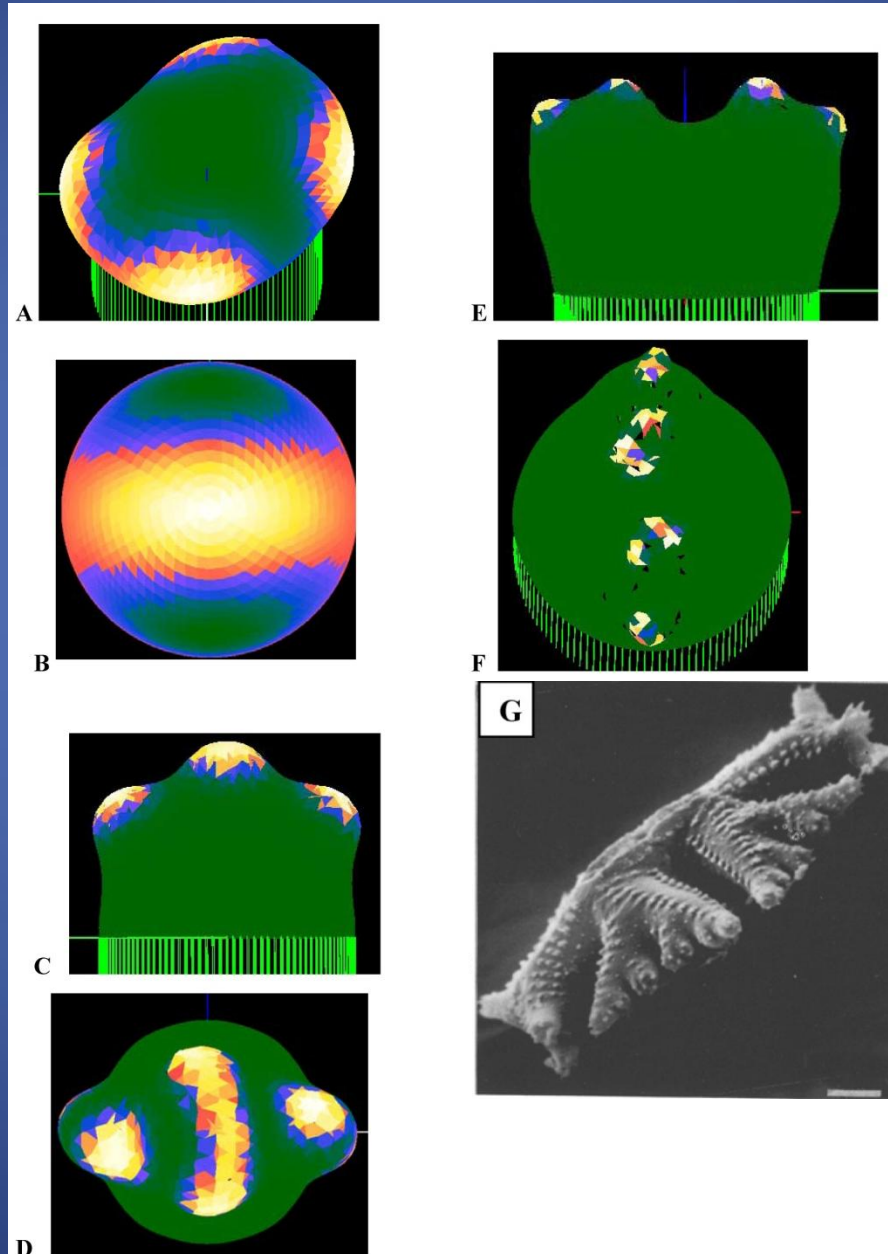
Bessel function on disk

# Maintaining Branching Plane

Successive branches tend to be at right angles. Geometry can not stop this.

A prepattern can define a plane.  
-(Y3,0 + Y3,2)

This creates planar branching, in the wing lobes but not in the polar lobe – like *Micrasterias*.



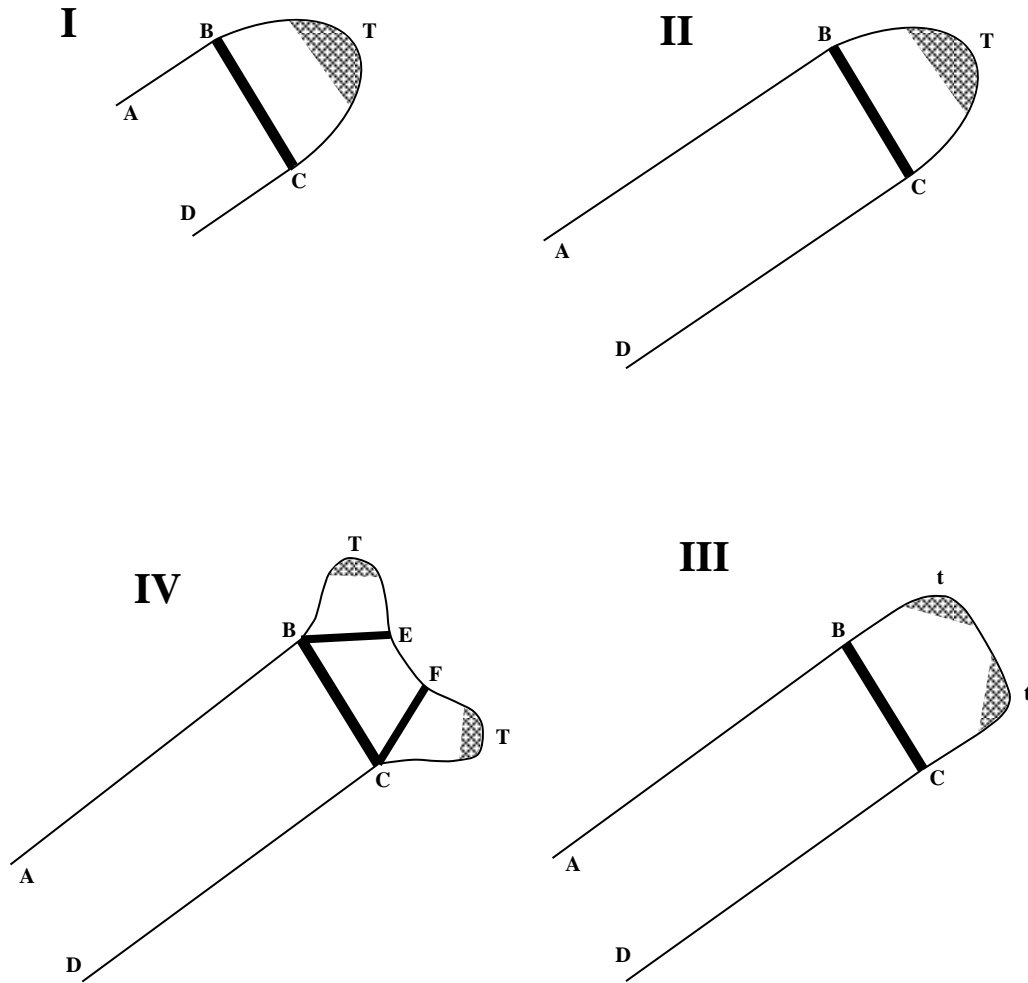
Plane begins to be lost in later branchings – like *Micrasterias*.

# A chemical mechanism for intrinsically controlling morphogenesis

- The  $X_{th}$  mechanism allowed us to characterize the growth-patterning interaction needed for morphogenesis. However, it involves an extrinsically controlled factor, the  $X_{th}$ .
- We have now developed a self-contained two Brusselator mechanism which successfully controls growth boundaries; it has no external cues.

# Controlling the boundaries with chemical dynamics

FIGURE 1

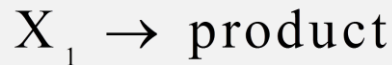
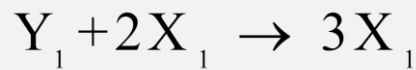
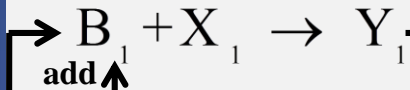
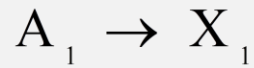


A

Dynamic  
Regulation of  
Growing Domains  
for Elongating and  
Branching  
Morphogenesis in  
Plants

Harrison, Adams,  
Holloway - in  
preparation

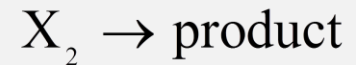
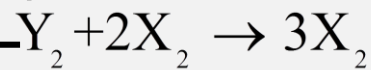
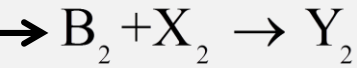
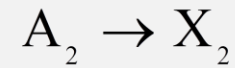
### Brusselator-1



$$B_{2 \text{ feedforward}} = n_1 \frac{Y_1}{Y_1 + E}$$

$$B_{1 \text{ feedback}} = n_2 \frac{Y_2}{Y_2 + F}$$

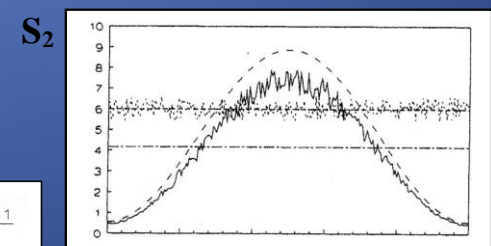
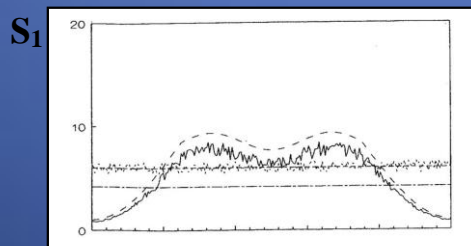
### Brusselator-2



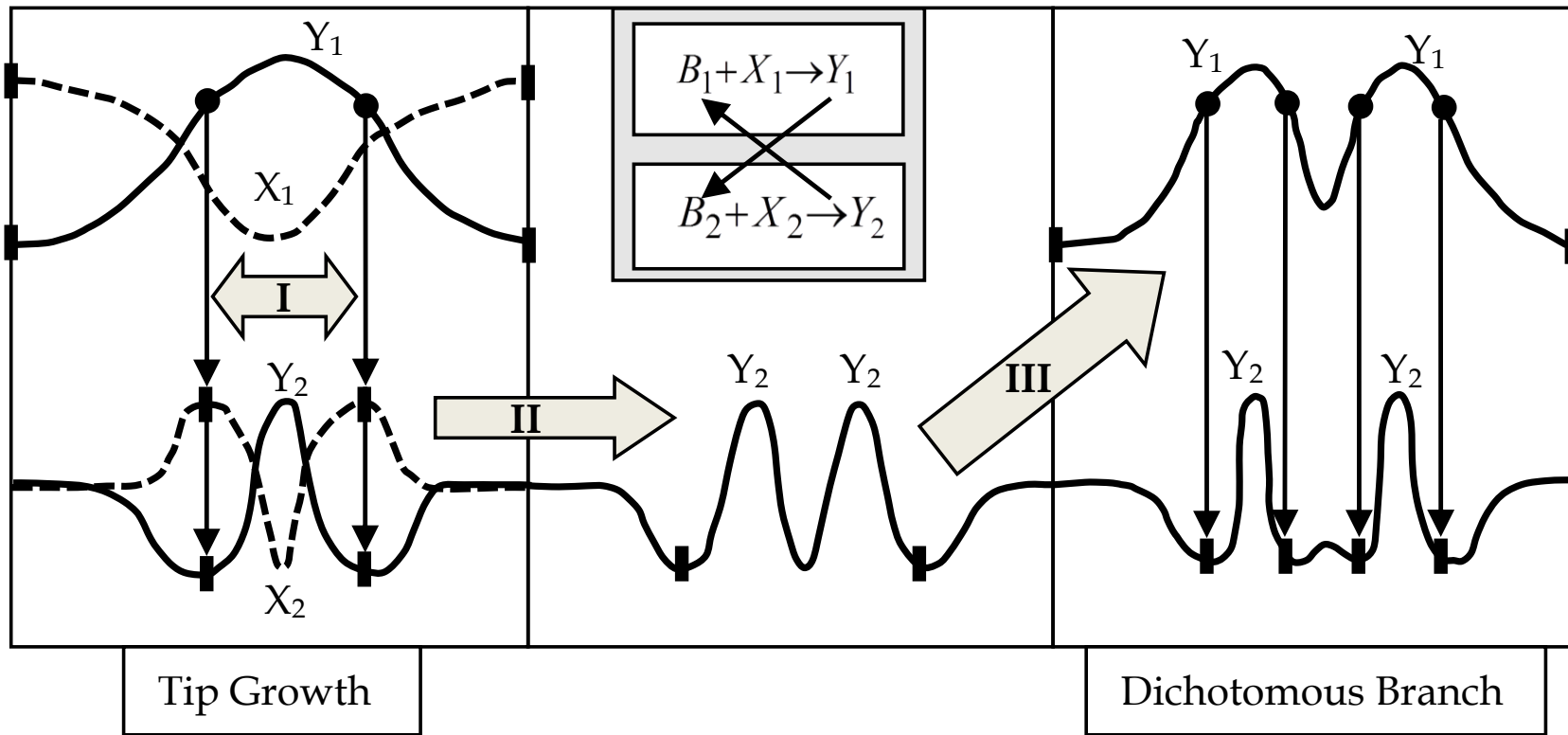
Local Growth

diffusion:  $D_{X1}, D_{Y1}, D_{X2}, D_{Y2}$

$B_{1 \text{ initial}}$  decays as  $e^{-t/\tau}$



add

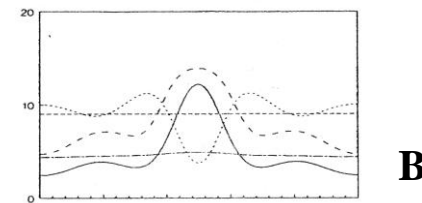
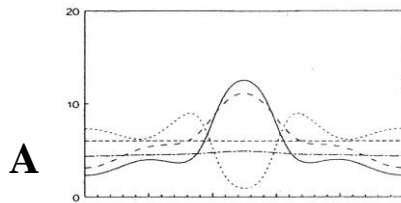


# Extending Tip Growth

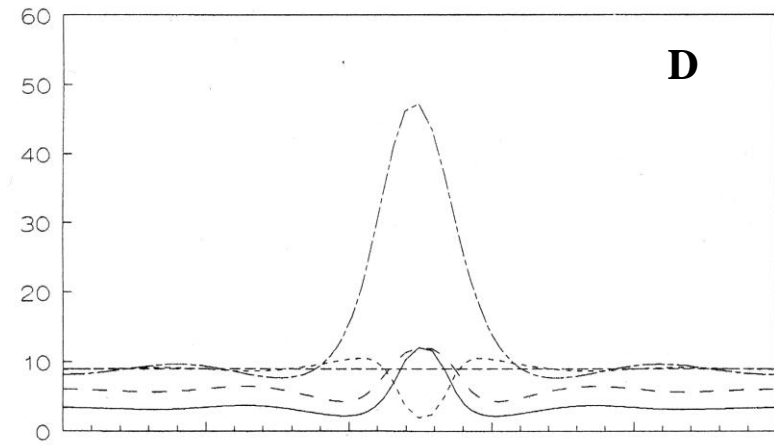
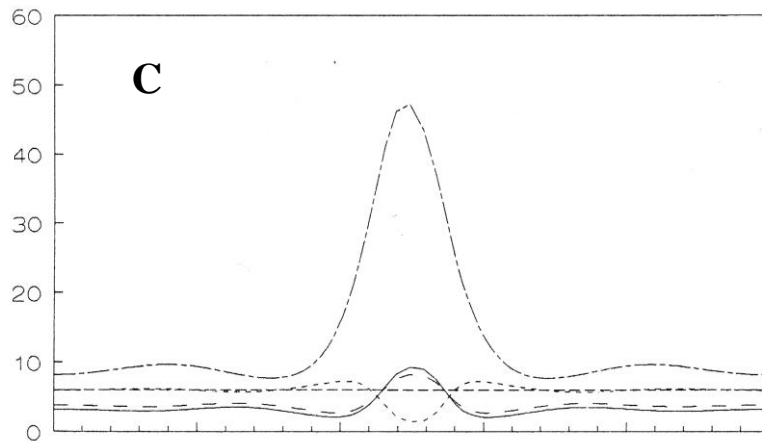
Brusselator-1

Brusselator-2

early



late



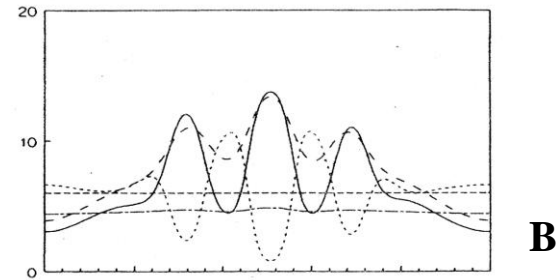
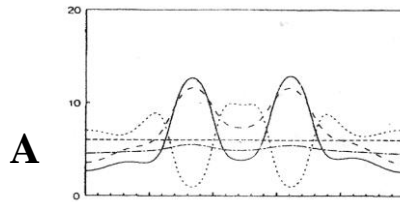
—  $Y_1$     - - -  $X_1$     ····  $A_1$     - · -  $B_1$     ---  $d_i$

—  $Y_2$     - - -  $X_2$     ····  $A_2$     - · -  $B_2$     ---  $d_i$

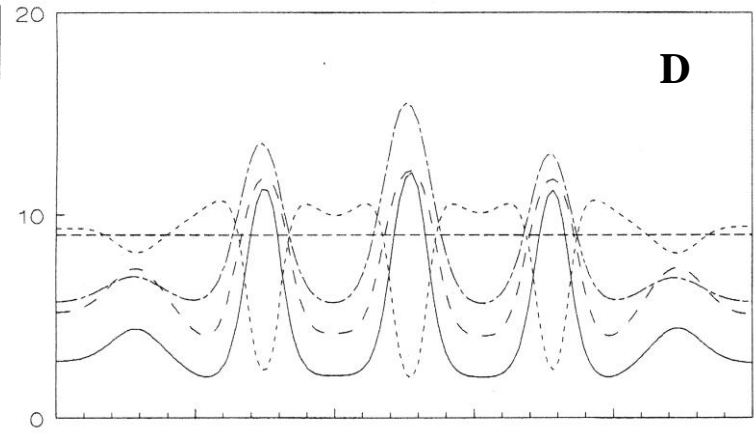
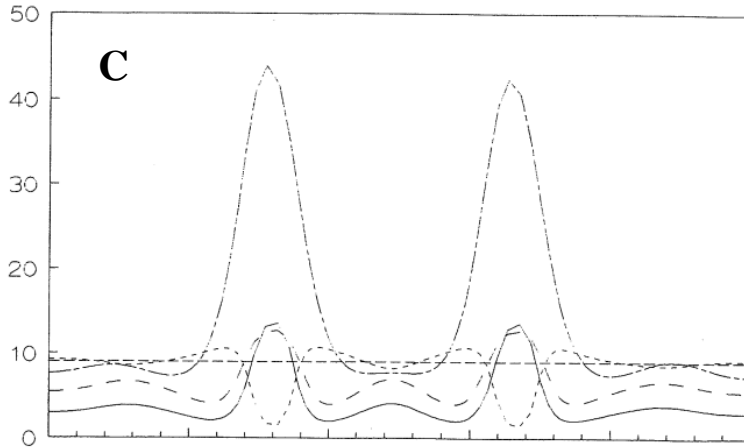


# Extending double and triple branches

early



late

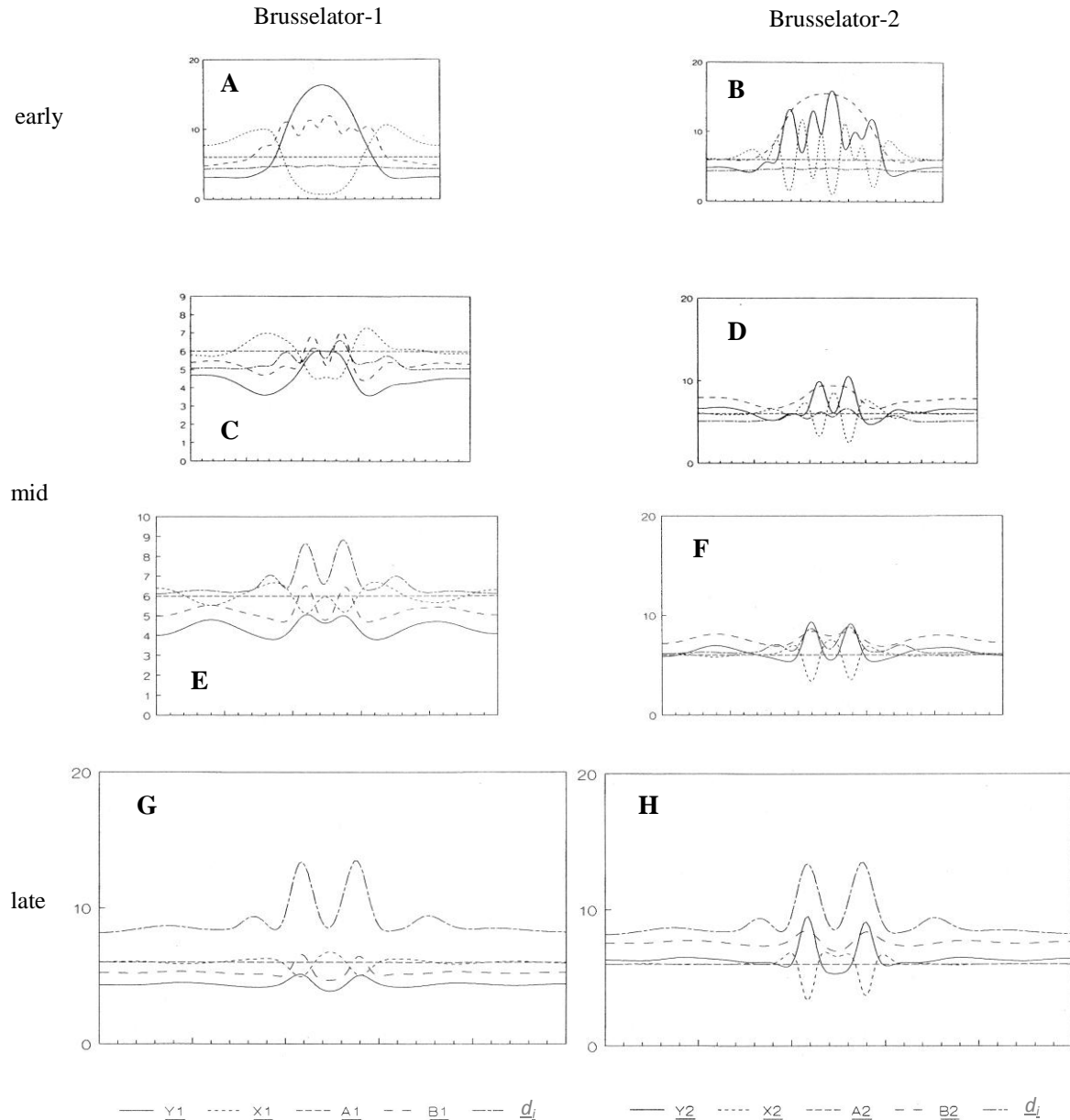


—  $Y_1$     - - -  $X_1$     - - -  $A_1$     - - -  $B_1$     - - -  $d_i$

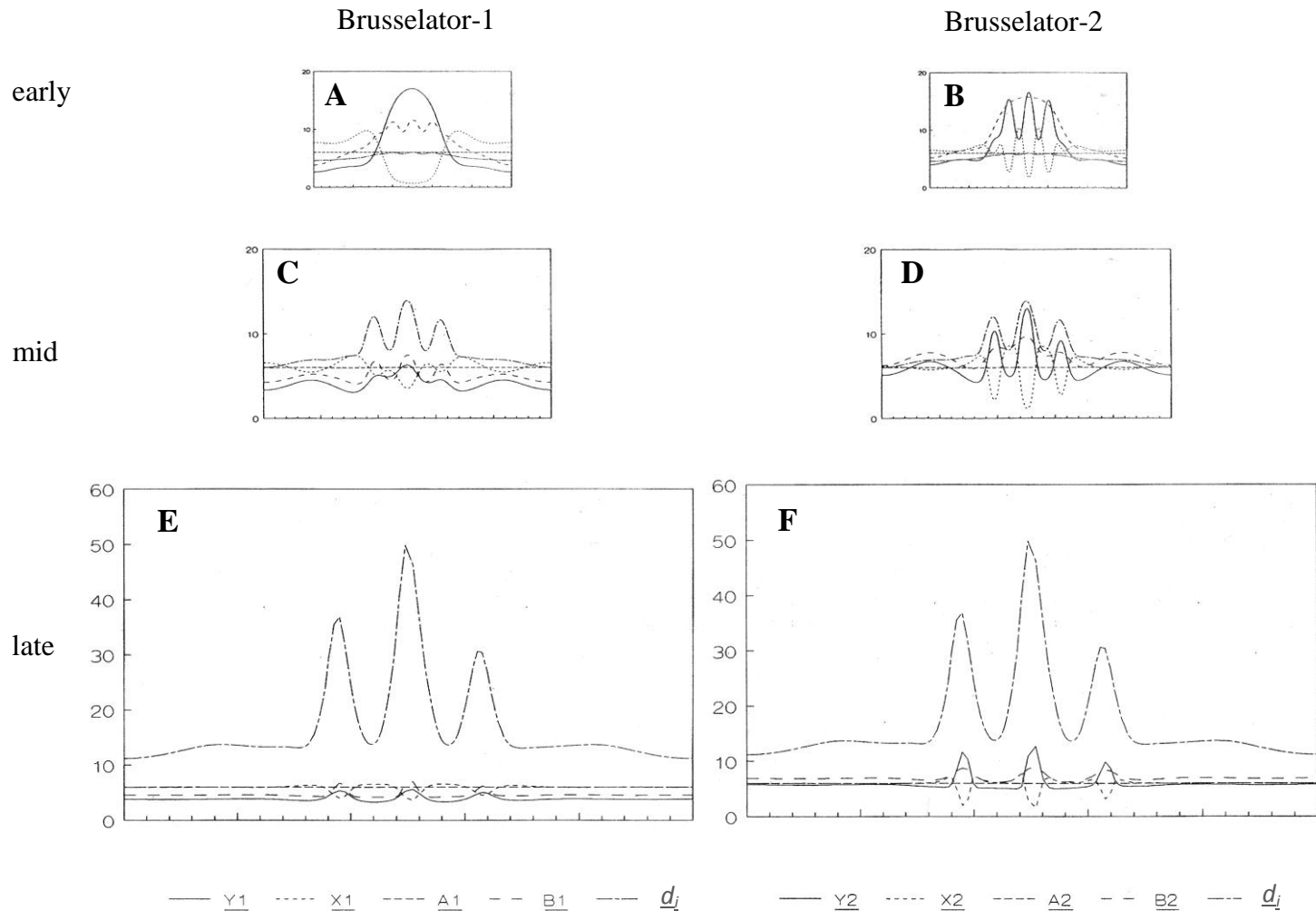
$d_i$

Dynamic  
boundary  
control:

Brusselator-2  
pattern (double-  
peak) alters  
Brusselator-1  
pattern,  
effectively  
segregating the  
pattern-forming  
regions and  
creating  
permanent  
branches.



# Dynamic boundary control for triple branch



# Double Brusselator conclusions:

- The growth-boundary control necessary for morphogenesis can be achieved by two mechanisms controlling the extent of each others' spatial patterning.

# Next

- Boundary control by Double Brusselator in 3D.
- Brusselator on mechanical (viscoplastic) surfaces.