



## Lecture 12: Growth Control

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MSc Computational Biology 2019/20

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## **Summary of Previous Lecture**

# Gompertz Law

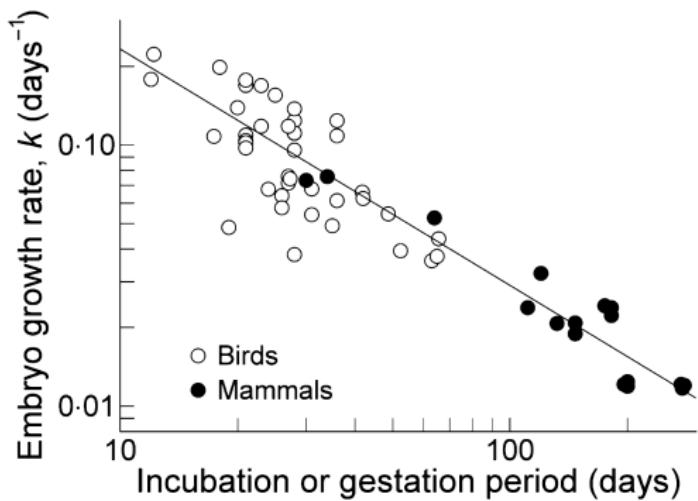
$$M = A \cdot \exp(-b \cdot \exp(-kt))$$

is the solution of

$$\begin{aligned}\frac{dM}{dt} &= \gamma M \\ \frac{d\gamma}{dt} &= -k\gamma.\end{aligned}$$

$M$  mass;  $A = M(t \rightarrow \infty)$  asymptotic mass;  $b = \ln(A/M_0)$  final fold-change in tissue size;  $k$  decline in growth rate;  $t$  time since onset of development.

# The decline in the Growth rate is inversely related to gestation time



Ricklefs, R. E. (2010). Embryo growth rates in birds and mammals. Functional Ecology 24(3): 588-596.

- Developmental Growth Rates decline continuously in a wide range of developmental systems (no counter-examples so far - not even solid cancers or stem cell based systems == organoids)
- The rate of decline is related to gestation time

What is the responsible mechanism?!

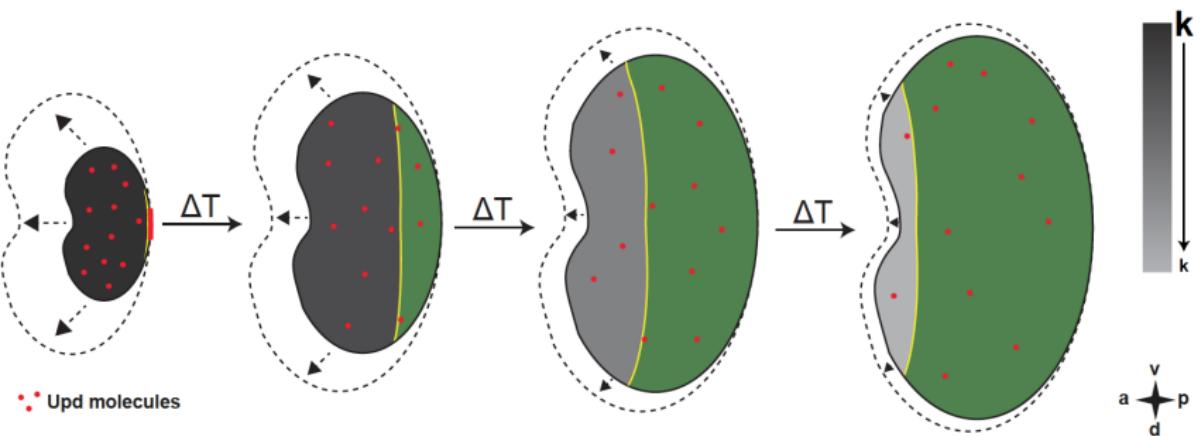
# **Models of Organ Growth Control**

# Rejected mechanisms for growth termination

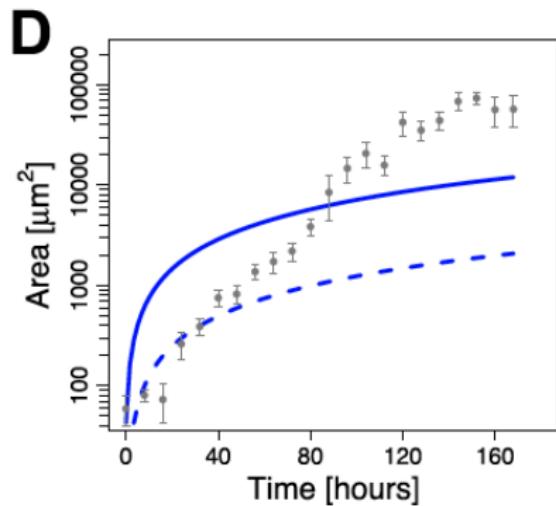
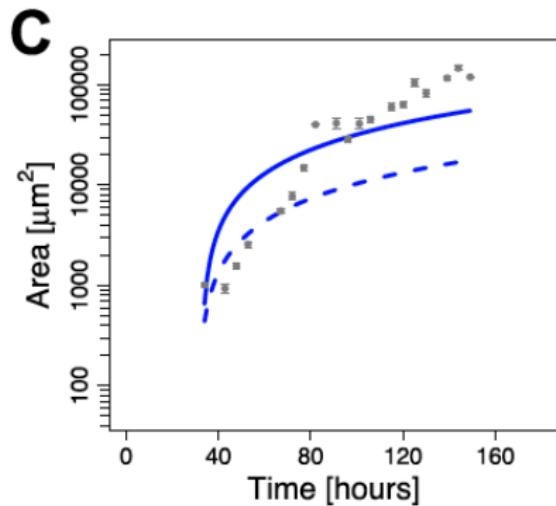
## Growth control

- by **surrounding tissue / larvae** ⇒ same size if discs develop outside larvae
- by **counting of cell divisions** ⇒ enhancing or blocking cell divisions does not alter final wing disc size
- **based on developmental time** ⇒ developmental delays do not matter for final size
- by **cell differentiation** ⇒ on its own, does not explain growth termination in the *Drosophila* eye disc

# Candidate Mechanism: Dilution of Upd



# Dilution cannot explain Growth Behaviour of *Drosophila* wing disc

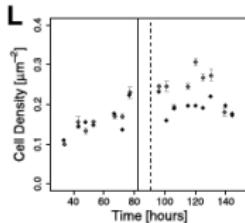
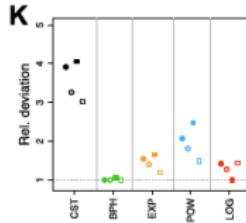
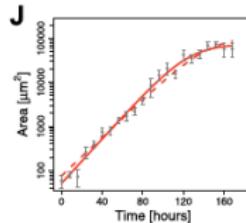
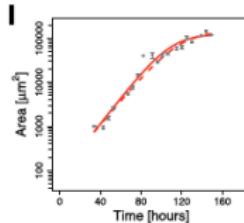
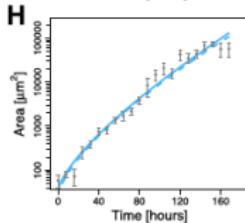
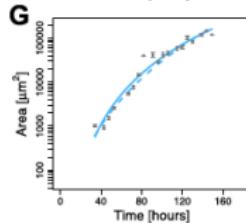
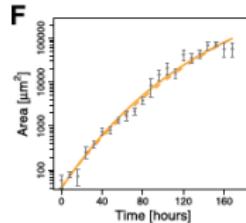
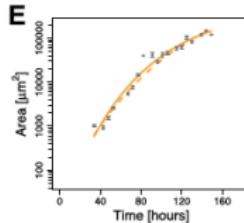
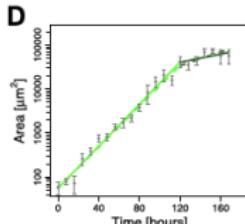
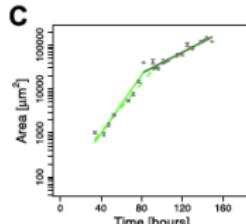
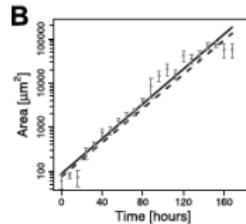
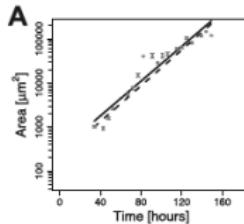


# Comparison of alternative Growth Models

*Review why we use*

$$\frac{\Sigma(x_i - \bar{x})^2}{S}$$

*to measure fitness*



Vollmer, J. & D. Iber (2016), Sci Rep 6: 39228.

# Candidate Mechanisms for Growth Control in the *Drosophila* wing disc

**Power-law model:** Fits the data but no biological mechanism (?)

**Exponential model:** Constant active removal of a growth factor (without dilution)

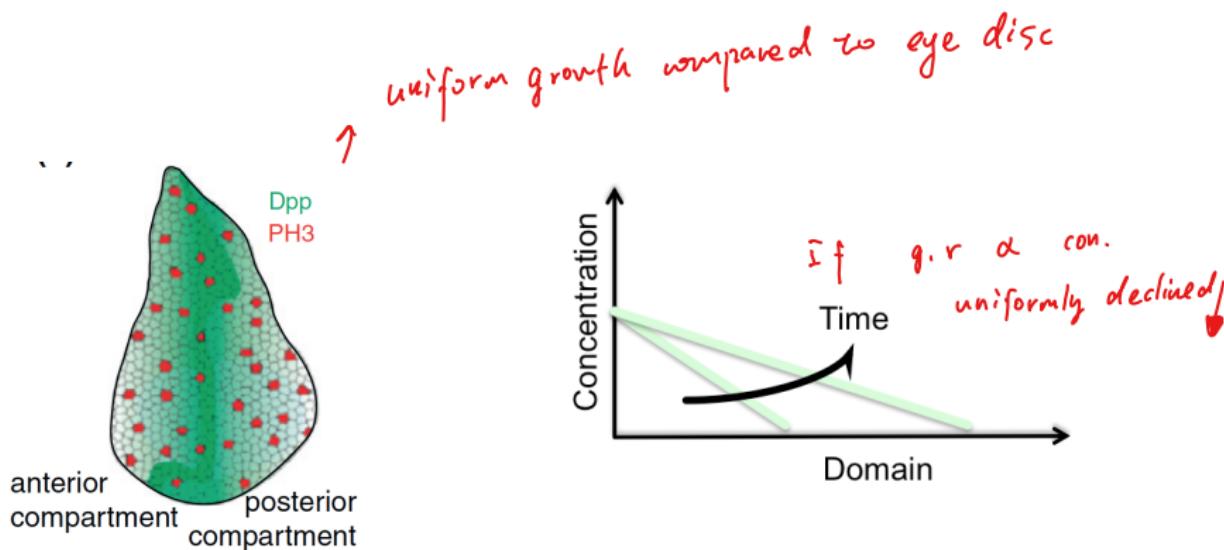
**Biphasic model:** something triggers a change in the growth rate

**Logarithmic growth model:** something defines a final size

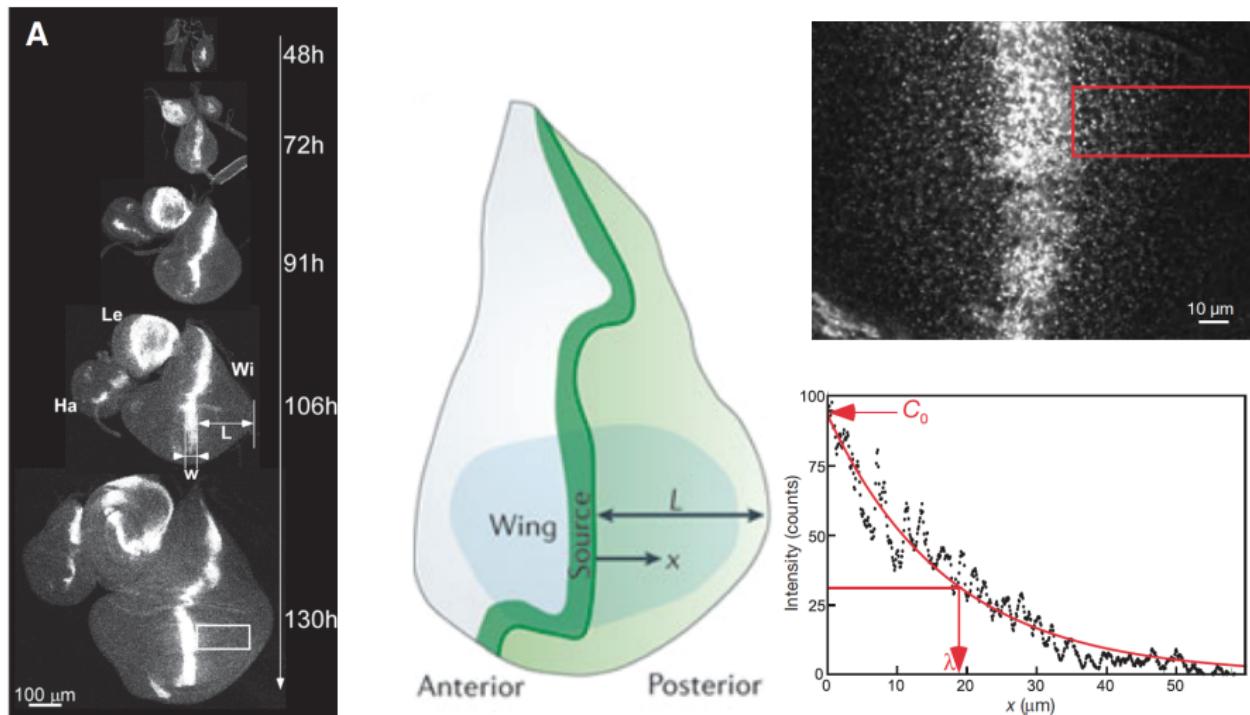
## Morphogen-based Models

Not only degradation  
but also growth rate - controlling.

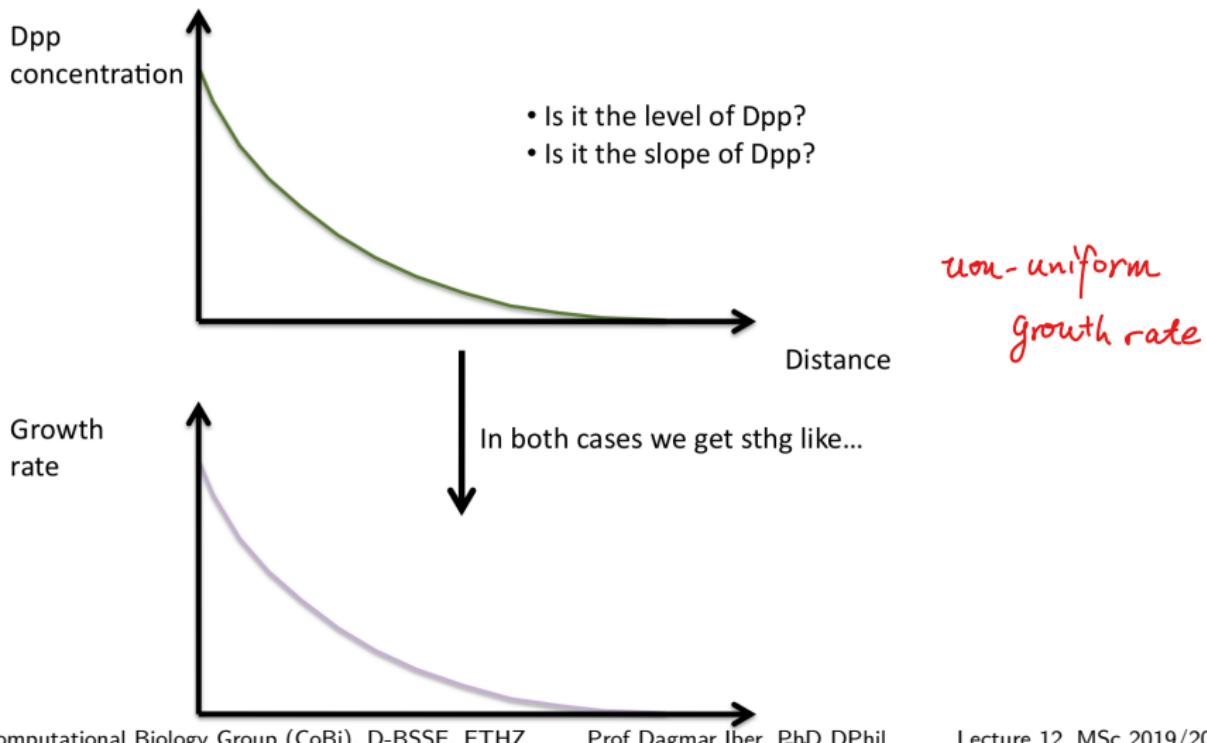
# Growth Rate dependent on steepness of linear morphogen gradients



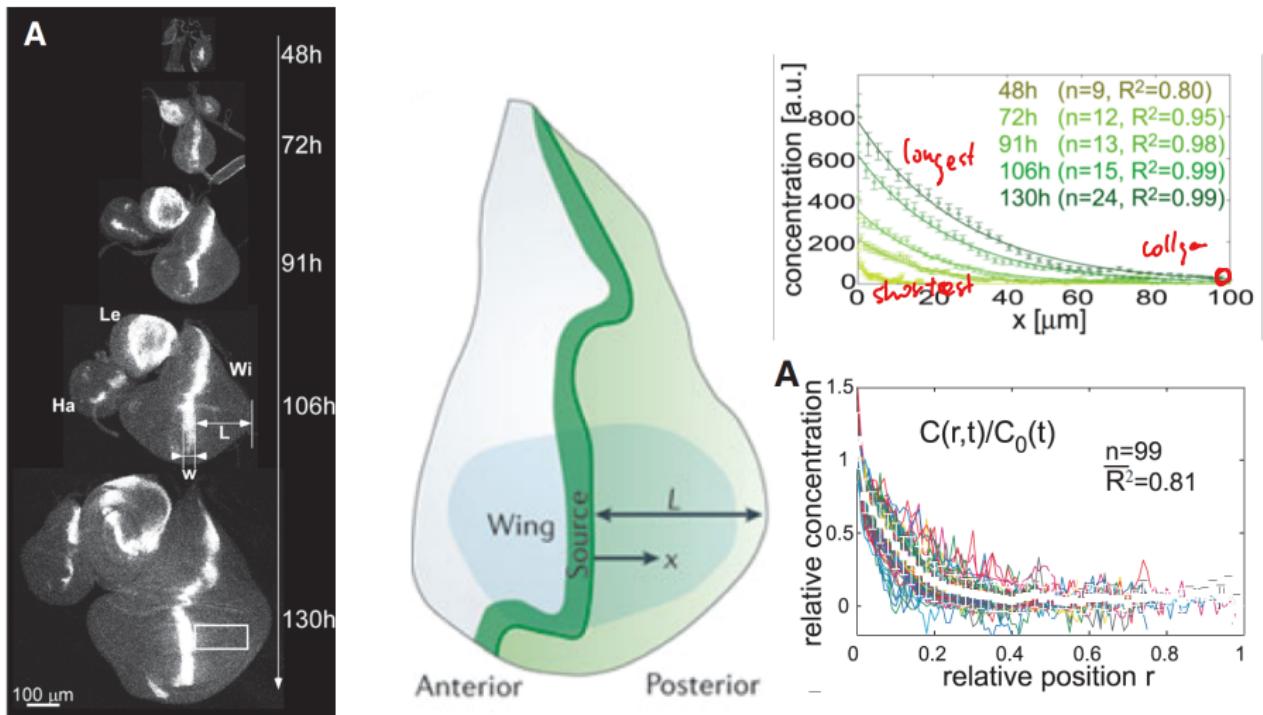
# The Dpp Gradient is of exponential shape



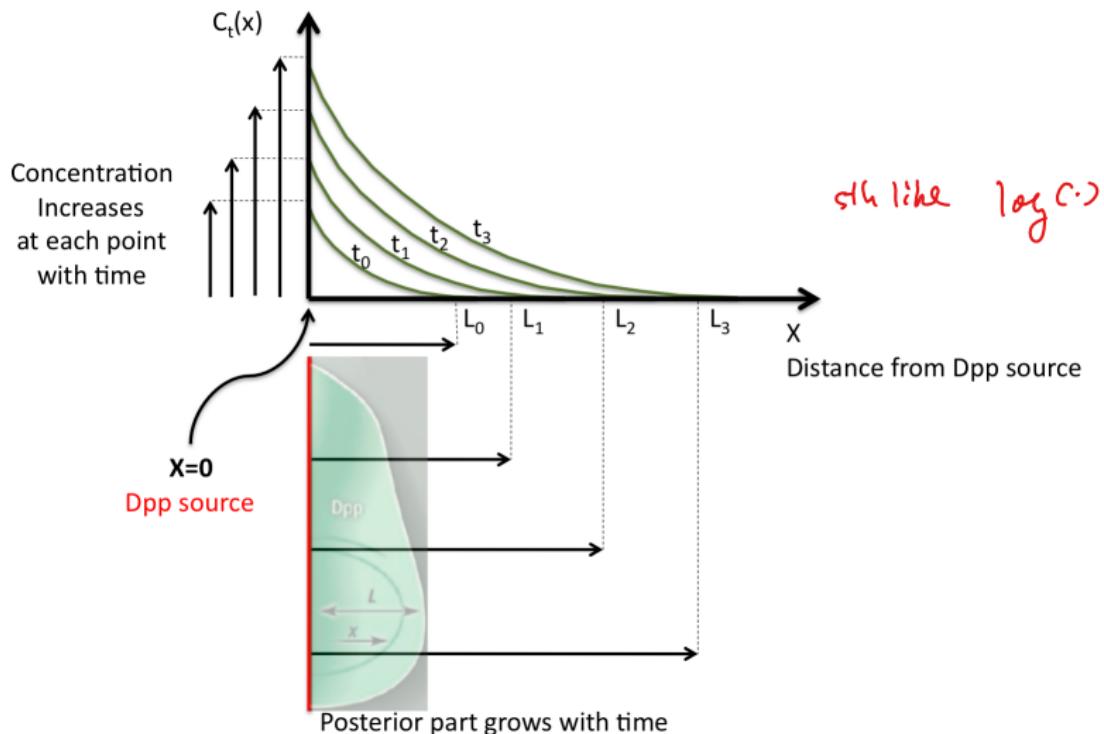
# How can uniform growth be controlled by an exponential gradient?



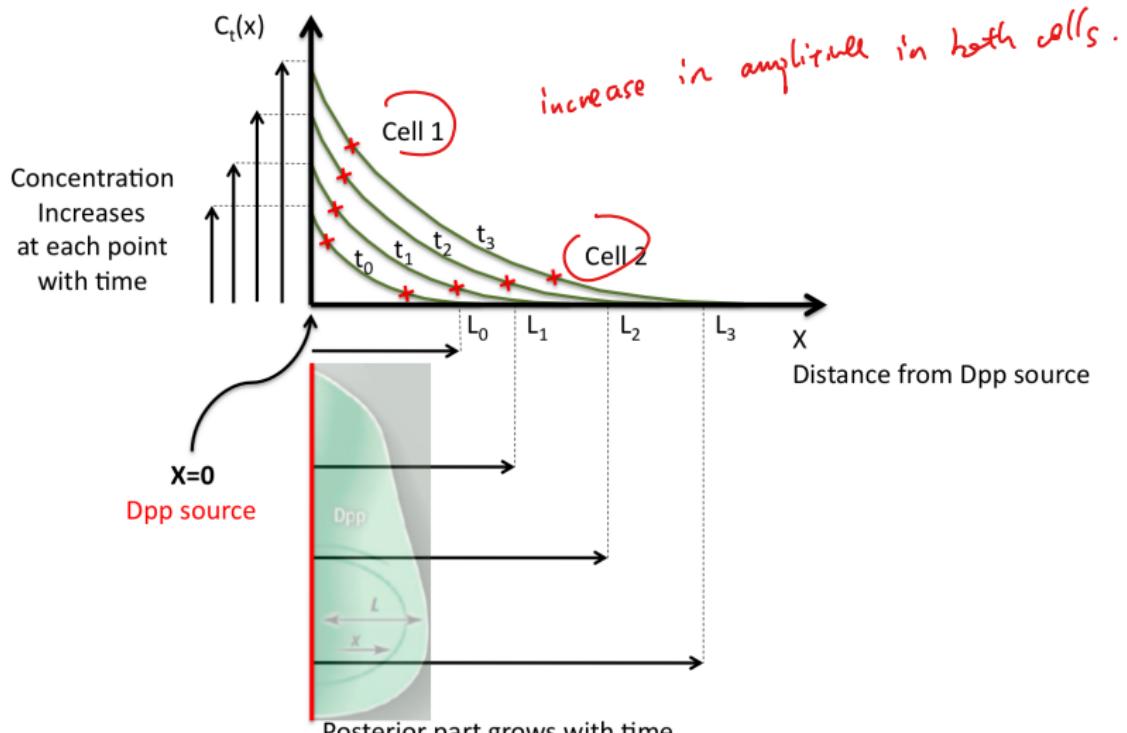
# The Dpp Gradient scales with the size of the Wing Disc



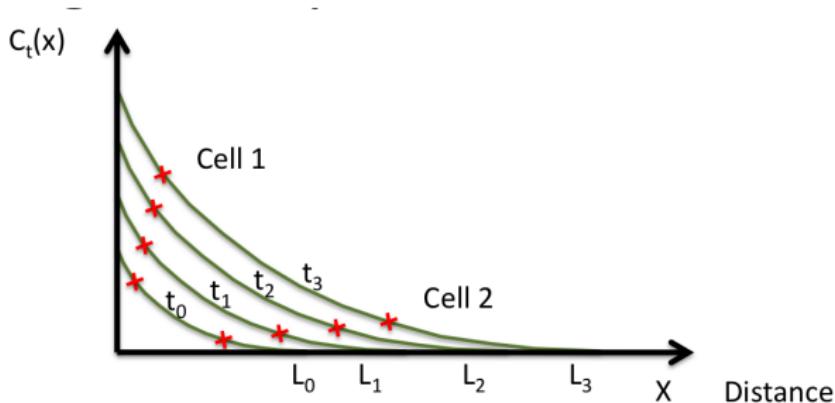
# The Dpp Gradient expands over time



# The Dpp concentration seen by cells over time.



# An exponential gradient



Observation:

$$\frac{\lambda}{L} = \text{constant} , \quad \lambda = \sqrt{\frac{D}{\kappa}}$$

For any t:

$$C_t(x) = C_0 e^{-\frac{x}{\lambda}}$$

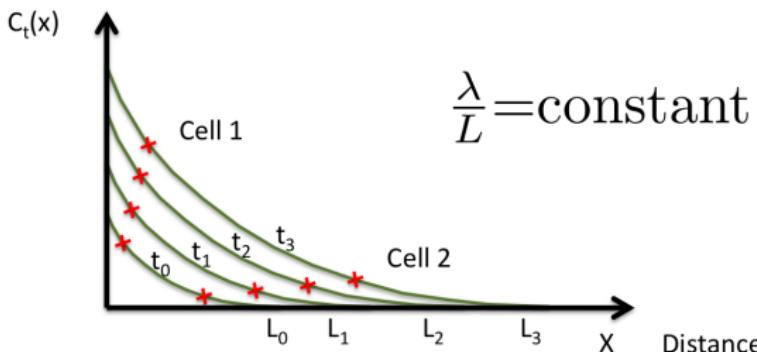
$$(t = t_0) : C_{t_0}(x) = C_{0,t_0} e^{-\frac{x}{\lambda_{t_0}}}$$

$$(t = t_1) : C_{t_1}(x) = C_{0,t_1} e^{-\frac{x}{\lambda_{t_1}}}$$

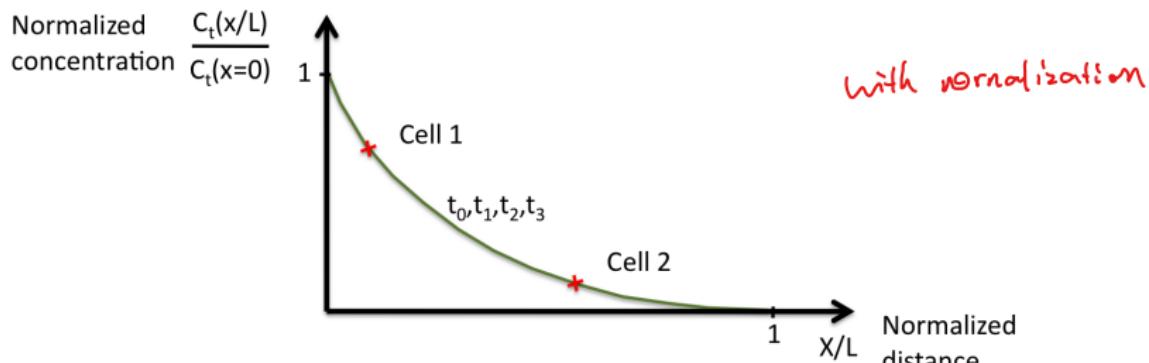
$$(t = t_2) : C_{t_2}(x) = C_{0,t_2} e^{-\frac{x}{\lambda_{t_2}}}$$

$$(t = t_3) : C_{t_3}(x) = C_{0,t_3} e^{-\frac{x}{\lambda_{t_3}}}$$

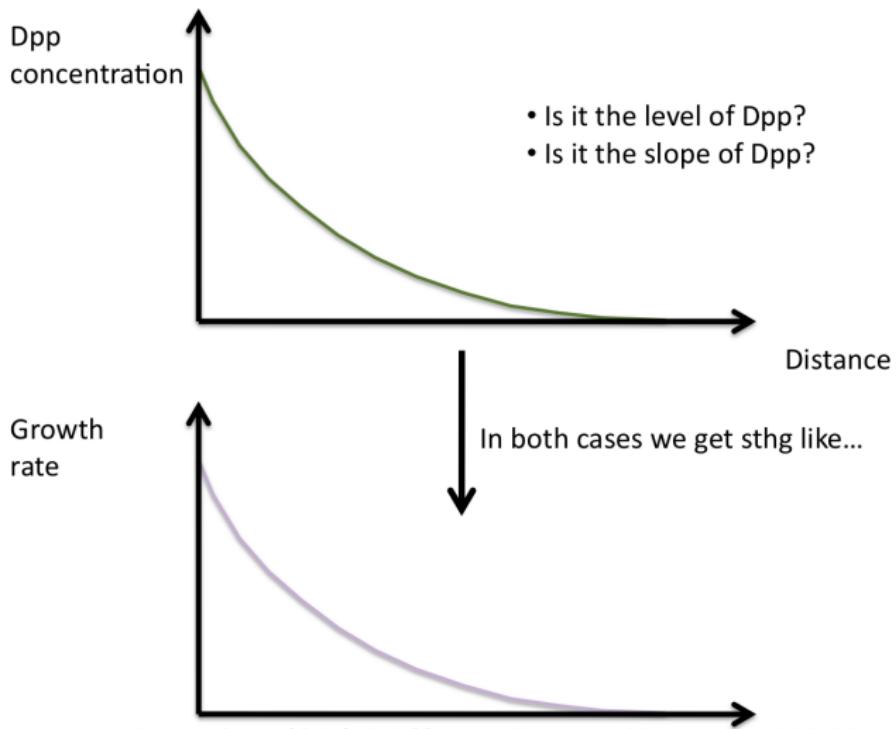
# The Dpp profiles scale on a growing domain



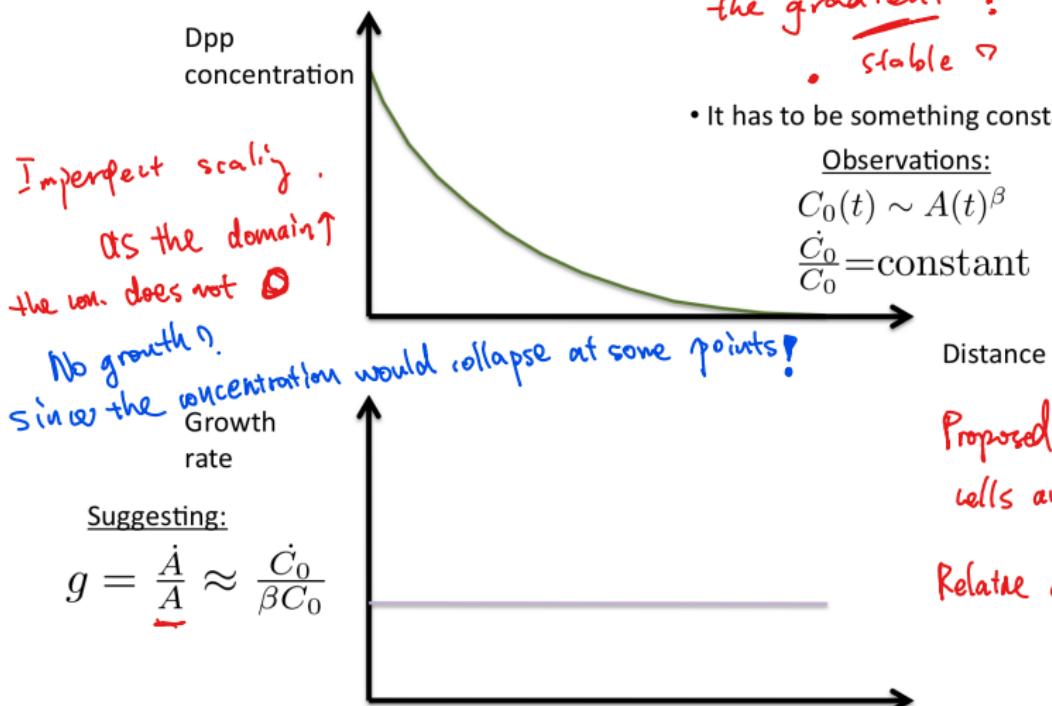
- ! Relative change
- remains the same
- !! since the curves are essentially the same



# How can uniform growth be controlled by an exponential gradient?



# Hypothesis: Cells read relative temporal change in gradient



what are the requirement for the gradient?

- stable ↗

- It has to be something constant...

Observations:

$$C_0(t) \sim A(t)^\beta$$

$$\frac{\dot{C}_0}{C_0} = \text{constant}$$

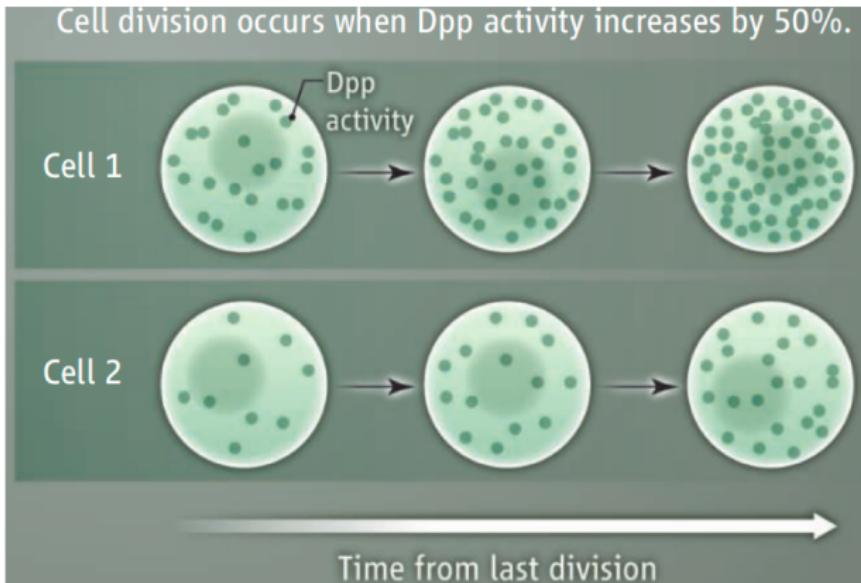
Distance

Proposed:

cells are const. the

Relative change in con.

# Hypothesis: Response to relative changes



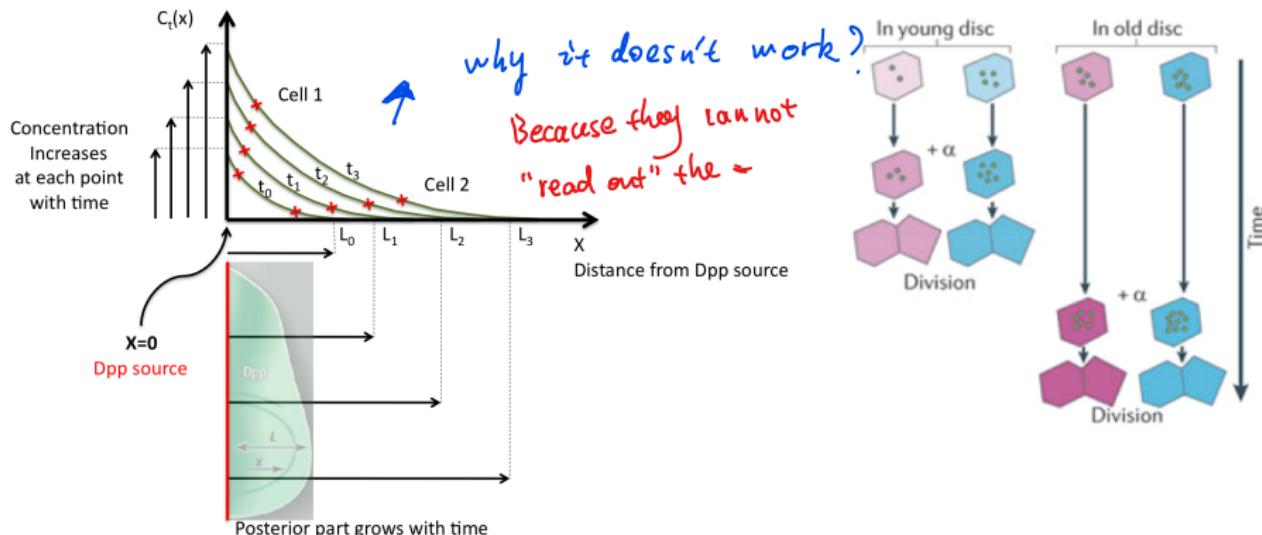
Relative increase of cellular  
Dpp during the cell cycle

$$\text{time : } \theta = \frac{\ln 2}{g}$$

$$\alpha = \frac{\Delta C_{cell}}{C_{cell}} \approx \beta \ln 2$$

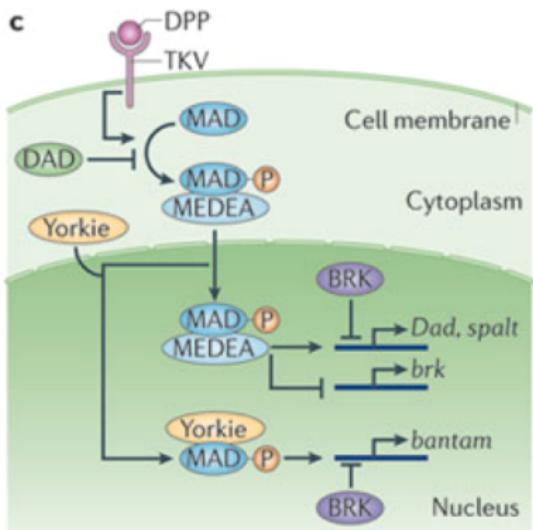
$$\alpha = 0.41, \alpha_s = 0.48$$

# Growth Control by a Scaling Dpp Gradient

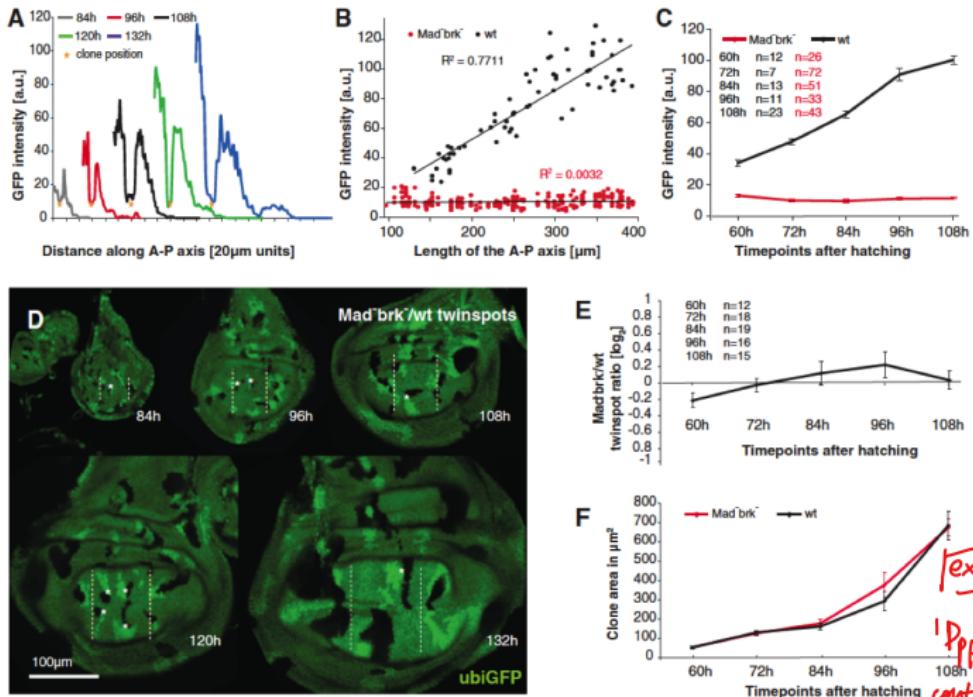


# Counter-Arguments

- Same growth rate in clones without the only Dpp transducer Mad and its downstream target transcription factor Brk (Schwank, G., et al. (2012). Comment on "Dynamics of Dpp Signaling and Proliferation Control". *Science* 335(6067): 401-401.)
- Same normal growth during latter half of larval development even when Dpp is removed using CRISPR-Cas9 genome editing (Akiyama, T. and M. C. Gibson (2015). "Decapentaplegic and growth control in the developing Drosophila wing." *Nature* 527(7578): 375-378.)
- Dpp gradient not required for lateral wing disc growth (Harmansa, S., et al. (2015). "Dpp spreading is required for medial but not for lateral wing disc growth." *Nature* 527(7578): 317-322.)
- Dpp scaling is not perfect (Fried, P. and D. Iber (2014). "Dynamic scaling of morphogen gradients on growing domains." *Nat Commun* 5: 5077; Fried, P. and D. Iber (2015). Read-Out of Dynamic Morphogen Gradients on Growing Domains. *PLoS ONE* 10(11): e0143226.)

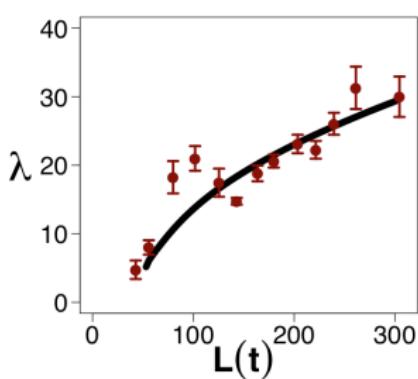


# Dpp Signalling not required for Growth

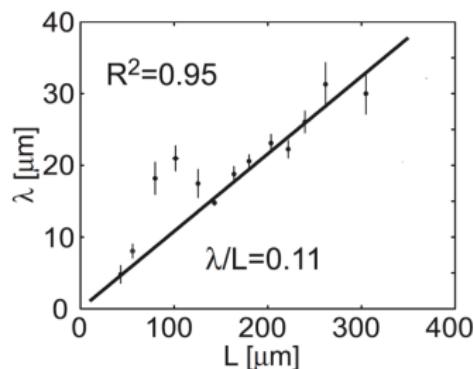


# Imperfect Scaling Mechanism explains Dpp data

Scaling because of pre-steady state dispersal of the Dpp gradient explains scaling based on Dpp dispersion without need for any further regulatory interactions.

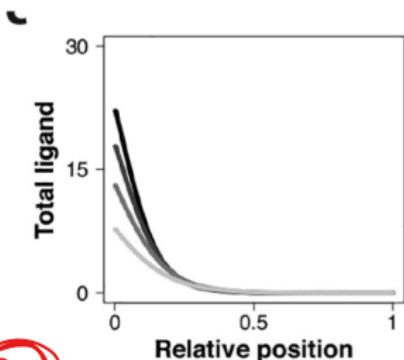
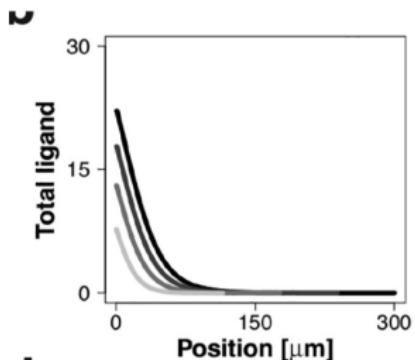


Fried and Iber, *Nat. Commun.*, 2014.

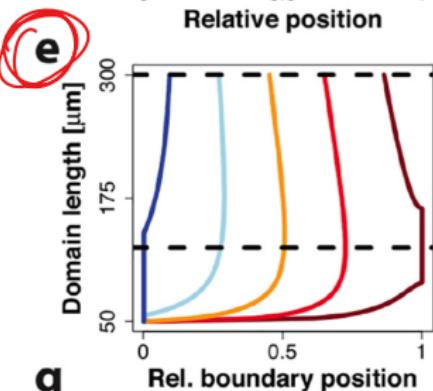
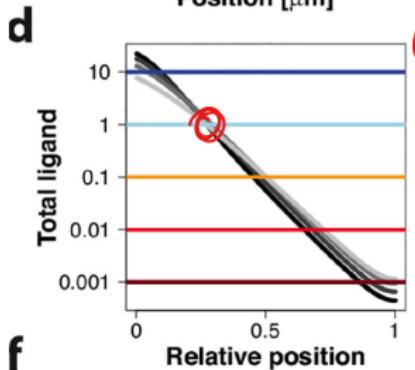


Wartlick et al., *Science*, 2011.

# Threshold-based Read-Out on the Dpp Gradient



All gradients cross in one relative position within the wing disc.

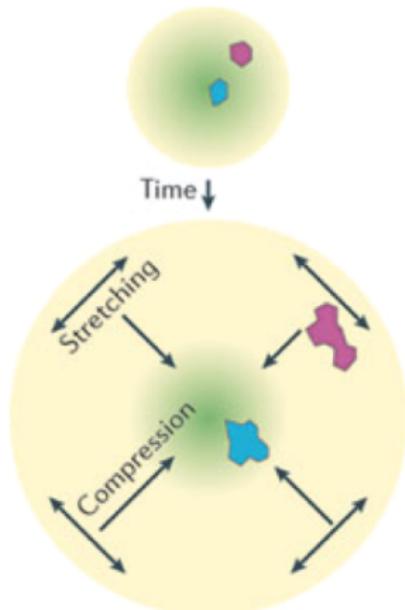


Accordingly, the Dpp dynamics cannot explain uniform growth.

## Models based on Morphogen Signalling & Mechanical Feedback

# Models based on Tissue Mechanics

- Enhanced growth because of Morphogen Signalling in the centre
  - Postulated Mechanical Feedback results in Compression in Centre
  - Stretching in lateral parts results in enhanced growth
- 
- Hufnagel, L., et al. (2007). On the mechanism of wing size determination in fly development. *Proceedings of the National Academy of Sciences of the United States of America* 104(10): 3835-3840.
  - Aegerter-Wilmsen, T., et al. (2007). Model for the regulation of size in the wing imaginal disc of Drosophila. *Mechanisms of Development* 124(4): 318-326.
  - Aegerter-Wilmsen, T., et al. (2012). Integrating force-sensing and signaling pathways in a model for the regulation of wing imaginal disc size. "Development 139(17): 3221-3231..

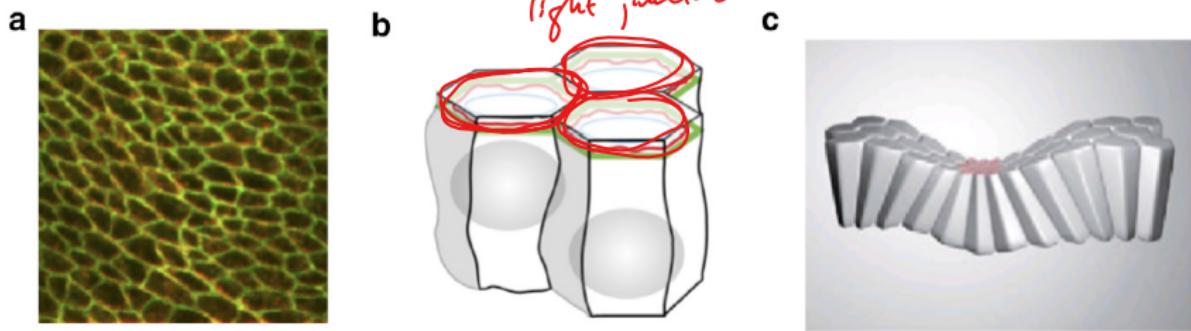


## Organization of Epithelial Tissue

# Organization of Epithelial Tissue

In an epithelium, each cell is tightly coupled to its neighbours by a ring of intercellular junctions which, in addition to maintaining the cohesion between cells, also forms a tight permeability barrier between cells.

The membrane boundaries of individual cells within an epithelium are, to a good approximation, polygonal in shape.

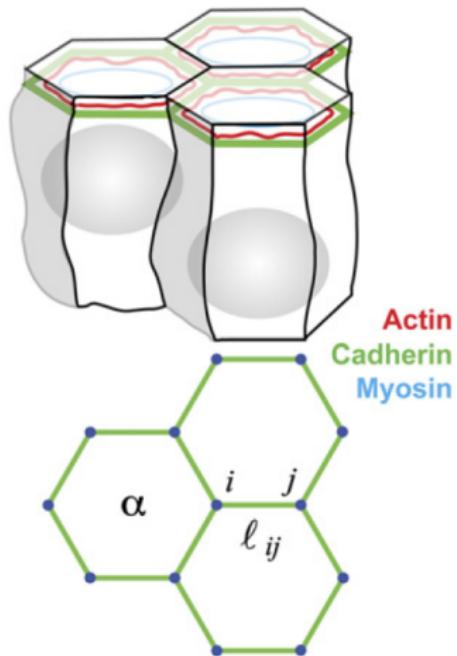


# Modelling an epithelial sheet

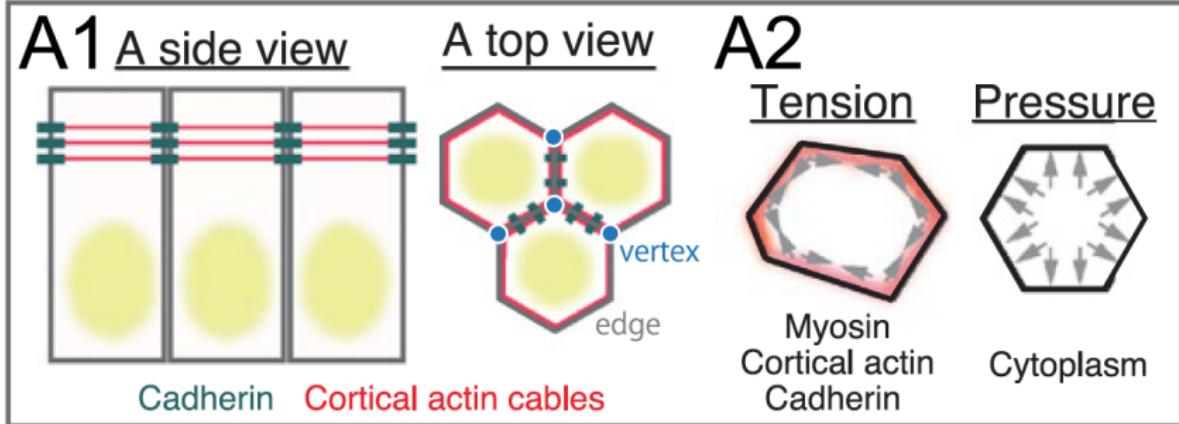
We will model an epithelium as a two-dimensional sheet of contiguous, coupled polygonal cells (i.e. as a collection of nodes connected by line segments).

We begin by describing a mechanical model for a single cell. To model an epithelium, it will then only be necessary to mechanically couple a suitable number of cells into a continuous two-dimensional sheet.

Further details in Weliky, M. and G. Oster (1990). Development 109: 373-386.



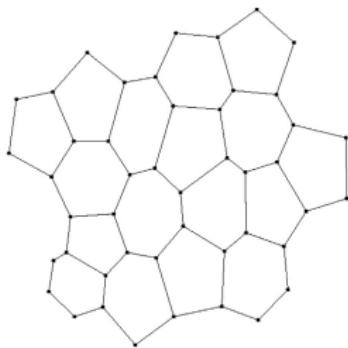
# Modelling an epithelial sheet



Ishihara & Sugimura (2012) Theor Biol

## Vertex Model

Representation of cells:



Fletcher et al., 2013

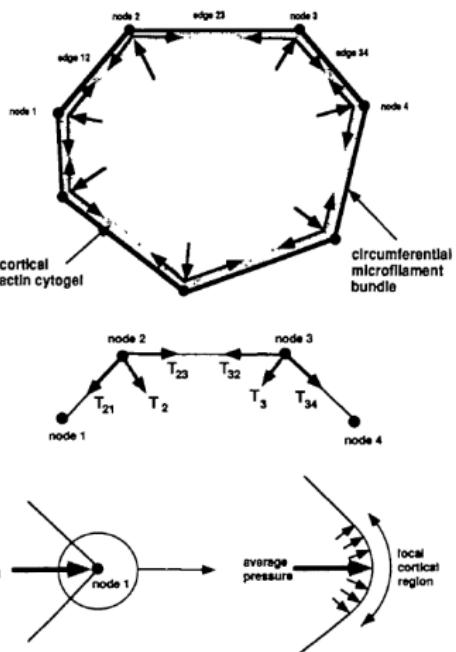
- cells represented by polygons
- neighboring cells share edges, intersection point = vertex

→ what are the disadvantages?  
why all convex

# Elastic tension forces

The elastic tension forces at cell node,  $n$ , are represented by a pair of two-dimensional vectors,  $\vec{T}_{(n,n-1)}$  and  $\vec{T}_{(n,n+1)}$ , applied to the node.

These vectors are directed along the line segments connecting this node to each of the two adjacent nodes, where  $\vec{T}_{(n,n-1)}$  denotes the vector directed towards the node in the counterclockwise direction and  $\vec{T}_{(n,n+1)}$  towards the clockwise node.



# Hooke's Law

According to Hooke's law, the force  $\vec{F}$  needed to extend or compress a spring by some distance  $\Delta \vec{x}$  is proportional to that distance. That is,

$$\vec{F} = k\Delta \vec{x}.$$

# Elastic tension forces

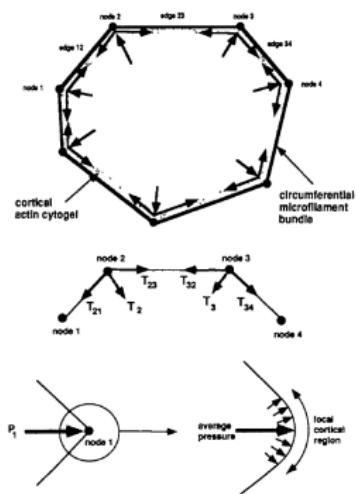
We will use the two-dimensional unit vectors  $\vec{t}_{(n,n-1)}$  and  $\vec{t}_{(n,n+1)}$  to describe the tension vectors' direction.

We can describe the elastic vector forces applied to each cell node as follows

$$\vec{T}_{(n,n+j)} = \vec{t}_{(n,n+j)} \cdot k_{elas} \cdot \Delta P$$

Here  $P$  is the cell perimeter, and  $k_{elas}$  is the elastic modulus of the cell cortex (i.e. the circumferential microfilament bundle and the cortical actin gel matrix).

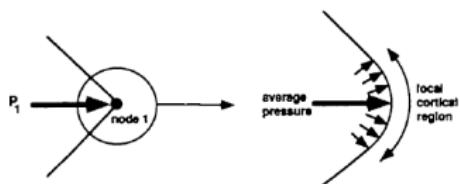
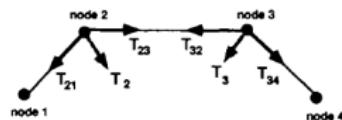
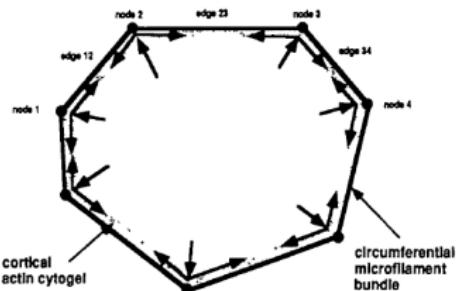
Larger values of  $k_{elas}$  reflect increasing contraction and/or density of microfilament bundles, while lower values reflect solation, which reduces the elastic modulus of the cortical actin gel matrix. Increasing the cell perimeter,  $P$ , stretches cortical fibers thus increasing their elastic restoring forces.



# Pressure

Intracellular pressure is assumed to be uniform within the cell.

The pressure force at each node is represented by the two-dimensional vector  $\vec{P}$  directed outward from the cell boundary and bisecting the angle between the two line segments joined at a node; that is, the pressure acts normal to the polygonal surface at each node.

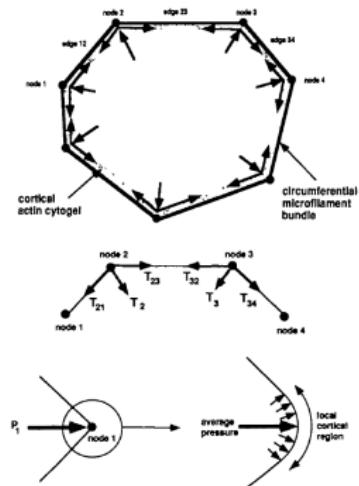


# Pressure

The two-dimensional unit surface normal vector  $\vec{n}$  describes the direction of the nodal pressure.

The standard assumption is that a change in cell volume (surface area) results in a change in the osmotic pressure difference such that the pressure difference is inversely proportional to the volume (surface area,  $A_n$ ):

$$\vec{P}_n = \frac{\text{const}}{A_n} \vec{n}$$



# The net force at a node

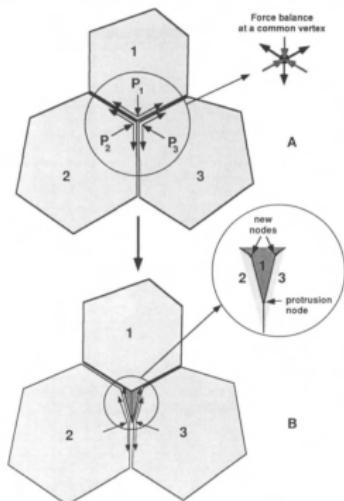
The net force acting at a cell node,  $n$ , is the vector sum of the nodal tensions and pressure:

$$\vec{F}_n = \vec{P}_n + \vec{T}_{(n,n-1)} + \vec{T}_{(n,n+1)}$$

The total force acting at an epithelial node is the sum of all net forces from all cells sharing this node plus the external forces:

$$\vec{F} = \sum_{n=1}^N \vec{F}_n + \vec{F}_{ext}$$

where  $N$  is the number of cells sharing the common junctional node.



# Viscous Drag Force

Instead of rigid body

Due to the movement through a viscous fluid, a frictional force acts on the vertices.

As the Reynolds number is much smaller than one,  $Re \ll 1$ , the viscous drag force can be estimated by Stokes' law,

$$\vec{F}_n^d := -6\pi\mu R \frac{d\vec{x}_n}{dt} \text{. velocity}$$

"Force from viscous fluid  
to stop the movement" (1)

Here,  $\mu$  refers to the dynamic viscosity of the cytoplasm,  $R$  to the radius,  $\vec{x}_n$  to the position of the vertex.

# Equations of Motion

According to Newtonian dynamics, each vertex accelerates in response to the sum of forces it experiences. Therefore, the deterministic equation of motion is described by:

$$m \frac{d^2 \vec{x}_n}{dt^2} := \vec{F} + \vec{F}_n^d, \quad \begin{matrix} \text{Tension} \\ \text{Vis.} \end{matrix} \quad (2)$$

where  $m$  is the mass of a vertex, which can be assumed to be equal for all vertices.

# Equations of Motion

Each second order ODE can be split into two first order ODEs

$$\frac{d\vec{x}_n}{dt} = \vec{u}_n$$

$$m \frac{d\vec{u}_n}{dt} = \vec{F} + \vec{F}_n^d,$$

where  $\vec{u}_n$  is the velocity of vertex  $n$ . As the mass  $m$  of a vertex is very small, we can exploit a quasi-steady state,  $m \frac{d\vec{u}_i}{dt} = 0$ , and obtain

*Why? Viscous Force.*  $\vec{F} = -\vec{F}_n^d := 6\pi\mu R \frac{d\vec{x}_n}{dt}$  . (3)

such that

$\frac{d\vec{x}_n}{dt} = \frac{1}{6\pi\mu R} \vec{F}$

*Tension*      *gnosis*  
*Steady State*      *Speed* (4)

# Equations of Motion

Rescaling  $t$  by  $6\pi\mu R$ , we have for the position vector of each node,  $\vec{x}_n$ ,

$$\frac{d\vec{x}_n}{dt} = \sum_{n=1}^N \vec{P}_n + \vec{T}_{(n,n-1)} + \vec{T}_{(n,n+1)} + \vec{F}_{ext}$$

The total number of equations is equal to the number of nodes in our simulation, which can be many hundreds. Since the equations are nonlinear, an analytical solution to this system is not possible, so we must apply numerical methods.

# Energy Functional

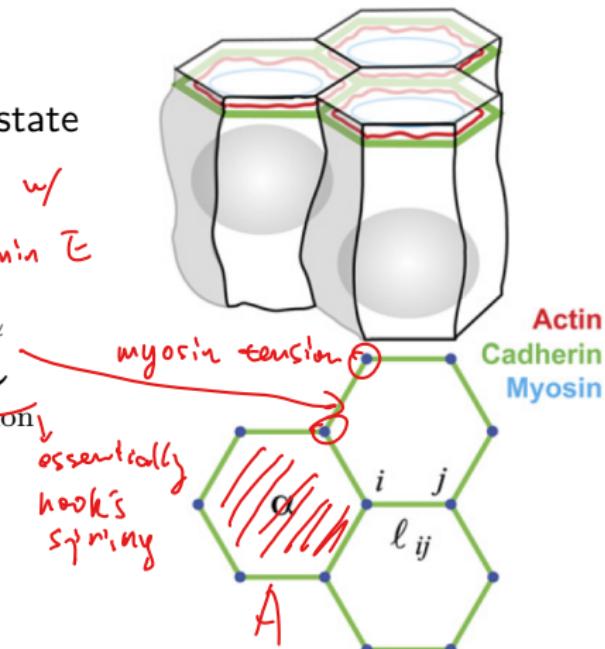
See lecture 6

Alternatively, we can use an energy functional and determine the steady state distribution:

Steady state w/  
min  $E$

$$E = \underbrace{\sum_{<i,j>} \lambda_{i,j} l_{i,j}}_{\text{surface tension}} + \underbrace{\sum_{\alpha} \gamma_{\alpha} \Pi_{\alpha}^2}_{\text{cortical tension}} + \underbrace{\sum_i \kappa_{\alpha} (A - A_0)^2}_{\text{area elasticity}}$$

size



# Relation between Forces and Energy-based View

*"optimization"*  
Force  $\rightarrow$  ODE

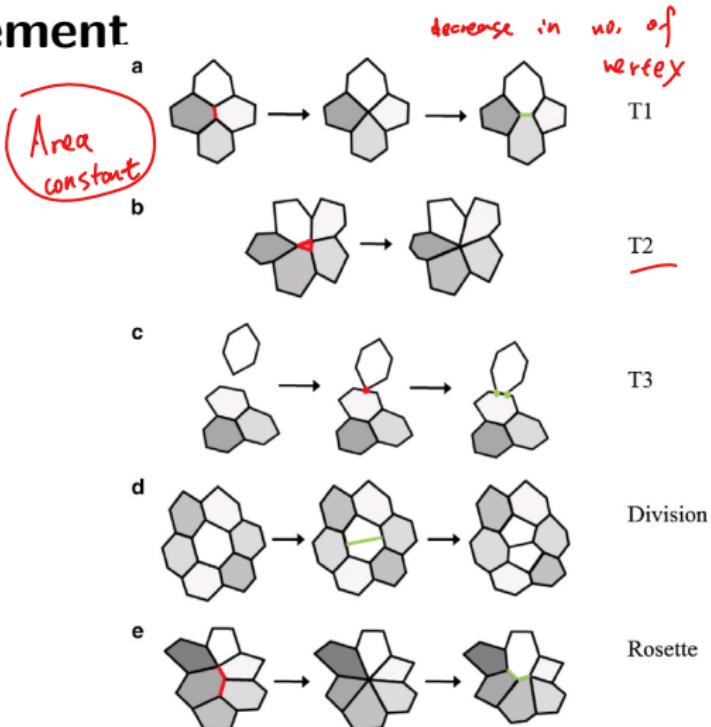
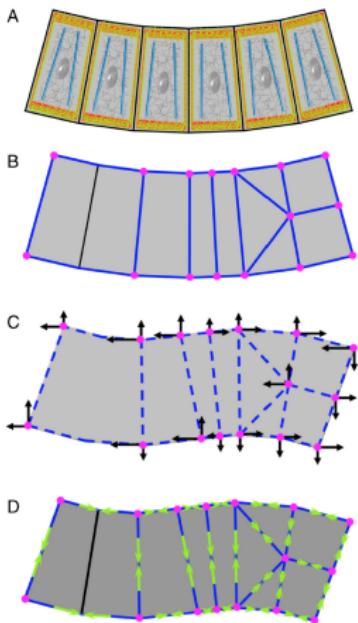
Evolution in time:

- vertex  $i$  moves according to:  $\mu \frac{d\mathbf{x}_i}{dt} = \mathbf{F}_i$
- forces derived from energy potentials:

$$\mathbf{F}_i = \underbrace{\frac{\partial E}{\partial \mathbf{R}_i}}_{E(\mathbf{R}_i)} = \underbrace{\sum_{\alpha} \frac{K_{\alpha}}{2} (A_{\alpha} - A_0)^2 + \sum_{<i,j>} \frac{\Gamma_{ij}}{2} (r_{ij} - l_{ij})^2}_{}$$

- term 1: area elasticity,  $K_{\alpha}$  = elasticity coefficient
- term 2: perimeter contractility,  $\Gamma_{ij}$  = contractility coefficient

# Junctional Rearrangement

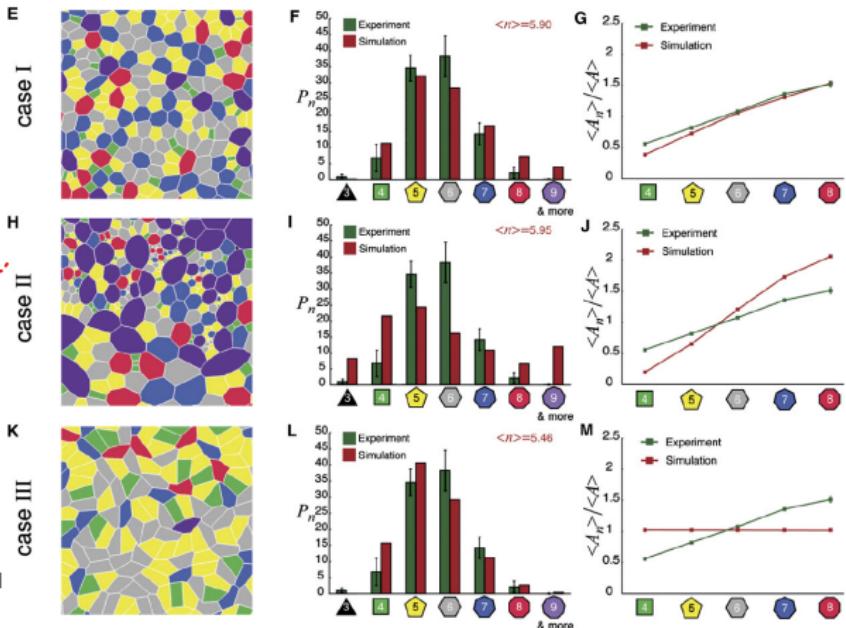
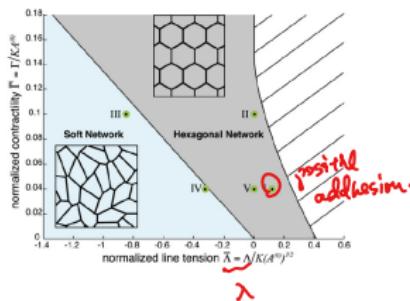


Brodland, G. W., et al. (2010) PNAS 107: 22111-22116.

Fletcher, A. G., et al. (2014). Biophys J 106: 2291-2304.

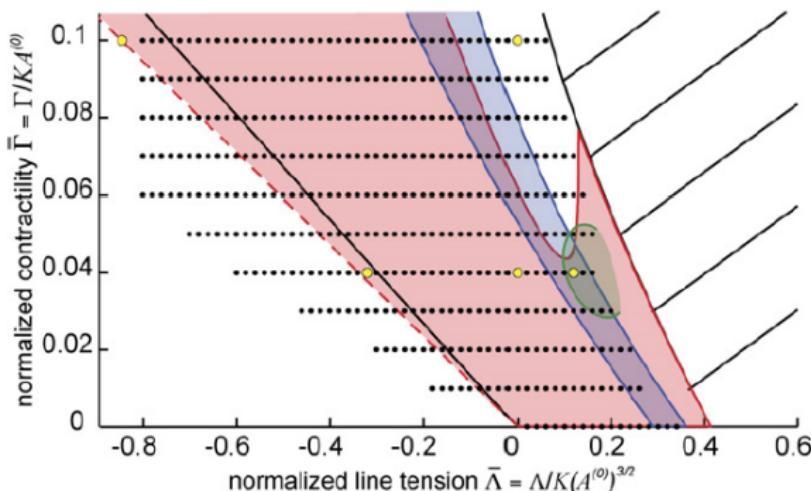
# Vertex Model of epithelial sheet

Membrane Tension versus Cell Junction Strength



Farhadifar, R., et al. (2007). Curr Biol

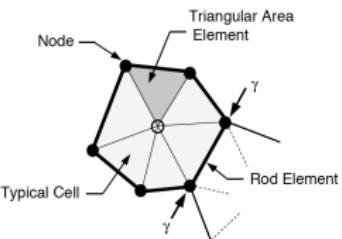
# Comparison to Wing Disc Data



same polygon distribution  
same area distribution  
same response to laser ablation

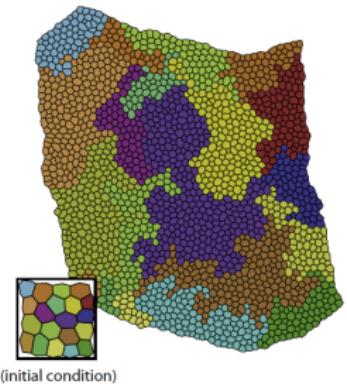
# FEM Tissue Simulations & longest axis division

Better match of polygon distribution with division perpendicular to longest axis.

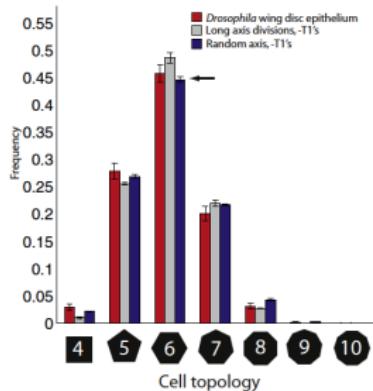


Gibson et al Cell (2011).

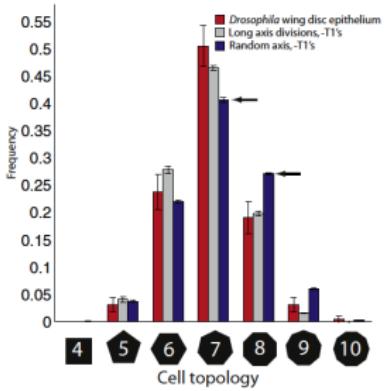
D Finite element simulator



E Global cell shape distribution



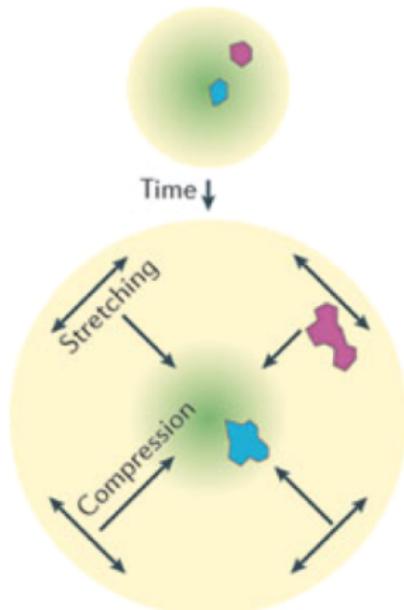
F Mitotic cell shape distribution



## Models based on Morphogen Signalling & Mechanical Feedback

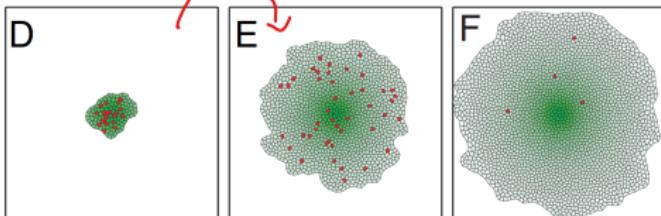
# Models based on Tissue Mechanics

- Enhanced growth because of Morphogen Signalling in the centre
  - Postulated Mechanical Feedback results in Compression in Centre
  - Stretching in lateral parts results in enhanced growth
- 
- Hufnagel, L., et al. (2007). On the mechanism of wing size determination in fly development. *Proceedings of the National Academy of Sciences of the United States of America* 104(10): 3835-3840.
  - Aegerter-Wilmsen, T., et al. (2007). Model for the regulation of size in the wing imaginal disc of Drosophila. *Mechanisms of Development* 124(4): 318-326.
  - Aegerter-Wilmsen, T., et al. (2012). Integrating force-sensing and signaling pathways in a model for the regulation of wing imaginal disc size. "Development" 139(17): 3221-3231..

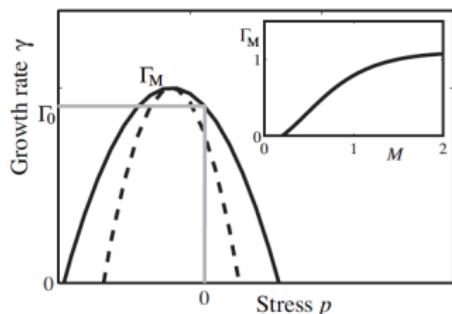


# Shraiman Model, PNAS 2007 - General Ideas

- 2D layer of cells is represented as a **polygonal tiling**, each cell corresponding to a polygon and in addition having certain **height**.
- The positions, shapes, and heights of the polygonal cells are determined by the **condition of mechanical equilibrium** that reconciles cell packing with their intrinsic volume
- For example, the effect of **lateral compression** will be a **reduction of cell area** and **increase in its height**, the latter deformation providing a visualization of the corresponding uniaxial stress.
- The simulation will determine these stresses and deformations and implement cell growth in accordance with the growth function  $\Gamma_M$ .
- $\Gamma_M$  depends on the stress in the tissue (figure) and on the exponential morphogen profile  $M(r) = m \exp(-r/\lambda)$ .
- Volume growth of divided cells is exponential:  $\frac{dV_{0,a}(t)}{dt} = \kappa_a$ .



- Rate of cell proliferation (in the model) depends on morphogen levels and mechanical strain.
- Dependence of the growth rate on the lateral stress  $p$  within the cell layer for a fixed morphogen level,  $M$ . Dashed line: stronger mechanical feedback.



- Note Inhibition of growth by sufficiently high stress.
- A thinner layer,  $P < 0$ ,  $\Rightarrow$  cells under tensile stress, whereas  $P > 0$   $\Rightarrow$  compression.
- Maximum growth rate at  $P < 0$  because tension promotes growth in epithelial cell cultures.
- (Inset) Maximal growth rate  $\Gamma_M(M)$  as a function of morphogen level. The essential feature here is the threshold  $M_0$  and the monotonic increase with  $M$ .

# Shraiman Model, PNAS 2007 - Tissue Growth

Dividing cells chosen with probability proportional to  $\gamma_a = \Gamma(\zeta, M)$ :

$$\Gamma(\zeta, M) = \Gamma_M(M)(1 - q(\zeta_a - \zeta_0)^2)$$

$$\text{with } \Gamma_M = (M - M_0)\theta(M - M_0)$$

$$\text{Morphogen } M(r) = m \exp\left(\frac{-r}{\lambda}\right)$$

Because deformation  $\zeta_a - 1$  and stress  $p$  are proportional to each other,  $p$  was replaced by  $\zeta_a$  in the parameterization of  $\gamma$  by  $\zeta_a$ .

Each cell division is followed by minimizing  $E$ , so that daughter cells approach the fixed "adult" cell volume in a single relaxation step after division:

$$\frac{dV_{0,a}(t)}{dt} = \kappa_a, \quad \text{with } V_{0,a}(0) \text{ being the volume of cell } a \text{ right after the division.}$$

# Shraiman Model - Energy Functional

$$E(r_i, \zeta_a) = \sum_a \left[ \rho_a + \underbrace{a(V_a - V_0)^2}_{\text{deviations from unstressed cell volume}} + b \underbrace{\sum_{\beta=\nu(a)} (\zeta_a - \zeta_\beta)^2}_{\substack{\text{height variations between neighbouring cells} \\ \text{in red underline}}} + \underbrace{c(\zeta_a - 1)^2}_{\text{deviations from unstressed cell height}} \right]$$

T similar height  
 in neighbours

$r_i$ : vertices of the cell

$\zeta_a$ : cell height

$\rho_a(r_i)$ : cell perimeter

$V_a = A_a \times \zeta_a$ : cell volume

$A_a(r_i)$ : cell apical area

$V_0$ : intrinsic cell volume

# Shraiman Model, PNAS 2007

## Cell Positions and Deformations from Energy Minimization

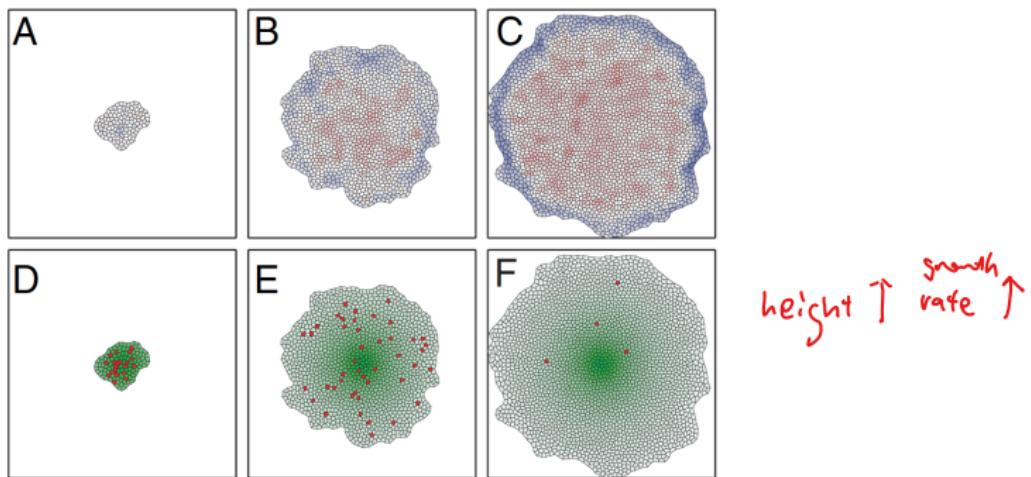
$$E(r_i, \zeta_a) = \sum_a \left[ \rho_a + a(V_a - V_0)^2 + b \sum_{\beta=\nu(a)} (\zeta_a - \zeta_\beta)^2 + c(\zeta_a - 1)^2 \right]$$

Minimization of  $E$  with respect to  $r_i$  and  $\zeta_a$  determines cell positions and deformations and corresponding local stresses.

In particular, the uniaxial compression of the cell,  $p_a$  is proportional to  $\zeta_a - 1$  and is therefore directly obtained from the minimization of  $E$ .

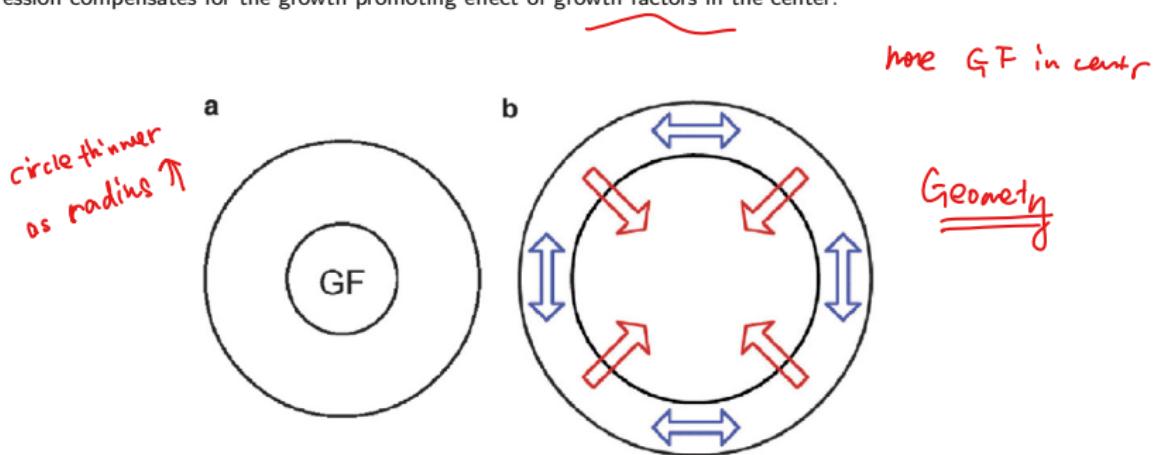
# Shraiman Model, PNAS 2007

Numerical simulation of the mechanical feedback model of disk size determination. (A?C) Snapshots of the simulated growth at different times with A corresponding to the start of the simulation, B the intermediate time, and C close to cessation of growth. Color code indicates layer deformation with red corresponding to lateral compression ( $\zeta - 1 > 0$ ) and blue corresponding to tension ( $\zeta - 1 < 0$ ). (D-F) Shown (green) is the level of morphogen  $M(r)$  peaked at its source cell. Cells that are about to divide are marked red (this is intended to emulate BrdU staining of mitotic cells). Note that cell proliferation is approximately uniform throughout the disk as is the case for in vivo observations. This uniformization of growth is a result of the mechanical feedback mediated by the build-up of compression (as seen in A-C), which compensates for the excess of morphogen in the central region. Shortly after the disk expands beyond the range where morphogen is above threshold, the build-up of stress arrests growth throughout the disk (F).



# Aegerter-Wilmsen Model, 2007

Initially growth occurs in the center where the growth factor concentration is high (GF in (a)). This growth causes the peripheral regions to stretch and the center to be compressed (b). The stretching in the peripheral regions induces growth there. Even though this growth reduces the stretching in the peripheral regions, some stretching remains. As a consequence, the center will still be compressed to some extent, which inhibits growth in this region. The wider the peripheral regions, the larger the compression becomes. Finally, growth stops when the inhibiting effect exhibited by compression compensates for the growth promoting effect of growth factors in the center.



Aegerter-Wilmsen, T., et al. (2007). Model for the regulation of size in the wing imaginal disc of Drosophila. Mechanisms of Development 124(4): 318-326.

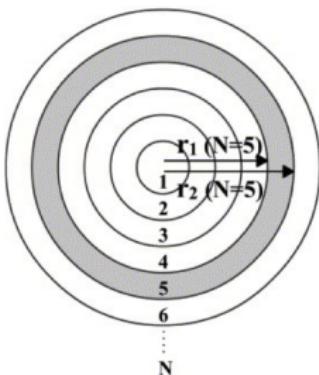
# Aegerter-Wilmsen Model, 2007

The model implementation is simpler than in the Shraiman model. The tissue is modelled as a set of rings. Each ring  $n$  has a different growth factor concentration:

$$Gf(n) = -0.5 * \tanh(c_7/r_2(N) \cdot r_2(n) - c_7 \cdot c_8) + 0.5,$$

Aegerter-Wilmsen et al., s

where  $r_2(N)$  is the  $r_2$  of the outer ring and therefore the radius of the disc.  $c_7$  and  $c_8$  are constants.



# Growth Rate

$$\text{Growth Factor } \Delta G_{gf} = c_1 \cdot \tanh(c_2 \cdot Gf) \cdot \Delta t$$

$$\text{Stretch } \Delta G_{str} = (|Grn| - c_3) \cdot c_4 \cdot \Delta t (\Delta G_{str} \geq 0)$$

$$\text{Compression } \Delta G_{comp} = -(c_5 \cdot \exp(Comp(n) * c_6) - c_5) \cdot$$

$$\text{TOTAL } \Delta G_{tot} = \Delta G_{gf} + \Delta G_{str} + \Delta G_{comp} (\Delta G_{tot} \geq 0)$$

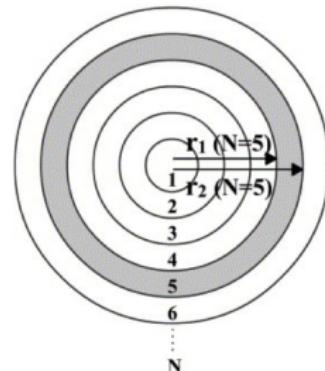
$c_1-c_6$  are constants.  $G_{tot}$  and  $G_{str}$  cannot be negative.  $Gf$  denotes the growth factor distribution

$$Gf(n) = -0.5 * \tanh(c_7 / r_2(N) \cdot r_2(n) - c_7 \cdot c_8) + 0.5,$$

$Grn$  denotes the growth needed to abolish stretching.  $Comp$  the compression exerted by stretching of the more peripheral rings. Initially,  $Grn$  and  $Comp$  are 0 everywhere.

# Calculation of new dimensions

The dimensions are calculated by redistributing the surface, starting from the center ring ( $n = 1$ ). Naturally, the  $r_1$  for one ring is equal to the  $r_2$  of the adjacent ring closer to the center ( $r_1(n) = r_2(n - 1)$ ).



For the  $r_2$  of ring  $n$  the following equation is used:

$$r_2(n) = \sqrt{(r_{2\text{old}}(n)^2 - r_{1\text{old}}(n)^2) \cdot (1 + \Delta G_{\text{tot}}(n))} + r_1(n)^2,$$

Aegerter-Wilmsen et al., s

where  $r_{2\text{old}}$  and  $r_{1\text{old}}$  are the  $r_2$  and  $r_1$  of a certain ring before the latest growth step. This corresponds to

$$\frac{r_2(n)^2 - r_1(n)^2}{r_{2\text{old}}(n)^2 - r_{1\text{old}}(n)^2} = 1 + \Delta G_{\text{tot}}(n),$$

# Calculation of stretching

surface of a certain ring at t=0       $sur_{t0}(n) = \pi(r2_{t0}(n)^2 - r1_{t0}(n)^2)$

width of the ring at t=0       $l_{t0}(n) = r2_{t0}(n) - r1_{t0}(n)$

present surface of the ring       $sur(n) = \pi(r2(n)^2 - r1(n)^2)$

present width of the ring       $l(n) = r2(n) - r1(n)$

STRETCHING       $Str(n) = \frac{(sur(n)/l(n)^2)}{(sur_{t0}(n)/l_{t0}(n)^2)} - 1,$

If cells have the same width and length at t=0 (no forces present), stretching basically means the difference between the width and the length of a cell.

# Calculation of compression

Once the stretching is calculated, the compression can be calculated as well:

$$\text{Comp}(n) = \sum_{m=n+1}^{m=N} \text{Str}(m) \cdot (r_2(m) - r_1(m)). \quad (5)$$

# Growth that would be needed to abolish the stretching in a certain ring (Grn)

Furthermore, the growth that would be needed to abolish the stretching in a certain ring (Grn) is calculated as well:

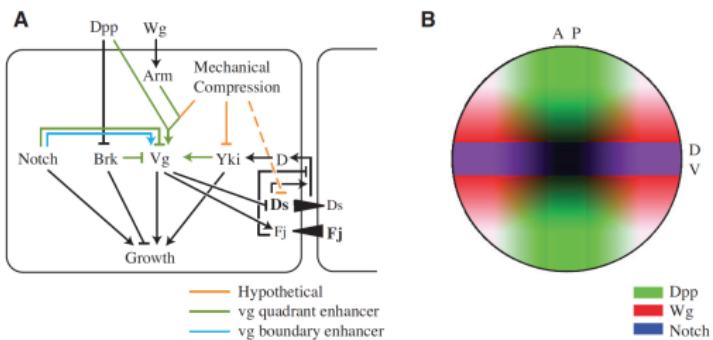
$$r_{2n}(n) = \frac{r_{2t0}(n)}{r_{1t0}(n)} \cdot r_1(n)$$

$$Grn(n) = \frac{r_{2n}(n)^2 - r_1(n)^2}{(r_2(n)^2 - r_1(n)^2)} - 1,$$

where  $r_{2n}$  denotes the  $r_2$  needed to abolish stretching, given the  $r_1$  of the ring.

# Aegerter-Wilmsen Model, Development, 2012

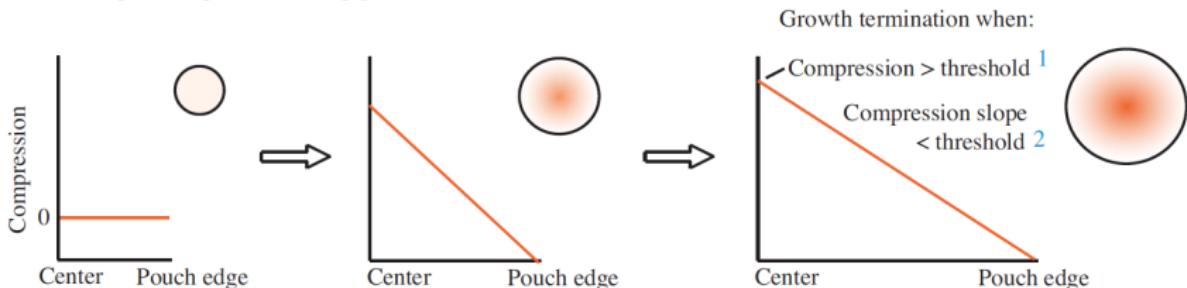
Main assumptions underlying the model. The regulatory network (A) represents protein activities and interactions that regulate these activities. The model does not distinguish between interactions at the transcriptional and protein activity level, but considers effects on net activities. All protein activities emerge from the network, except for those of Dpp, Wg and N, which are implemented in the model as depicted in B. In the regulatory network, differences in Ds and Fj concentrations between neighboring cells lead to activation of D by changing its intracellular localization. For simplicity, the resulting asymmetric localization is not depicted. In addition to the assumptions shown, it is assumed that apical cell shapes can be found by minimizing Eqn 2, that a weighted average of the area of a cell and its neighbors is a good readout for mechanical stress, that cells do not rearrange when exposed to mechanical tension, and that the planar polarization of D imposes a bias on the direction of the division plane. The interactions shown in orange are hypothetical and form the main untested assumptions underlying the model. The regulation of ds by mechanical compression is dotted, as this regulation is not essential for the principle behind size regulation in the model, but improves the fit of simulation results with experimental data. AP, anteroposterior boundary; DV, dorsoventral boundary.



# Aegerter-Wilmsen Model, Development, 2012

The size regulation mechanism can be understood in terms of a compression gradient model. General principle: compression increases in the center during growth. Growth ceases when compression is higher than a certain threshold in the center and the compression gradient is lower than a threshold level in the rest of the disc.

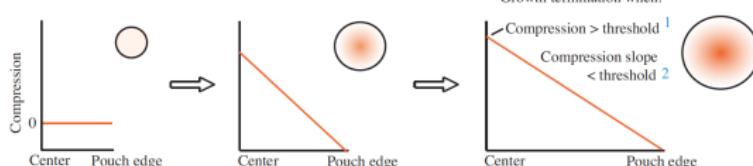
Build-up of compression during growth



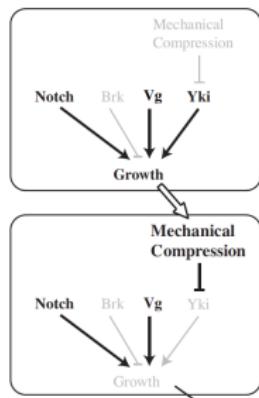
Aegerter-Wilmsen, T., et al. (2012). Integrating force-sensing and signaling pathways in a model for the regulation of wing imaginal disc size." Development 139(17): 3221-3231.

# Combining Signalling & Mechanical Feedbacks

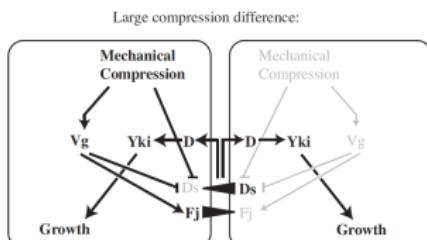
**A** Build-up of compression during growth



**B** <sup>1</sup> Center

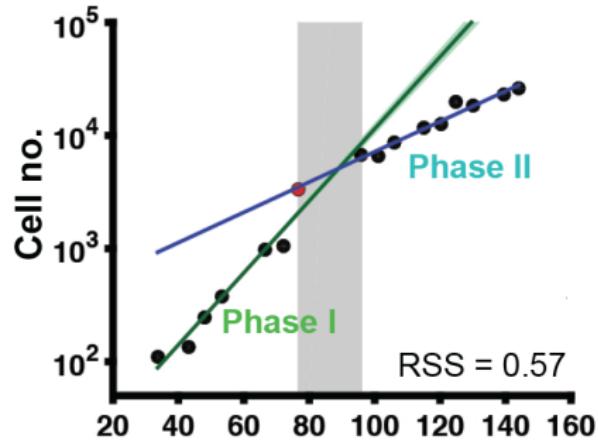
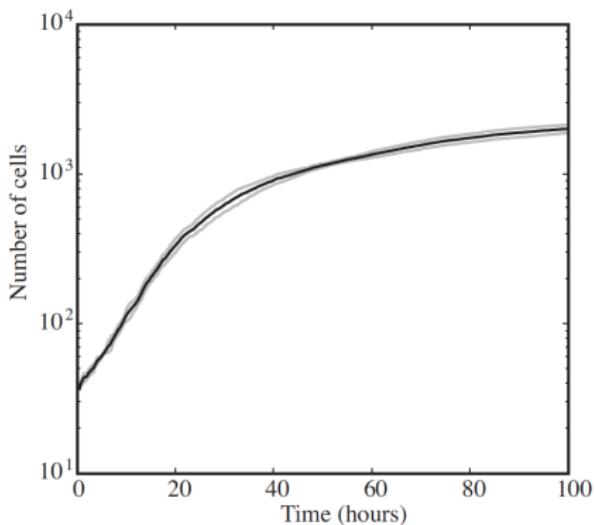


**C** <sup>2</sup> Compression slope read-out



- Growth Rate depends on Signalling Inputs
- Bias in Cell division plane because of Ds/Fj signalling
- Cell shapes are calculated by minimizing an energy function comparable to the one used by Shraiman, based on Vertex Model without periodic boundary conditions

# Growth Kinetics: cell numbers lower than in data - Feedback too strong?



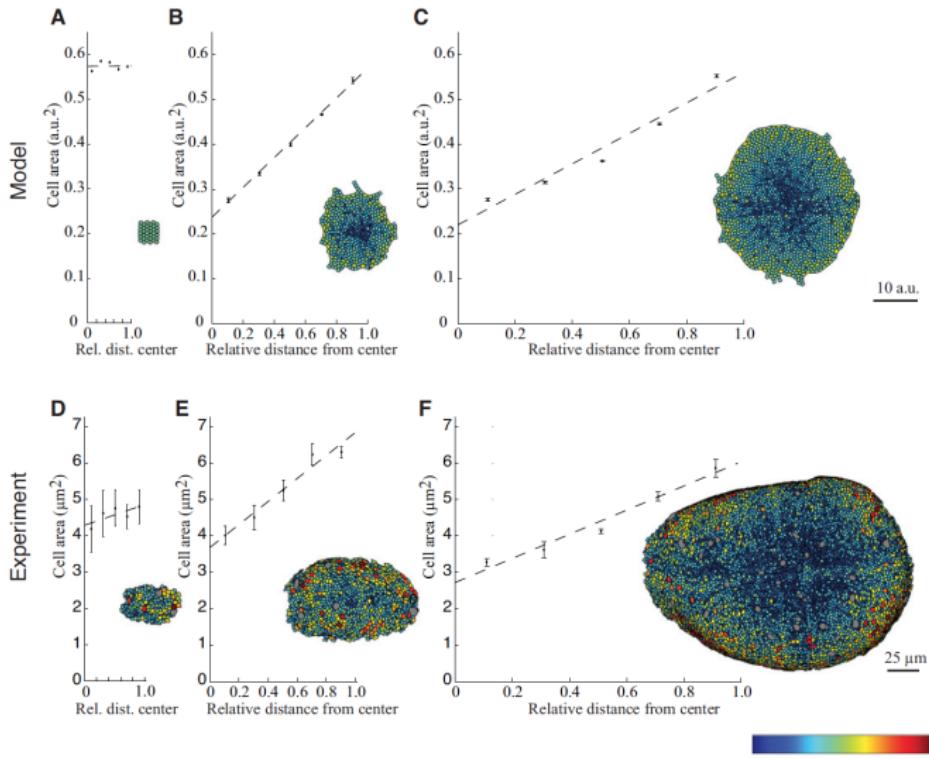
# Apical cell areas become smaller in center of domain.

(A-C) The model predicts an initial build-up of a cell area gradient (A,B), which is flattened at later stages (C).

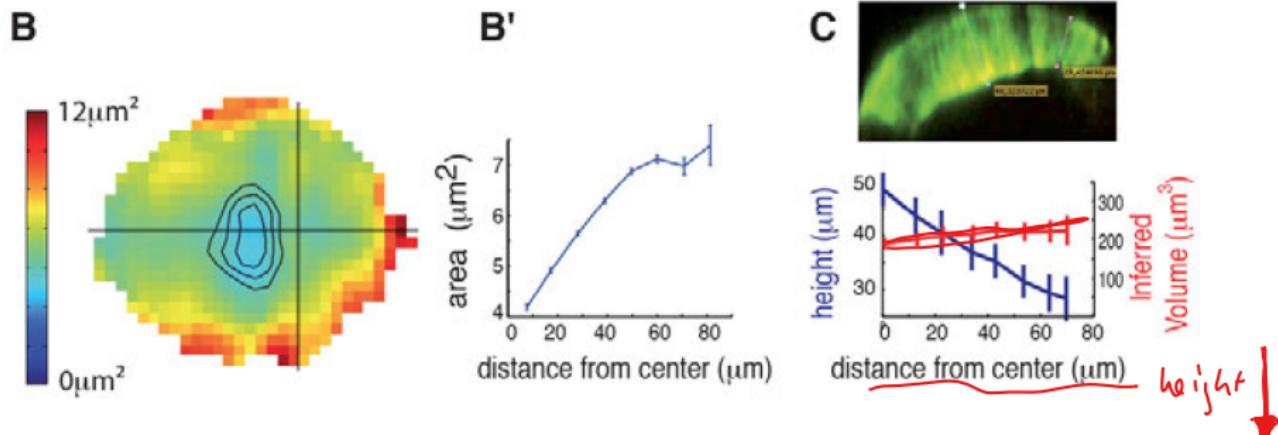
(D,F) Initial conditions (A) are compared with wing discs in which the ring of wg expression has not yet formed (D), and final simulated distributions (C) are compared with late third instar discs (F).

(E) The pouches of the mid third instar discs contain about one quarter the number of cells of those in the late third instar and are compared with simulated discs (B) that contain roughly the same factor-less cells than the final simulated disc.

Average results  $\pm$ s.e.m. of three discs are shown. Dotted lines are linear least square fits through the means.

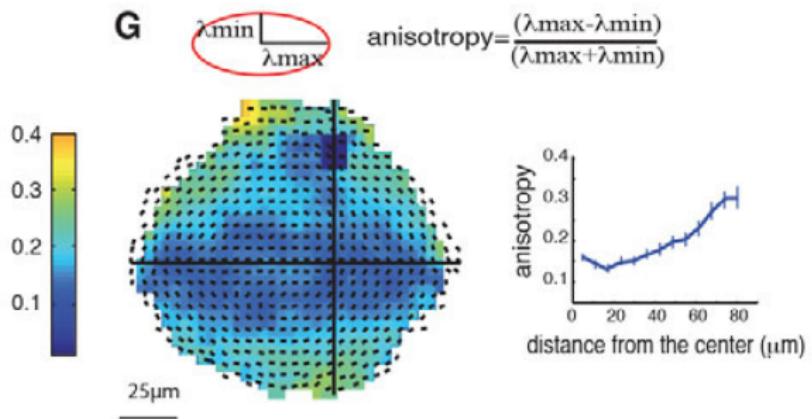
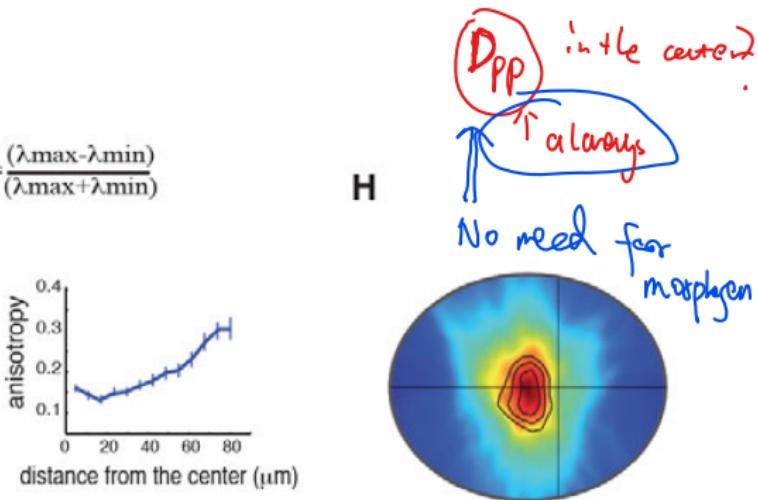


# Cell Area & Volume Distributions in the Drosophila Wing Disc



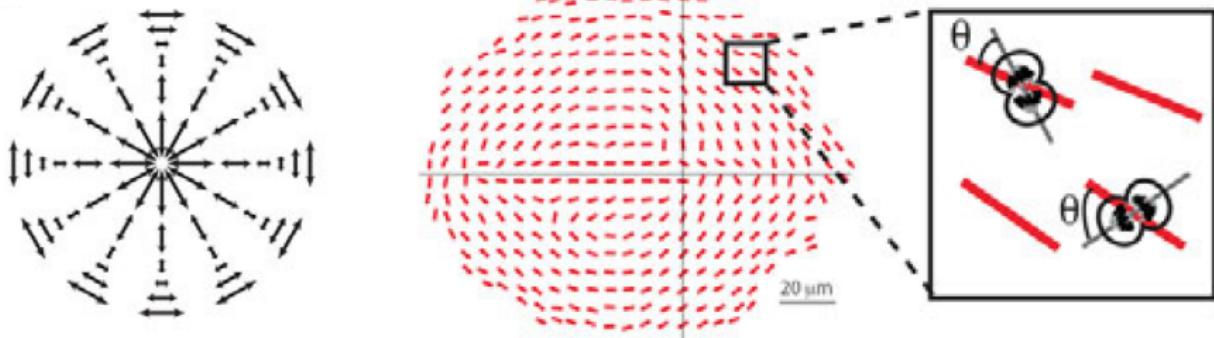
Legoff, L., et al. (2013). A global pattern of mechanical stress polarizes cell divisions and cell shape in the growing Drosophila wing disc."Development 140(19): 4051-4059.

# Tissue Anisotropy in the Drosophila Wing Disc

**G****H**

Legoff, L., et al. (2013). "A global pattern of mechanical stress polarizes cell divisions and cell shape in the growing Drosophila wing disc." *Development* 140(19): 4051-4059.

# Distribution of Cell Division Angles in the Drosophila Wing Disc



Legoff, L., et al. (2013). "A global pattern of mechanical stress polarizes cell divisions and cell shape in the growing Drosophila wing disc." *Development* 140(19): 4051-4059.

# Short-Comings of Tissue Mechanics Models

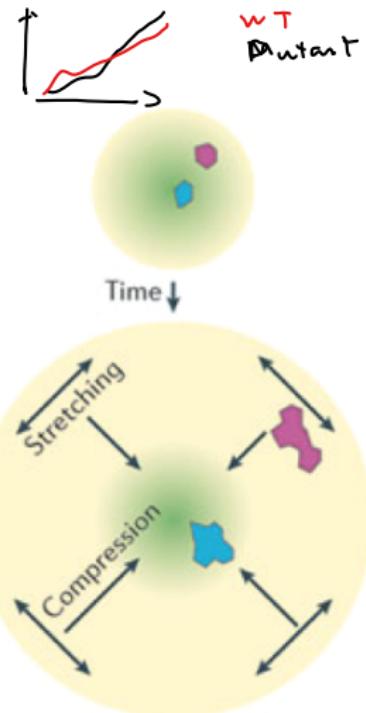
*The central problem*

- Current models require **enhanced medial growth as a result of Morphogen Signalling**. However, normal growth during latter half of larval development even when Dpp is removed using CRISPR-Cas9 genome editing

(Akiyama, T. and M. C. Gibson (2015). "Decapentaplegic and growth control in the developing Drosophila wing." *Nature* 527(7578): 375-378.)

- Current models cannot recapitulate the measured growth kinetics

(Aegerter-Wilmsen, T., et al. (2012). "Integrating force-sensing and signaling pathways in a model for the regulation of wing imaginal disc size." *Development* 139(17): 3221-3231.).



## Current Status: Certain ideas have been ruled out

- Growth control by **counting cell divisions** ⇒ enhancing or blocking cell divisions does not alter final wing disc size
- Growth control by **surrounding tissue / larvae** ⇒ same size if discs develop outside larvae
- Growth control **based on developmental time** ⇒ developmental delays do not matter for final size
- Growth Control solely by **cell differentiation**
- Growth control by **steepness of linear morphogen gradients** ⇒ Dpp gradient is of exponential shape
- Growth control by **relative change of Dpp amplitude** ⇒ Dpp signalling not necessary for normal growth; imperfect scaling of the Dpp gradient
- Growth control **based on combined Dpp morphogen signalling and mechanical feedback** ⇒ Dpp signalling not necessary for normal growth.

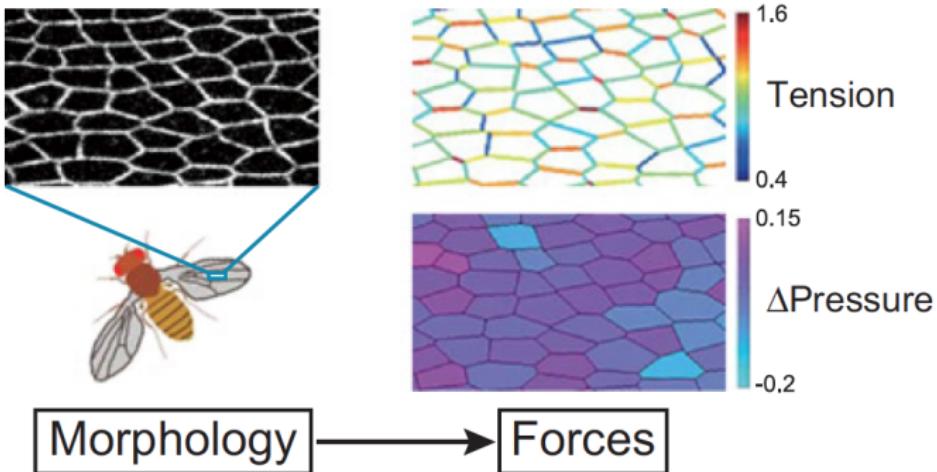
## Current Status: Possible Mechanisms

- Growth Control by Dilution: can explain growth behaviour in the *Drosophila* eye disc, but not in the wing disc
- some form of mechanical feedback

## **Inference of cellular properties from images**

# Inference of cellular properties from images

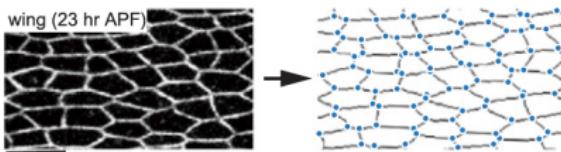
## Estimating the dynamics of forces



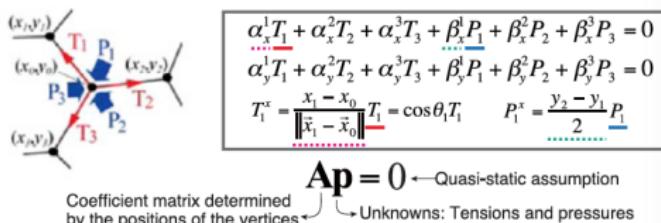
Ishihara, S. & Sugimura, K. (2012) Bayesian inference of force dynamics during morphogenesis. J Theor Biol 313, 201-211

# Inference of cellular properties from images

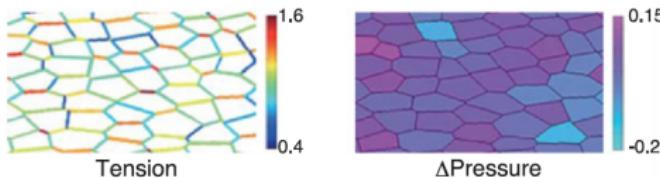
## B1 Input: an image of cells (positions of vertices)



## B2 Balance of forces at each vertex



## B3 Output: relative values of tension and pressure



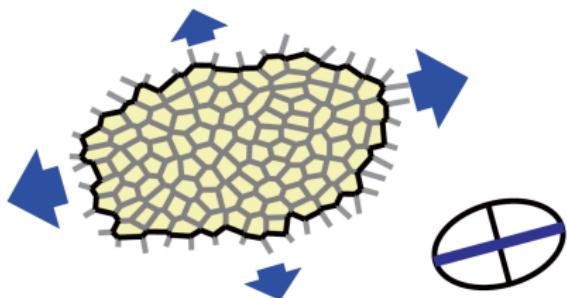
Ishihara, S. & Sugimura, K. (2012) Bayesian inference of force dynamics during morphogenesis. J Theor Biol 313, 201-211

# Batchelor Stress Tensor

$$\sigma = \frac{1}{A} \left( - \sum_i P_i A_i \mathbf{I} + \sum_{[ij]} T_{ij} \frac{\mathbf{r}_{ij} \otimes \mathbf{r}_{ij}}{|\mathbf{r}_{ij}|} \right) \quad (6)$$

where  $\mathbf{I}$  is the two-dimensional identity matrix and  $A = \sum_i A_i$  is the total tissue area.

Note that the scale of  $\sigma$  is undetermined. But quantities like the maximal stress direction can be obtained.

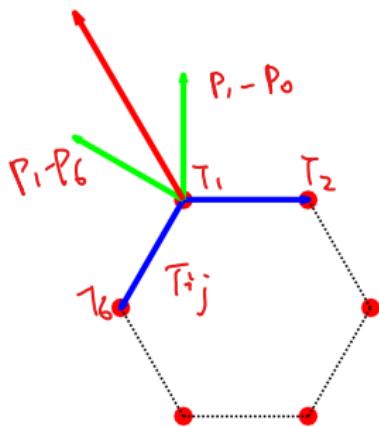


$$N_{xx} = \left[ - \sum_{cell:i} \Delta P_i A_i + \sum_{edge:j} T_j \frac{\Delta x_j \Delta x_j}{|\Delta \vec{x}_j|} \right] / \sum_{cell:i} A_i$$

$$N_{yy} = \left[ - \sum_{cell:i} \Delta P_i A_i + \sum_{edge:j} T_j \frac{\Delta y_j \Delta y_j}{|\Delta \vec{x}_j|} \right] / \sum_{cell:i} A_i$$

$$N_{xy} = N_{yx} = \left[ \sum_{edge:j} T_j \frac{\Delta x_j \Delta y_j}{|\Delta \vec{x}_j|} \right] / \sum_{cell:i} A_i$$

# Forces acting at a given node



$\vec{r}_i^\perp = (-y_i, x_i)$ :  
orthogonal vector

One vertex

- $\vec{r}_i = (x_i, y_i)$ : positions of vertices
- $T_{ij}$ : tension of the vertex that connects the  $i$ -th and  $j$ -th vertices
- $P_i$ : pressure of the  $i$ -th cell

Over-determined system

?

too many sol. for

( $P_i$ )

$$\vec{F} = \frac{\vec{r}_2 - \vec{r}_1}{\|\vec{r}_2 - \vec{r}_1\|} T_1 + \frac{\vec{r}_6 - \vec{r}_1}{\|\vec{r}_6 - \vec{r}_1\|} T_6 + \frac{1}{2} \left( \frac{[\vec{r}_2 - \vec{r}_1]^\perp}{\|\vec{r}_2 - \vec{r}_1\|} \|\vec{r}_2 - \vec{r}_1\| (P_1 - P_0) \right) + \frac{1}{2} \left( \frac{[\vec{r}_1 - \vec{r}_6]^\perp}{\|\vec{r}_1 - \vec{r}_6\|} \|\vec{r}_1 - \vec{r}_6\| (P_1 - P_0) \right)$$

NO Area !

Area

pressure

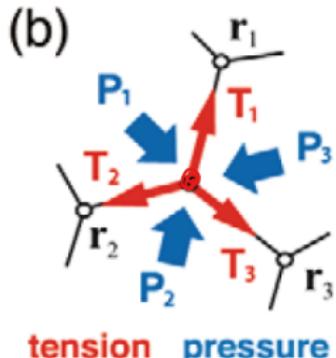
# Forces: component notation

- $r_i = (x_i, y_i)$ : positions of vertices
- $T_{ij}$ : tension of the vertex that connects the  $i$ -th and  $j$ -th vertices
- $P_i$ : pressure of the  $i$ -th cell

Let us consider the force acting on the 0-th vertex at the origin  $r_0 = (0, 0)$ . The forces in the  $x$  and  $y$  directions are given by

$$F_0^x = \sum_{i=1}^3 \frac{x_i}{|\vec{r}_i|} T_i - \sum_{i=1}^3 \frac{y_i}{2} (P_i - P_{i+1})$$

$$F_0^y = \sum_{i=1}^3 \frac{y_i}{|\vec{r}_i|} T_i + \sum_{i=1}^3 \frac{x_i}{2} (P_i - P_{i+1})$$



Ishihara, S. et al. (2013) The European physical journal. E, Soft matter 36, 9859

# Forces: component notation

# conditions > # cells

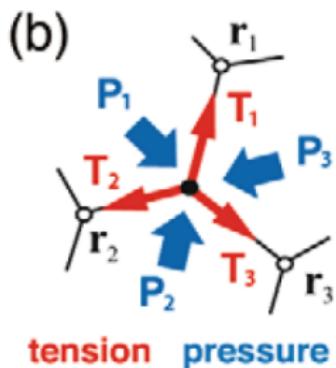
$$F_0^x = \sum_{i=1}^3 \frac{x_i}{|\vec{r}_i|} T_i - \sum_{i=1}^3 \frac{y_i}{2} (P_i - P_{i+1})$$

$$F_0^y = \sum_{i=1}^3 \frac{y_i}{|\vec{r}_i|} T_i + \sum_{i=1}^3 \frac{x_i}{2} (P_i - P_{i+1})$$

Orientation of the edge:  $\theta_{ij} = \tan^{-1} \left( \frac{y_{ij}}{x_{ij}} \right)$

$$\Rightarrow \frac{x_i}{|\vec{r}_i|} = \cos(\theta_{ij}); \quad \frac{y_i}{|\vec{r}_i|} = \sin(\theta_{ij})$$

Pressures act along the sides of the cells in their normal direction. Projection to the x- and y-axes gives the pre-factors  $-y_i$  and  $x_i$ , respectively, of the normal force. Half of the force acts on each end-point.



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# Full system of Equations

Repeating the same derivation of force-balance equations for every vertex, we obtain a vector

$$\vec{F} = (\vec{F^x}, \vec{F^y})^T \quad (7)$$

that represents the forces acting on vertices in the  $x$  and  $y$  directions as

$$\vec{F} = \mathbf{A_T} \vec{T} + \mathbf{A_P} \vec{P} = \mathbf{A} \vec{X}. \quad (8)$$

Here,  $\vec{T}$  and  $\vec{P}$  are column vectors composed of  $T_{ij}$  and  $P_i$ , respectively.  $\vec{X} = (\vec{T}, \vec{P})^T$  represents the unknown variables to be inferred.  $\mathbf{A_T}$  and  $\mathbf{A_P}$  represent matrices with the coefficients of  $T_{ij}$  and  $P_i$ .

# Dimension of Full system of Equations

$$\vec{F} = \mathbf{A}_T \vec{T} + \mathbf{A}_P \vec{P} = \mathbf{A} \vec{X}.$$

Suppose we have an image, in which  $N$  cells are surrounded by  $R$  cells:

- $E$ : numbers of cell contact surfaces (edges)
- $V$ : number of vertices

## Dimensions

- $\vec{T}$ :  $[E \times 1]$ ,  $\vec{P}$ :  $[N + R \times 1]$ ,  $\vec{X}$ :  $[E + N + R \times 1]$
- $\vec{F}$ :  $[2V \times 1]$
- $\mathbf{A}_T$ :  $2V \times E$ ,  $\mathbf{A}_P$ :  $[2V \times (N + R)]$ ,  $\mathbf{A}$ :  $[2V \times (E + N + R)]$

# Quasi-steady State Assumption

Under the assumption of quasi-static cell shape changes, the force-balance equation becomes

$$\vec{F} = \mathbf{A}_T \vec{T} + \mathbf{A}_P \vec{P} = \mathbf{A} \vec{X} = 0 \quad (9)$$

This gives us a relationship between the observable geometry (angles and lengths) of cells and the unknown tensions  $\vec{T}$  and pressures  $\vec{P}$  to be determined.

Note that we can only determine relative forces and pressure differences.

$T_{i+1}$   
 $P_i - P_{i+1}$

# Overdetermined System

$$\vec{F} = \mathbf{A}_T \vec{T} + \mathbf{A}_P \vec{P} = \mathbf{A} \vec{X} = 0 \quad (10)$$

Number of unknowns = length of  $\vec{X}$  = number of cells ( $N + R$ ) plus  
cell contact surfaces ( $E$ )

**why?**

Number of conditions =  $2 \times$  number of vertices

$$\begin{cases} F_x = T \\ F_y = \end{cases}$$

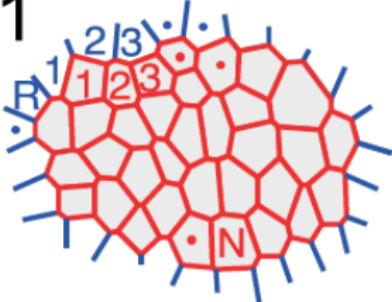
Number of unknowns is **smaller** than the number of the conditions for  
a sufficient number of cells.

To get plausible and unique estimates, the inverse problem must be  
formulated to handle this indefiniteness.



# The Inverse Problem

C1

 $N + R$  cells $e + 2R$  edges

$$e = 3(v + f)/2 - f$$

$$v - e + N = 1$$

 $v + R$  vertices $f$  four-way junctionsequality for  $e, v$ , and  $f$ 

topological constraint

→  **$R + 1 + f$  indefiniteness**  
*(boundary + Phs + four-way j)*

C2 Inverse problem

$$P(\mathbf{p}|\mathbf{b}) \propto P(\mathbf{b}|\mathbf{p}) \pi_T(\mathbf{p})$$

Posterior distribution

Likelihood

Prior

$$\exp\left[-\frac{1}{2\sigma^2}\|\mathbf{Ap} - \mathbf{b}\|^2\right]$$

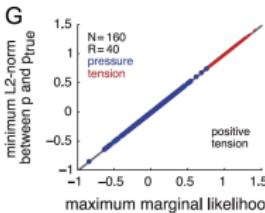
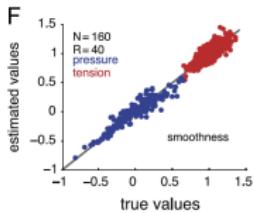
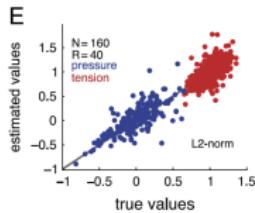
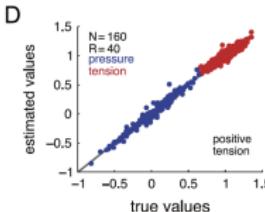
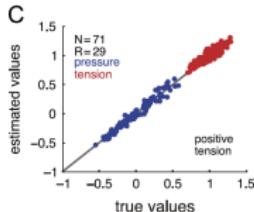
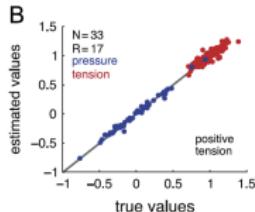
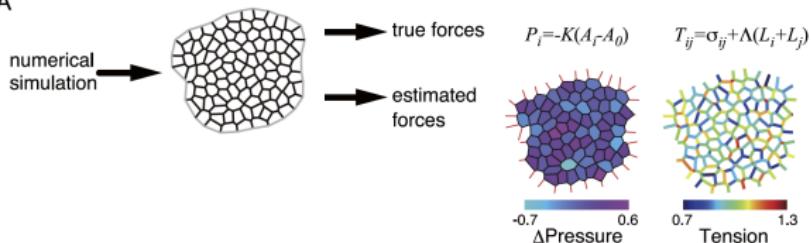
Force balance

$$\exp\left[-\frac{1}{2\omega^2}\sum_{[ij]}\left(T_{ij} - T_e\right)^2\right]$$

Positive tension

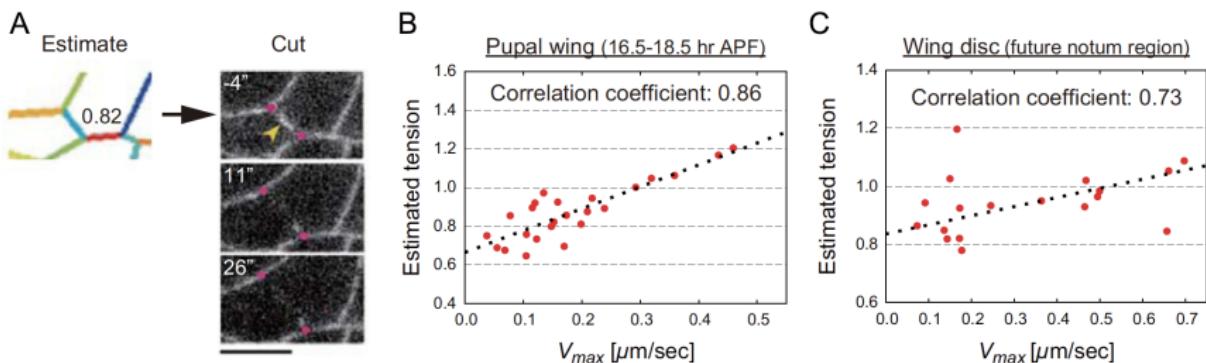
# Estimated relative pressures and tensions against their true values

A



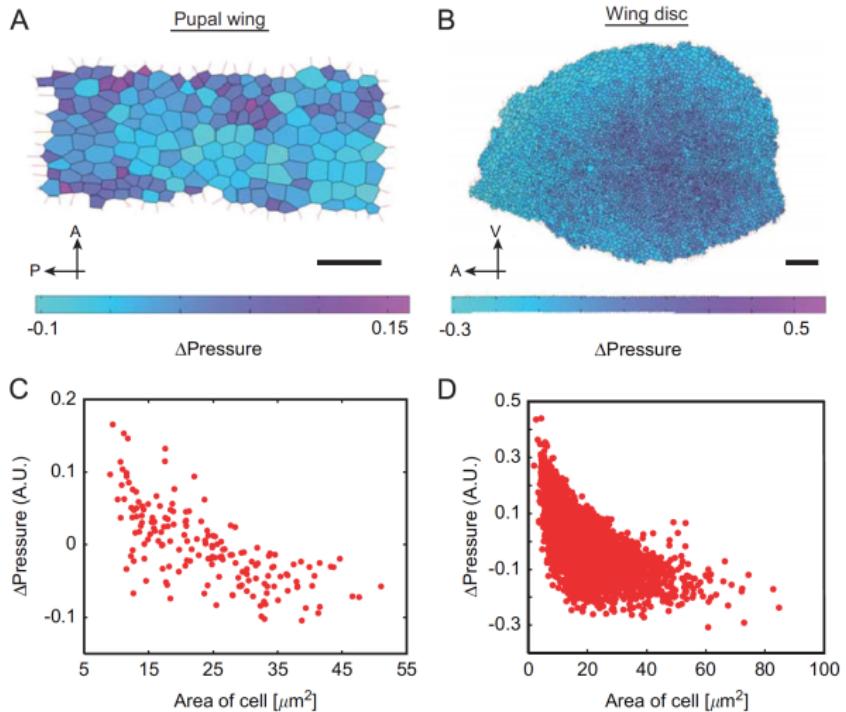
Ishihara, S. & Sugimura, K.  
Bayesian inference of force dynamics during morphogenesis. J Theor Biol 313, 201-211,  
doi:10.1016/j.jtbi.2012.08.017  
(2012).

# In vivo Validation



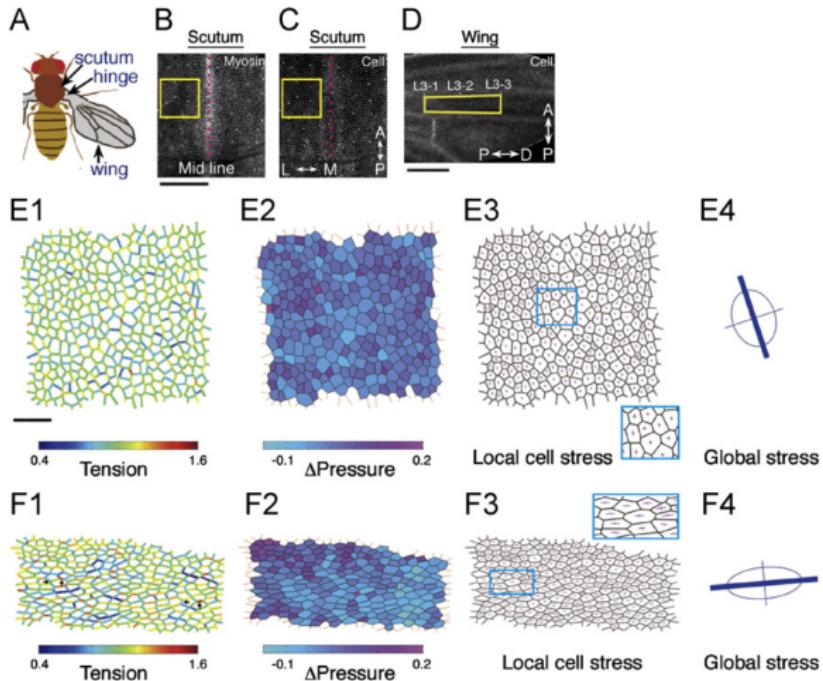
Ishihara, S. & Sugimura, K. Bayesian inference of force dynamics during morphogenesis. J Theor Biol 313, 201-211, doi:10.1016/j.jtbi.2012.08.017 (2012).

# Inferred Pressure & Tension in the Wing Disc



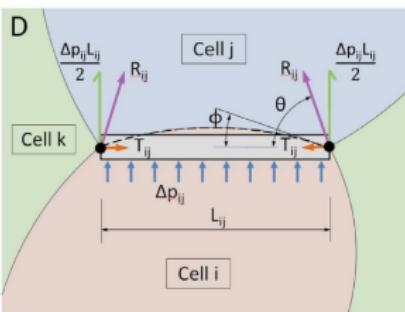
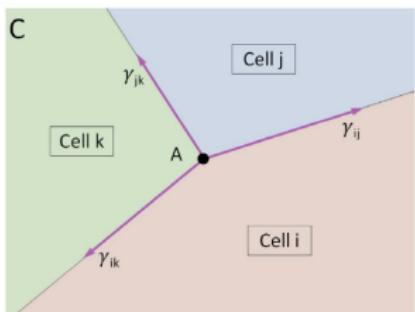
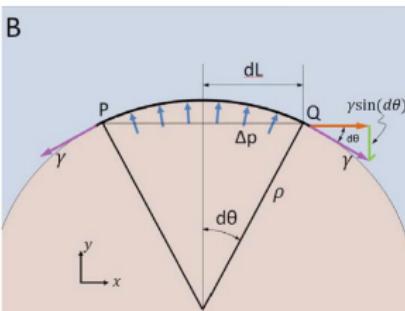
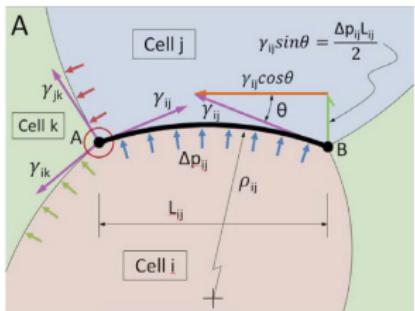
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# Inferred Pressure & Tension in the Wing Disc



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 (2012).

# CELLFit: Segment also Curvature of Membranes



**Laplace Equation:**

$$\Delta p_{ij} = \frac{\gamma_{ij}}{\rho_{ij}} \quad (11)$$

$\rho_{ij}$ : radius of curvature

$\gamma_{ij}$ : tension

Brodland, G. W. et al (2014) Plos One

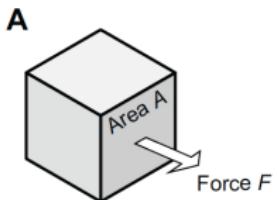
unstable .

# Summary

## Inference of Epithelial Properties:

- Images of epithelia can be used to infer hydrostatic pressure and membrane tension
- Challenge to deal with overdetermined system and low quality data
- What is the correct model?

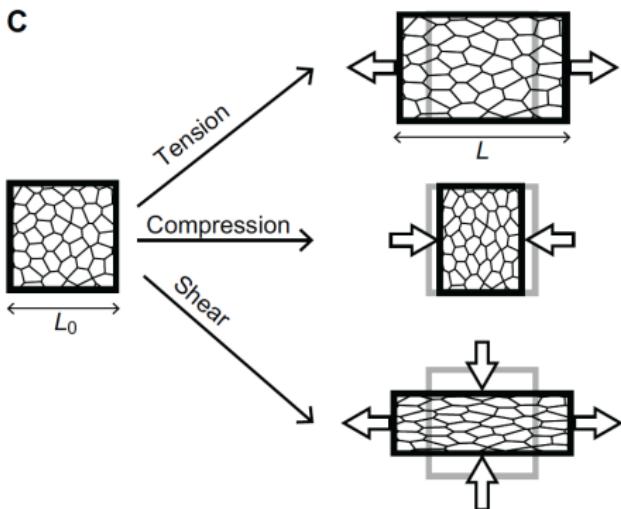
# Definition of mechanical terms



**B**

$$\text{Stress } \sigma = \frac{F}{A}$$

$$\text{Deformation } \epsilon = \frac{L-L_0}{L_0}$$



**Stress.** A coarse-grained description of the forces within a tissue. When a piece of tissue experiences a force from a neighboring tissue region (A), mechanical stress ( $\sigma$ ) is defined as the ratio of the force ( $F$ ) to the area of contact ( $A$ ) with that region (B, top equation).

**Tension** and **compression** correspond to forces pointing respectively outwards from and inwards to the body.

**Deformation** (also called 'strain') is the relative change in size of an object subjected to a force. In one dimension, it is a dimensionless number,  $\epsilon$  (B, bottom equation): the fraction of change in the object length (where  $L$  is the new length and  $L_0$  the original length), which would be positive for elongation or negative for contraction.

# Thanks!!

**Thanks for your attention!**

Slides for this talk will be available at:

<http://www.bsse.ethz.ch/cobi/education>