The Genotype-Phenotype Map for Shape:

Insights from Morphogenesis

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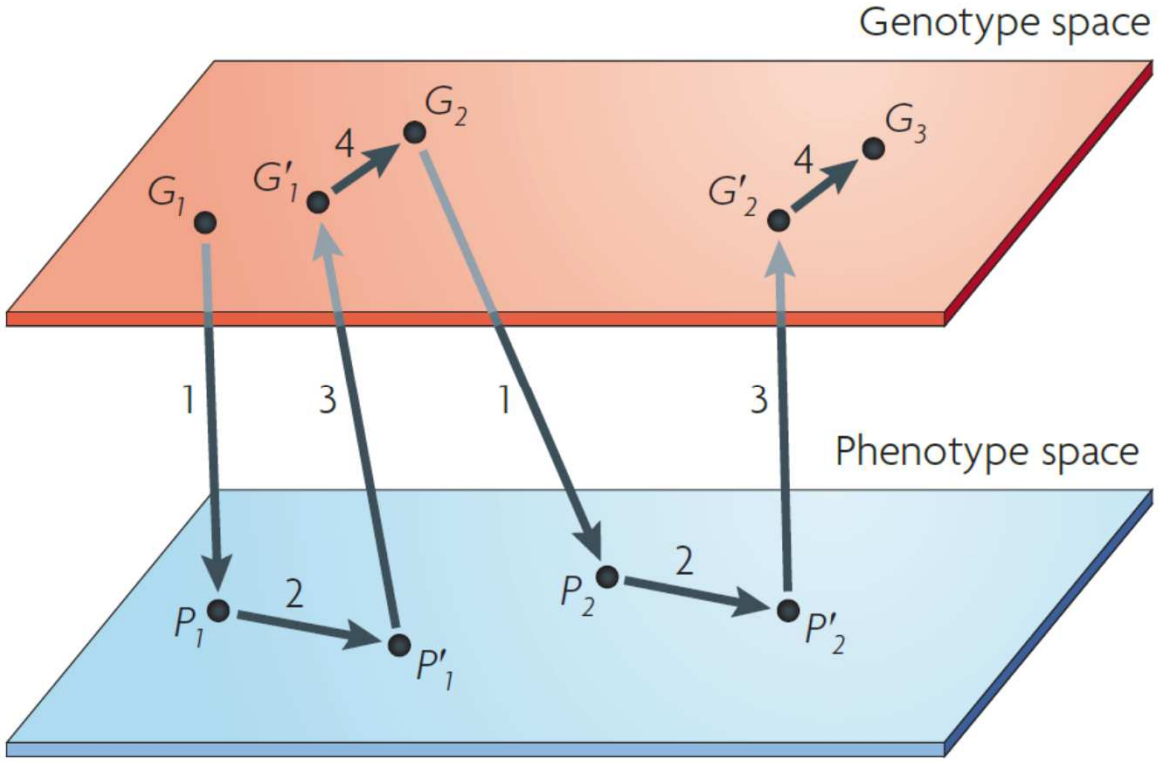
The metaphor of the genotype-phenotype map

Popularized by Richard Lewontin (1974)



1: development creates the phenotype using genetic information + the environment.

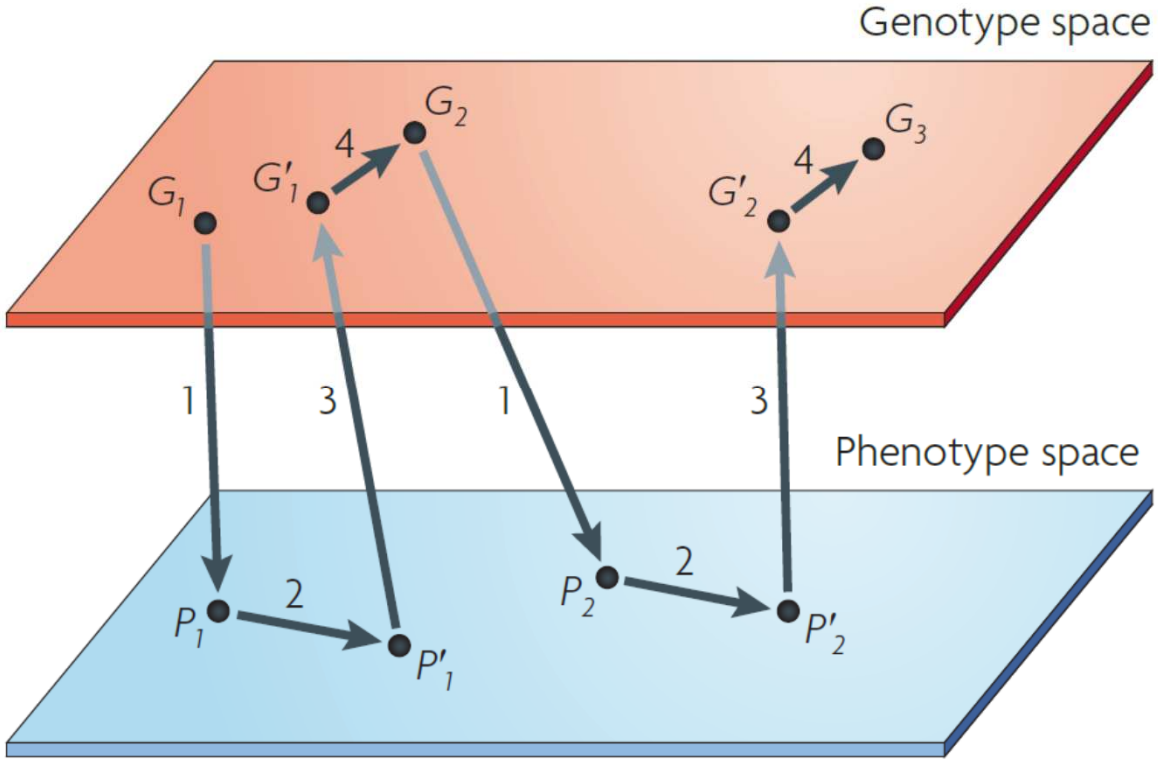
2: natural selection acts on phenotypes and alters mean position on P space.

3: the identity of phenotypes determines wich genotypes are preserved.

4: mutation and recombination alter position in G space.

Houle et al. 2010

The metaphor of the genotype-phenotype map



Genetics

Development

Ecology

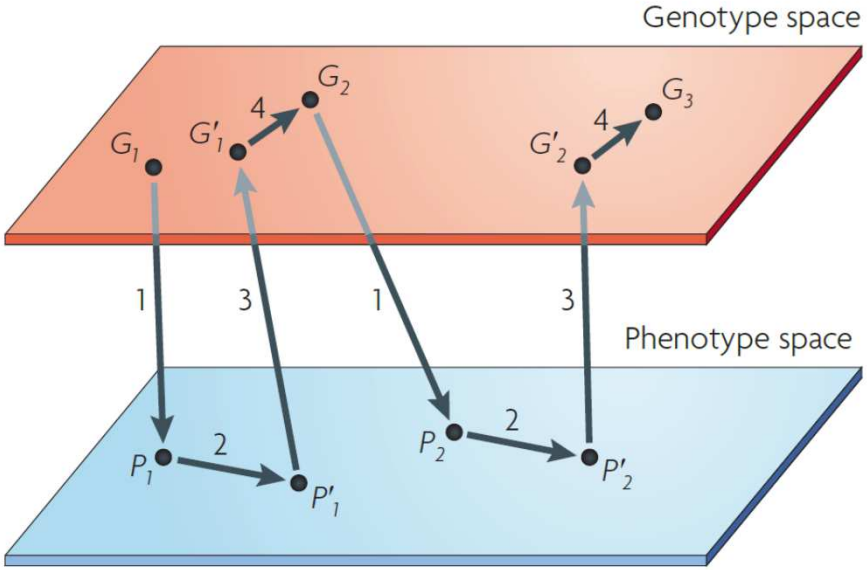
Should we care about development in the study of the genotype-phenotype map ?
(e.g. Albertson et al. 2018)

Evolutionary genetics: mapping genetics variants into phenotypic variants.

- Very successfull research program in finding where do mutation occur.
- e.g. Gephebase contains more than 1600 entries.
- But limitations

Should we care about development in the study of the genotype-phenotype map ?

- Causation vs. association
- Complex genetic architectures



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PLOS | GENETICS

Modeling 3D Facial Shape from DNA

Peter Claes[1], Denise K. Liberton[2], Katleen Daniels[1], Kerri Matthes Rosana[2], Ellen E. Quillen[2],

2014

"…our methods provide the means of identifying the genes that affect facial shape and for modeling the effects of these genes to generate a predicted face."

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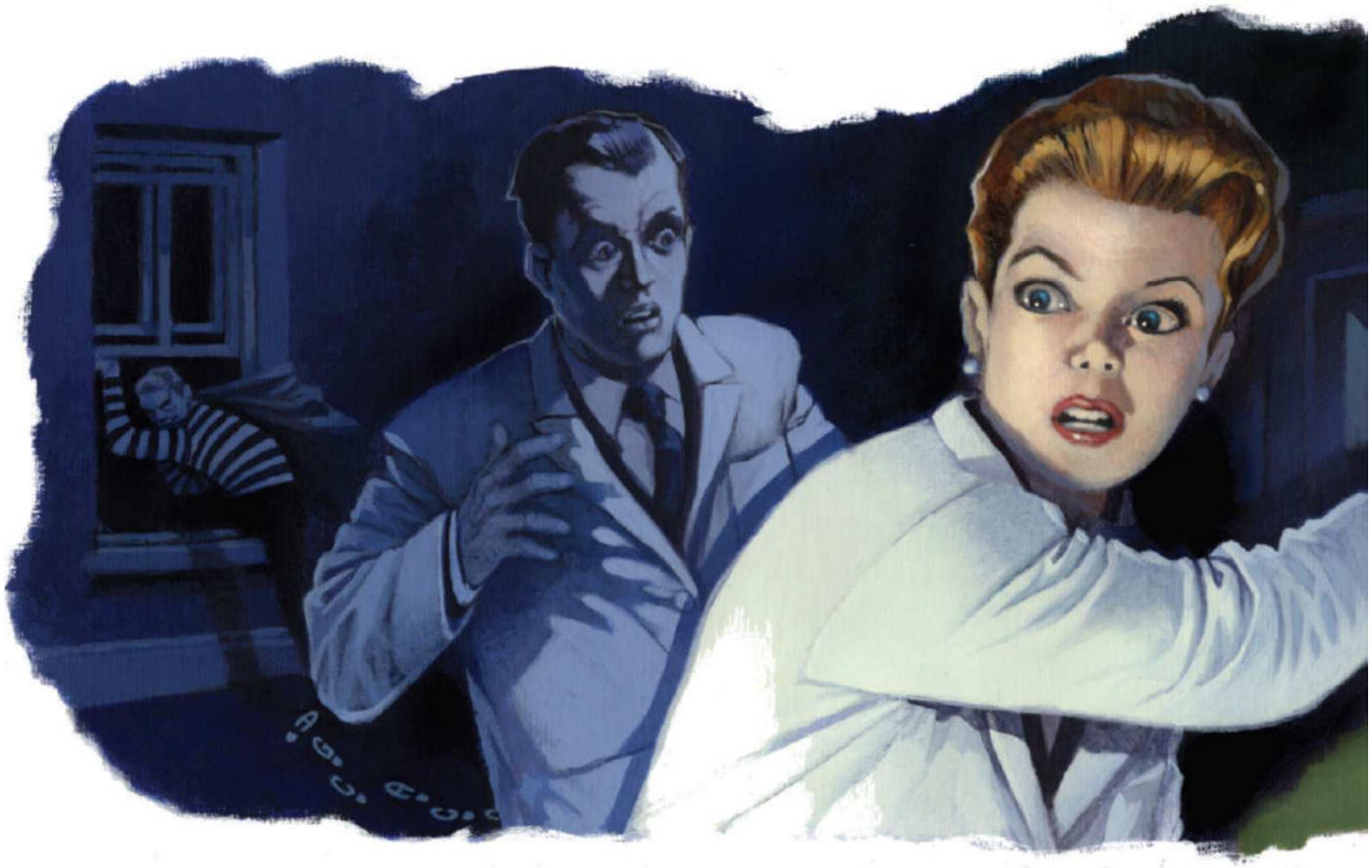
Formal Comment

Let's Face It—Complex Traits Are Just Not That Simple

Benedikt Hallgrimsson[1]*, Washington Mio[2], Ralph S. Marcucio[3], Richard Spritz[4]

"The second, and deeper, issue, though, is whether genomic prediction of complex morphologies is even feasible."



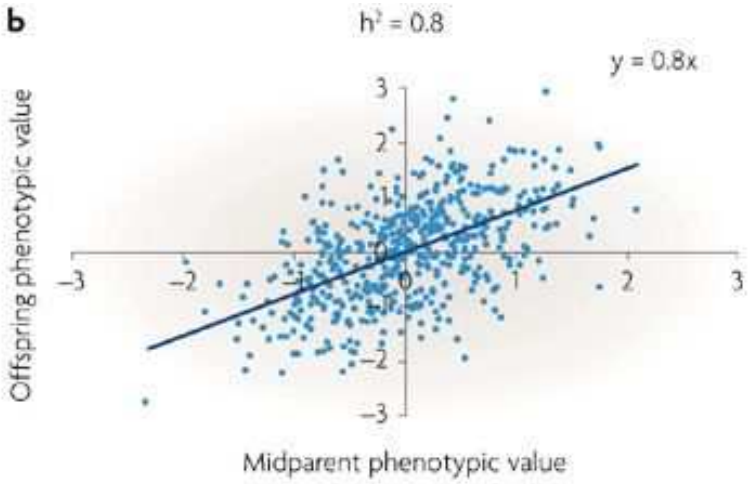
The case of the missing heritability

When scientists opened up the human genome, they expected to find the genetic components of common traits and diseases. But they were nowhere to be seen. **Brendan Maher** shines a light on six places where the missing loot could be stashed away.

Some diseases and traits are known since a long time to be highly heritable but observed genetic variation do not account for the heritability

Example: human height

Heritability = 80 %



Visscher et al. 2008

Genome-wide data from 253,288 individuals

- 697 loci associated with human height variation
- Explain 16% of the phenotypic variance
- Account for 25 % of the heritability

Wood et al. 2014

Genome-wide data from 253,288 individuals

Re-analysis by Boyle et al. 2017

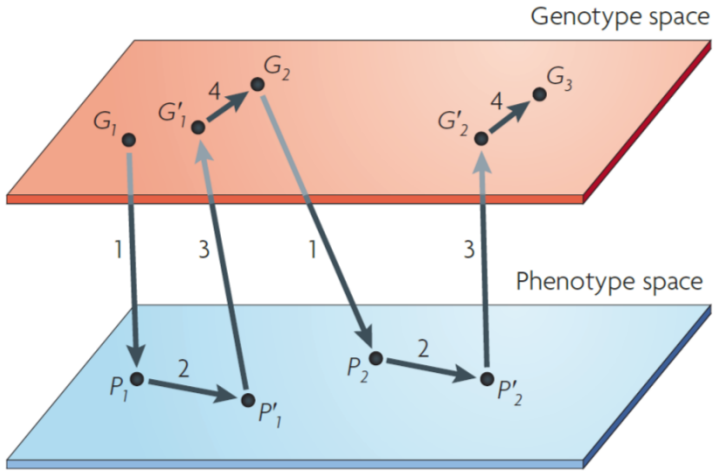
- More than 100,000 SNPs exert independent causal effects on height
- Causal variants are spread very widely across the genome
- A substantial fraction of all genes contribute to trait variation

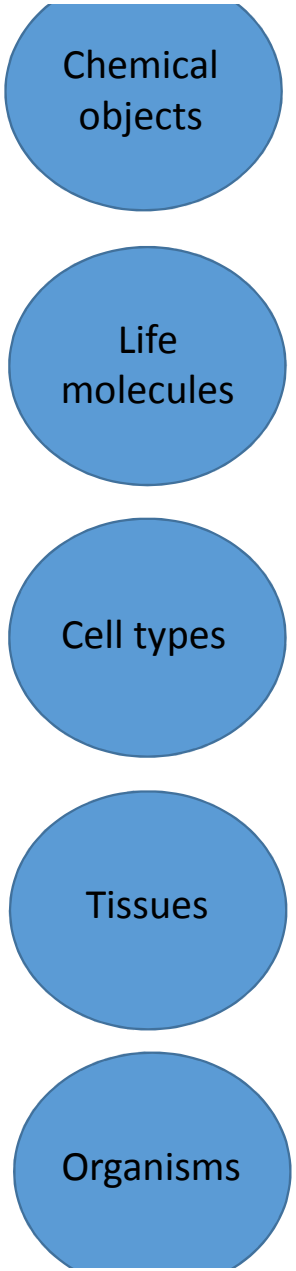
(i.e. not specific biologically relevant genes and pathways)

Traits having complex genetic architecture make it very difficult to predict phenotypic variation on the basis of genetic variation

One possible solution to this problem:

Look at another level of the genotype-phenotype map





Biology [..] deals with a complex hierarchy of objects ranging from cells to populations [...] The objects which exist at each level constitute a limitation of the total possibilities offered by the simpler level.

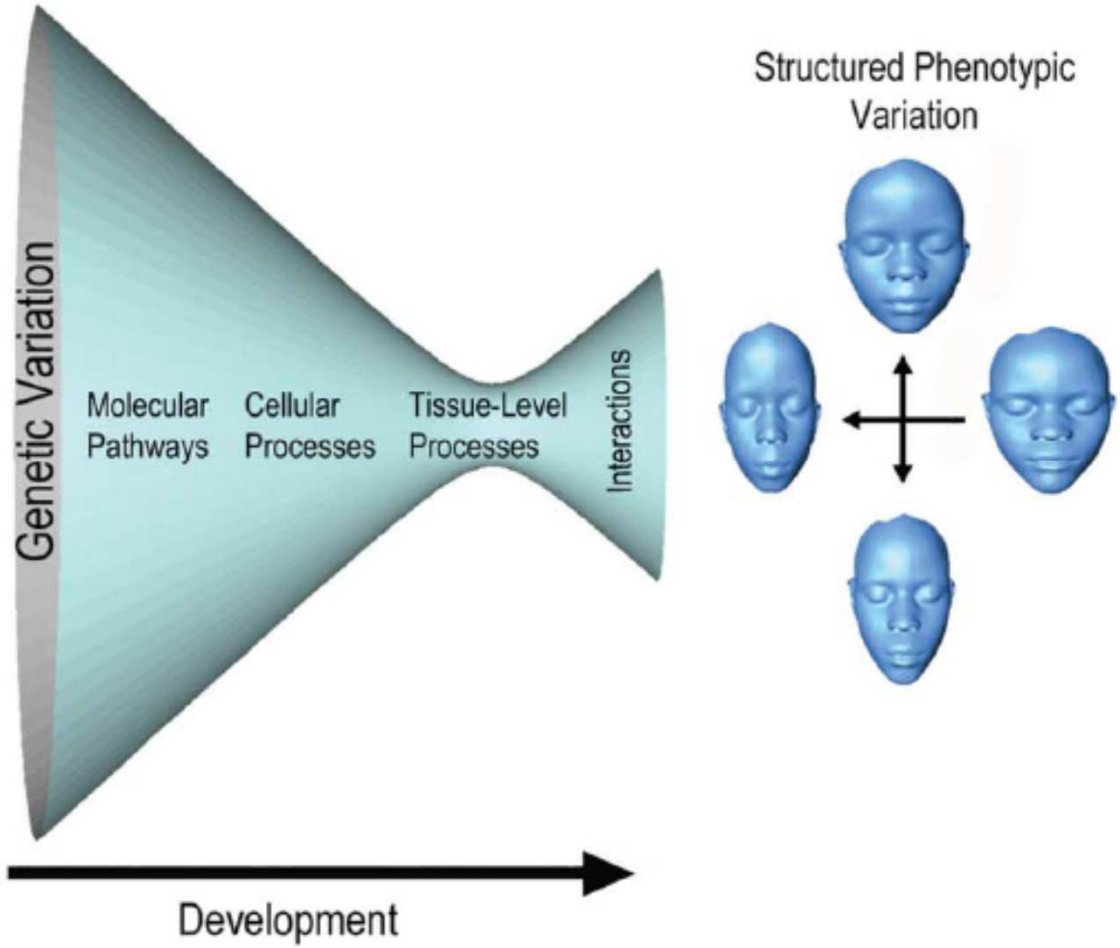
Each system at a given level uses as ingredients some systems of the simpler level, but some only. The hierarchy in the complexity of objects is thus accompanied by a series of restrictions and limitations. At each level, new properties may appear which impose new constraints on the system.

Evolution and Tinkering

François Jacob

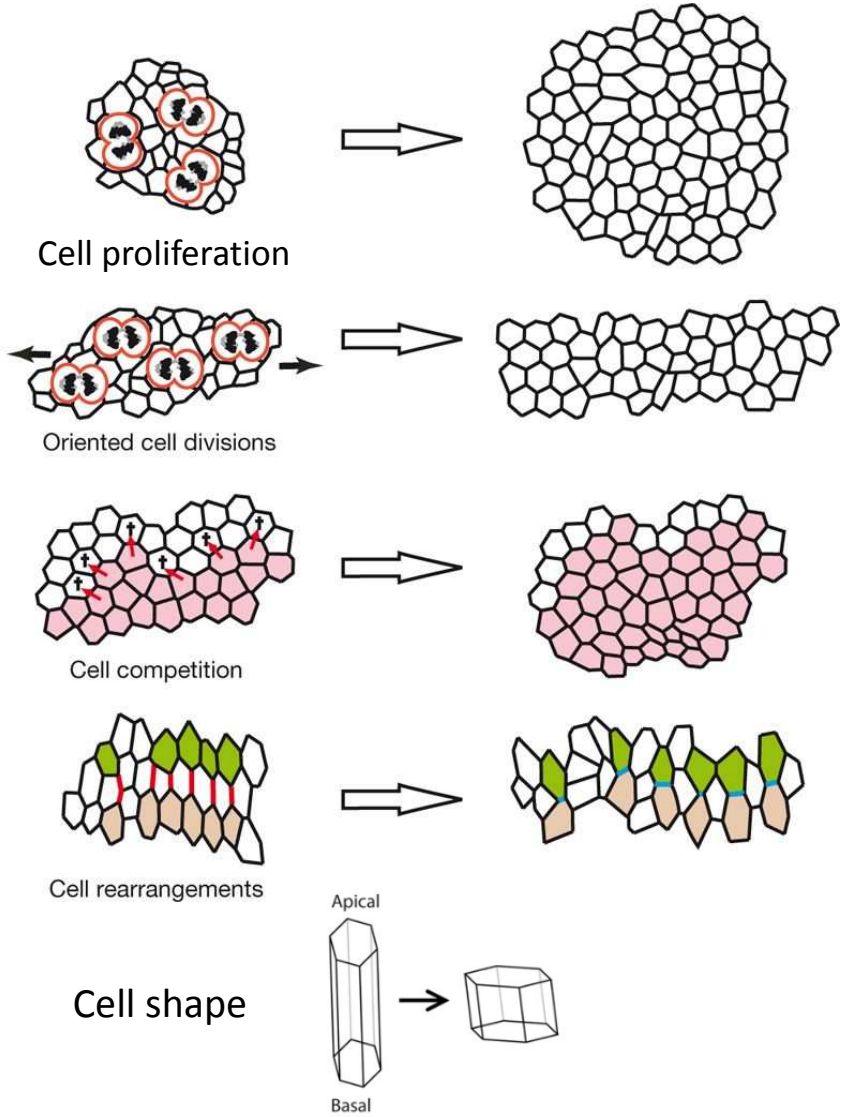
10 June 1977, Volume 196, Number 4295

SCIENCE



Hallgrimsson et al. 2014.

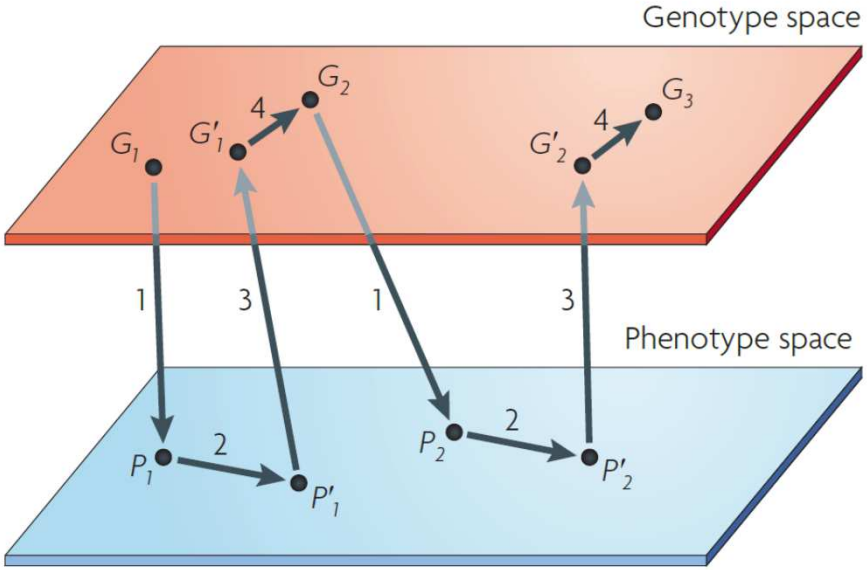
Cellular mechanisms of tissue size and shape



Lecuit and Legoff 2007

Should we care about development in the study of the genotype-phenotype map ?

- Causation vs. association
- Complex genetic architectures
- Developmental constraints

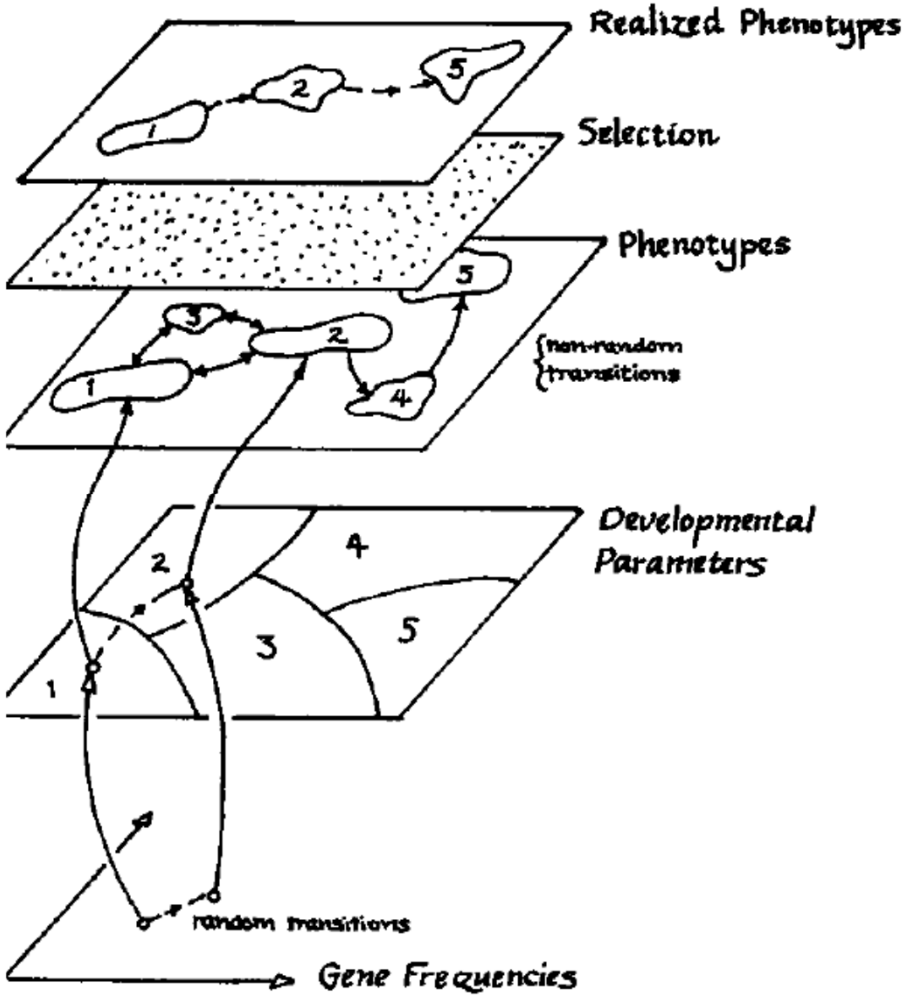


Pere Alberch
(1954 - 1998)

"Even if mutations affect the parameters randomly […] the developmental outcome will not be random."

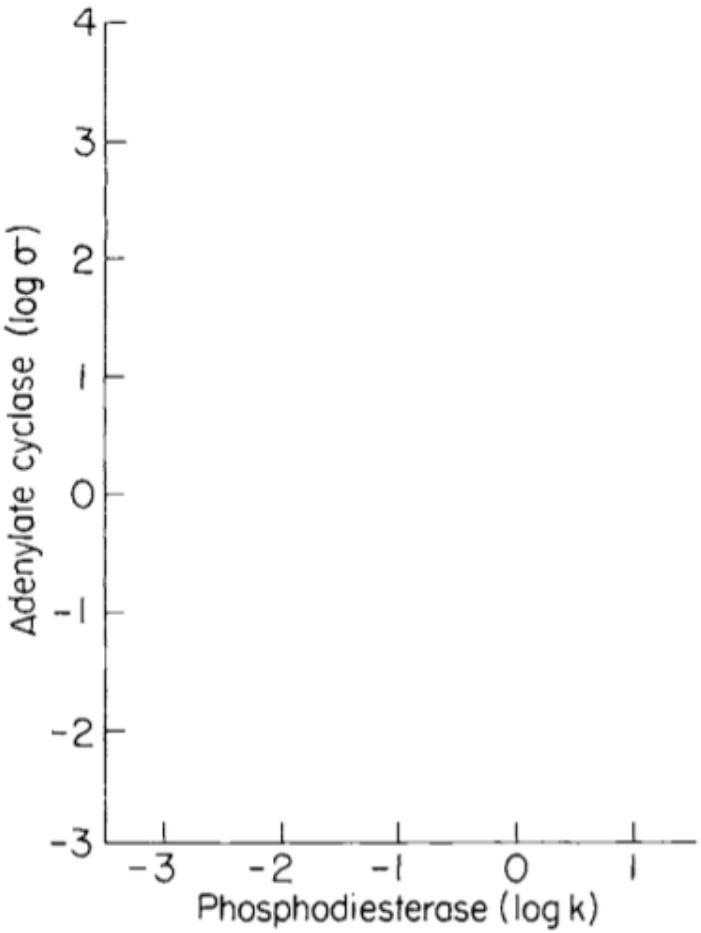
"Biases on the production of variant phenotypes or limitations on phenotypic variability caused by the structure, character, composition, or dynamics of the developmental system."

Maynard-Smith et al. 1985.

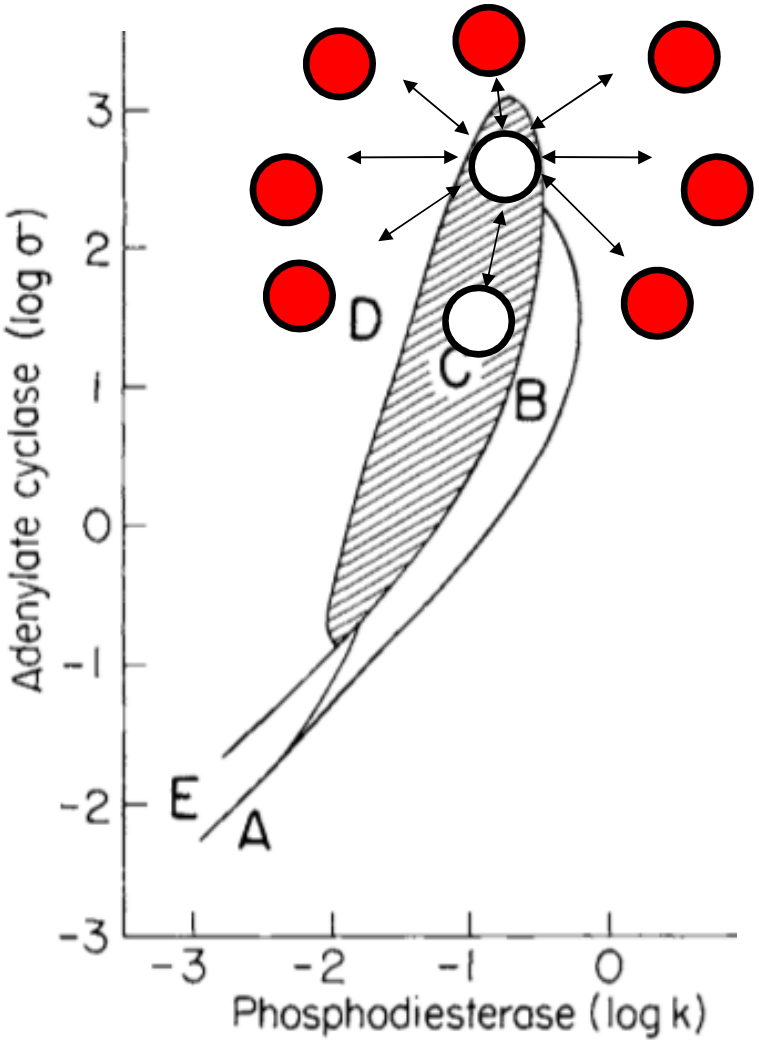


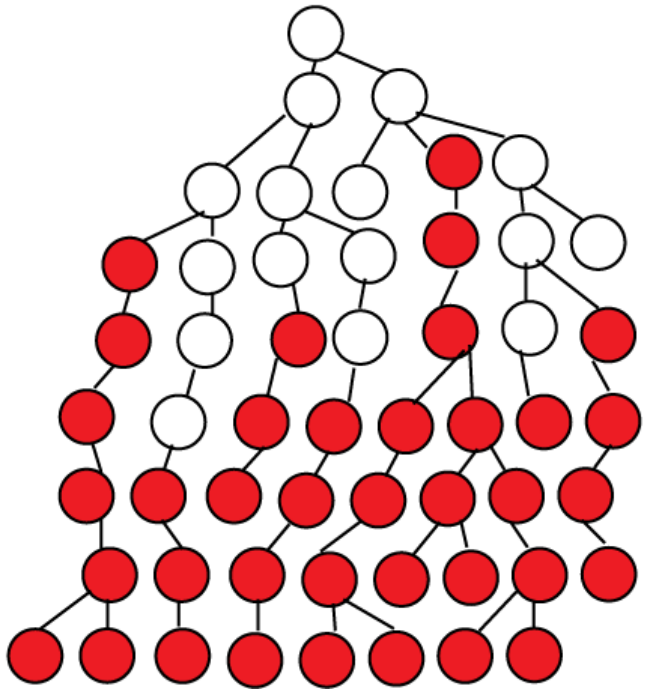
Oster & Alberch 1982

Dictyostelium discoideum



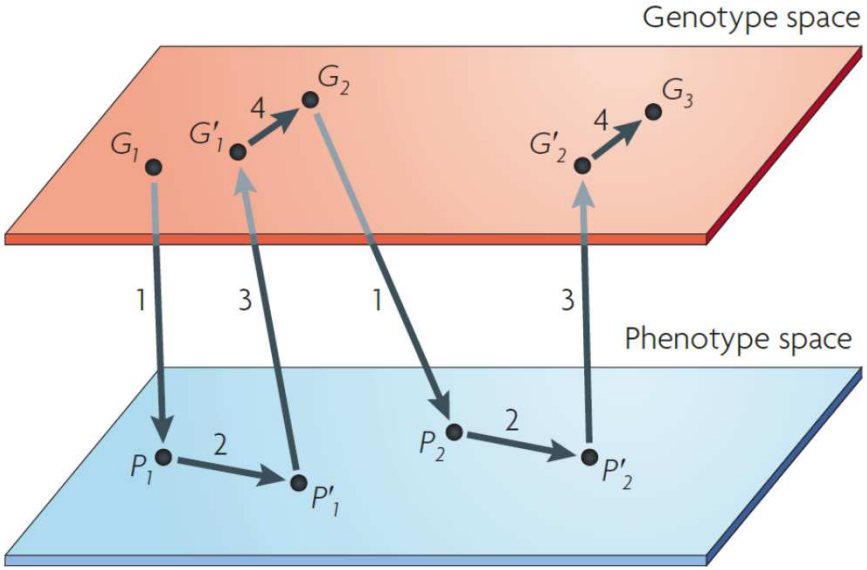
Oster & Alberch 1982

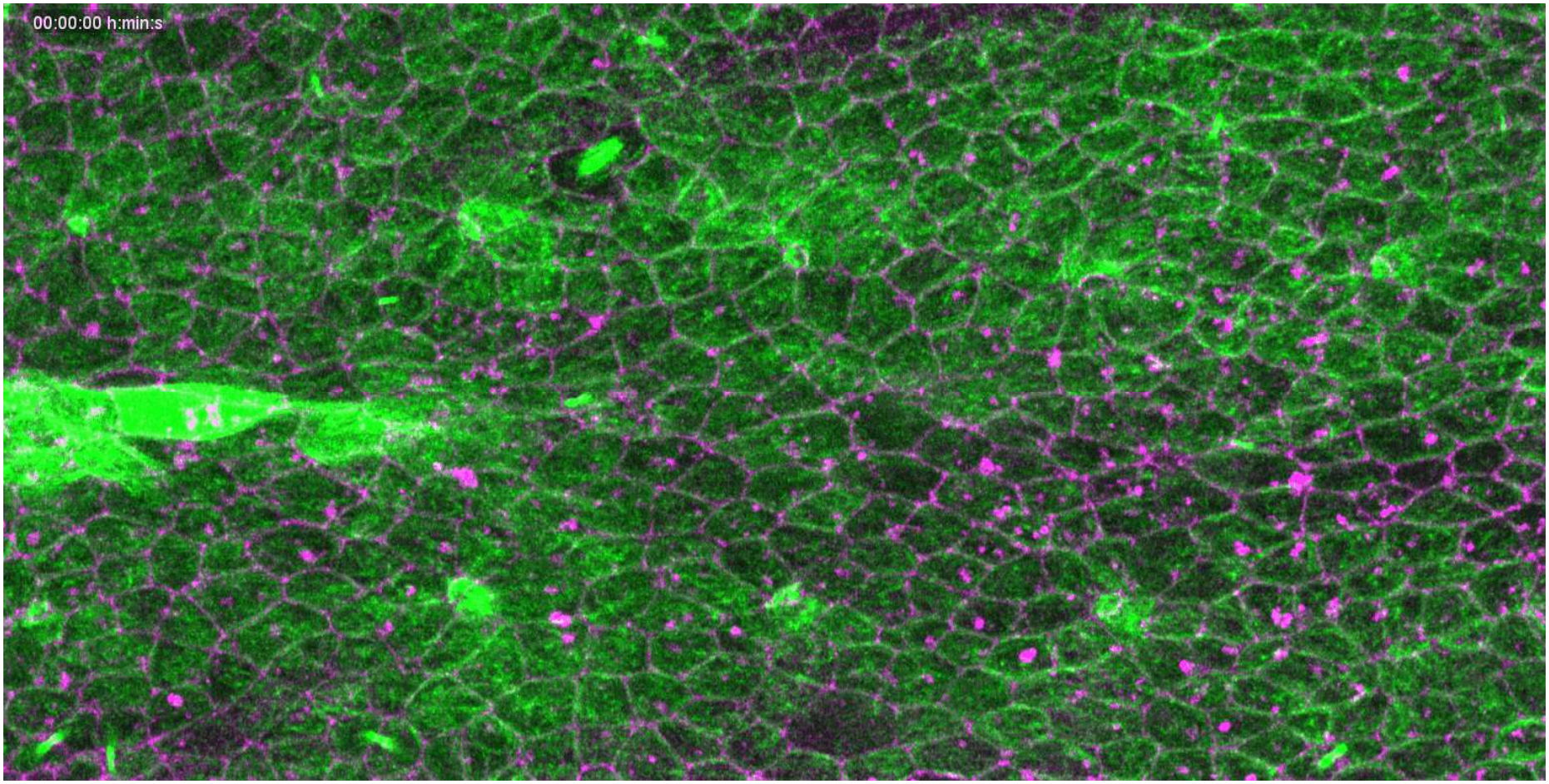

Adenylate cyclase (log σ)
3
2
1
0
-1
-2
-3
-3
-2
-1
0
Phosphodiesterase (log k)
D
C
B
E
A



Should we care about development in the study of the genotype-phenotype map ?

- Causation vs. association
- Complex genetic architectures
- Developmental constraints
- Morphogenesis is beautifull !





Alexis Villars

Should we care about development in the study of the genotype-phenotype map ?

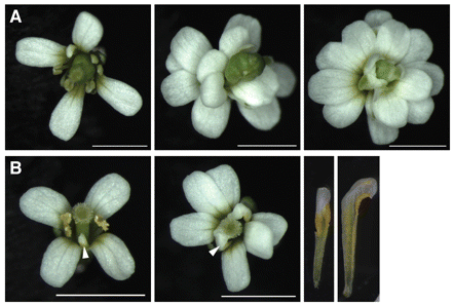
Certainly but ….

- **It remains difficult to study morphogenesis in non model species.**

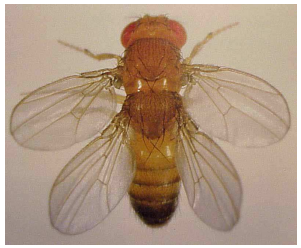
- **Developmental biology has long focused on qualitative features**

« Variation » in developmental biology

Spemann's siamese tadpole

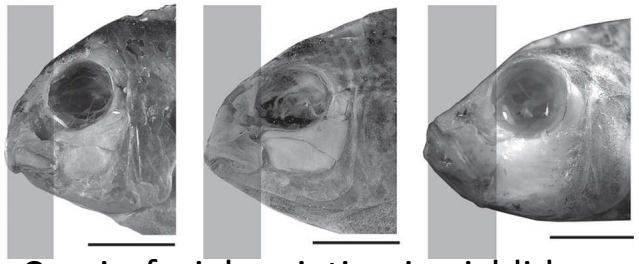


ABC model for floral organs identity



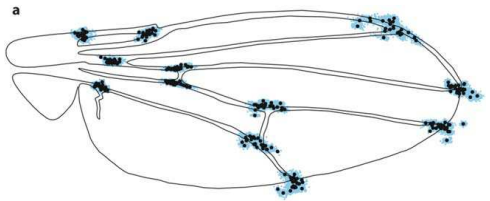
Ultrabithorax mutant fly

« Variation » in evolutionary biology



Cranio-facial variation in cichlids

Corolla shape variation in *Erysimum* sp.



Variation in *Drosophila* wing shape

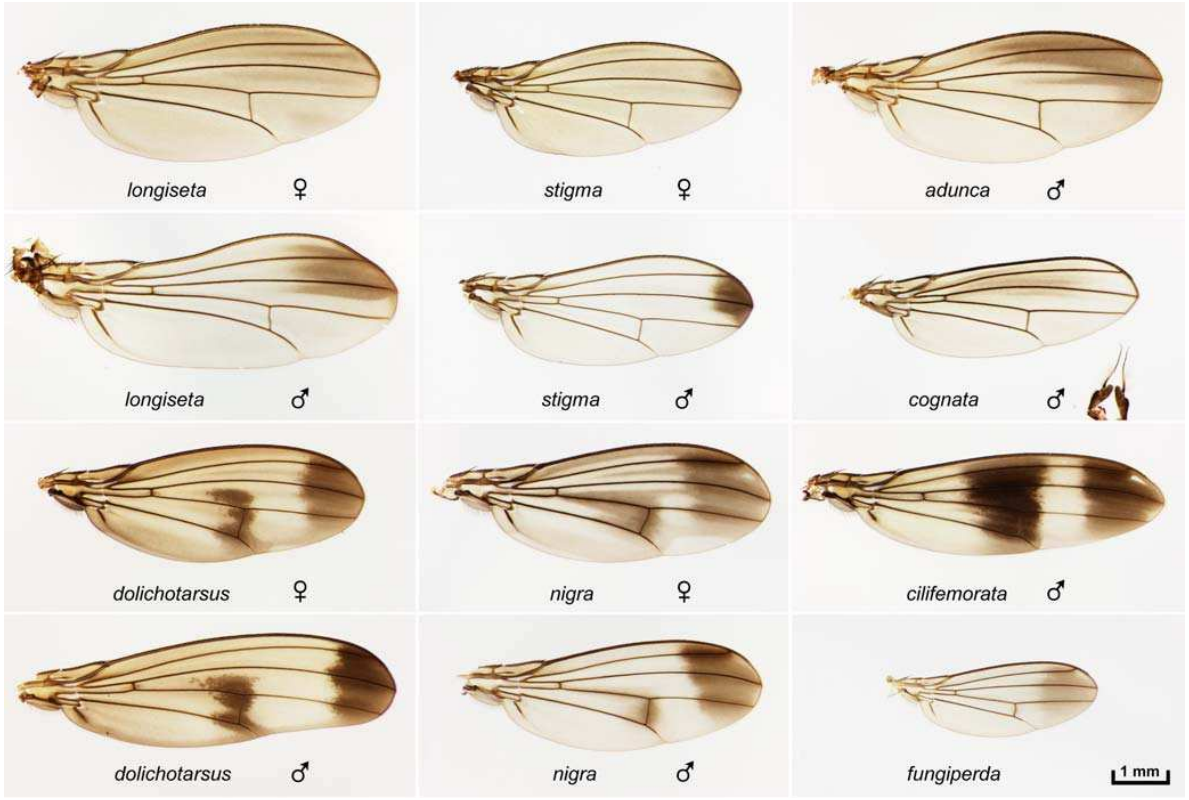
Morphogenetic bases of organ shape variation

Example 1: wing shape in Drosophila.
Example 2: tooth shape in mammals.
Example 3: ovipositor length in Drosophila.
Example 4: digit loss in mammals.

Strategy:

Understand how the organ is formed in a model species and then use this knowledge to study natural variation in this organ.

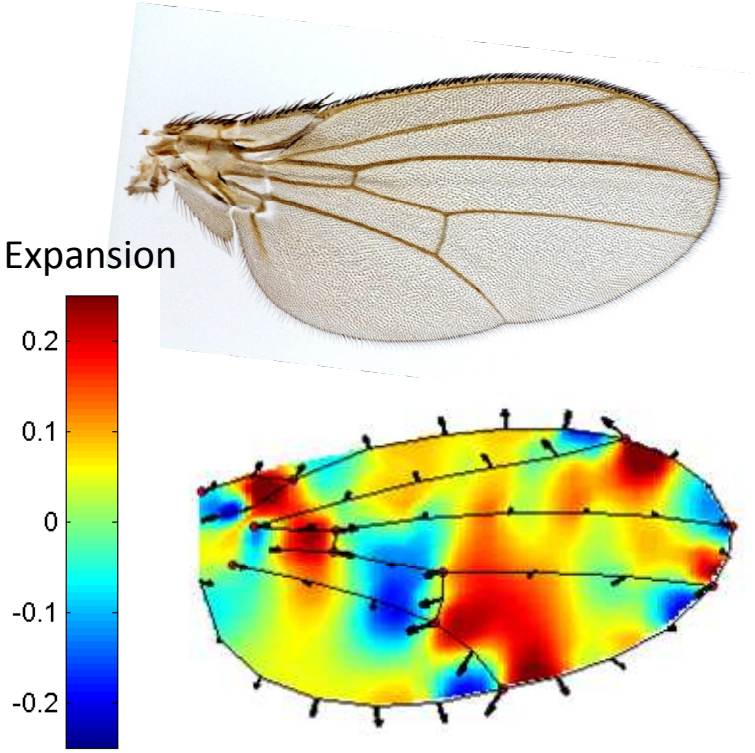
Diversity of *Drosophila* wing shape and size



Hawaiian *Drosophila* (Edwards et al 2007)

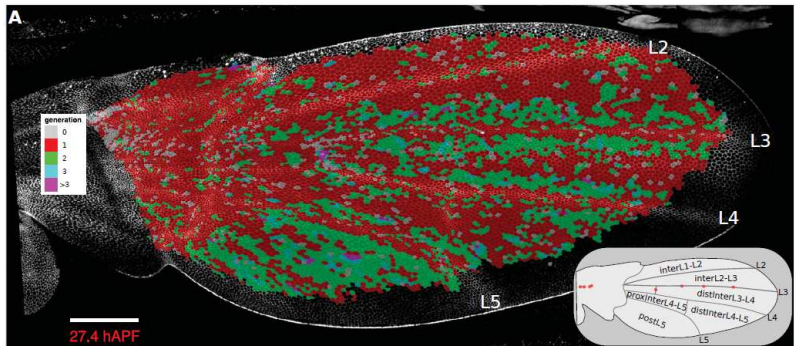
Drosophila wing shape

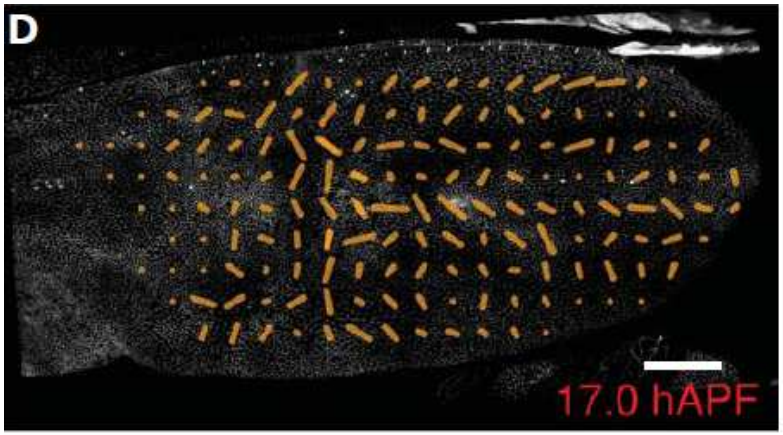
Shape variation and cellular processes can be finely quantified



Variation within a population of
D. melanogaster

David Houle lab.





Patterns of cell proliferation and of cell division orientation (22 to 31 h APF)

Etournay et al. *Elife* 2016.

Where are the QTNs for wing shape in *Drosophila* ? Dozens of QTLs have been mapped. Despite hundreds of person-years of effort and all the resources available for the preeminent model insect, there are no mapped QTNs.

The best candidate is a non-coding SNP in the promoter of the *Egfr* gene, mapped by association. This SNP explains less than 1% of the trait variance in one population and none in another and may simply be a marker in LD with the causal variant. What if *Drosophila melanogaster* wing shape is a typical complex trait?

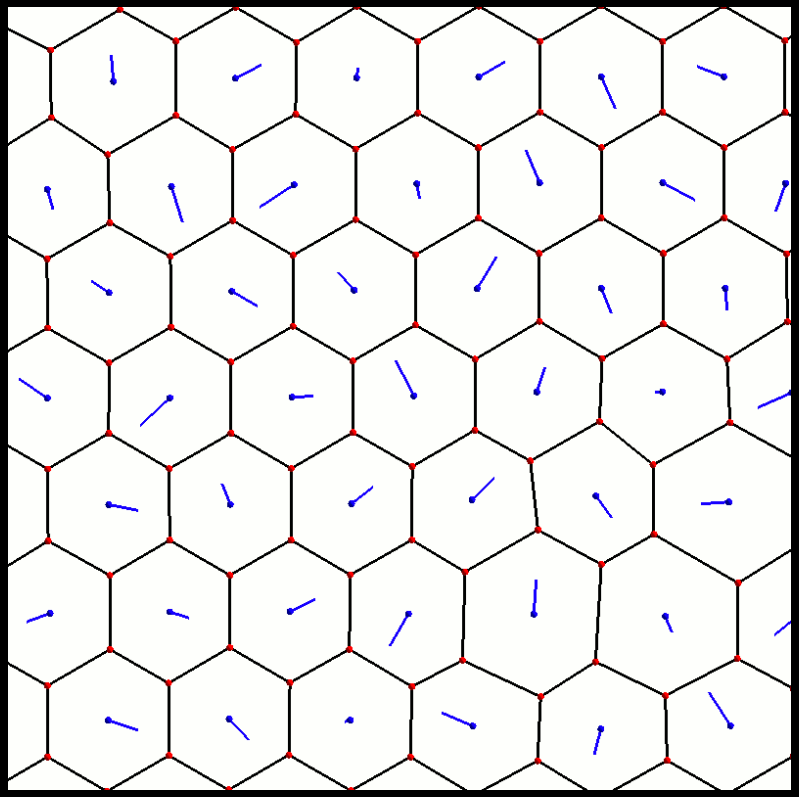
Rockman 2011

Live imaging of wing morphogenesis

Tissue Shape Changes During Hinge Contraction

Aigouy et al. 2010

Vertex models



Honda et al. 2004. *J. Theor. Bio.*
Farhadifar et al. 2007. *CB*
Canela-Xandri et al. 2011. *PlosCompBiol*
Mao et al. 2011. *Genes & Dev.*
Fletcher et al. 2014. Biophys. J.

Cell signalling

Cell polarity

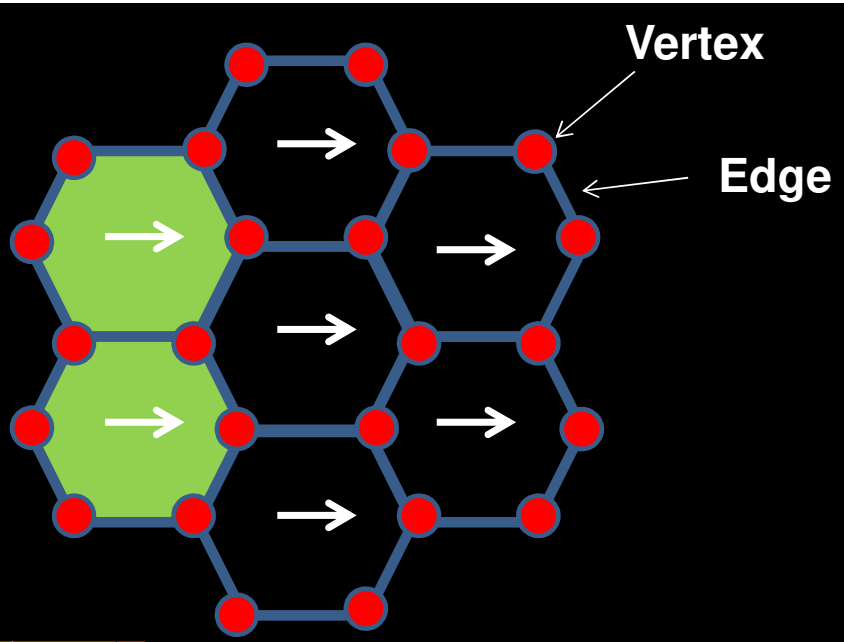
Cell division

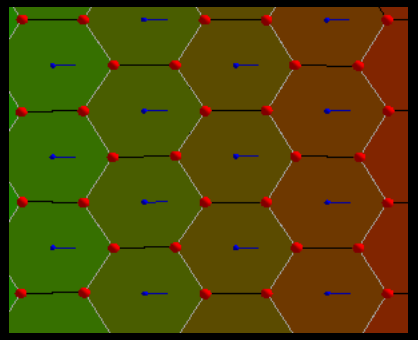
Cell growth

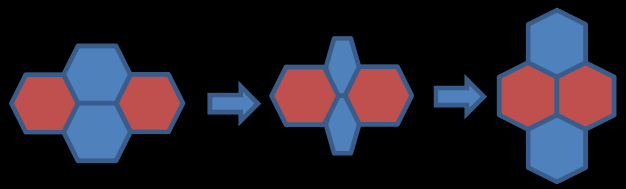
Cell intercalation

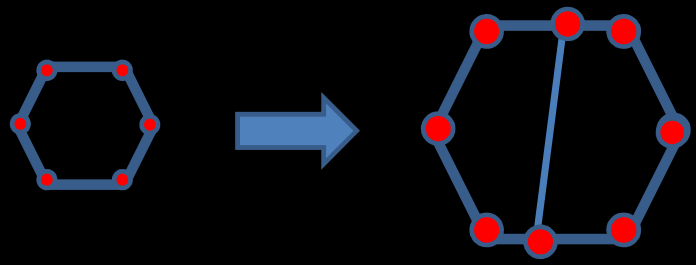
Cell cycle

Cell apoptosis





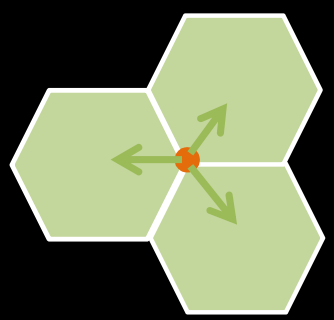


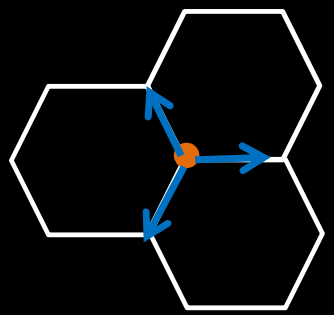


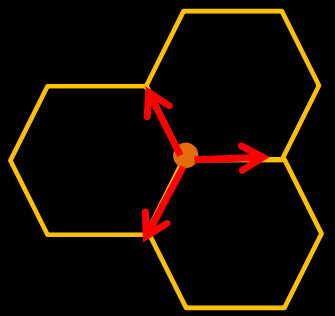
Cell pressure

Tension

$$E(R_i) = \sum_{\alpha} \frac{K_\alpha}{2} \left(A_\alpha - A_\alpha^{(0)}\right)^2 + \sum_{(i,j)} \Lambda_{ij} \cdot l_{ij} + \sum_{\alpha} \frac{\Gamma_\alpha}{2} \cdot L_\alpha^2$$



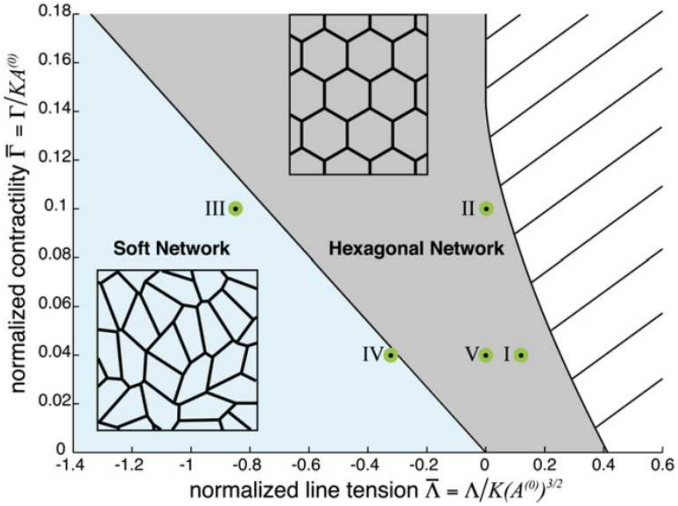


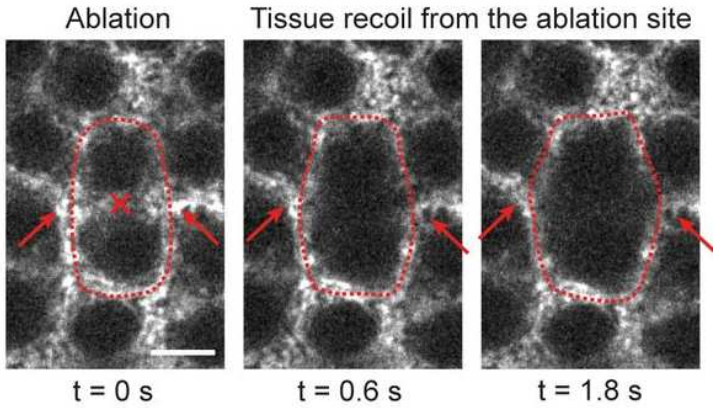


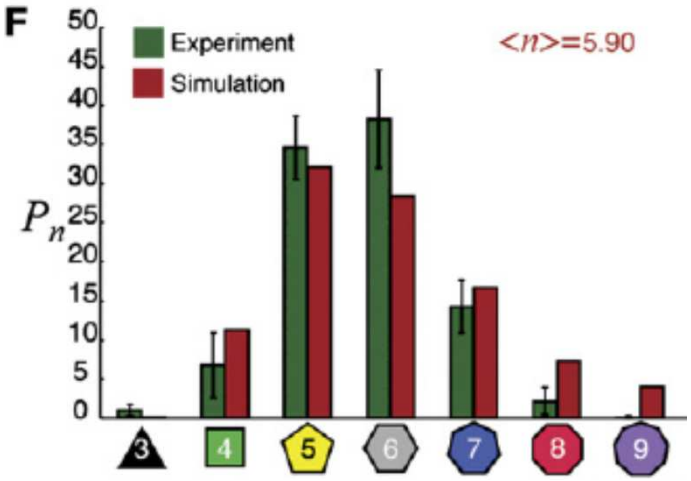
Experimental estimation of the parameters

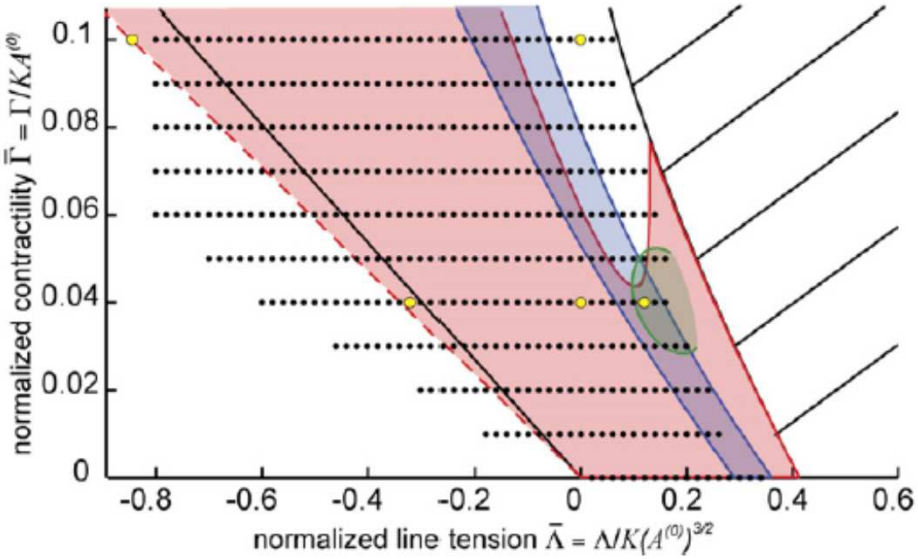
Farhadifar et al. 2008

$$E(\boldsymbol{R}_i) = \sum_{\alpha} \frac{K_\alpha}{2}\left(A_\alpha - A_\alpha^{(0)}\right)^2 + \sum_{<i,j>} \Lambda_{ij}\ell_{ij} + \sum_{\alpha} \frac{\Gamma_\alpha}{2} L_\alpha^2$$

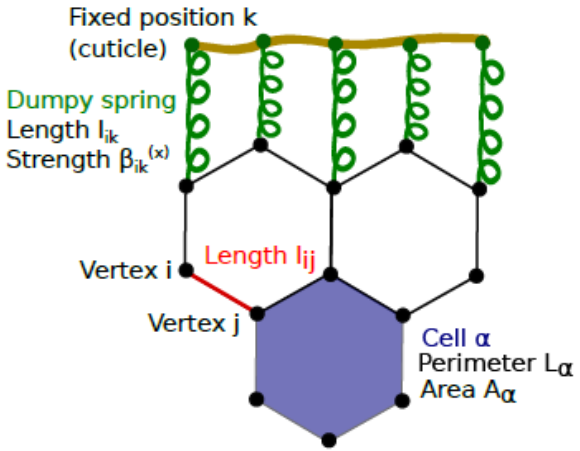


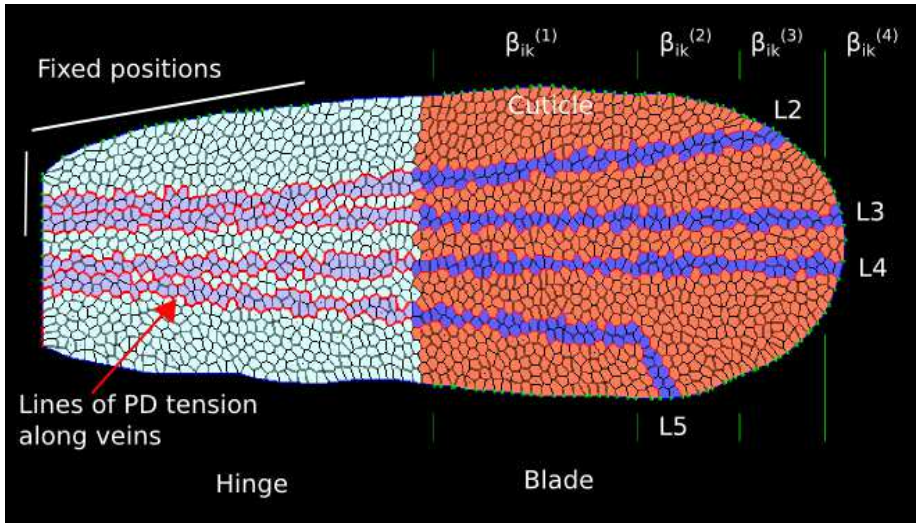




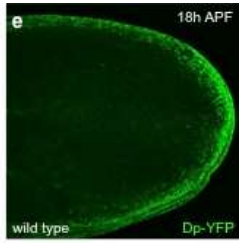


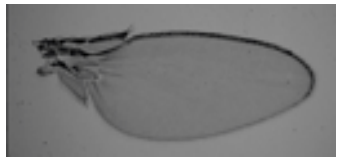
Model



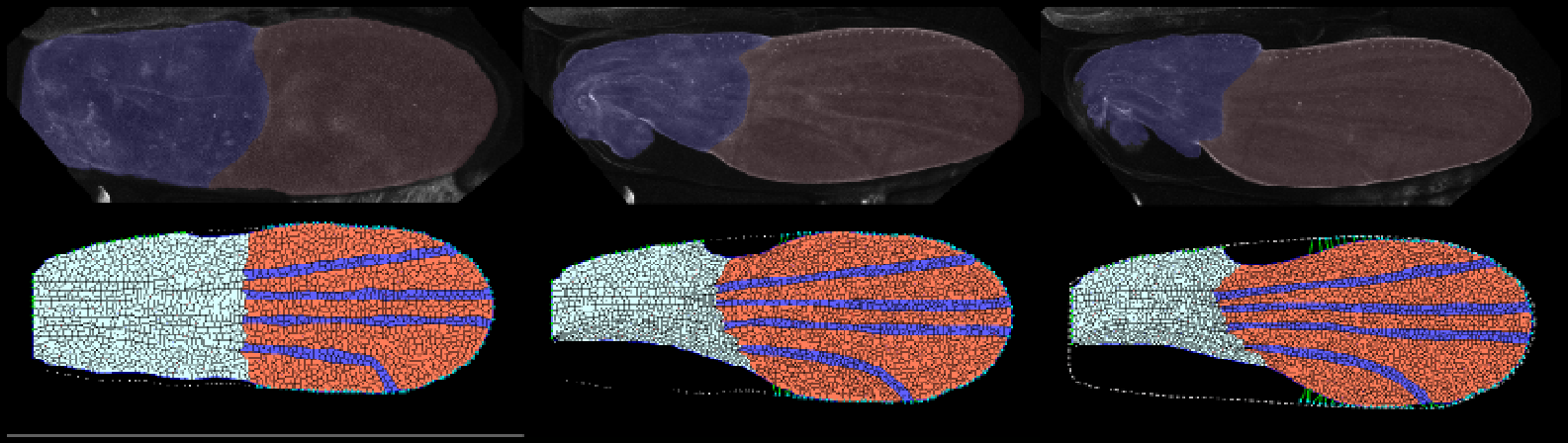


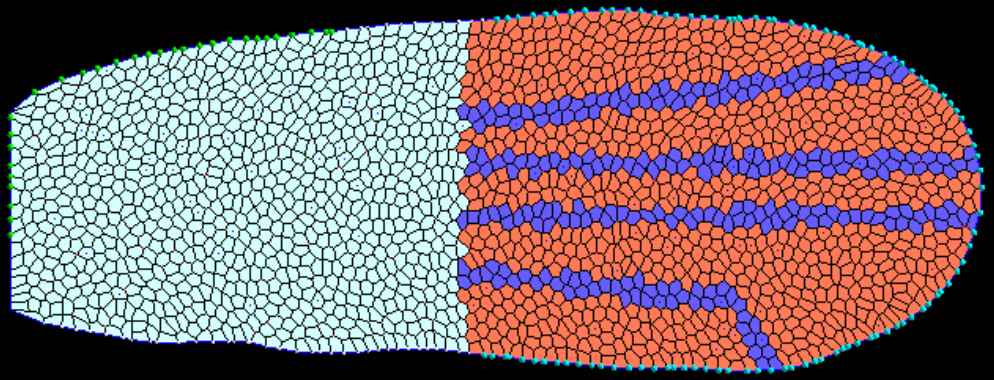
- Two fold reduction of cell area (for all cells).
- One round of mitosis (blade cells only) – Hertwig rule
- Graded distribution of Dumpy along the PD axis
- Higher line tension and cortical contractility for hinge cells.
- PD tension along the vein cells
- Fixed positions for anterior/proximal hinge margin



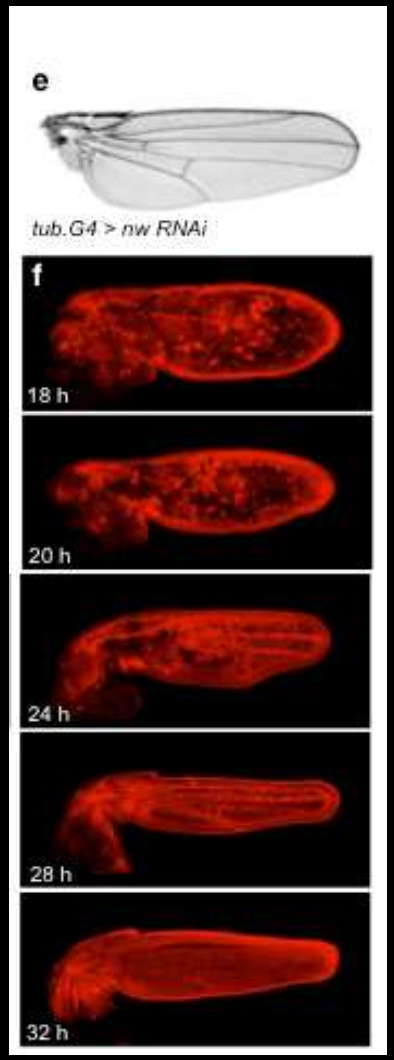


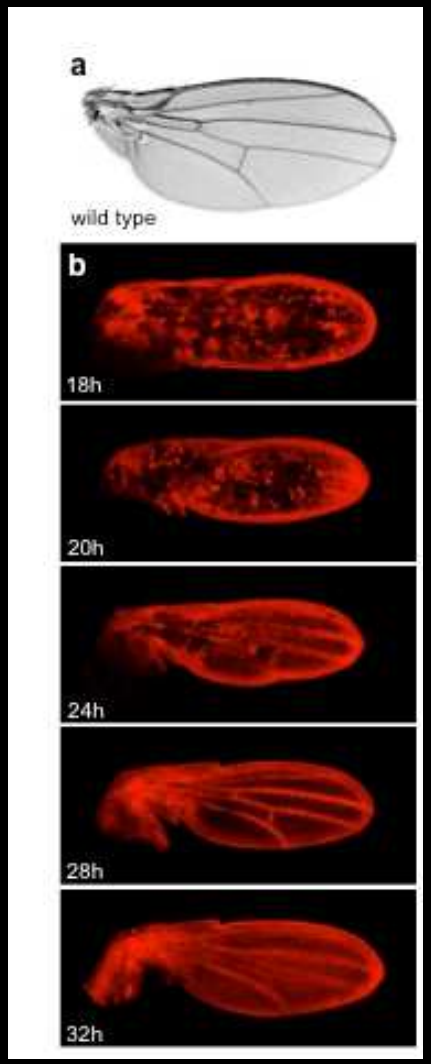
Simulation of a wild-type wing

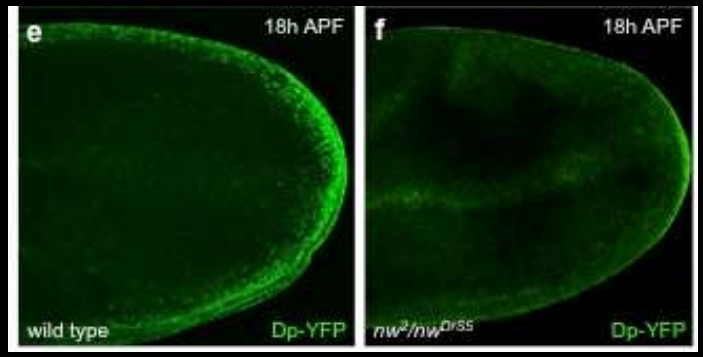




Manipulation of the *narrow* gene affects the pattern of Dumpy, and wing shape.

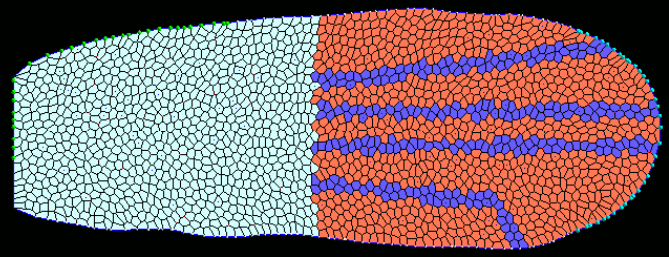


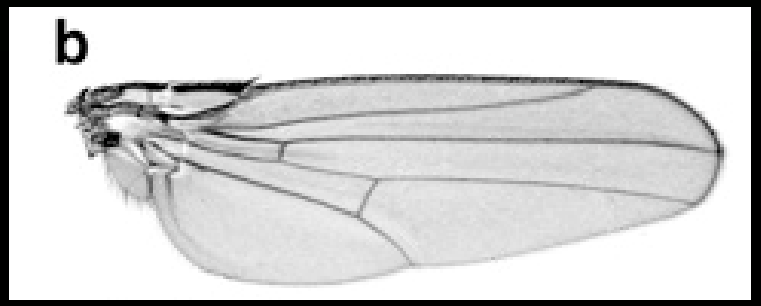


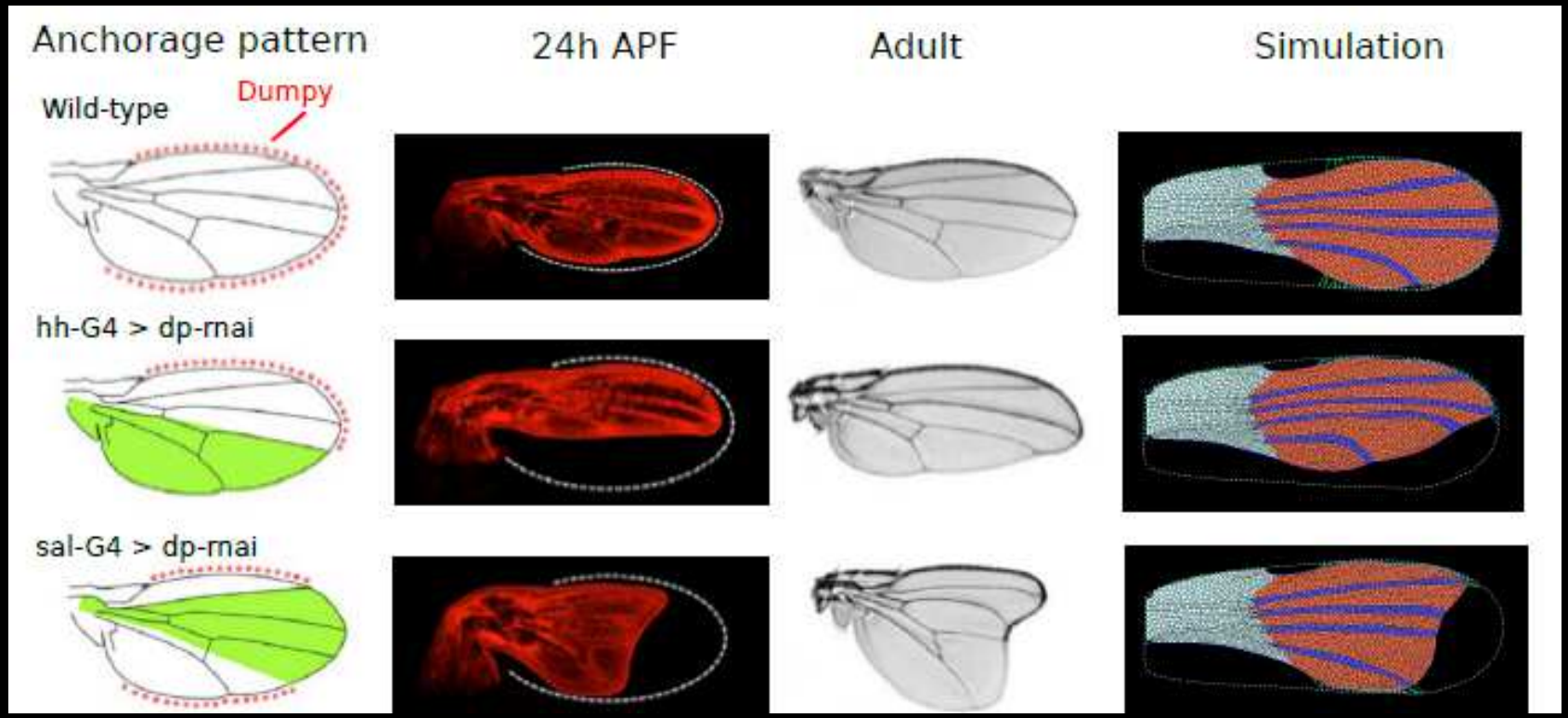


wild-type

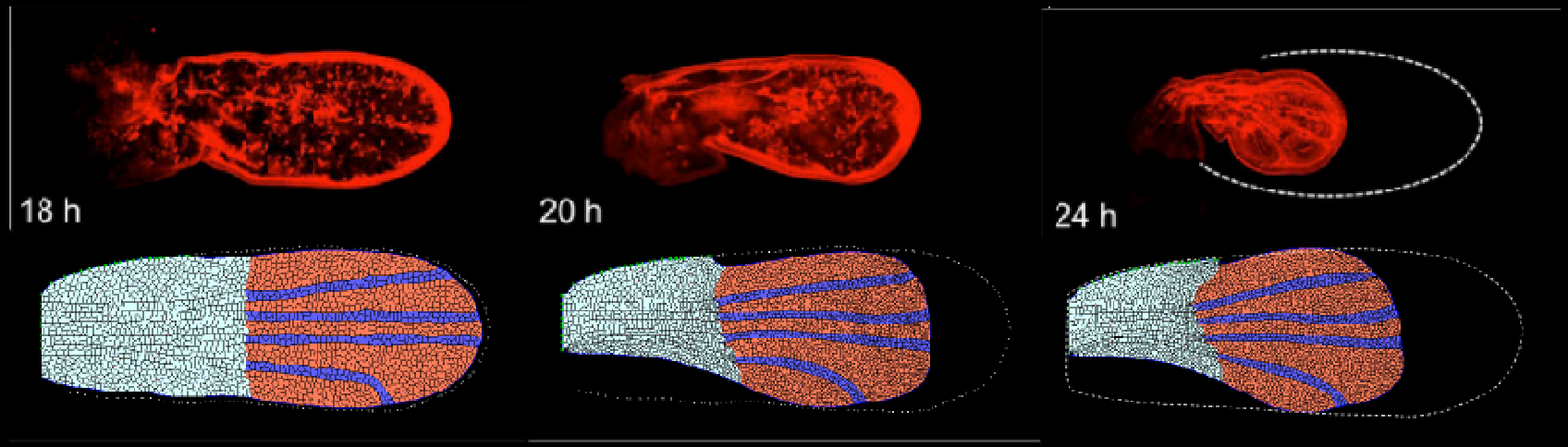
tub-gal4 > nw-RNAi





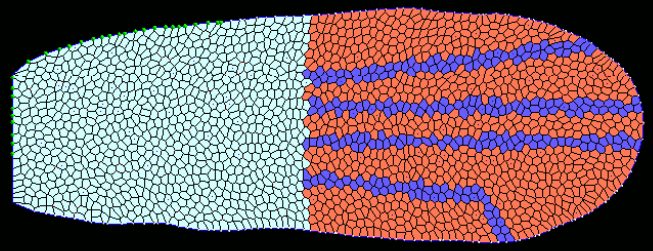


Ray, Matamoro-Vidal et al. Dev. Cell. 2015

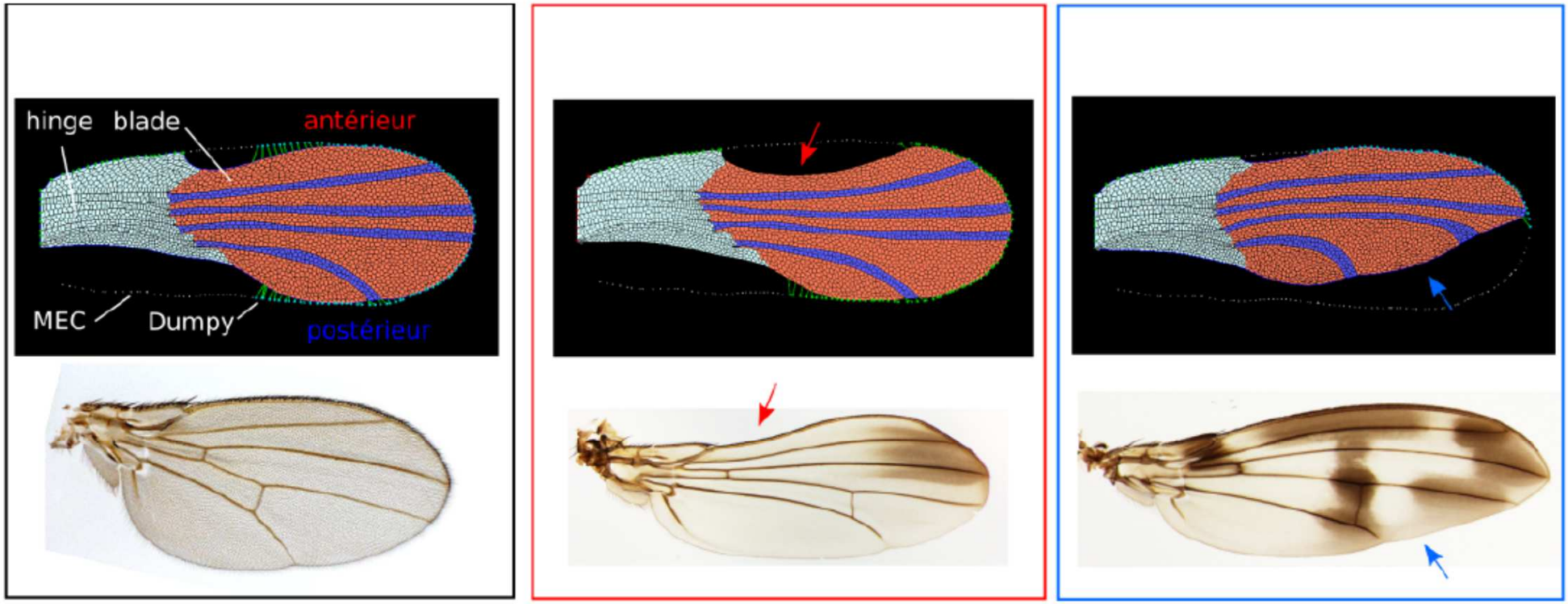


wild-type

nub-gal4 > dp-RNAi



The model recapitulates qualitatively the variation observed in nature



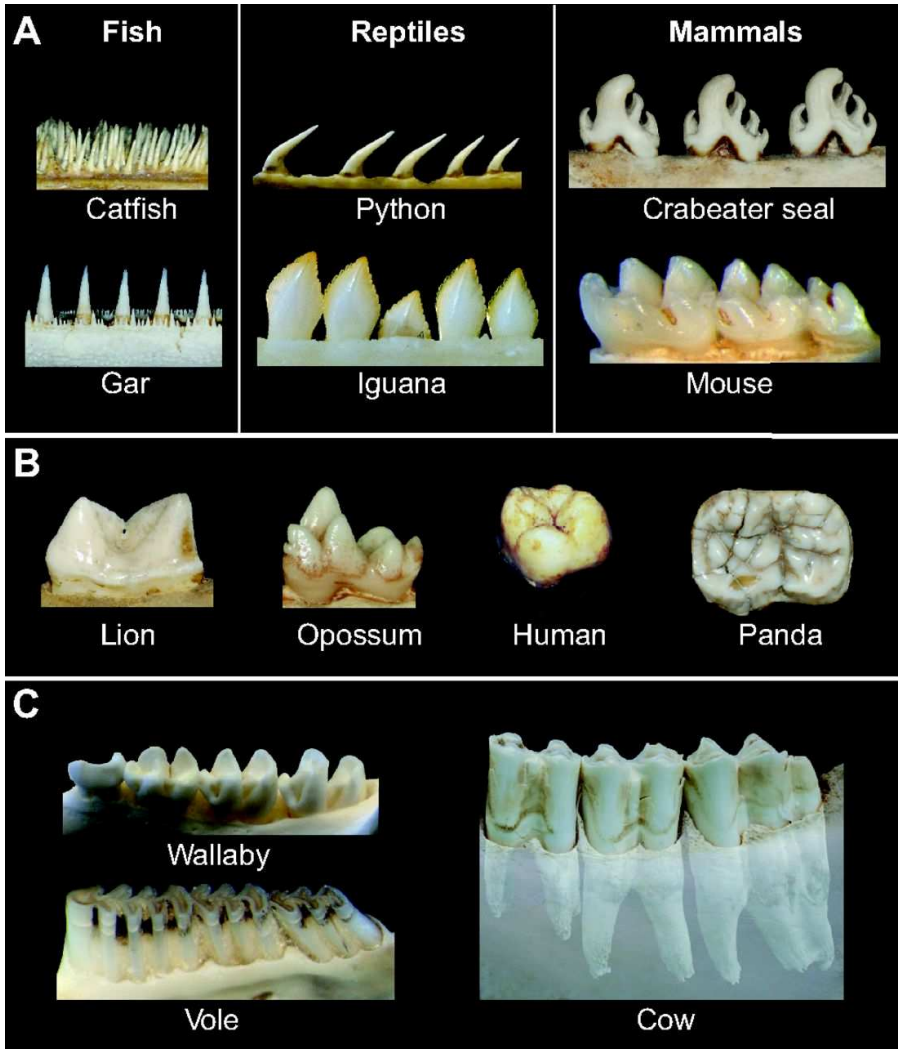
- On how to reconciliate developmental and evolutionary views of variation.

- They are certainly many mutations that can alter wing shape during pupal morphogenesis, but they must do so by altering the mechanical force shaping the wing.

- Modelling this force captures the variation that can be potentially obtained by mutating many genes.

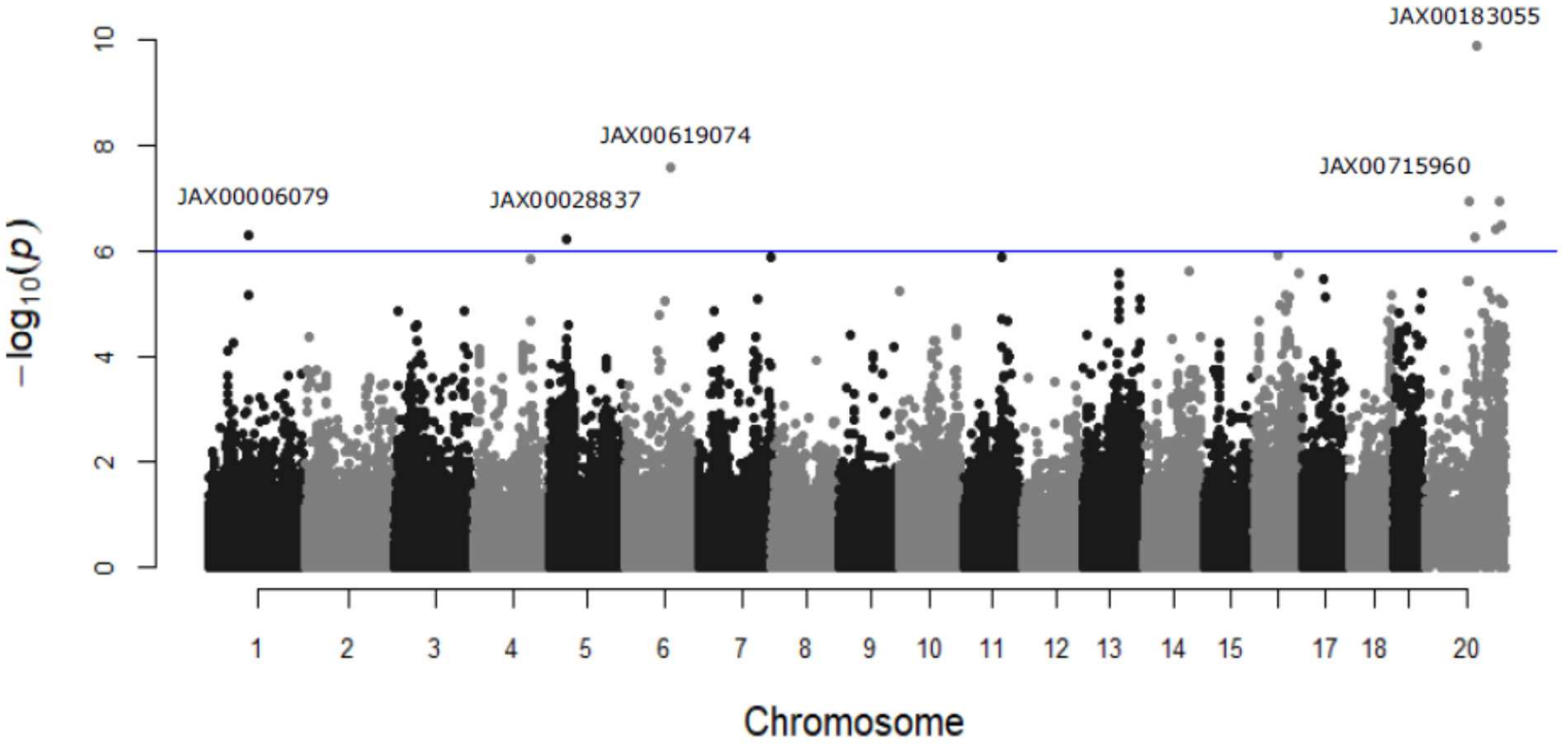
- 3 years of work just to understand this aspect of morphogenesis.
    - Check other developmental stages
    - Test model predictions regarding natural variation

Example 2: vertebrate teeth



Jernvall & Thesleff 2014

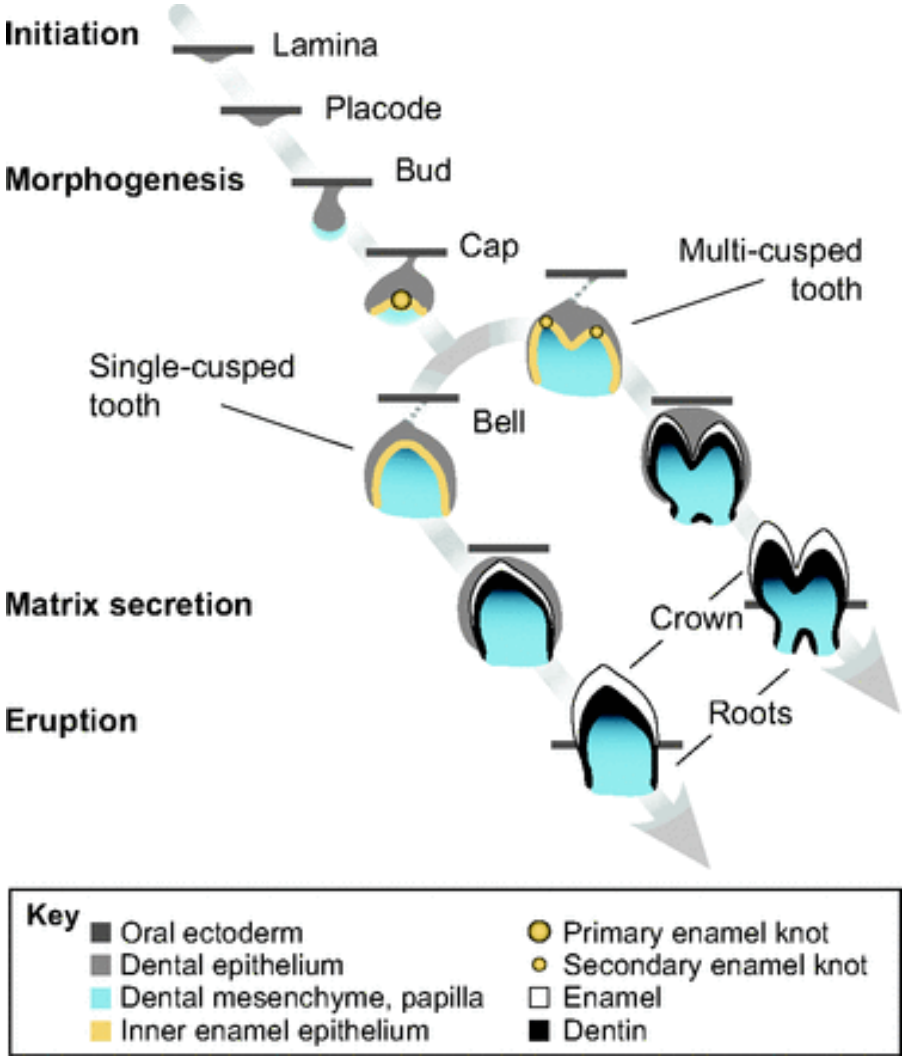
QTL mapping on tooth shape between *Mus musculus domesticus* and *Mus musculus musculus*



Together, the loci explain ~10% of molar shape variation, with individual effects ranging from 1% to 3%

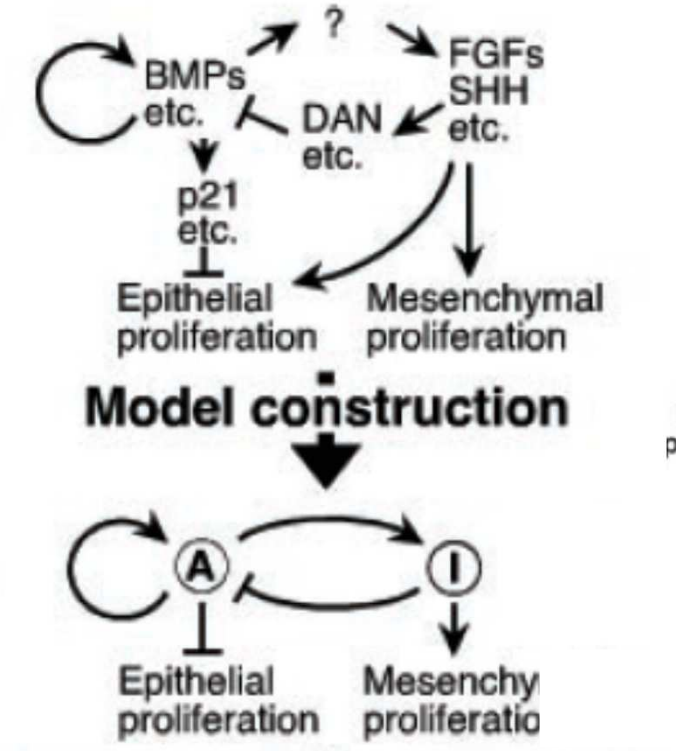
Pallares et al. 2017.

QTL	Chr	Position	Best SNP	p-value	Effect size
Mo.1	chr1	84306638	JAX00006079	5.11E-07	1.1%
Mo.5	chr5	36723779	JAX00128837	5.79E-07	3.2%
Mo.6	chr6	97980057	JAX00619074	2.71E-08	2.8%
Mo.X.1	chrX	92638616	JAX00715960	1.18E-07	1.6%
Mo.X.2**	chrX	104533418	JAX00183055	1.28E-10	2.2%

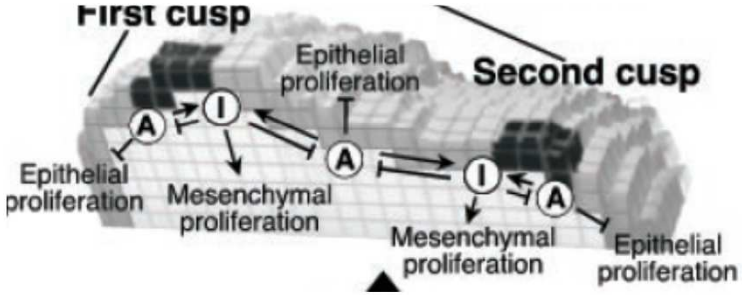


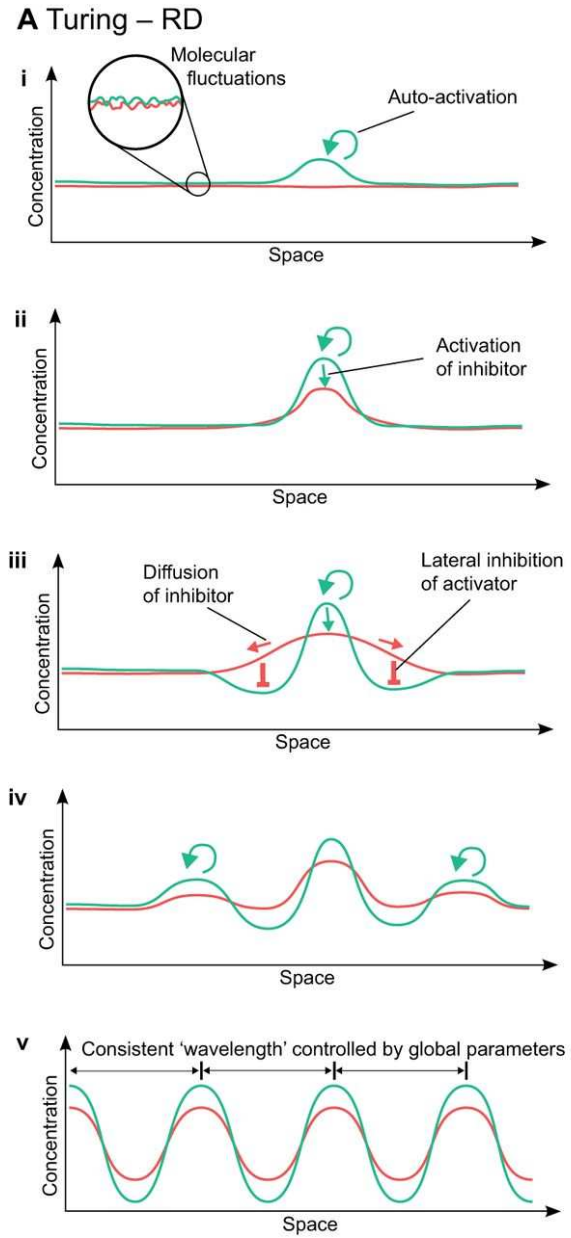
Jernvall & Thesleff 2014

Modelling tooth morphogenesis

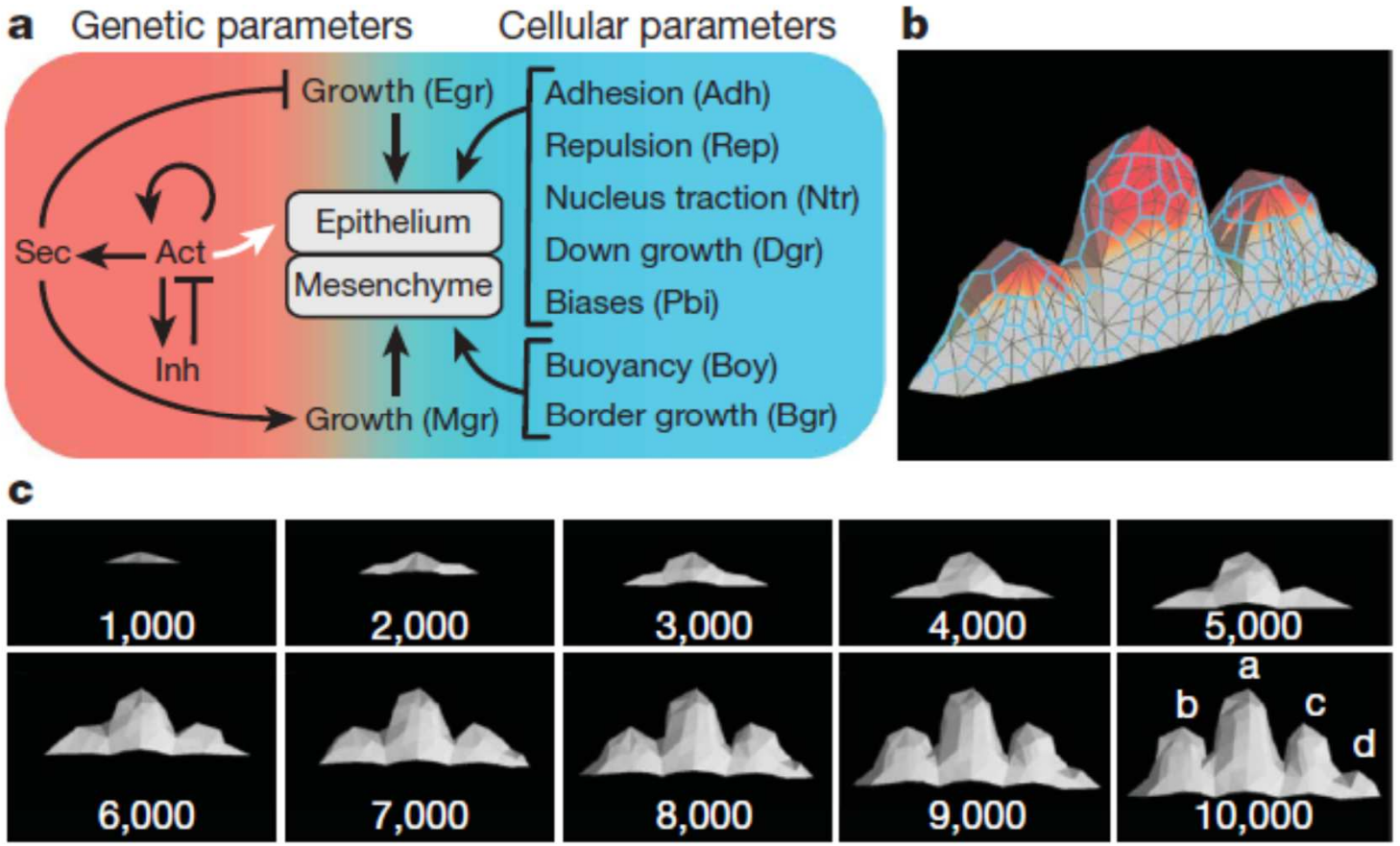


Salazar-Ciudad & Jernvall 2003



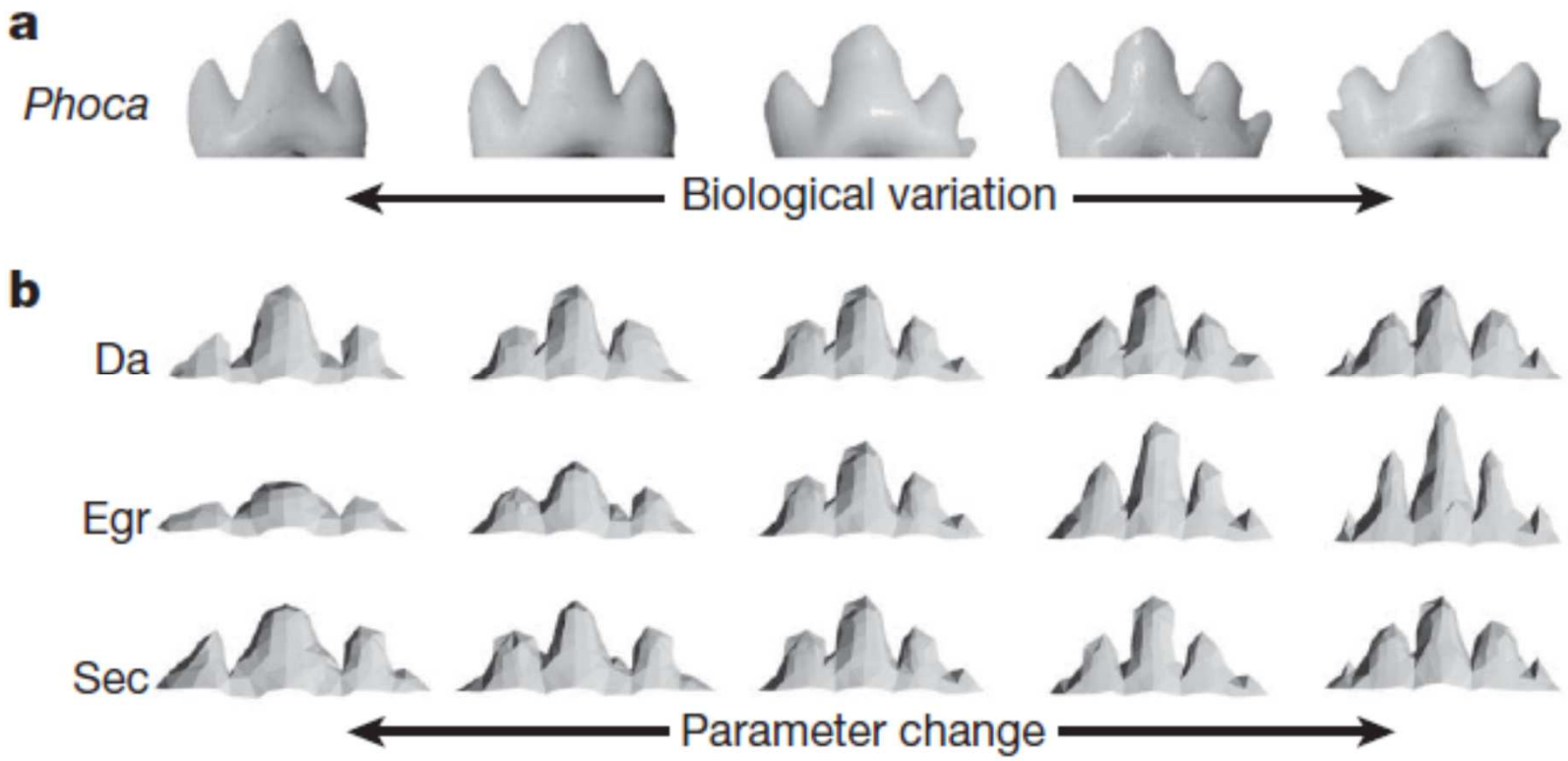


Green & Sharpe 2015

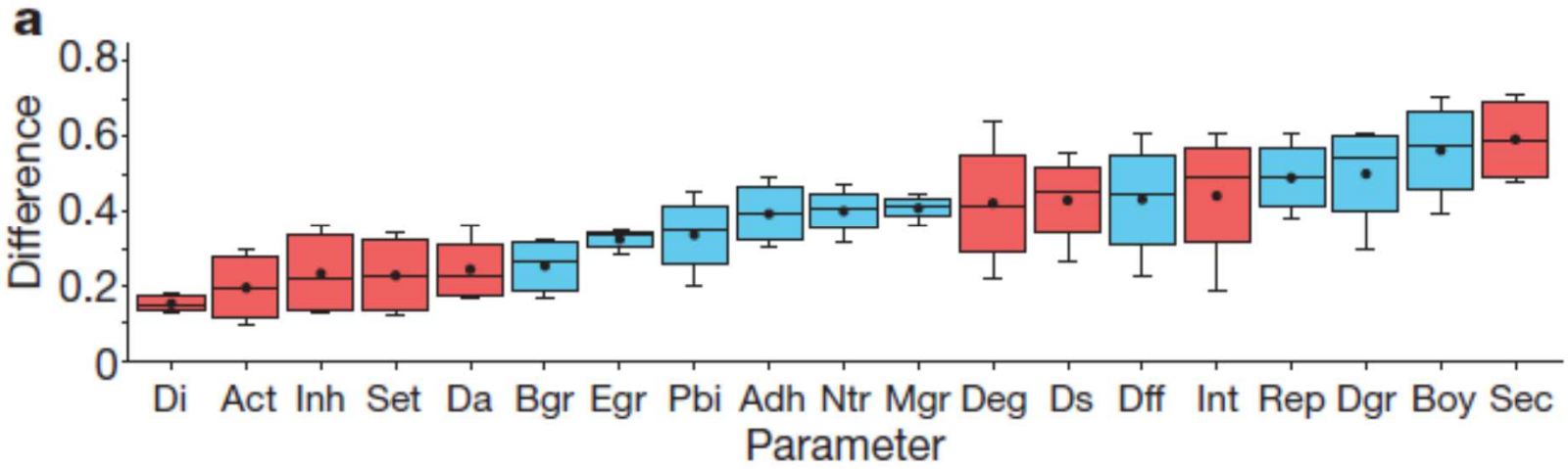


Salazar-Ciudad & Jernvall 2010

The model was orginally designed from rodents data but it reproduces variation observed in seals



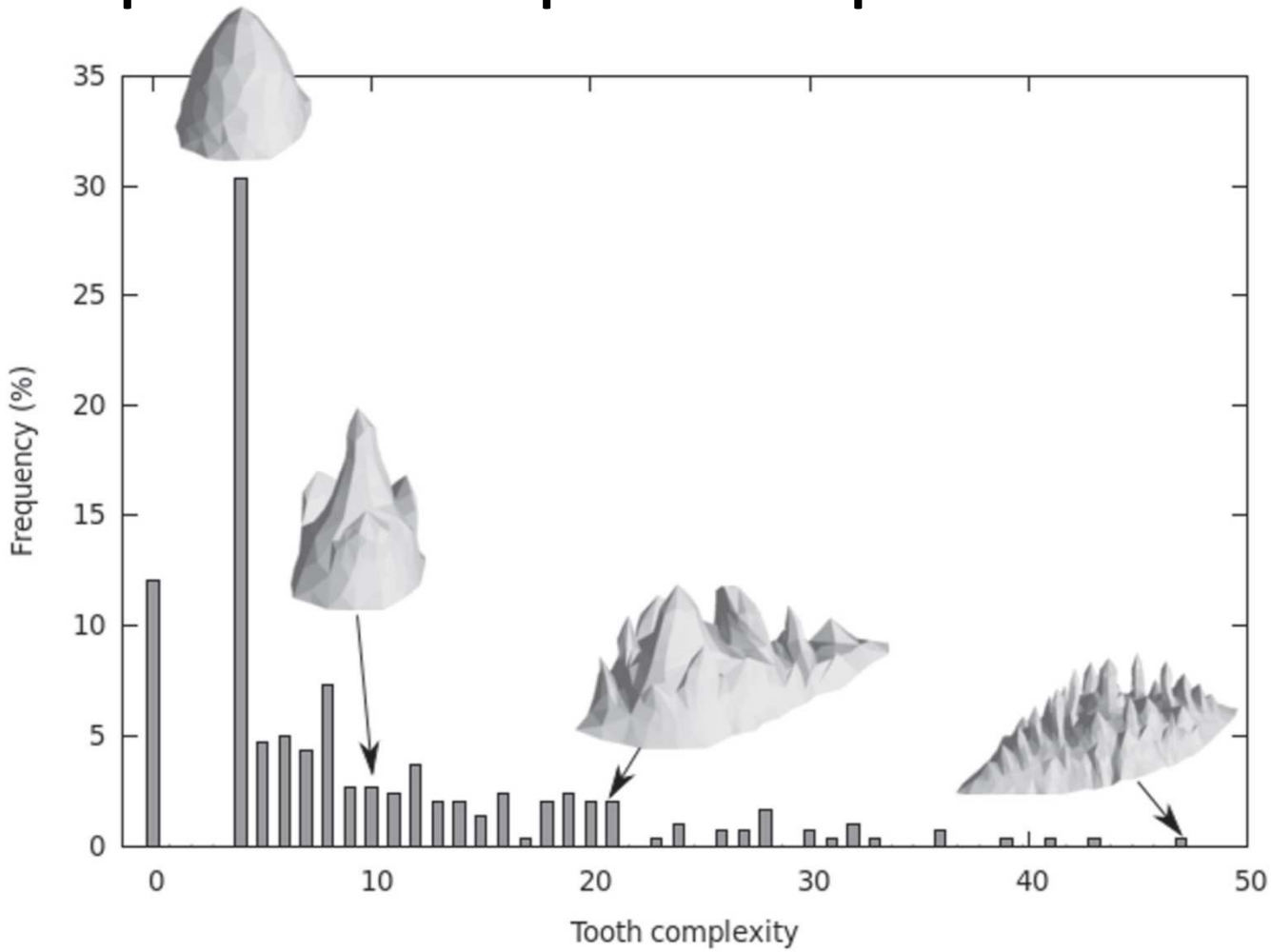
Salazar-Ciudad & Jernvall 2010



Parameters that produce the most realistic variation:
Activator self-regulation (Act)
Activator diffusion (Da)
Inhibitor strength (Inh)
Inhibition diffusion (Di)
Secondary signal threshold (Set).

Salazar-Ciudad & Jernvall 2010

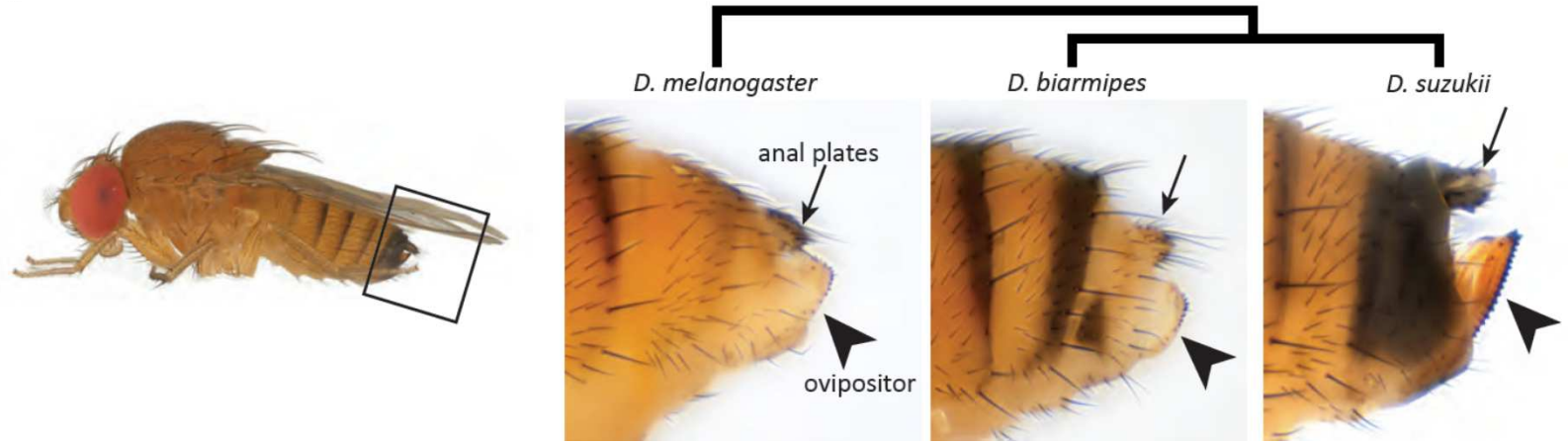
Exploration of the parameter space with the model



Morphogenesis based modelling of phenotypic variation allows to establish null hypotheses on the possible phenotypes and of their relative frequencies.

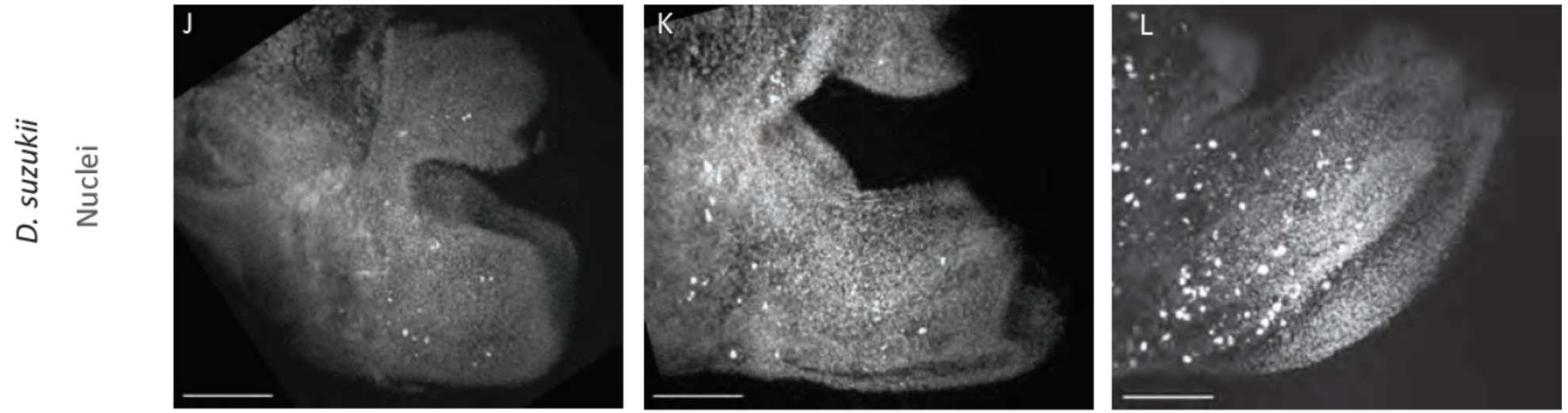
Salazar-Ciudad & Marin-Riera 2013

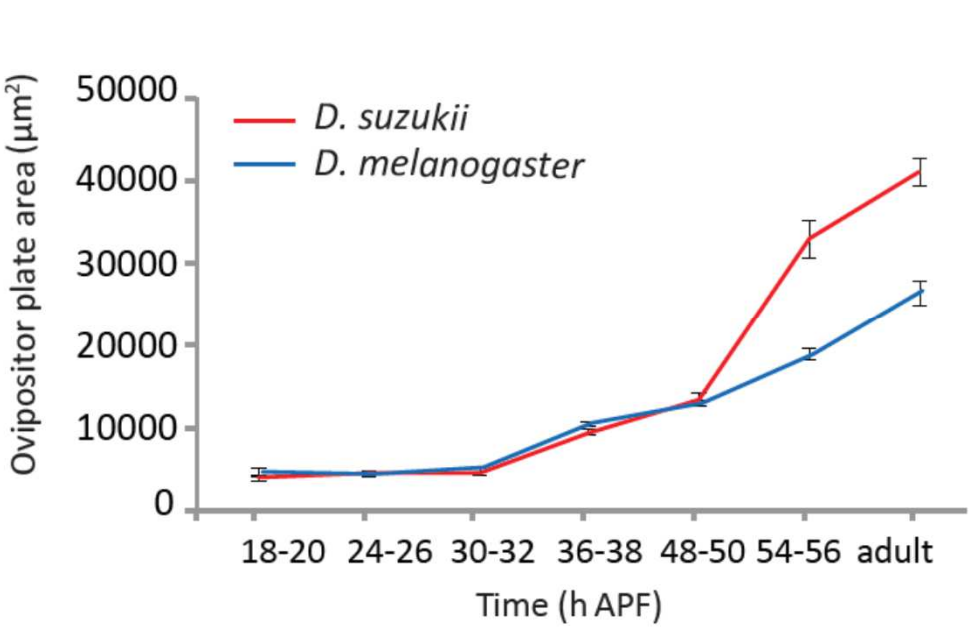
Example 3: evolution of ovipositor length in *D. suzukii*

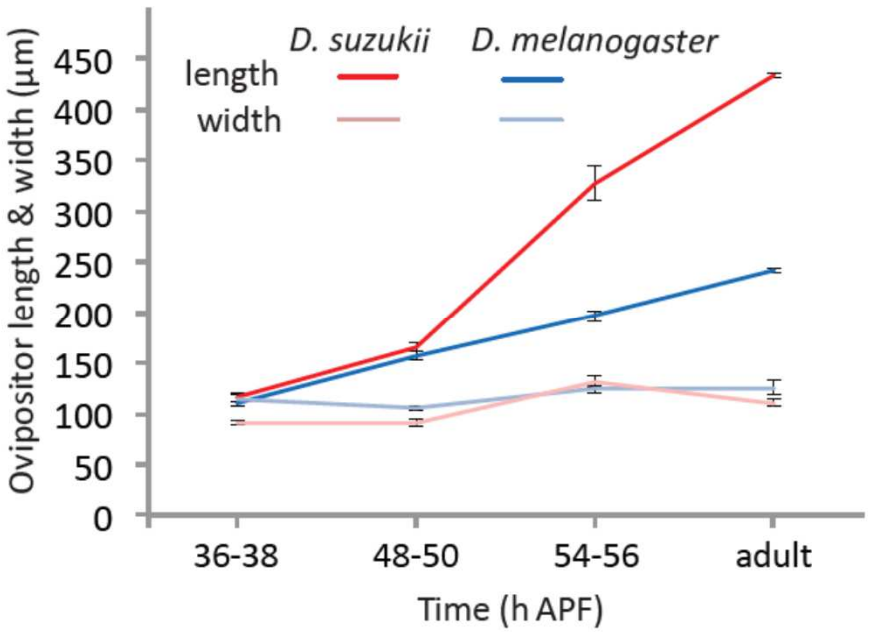


Green et al. 2018.

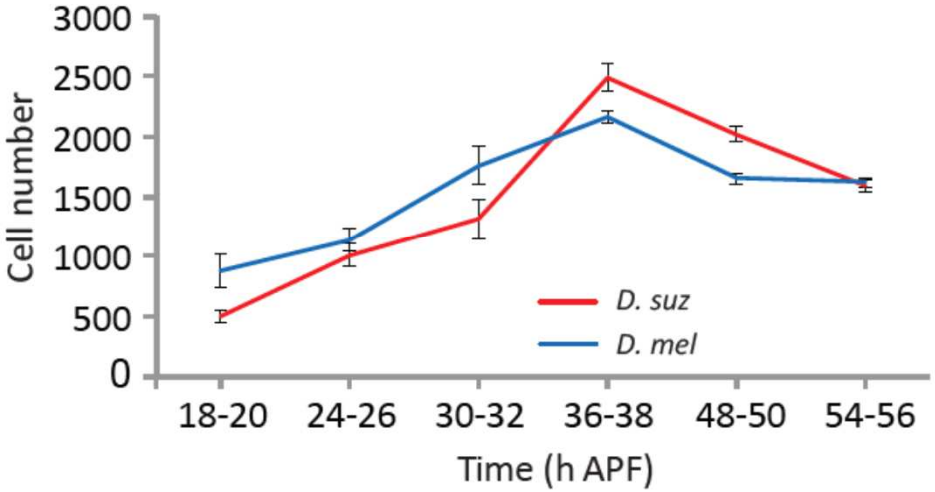
Difference in ovipositor area and shape appears after 48 h APF

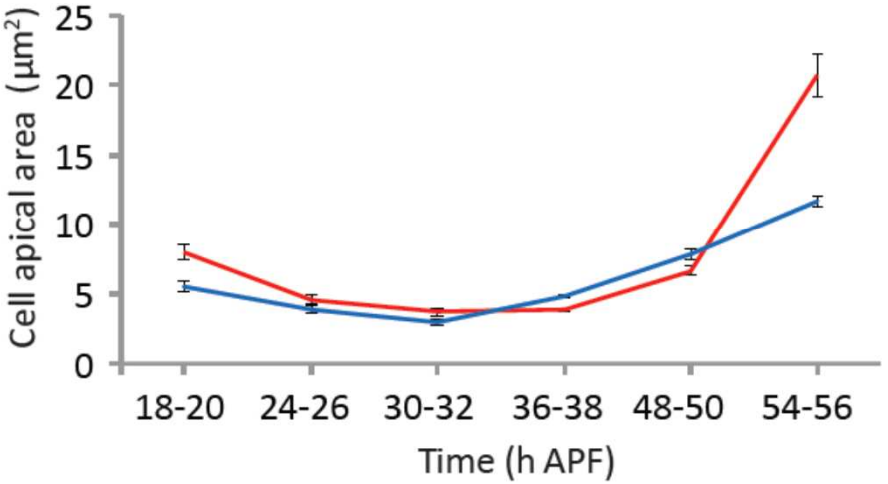


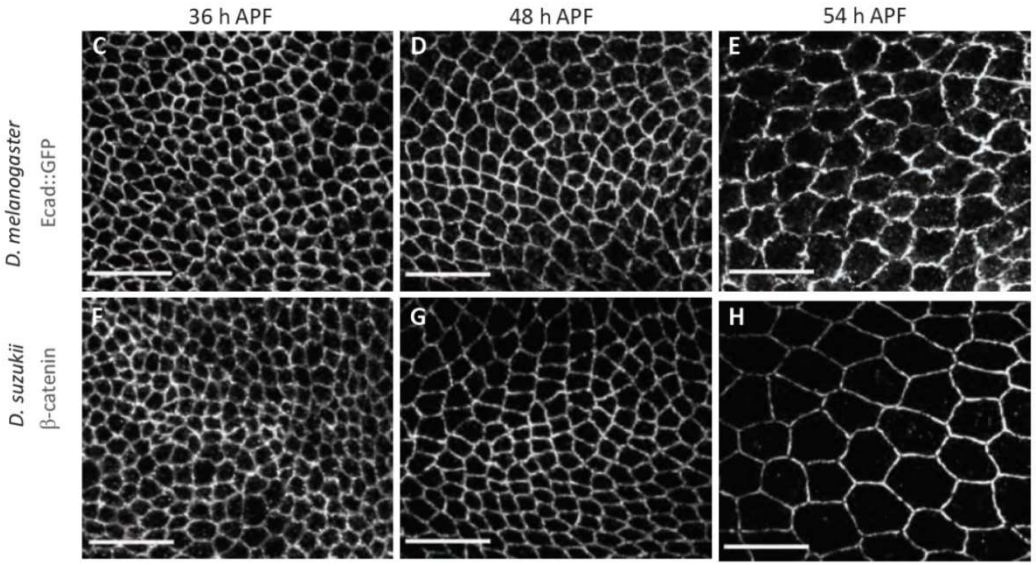




Inter-specific difference in cell area but not cell number

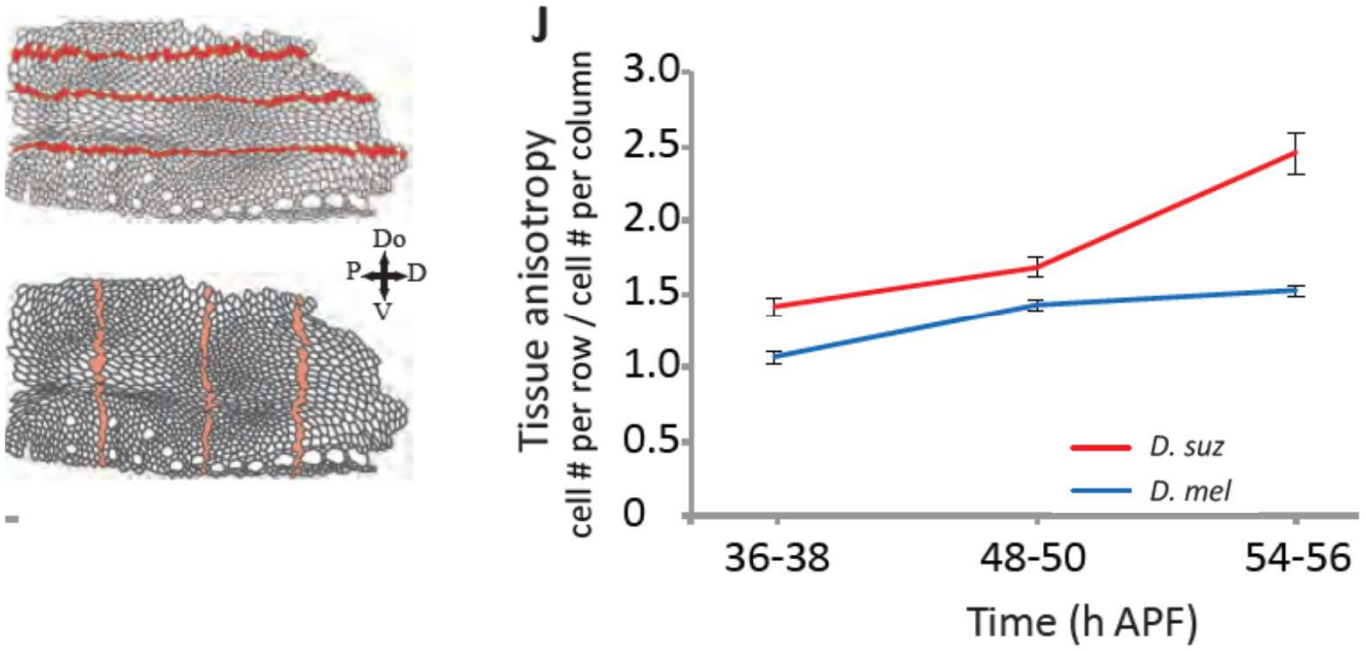


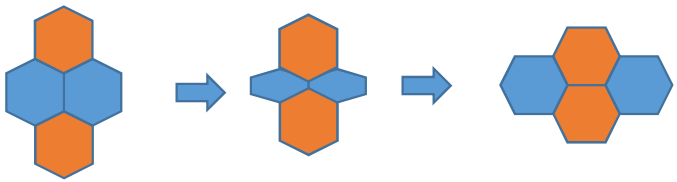




Explains difference in organ size but not difference in organ shape.

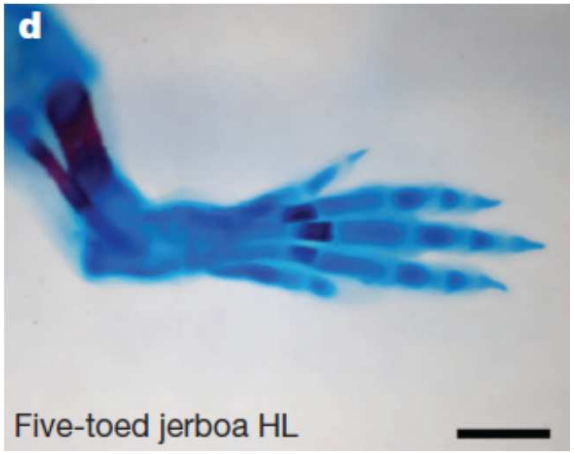
Anisotropic change in cell number explains difference in tissue shape

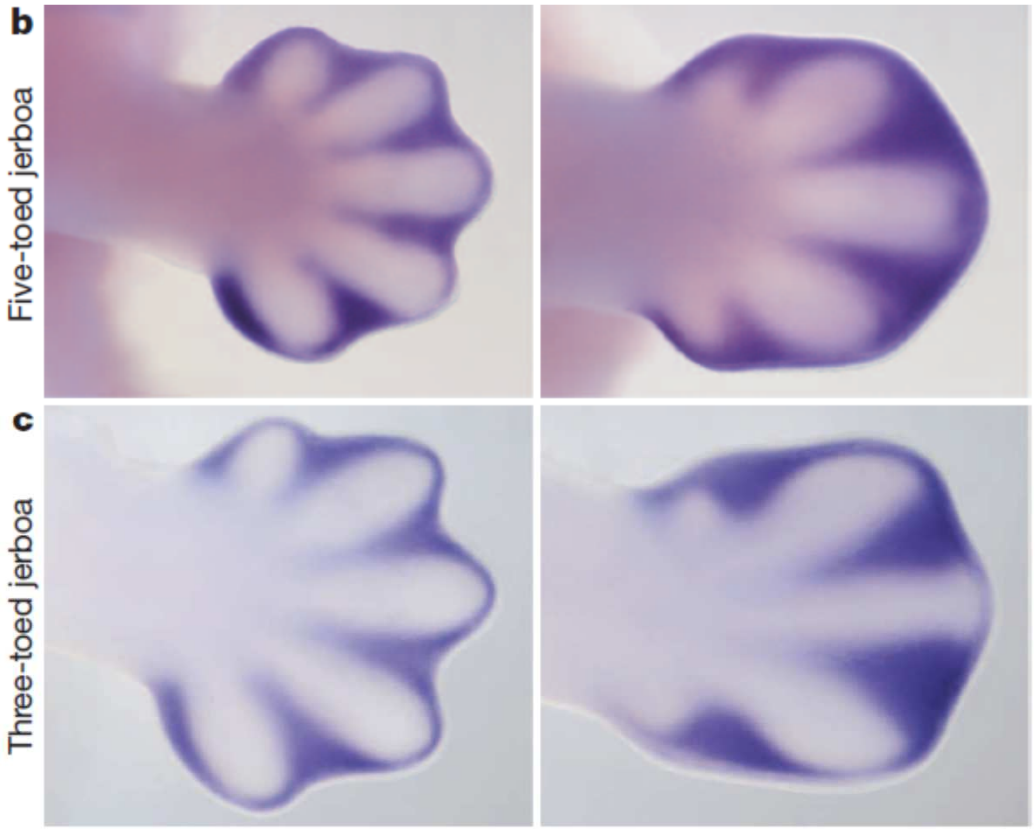




Oriented cell intercalation or patterned cell death explains evolution of tissue shape

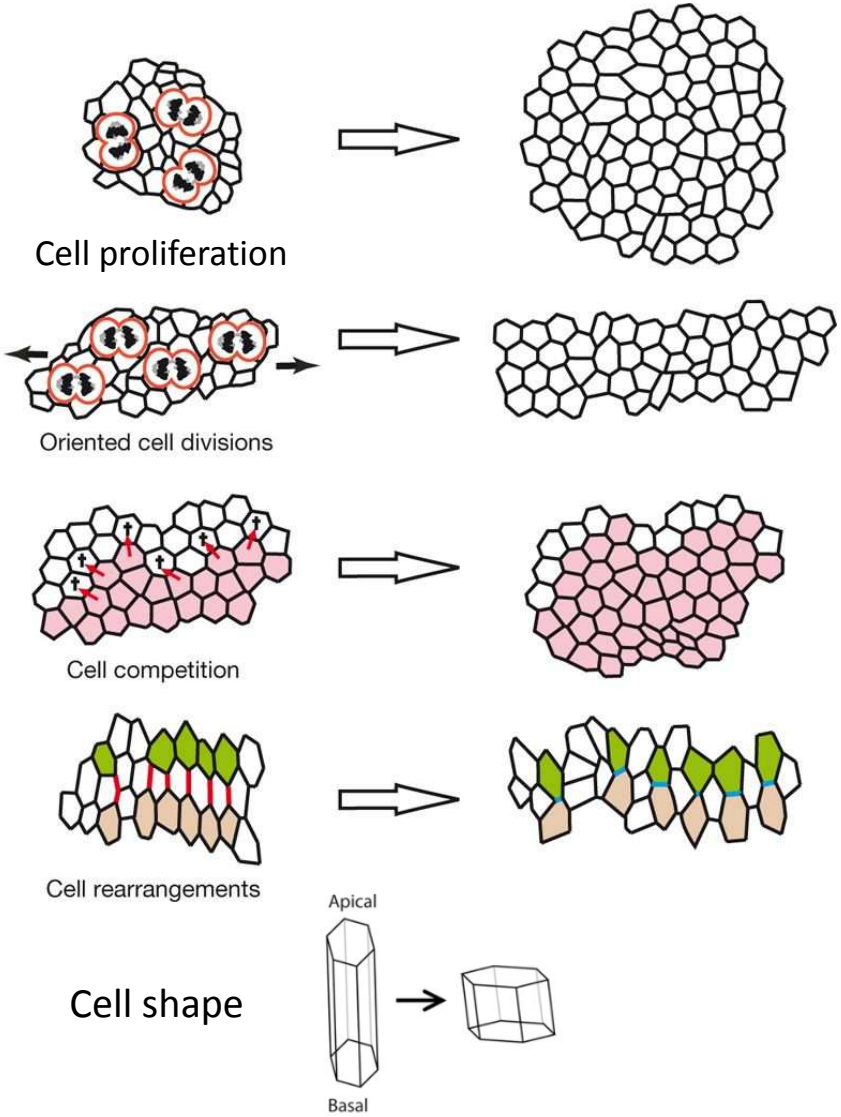
Example 4: digit loss in mammals





Cooper et al. 2014

Cellular mechanisms of tissue size and shape



Vertebrate tooth

Butterfly wing shape evolution

Drosophila wing shape ?

Drosophila ovipositor ?

Digit loss in mammals

Drosophila wing shape ?

Drosophila ovipositor

Drosophila ovipositor

Should we care about development in the study of the genotype-phenotype map ?

- Causation vs. association
- Complex genetic architectures
- Developmental constraints
- Morphogenesis is beautifull !