

**modeling development (1)**

**classical models of pattern formation**

**segmentation patterns**

# One more level between genotype and phenotype: Modeling development (and its evolution)

---

pattern formation (dependent on shape)

Pattern formation ————— > shape

pattern formation <———— > shape

TODAY: Classical models of pattern formation / segmentation

*Supervised modeling*

Top down modeling:

- Given observed pattern/behaviour X and assumptions A  
CAN A ————— > X (AND does it generate X++)
- Data driven models , quantitative fitting

*Theme: specific and/or general mechanisms  
and/or specific instantiations (?)*

# **development: cell differentiation, pattern formation and morphogenesis ss**

---

classically most studied: pattern formation

prepattern --> cell differentiation --> morphogenesis ss

3 most discussed general mechanisms for stationary pattern formation for development

Turing patterns (Turing 1952)  
introduced term 'morphogen'

Positional information (Wolpert 1969)  
morphogen gradient - coordinate system

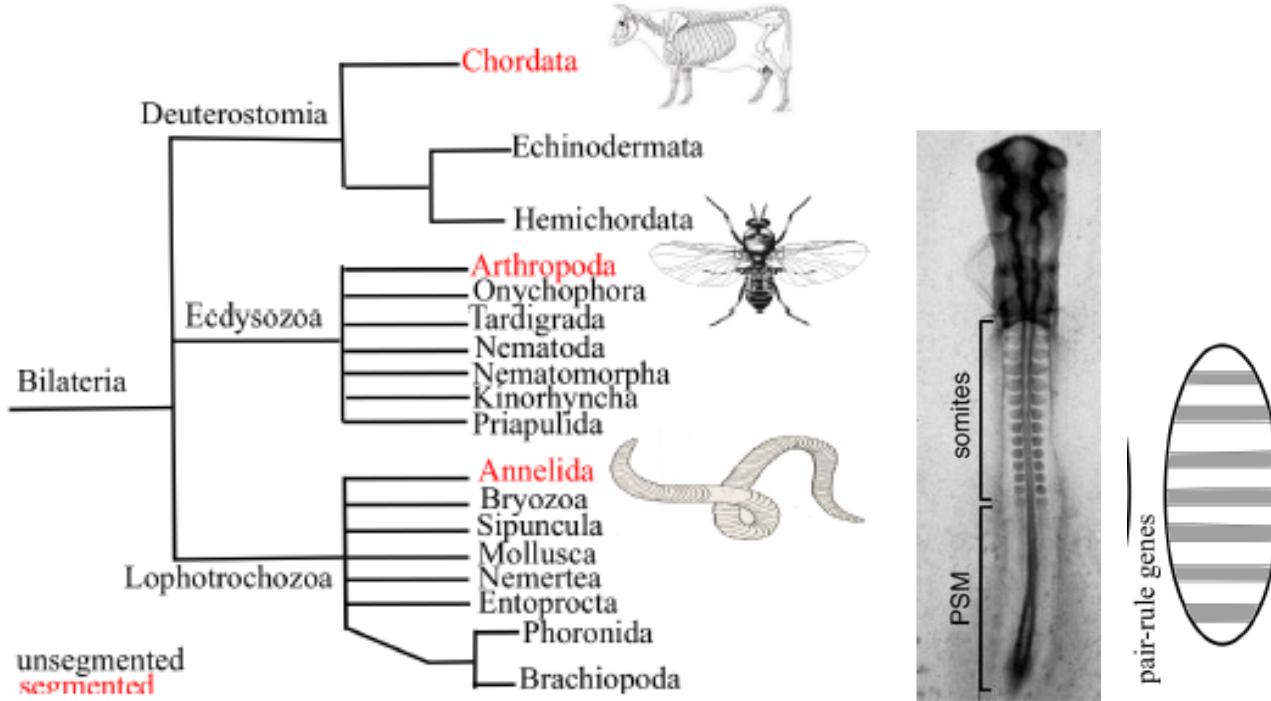
"Clock and waveform" Cook and Zeeman 1976 temporal oscillation --> spatial pattern

compare: "pattern is 'default'"

however here specific positioning/orientation  
in continuous medium

# Segmented bodyplans

A Overt body segmentation in the Bilaterian tree



from Ten Tusscher EPJE

reinventions (?)  
generic mechanism?  
homologous at molecular, pathway level?

# A generic regular pattern formation mechanisms

## Turing Patterns

---



*Can DIFFUSION create patterns from homogeneous state?*

- 2 interacting substances
- stable homogeneous equilibrium in absence of diffusion
- unstable for spatial heterogeneous perturbations
- with diffusion: stable (+ regular) stable patterns

## Turing patterns: formal requirements

---

$$\begin{cases} \frac{\partial A}{\partial t} = D_a \Delta A + f_1(A, I) \\ \frac{\partial I}{\partial t} = D_i \Delta I + f_2(A, I) \end{cases}$$

without diffusion stable:

$$tr J = a_{11} + a_{22} < 0$$

$$det J = a_{11} * a_{22} - a_{21} * a_{12} > 0$$

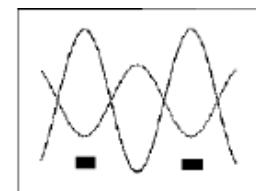
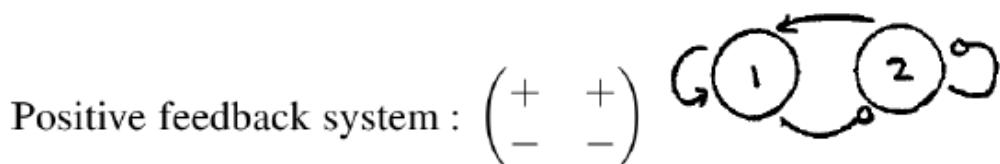
with diffusion unstable

$$\begin{cases} a_{11} + a_{22} < 0 \\ a_{11} * a_{22} - a_{21} * a_{12} > 0 \\ D_a a_{22} + D_i a_{11} > 2 \sqrt{D_a D_i * (a_{11} * a_{22} - a_{21} * a_{12})} > 0 \end{cases}$$

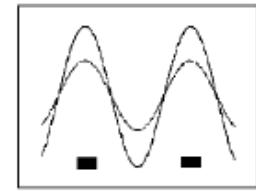
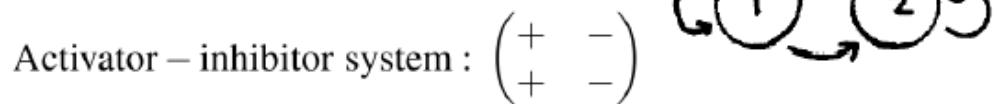
## simplified requirements

$$\left\{ \begin{array}{l} a_{11} + a_{22} < 0 \\ a_{11} * a_{22} - a_{21} * a_{12} > 0 \\ D_a a_{22} + D_i a_{11} > 0 \end{array} \right. \quad a_{11} > 0 \text{ and } a_{22} < 0 \quad \frac{D_i}{|a_{22}|} > \frac{D_a}{|a_{11}|}$$

Diffusion I  $\gg$  Diffusion A:  
short range activation, long range inhibition



Variables vary over space in phase:



## Turing patterns

---

In 2D:



NB wavelength

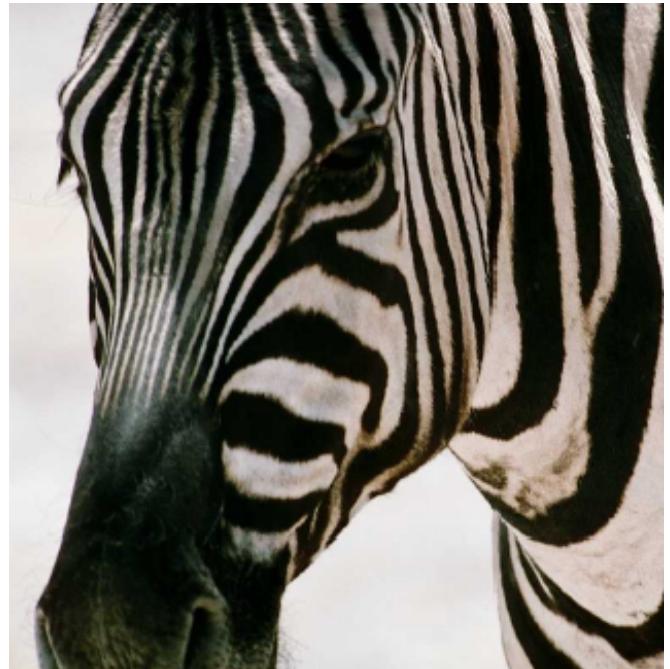
## regular patterns seen in e.g. coat patterns

---

Not only regular patterns,  
but also domain dependence  
shifting with irregular domains

Zebra: 'face recognition'

However sometimes “wrong”  
small domain: spots;  
large domain only 2 phases



*“the stripes are easy, but what about the horse part?”, Turing*

## **applicable in Biology? If so HOW?**

---

Strictly speaking:

Needs homogeneous initial state;

Needs diffusion

Needs large difference in diffusion;

*HAS been sought but NOT BEEN FOUND*

Less strictly speaking

Needs SOME mechanism of  
local activation / longer range inhibition

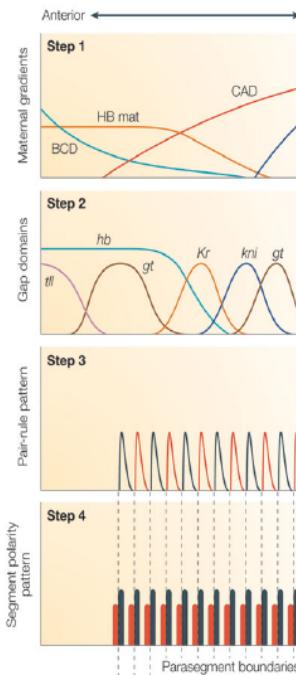
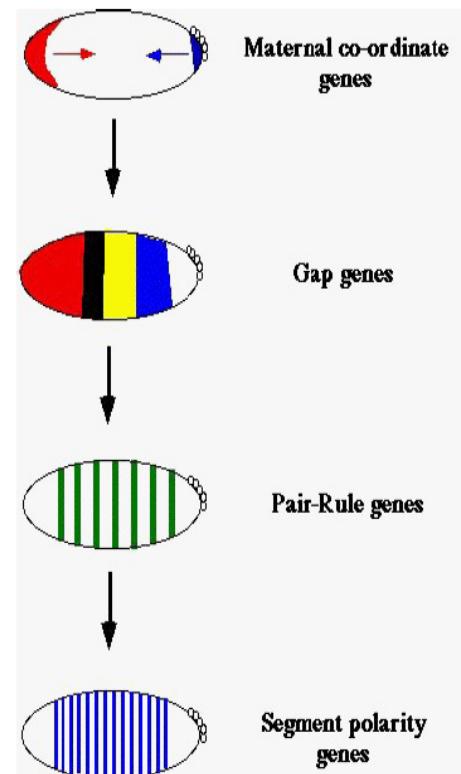
# Classical Modeling Fallacy

## Drosophila stripes as Turing patterns

## Observe stripes

Turing instability ->  
stripes

Hence == Turing pattern

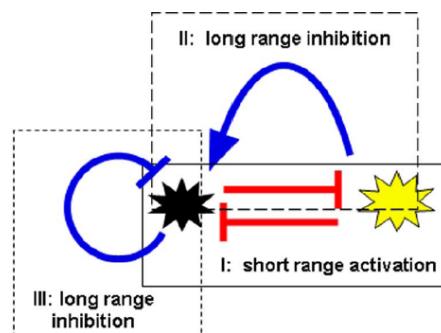
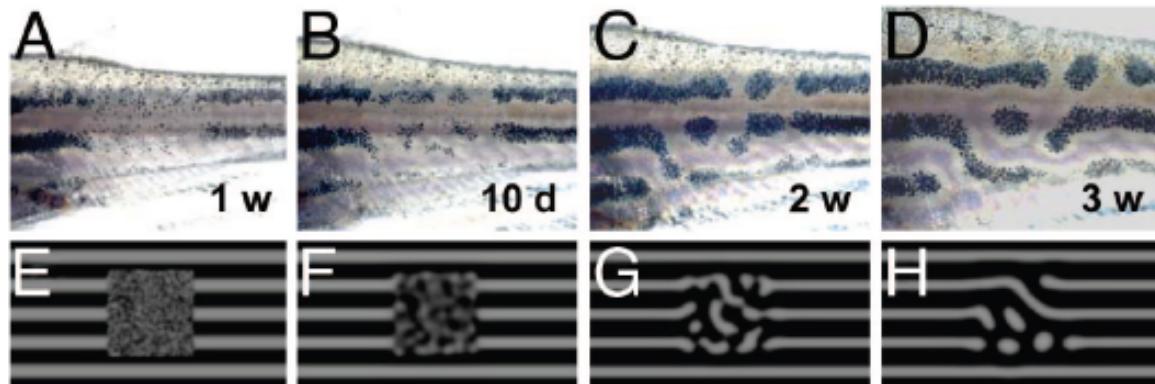


## Activation/inhibition scheme: fish stripes, Kondo-group

“looks like Turing patterns” (stripes)

“looks like turing patterns after ablation”

“short range activation, long range inhibition demonstrated by ablation experiments in pigment cells (no molecular interactions known)”



$$\begin{array}{c} \text{u} \xrightarrow{\text{w}} \text{v} \\ \sim \end{array} \quad \begin{array}{c} \text{u} \xrightarrow{\text{w}} \text{v} \\ \text{v} \xrightarrow{\text{w}} \text{u} \end{array} = \begin{pmatrix} + & - \\ + & - \end{pmatrix}$$

## Conclusions Turing Patterns

---

Elegant, very general

beyond Original diffusion – > pattern

However Stripes: too degenerate pattern to infer anything  
(needs ++)

Domain / disturbance variations more informative

However random positioning - but may be tweaked

Often invoked, eg. limb– > digits

Also for vegetation patterns

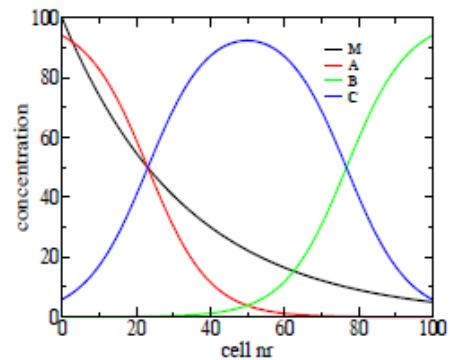
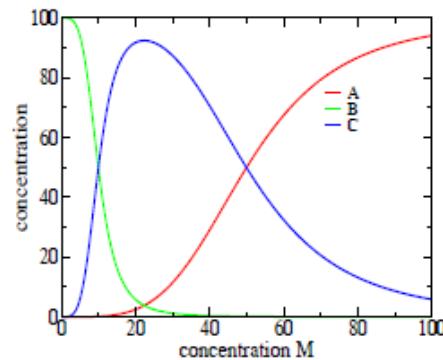
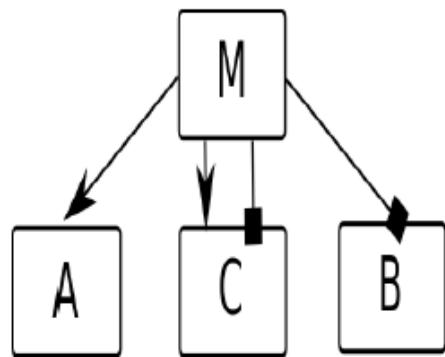


# Positional information/ french flag model

Wolpert 1969

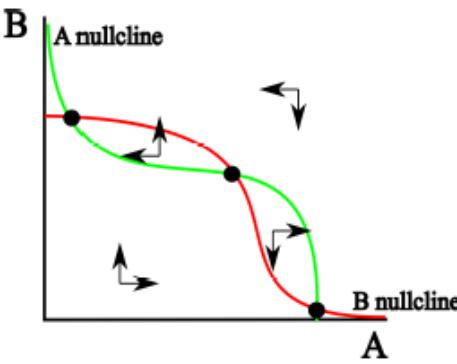
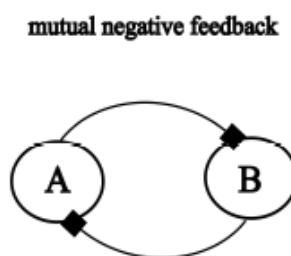
**“French flag”:**

different morphogen concentrations → activate different genes



**Alternative attractors:**

maintain expression domains when morphogen gradient disappears



## **Positional information/ french flag problem**

### **Wolpert 1969**

---

Source/sink/diffusion for gradient formation  
'read-out' of concentration – > cell differentiation  
(stabilization by mutual inhibition)

french flagproblem: how to be scale invariant?

source/sink diffusion is scale invariant!  
(but not a likely solution...)

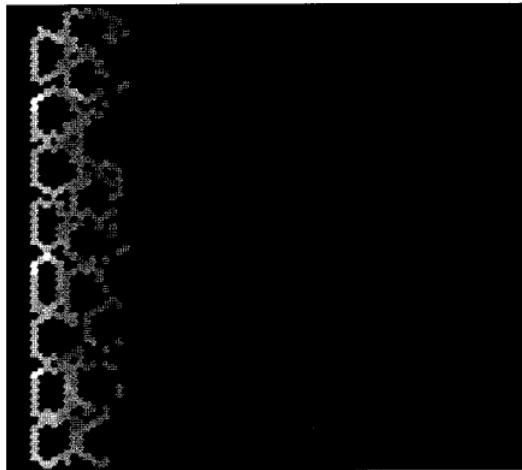
problems: spatial/temporal scaling of diffusion  
in tissue: cell boundaries may not allow gradients  
how to have precise quantitative readout?  
“simple mechanism may not be simple”  
noise

*“pathways which produce and use positional information”*

## receptors disturb gradient cf Kerzberg and Wolpert 1998

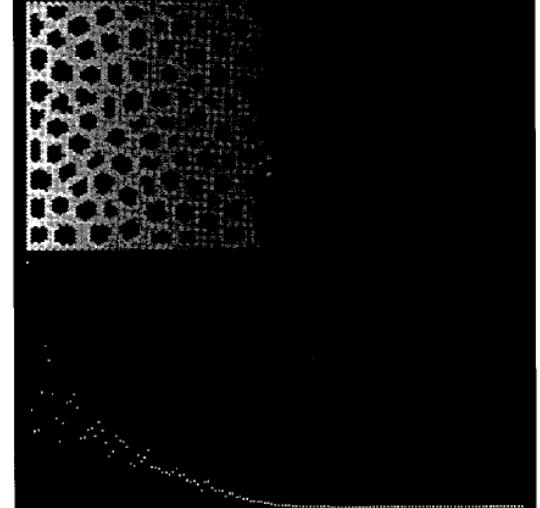
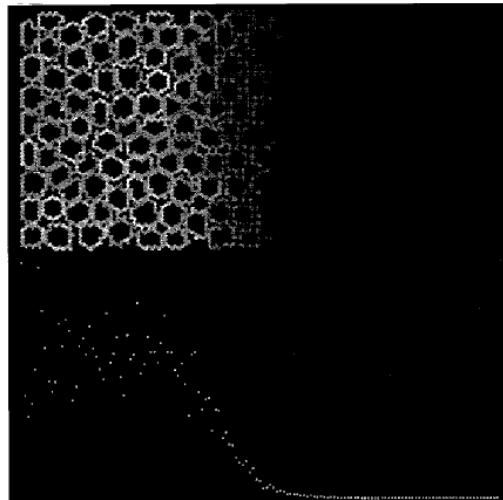
---

(a)



Diffusion

(b)



*several potential solutions proposed*

## early patterning in Drosophila

### Model 1: gap gene expression in Drosophila (pre-gastrulation / pre cellularizatrion)

---

*paradigm system for positional information*

Maternal gradient (Bicoid) (measured)

In syncytium stage (no cell walls to pass)

*paradigm system for data driven quantitative modeling*

Very precise description of pattern in space/time available

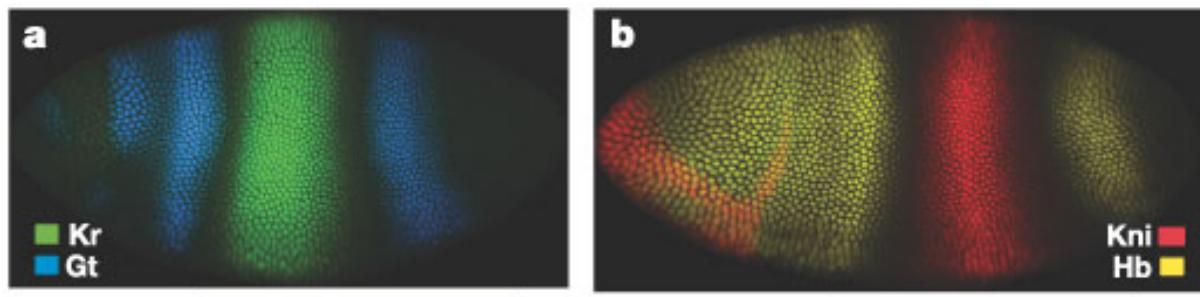
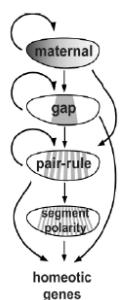
Much experimental knowledge about genes involved and their interactin

many papers main authors J. Reinitz anf J. Jaeger; here used:

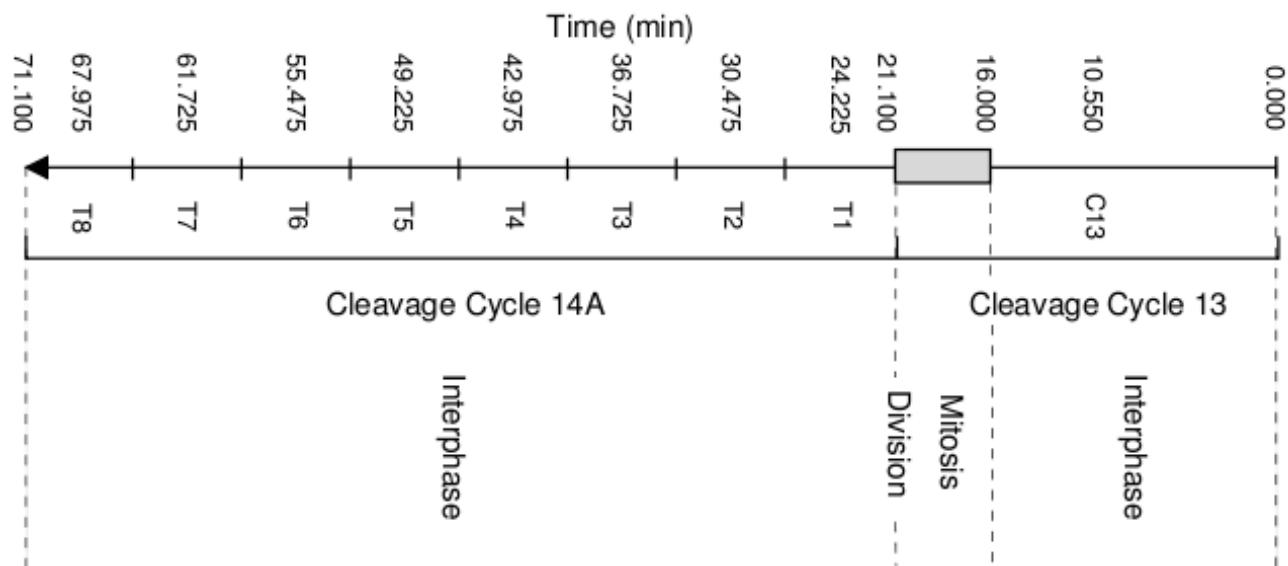
Manu, .... Reinitz 2009 Canalization of Gene Expression in the Drosophila Blastoderm by Gap Gene Cross Regulation, Pos Biology

J.Jaeger .. Reinitz 2004. Dynamic control of positional information in the early Drosophila embryo Nature

## modelled space-time frame



gap gene expression in late stage: black line: modeled area



## modeling gene regulation: ODE for each nucleus

---

$$\begin{aligned}\frac{dv_i^a}{dt} = & R^a g \left( \sum_{b=1}^N T^{ab} v_i^b + m^a v_i^{\text{Bcd}} + \sum_{\beta=1}^{N_e} E^{a\beta} v_i^\beta(t) + h^a \right) \\ & + D^a(n) [(v_{i-1}^a - v_i^a) + (v_{i+1}^a - v_i^a)] - \lambda^a v_i^a.\end{aligned}$$

T interaction between gap genes; m interation with biccid;  
E interaction of gap genes with time varying external factors;  $\lambda$  decay; D diffusion

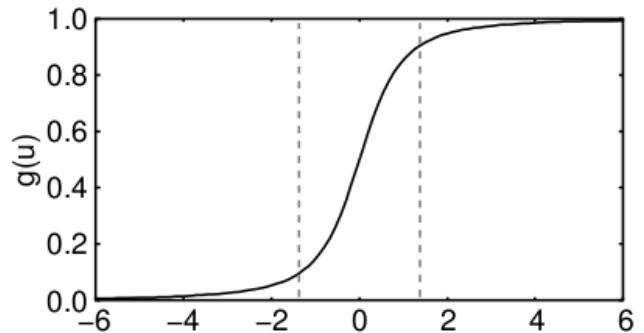
interphase: production, diffusion and decay;

mitosis: only diffusion and decay

division: nuclei divide, inherit state,  
distance between them halved

transcription:

$$g(u^a) = \frac{1}{2} \left[ \left( u^a / \sqrt{(u^a)^2 + 1} \right) + 1 \right]$$



## **“data driven modeling”: massive fitting using simulated annealing**

---

use: 'known genes', initial conditins, spatial/temporal variaton of non-regulated regulators.

Fit model output in all M nuclei, for all genes, at all N timepoints for which data are available.

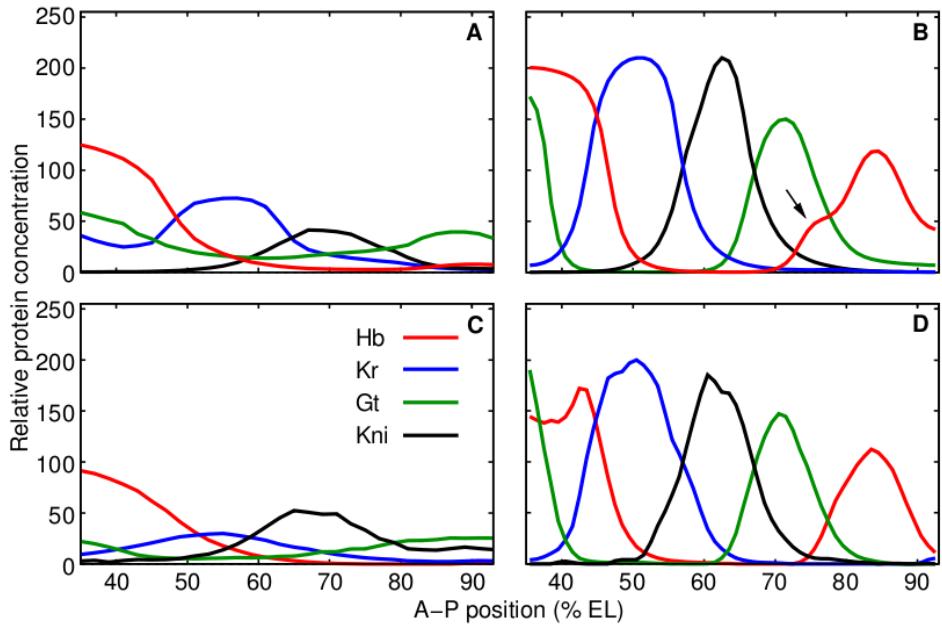
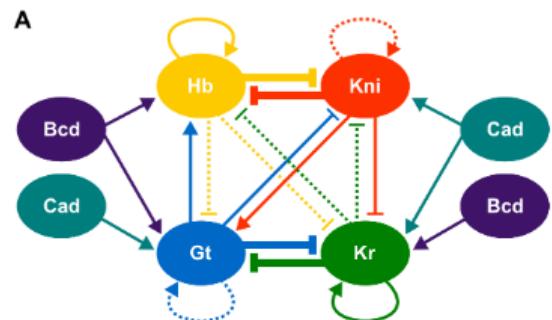
$$E = \sum_{\substack{\text{all } a, i, t, \text{ and} \\ \text{genotypes for} \\ \text{which data exists}}} (v_i^a(t)_{\text{model}} - v_i^a(t)_{\text{data}})^2 + (\text{penalty terms})$$

Do this Z=65 times gives Z different outcomes; and select good fits, no major patterning defects, no known regulatory mistakes (23/65) similar networks

## used example of 'good' network

Target gene <i>a</i>	Regulator gene <i>b</i>						
	<i>bcd</i>	<i>cad</i>	<i>tll</i>	<i>hb</i>	<i>Kr</i>	<i>gt</i>	<i>kni</i>
<i>hb</i>	0.025	0.004	0.003	0.021	-0.001	0.022	-0.112
<i>Kr</i>	0.118	0.021	-0.203	-0.026	0.035	-0.042	-0.062
<i>gt</i>	0.256	0.023	-0.011	-0.028	-0.202	0.007	0.003
<i>kni</i>	0.012	0.020	-0.187	-0.082	0.000	-0.017	0.013

Parameter	Gene <i>a</i>			
	<i>hb</i>	<i>Kr</i>	<i>gt</i>	<i>kni</i>
$R^a$	15.000	10.354	15.000	15.000
$D^a$	0.166	0.200	0.103	0.200
$t_{1/2}^a$	9.529	15.908	9.438	13.062



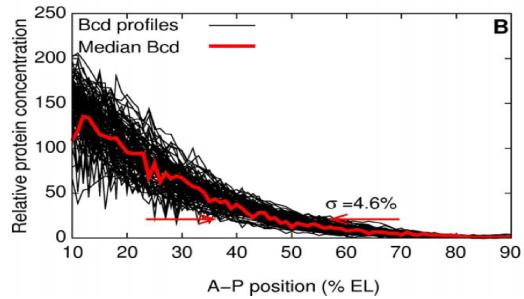
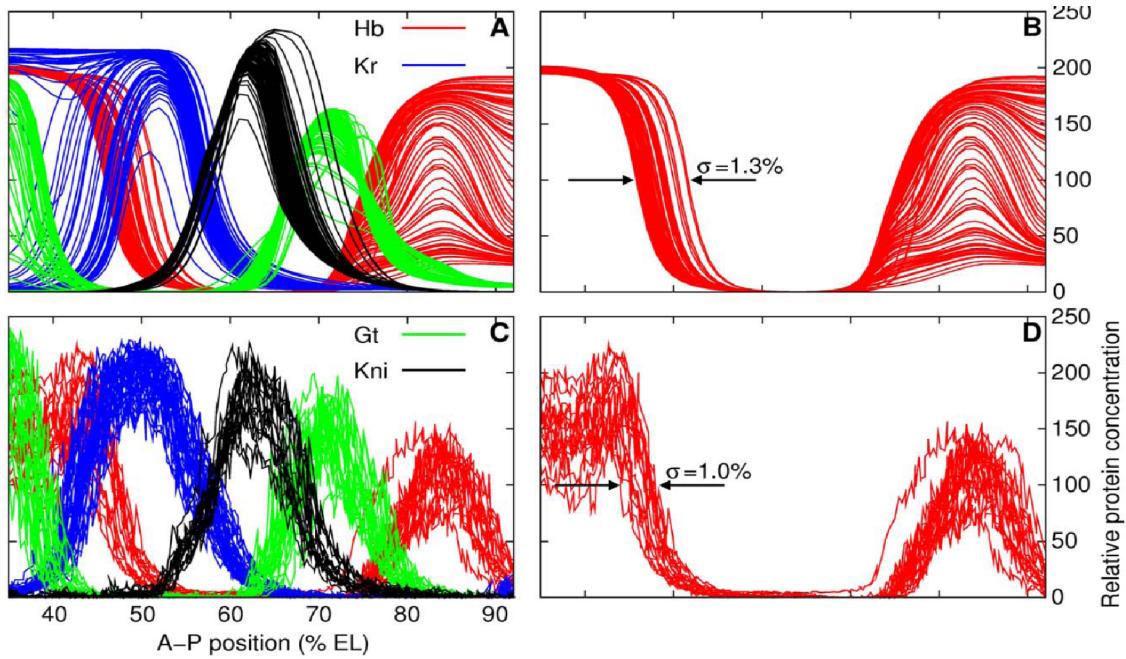
above: model: early - late; below av. exp. early-late

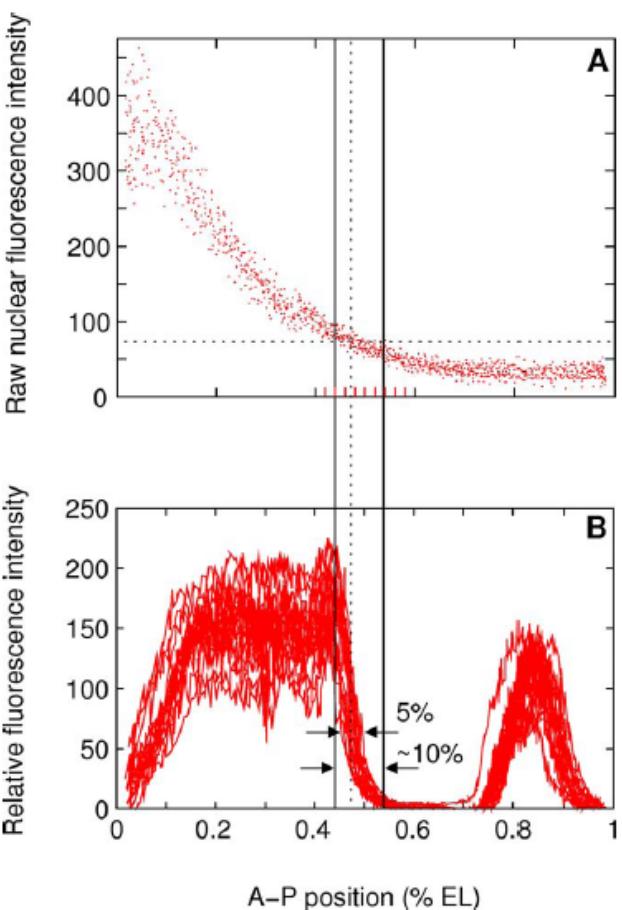
**classical question**  
**developmental patterning very precise, despite differences in**  
**e.g. size of embryo or gradient noise**

---

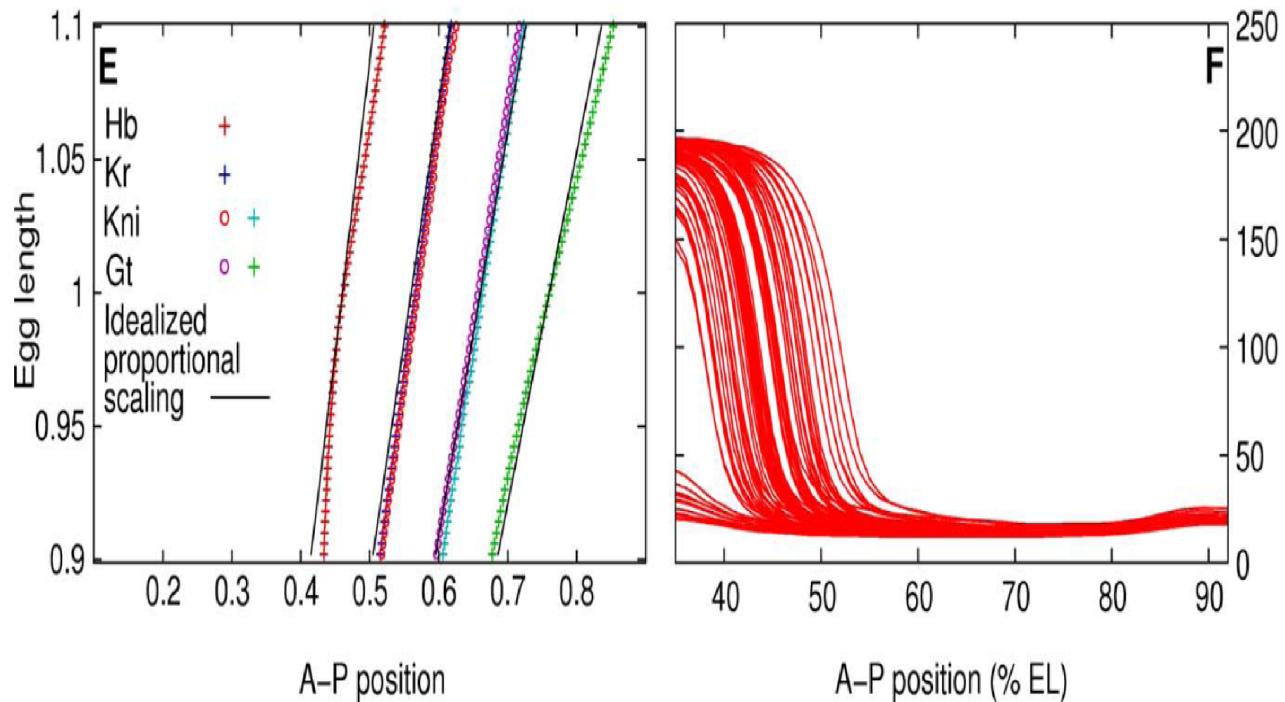
Manu et al 2009: is due to regulatory circuit.

# robustness to variation in bicoid gradient





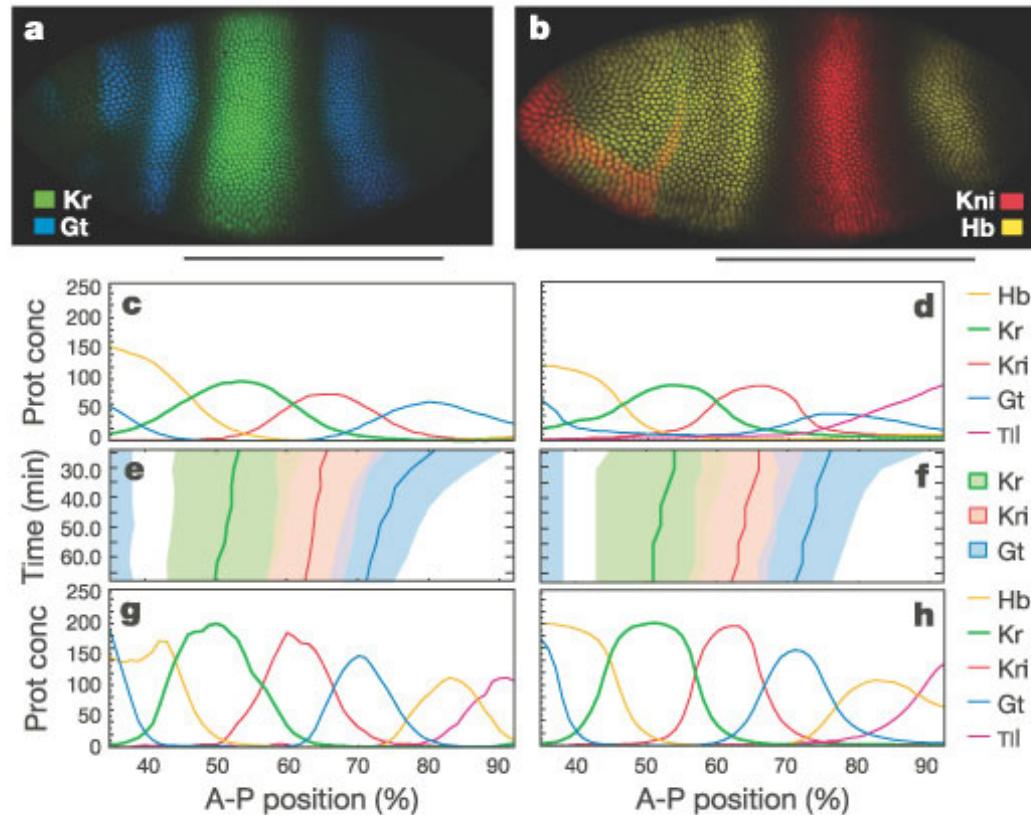
## robustnes to size varation (20%)



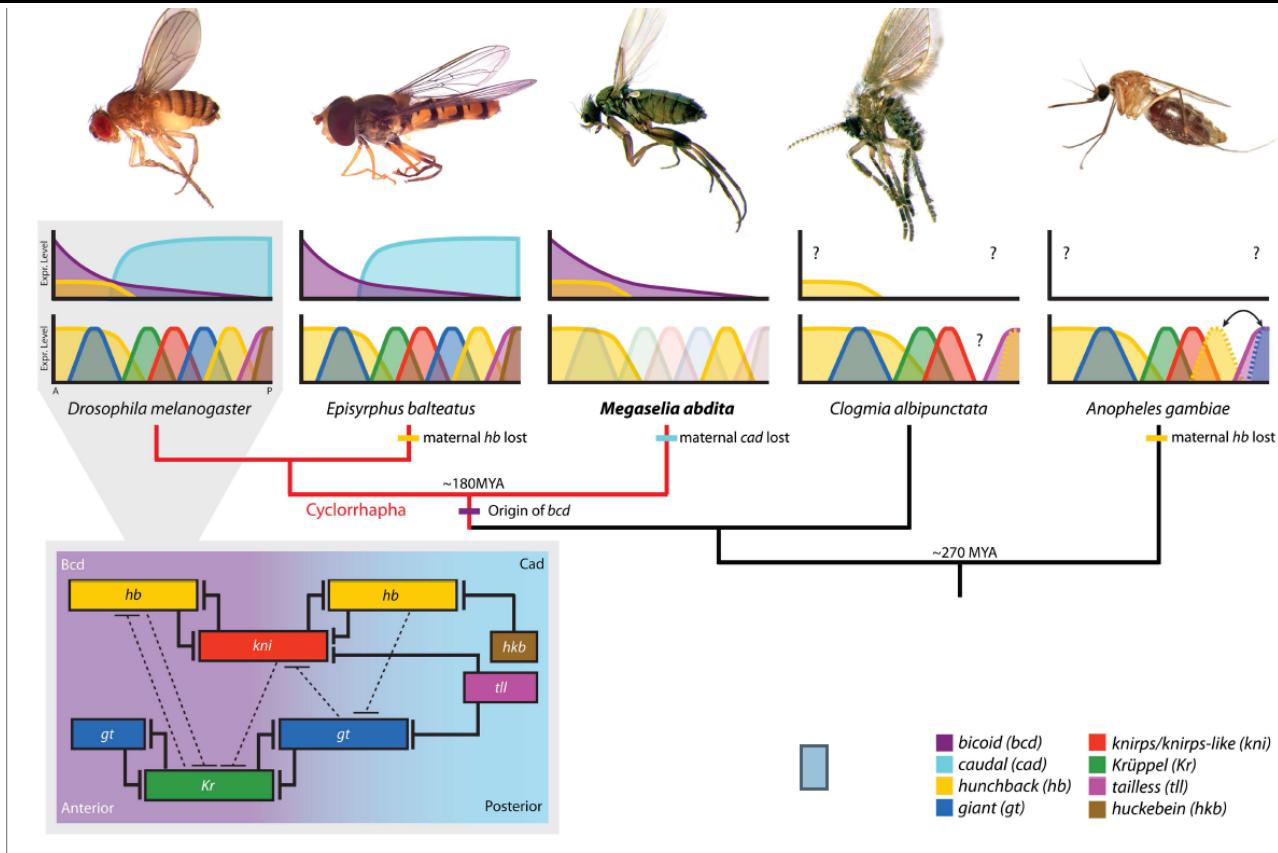
without cross regulation gap-genes

model also reproduces shifts in expression patterns over time

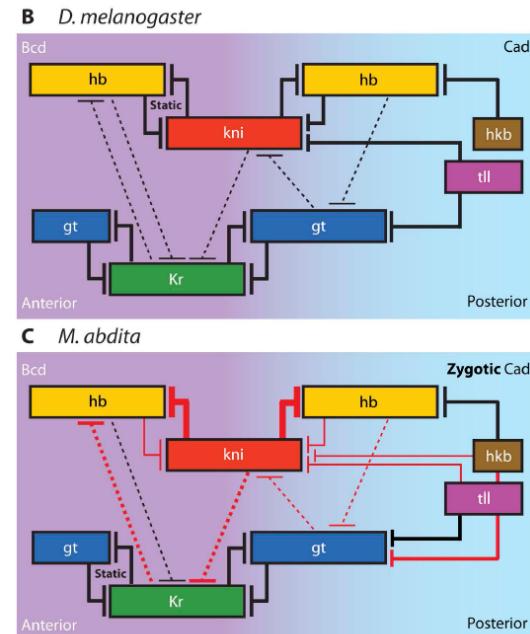
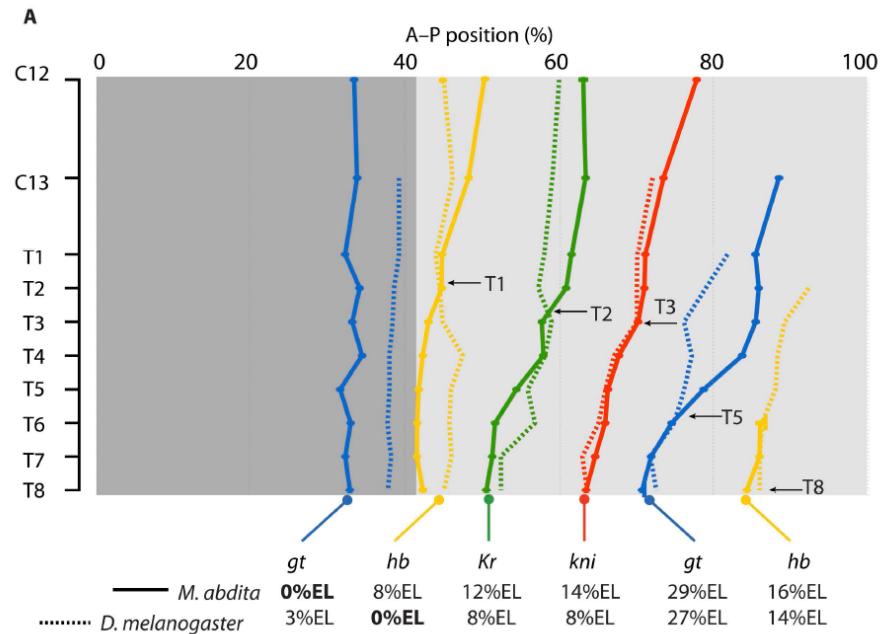
Jaeger et al 2004 op.cit



“Quantitative system drift compensates for altered maternal inputs to the gap gene network of the scuttle fly *Megaselia abdita*” Wotton et al eLife 2015



# Some, but only tiny differences in expression patterns



## discussion/conclusions

---

Fitting not very robust:  
alternative “as good” fits with even opposite signs of interaction  
(filtered to agree with experimental knowledge)

*because of shifting “better” fitting because less degenerate*

supervised models: Fits

++ = scaling property and noise reduction

++ insight in evolutionary drift / compensation in conserved patterning

## **Positional information (?):**

---

yes - gradient given and provides “coordinate system”

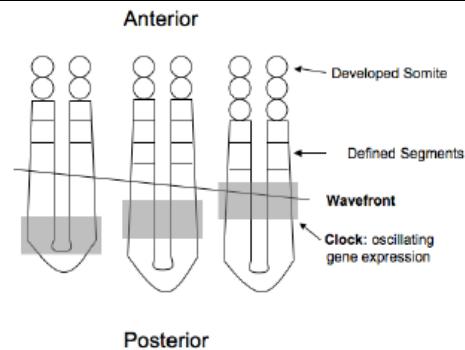
no - not simple concentration readout  
readout itself ‘makes the pattern’

scale invariant (tolerant) because of regulation / not invariant  
bicoid gradient

# However there appears to be a common mechanism in segmentation development in many organisms

## clock and wavefront mechanisms from temporal to spatial pattern

### Cooke and Zeeman 1976

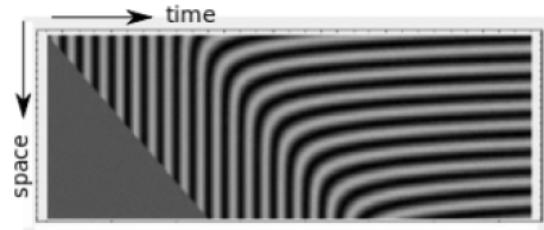


**clock:**

internal cellular oscillations, phase synchronized between cells

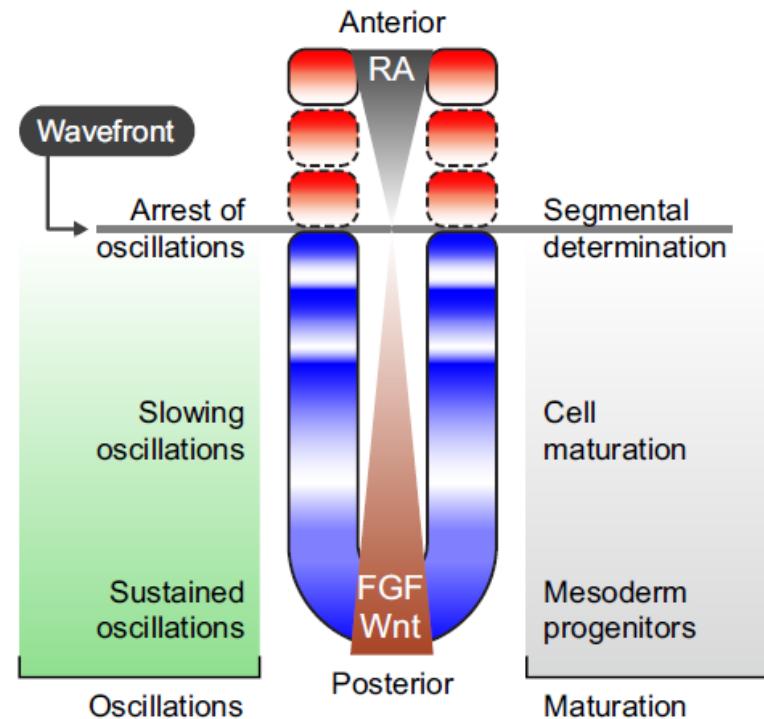
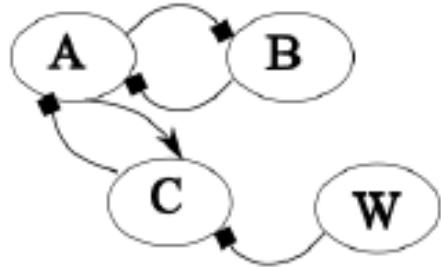
**wavefront:**

competence wave moving from anterior to posterior at constant speed



# gradients which appear to play a role

“arrest” can be autonomous  
(Hopf or other bifurcation  
or extern  
because of bistability  
Goldbeter 20..



similar result .

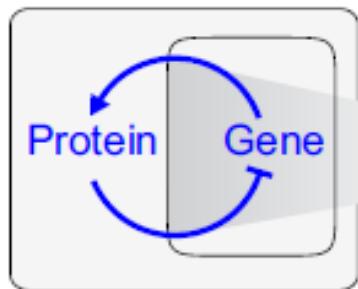
resistant to noise

distance governed by posterior rate of growth.

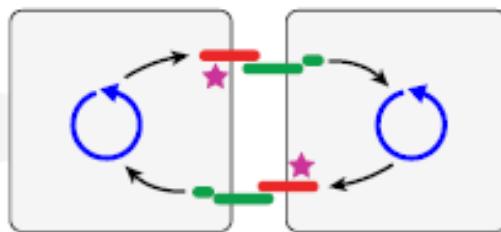
# proposed “implementation” as 3 tier mechanism in somitogenesis

---

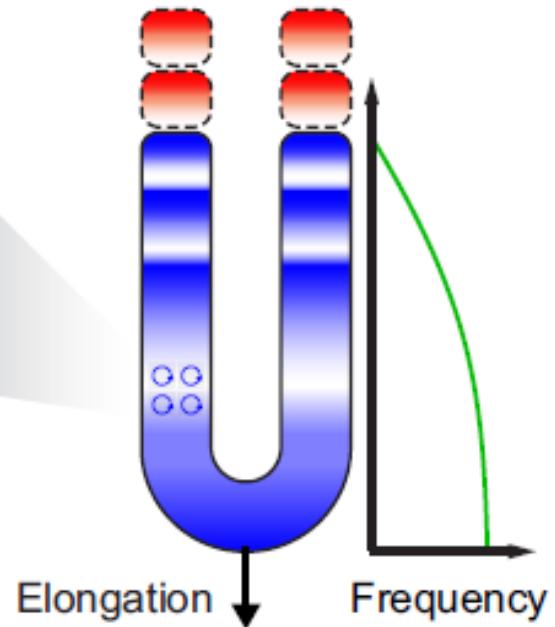
**A** Bottom tier: single cell oscillators



**B** Middle tier: local synchronization

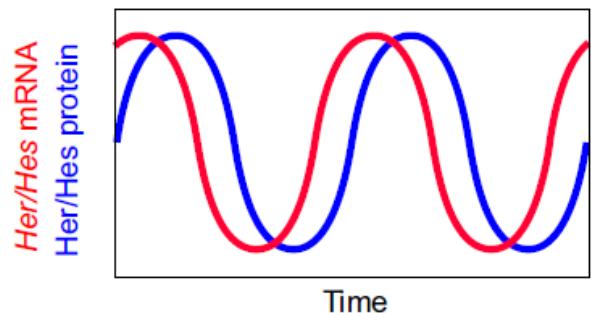
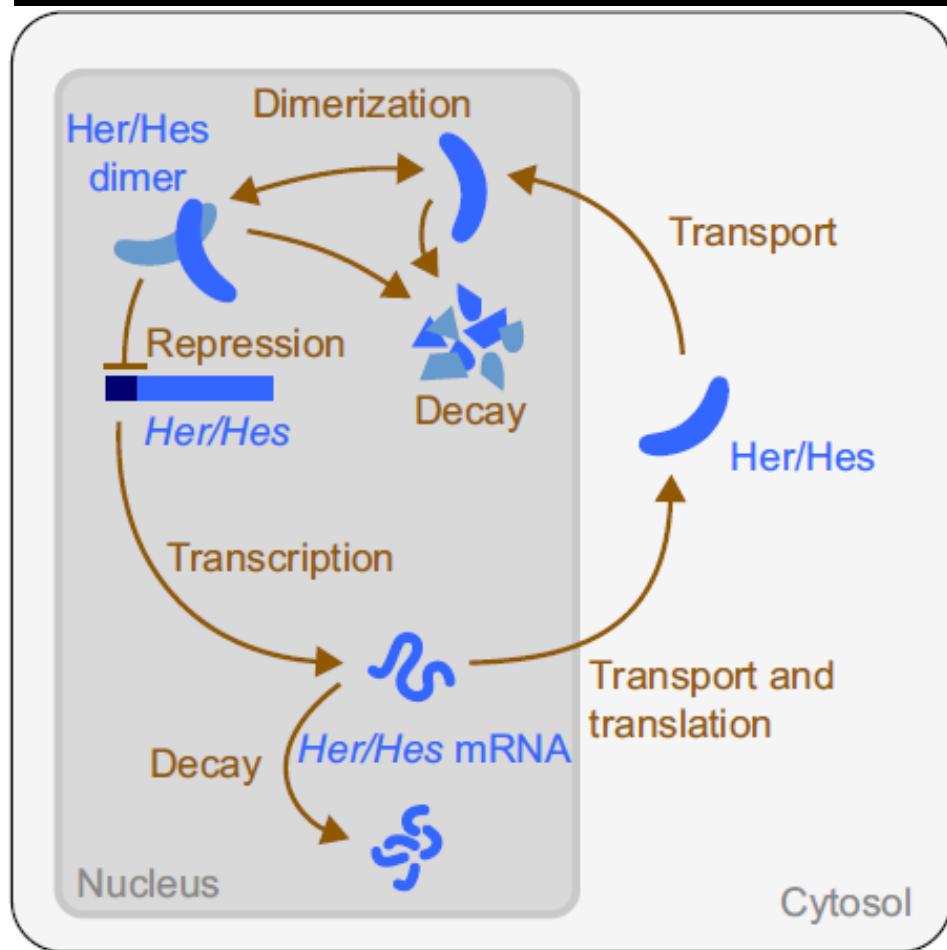


**C** Upper tier: global control of slowing and arrest



cf Oates et al 2012, Development, Morelli et al 2008

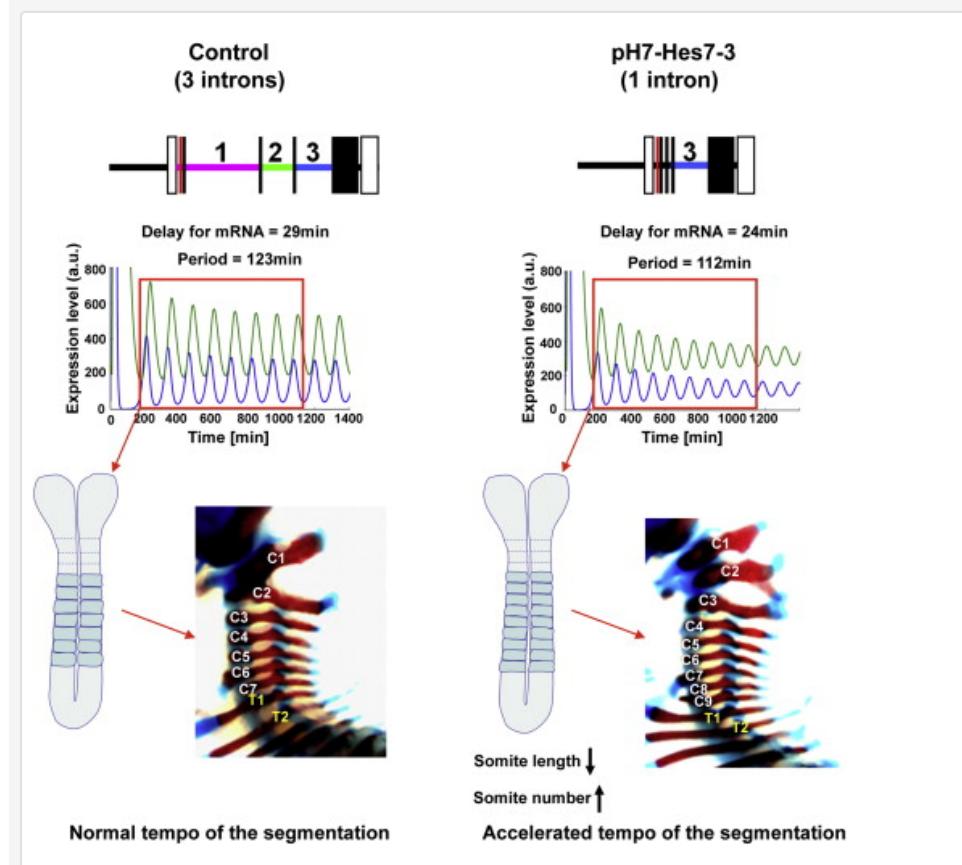
# single cell oscillator: delayed auto-feedback systems



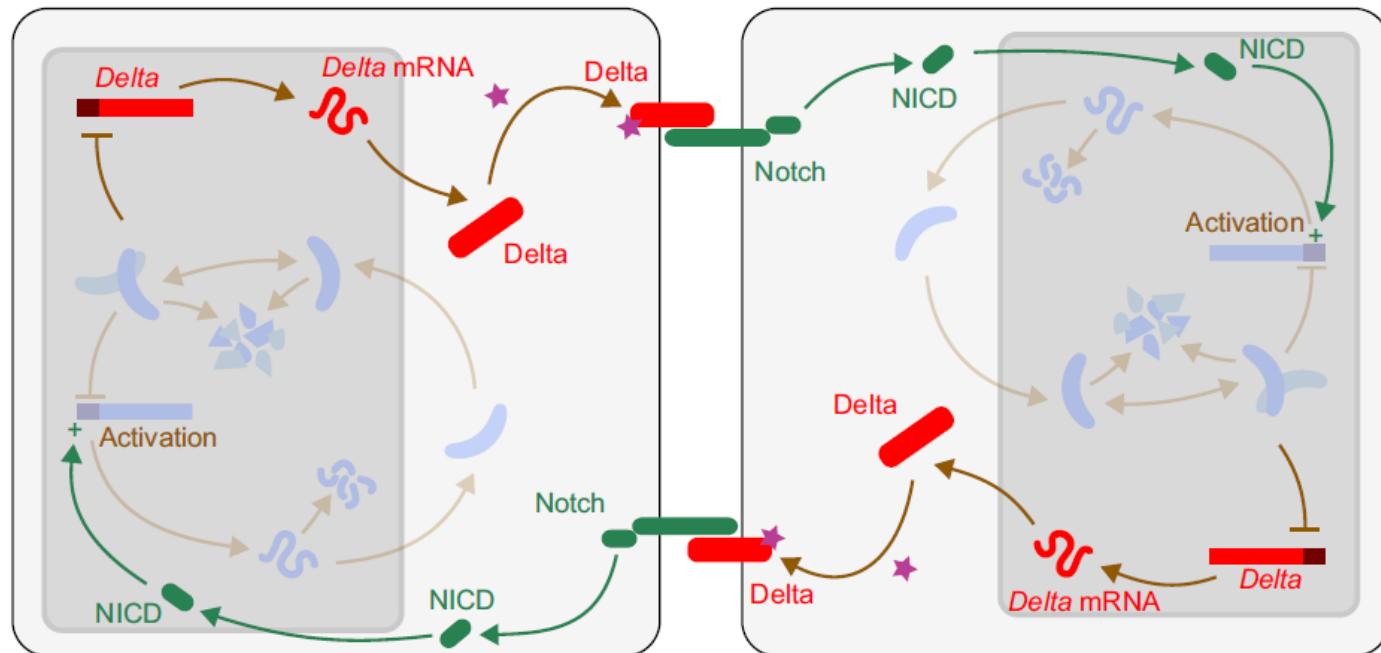
# delay determines number of segments

indeed: intron deletion speeds up the clock

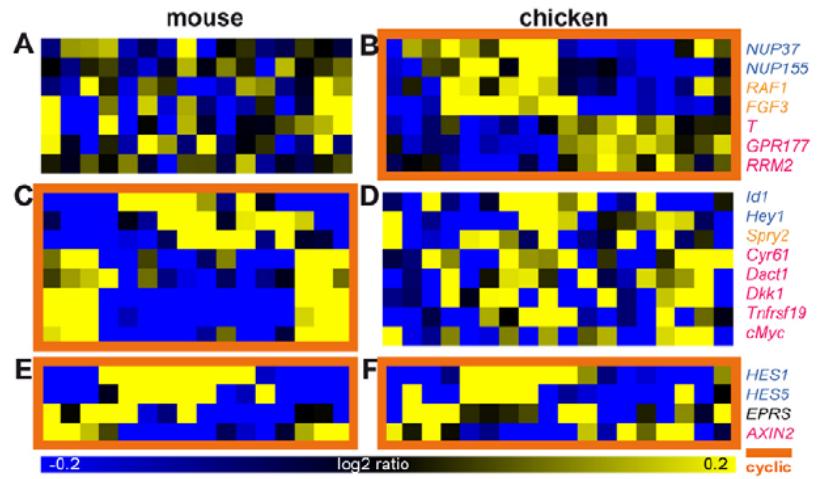
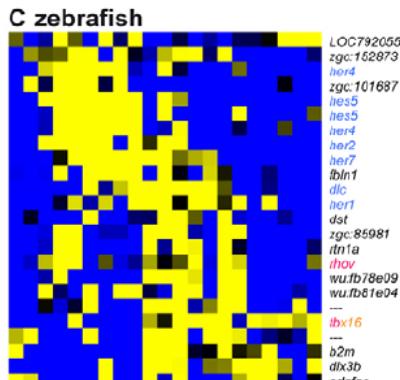
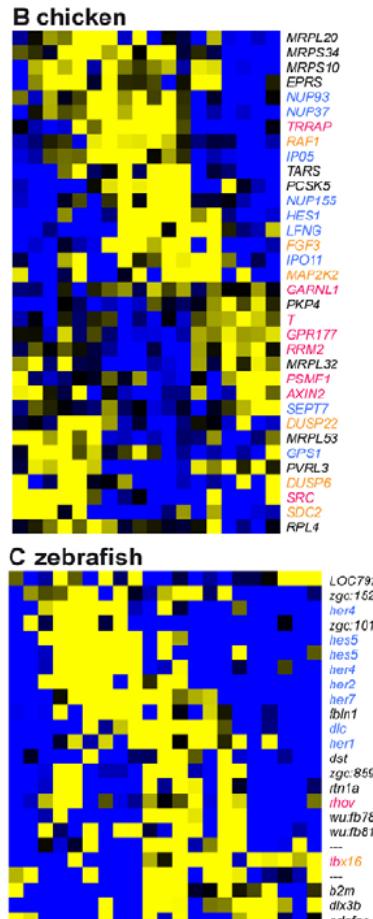
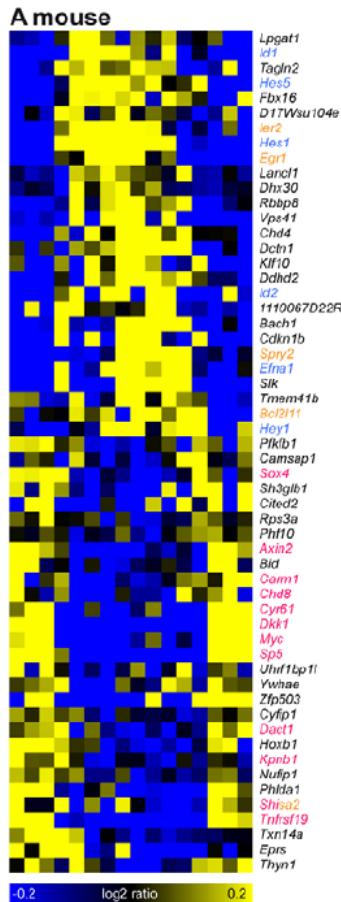
Harima et al Cell 2012



## neighbour synchronization: with delay: longer period

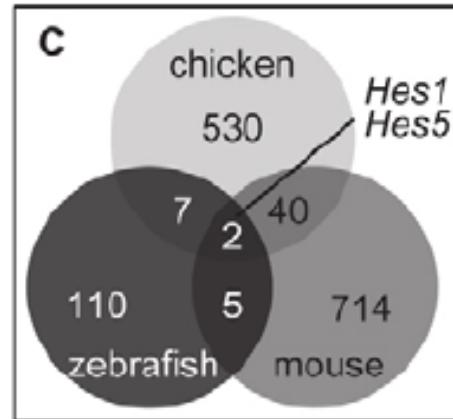


# reinvented or conserved, which genes oscillate? GO terms: signalling and transcription



# Only 2 overlapping orthologs involved in segmentation clock

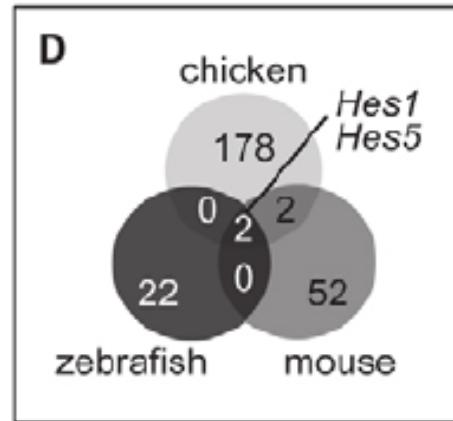
---



first estimate:

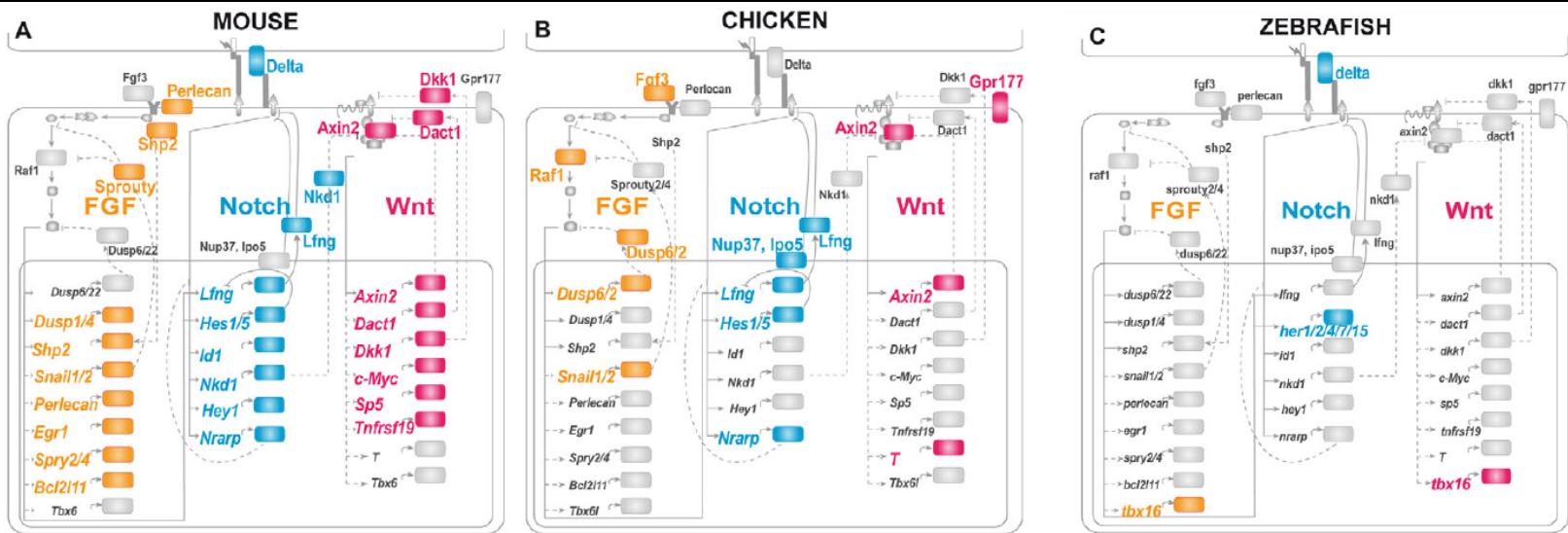
---

---



after filtering:

# Only 2 orthologs: but members of 3 pathways in all



(this analysis first to find member WNT pathway)

## **conclusion: very high plasticity!**

---

Only small subset of the 3 pathways oscillate:  
enough for functional oscillations?  
“just in time assembly” )

Similar (non) conservation pattern in cell cycle mechanisms  
yeast and pombe

Conserved HER/HES delayed oscillator also in  
medaka, Xenopus, and invertebrates (e.g. cockroach)!!

Segmentation lost? reinvented?

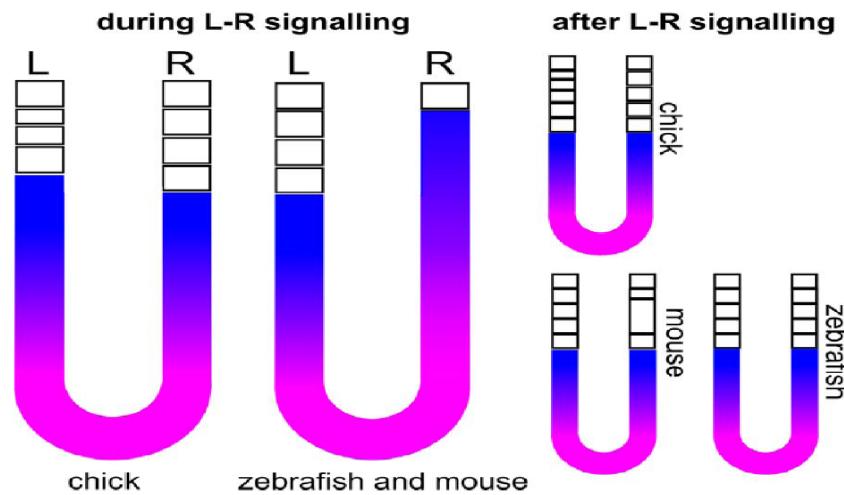
# Is segmentation “the same” in the different organisms??

---

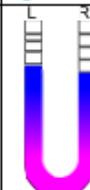
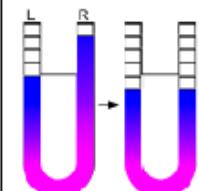
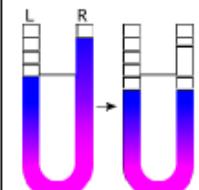
*RA knockout leads to asymmetric somatogenesis  
which is different for different vertebrate species  
HOW/WHY??*

*Model in more detail to find out which difference in regulatory network  
may explain difference in phenotype of RA knockouts*

*R.M.A. Vroomans, K.H.W.J. ten Tusscher*



**Table 3.1.** phenotypes of model organisms during somitogenesis

genetic properties			left-right asymmetry in absence of RA					
organism	pErk dynamics	oscillating pathways	left-right phenotype	Slower osc	FGF8	delay (somite nr)	somite size diff	return to symmetry
chick	smoothly retracting	FGF, Wnt, Notch		right side	symmetric, more anterior	no; left somites smaller	yes	unclear
zebrafish	retracts in jumps	Notch		right side	right side more anterior	right side 2-3 somites delayed	no	yes
mouse	oscillates	FGF, Wnt, Notch		right side	right side more anterior	right side 2-3 somites delayed	sometimes	yes

Vroomans & ten Tusscher 2017, Modelling asymmetric somitogenesis: Deciphering the mechanisms behind species differences

## **Generic mechanism vs species specific differences neutral drift or functional significant???**

---

Vroomans & ten Tusscher 2017:

*Indeed, our results suggest that rather than focussing on a catch-all mechanism in all vertebrate species and assuming that species differences merely reflect neutral developmental systems drift, we should keep an open mind for the possibility of functionally significant species differences.*

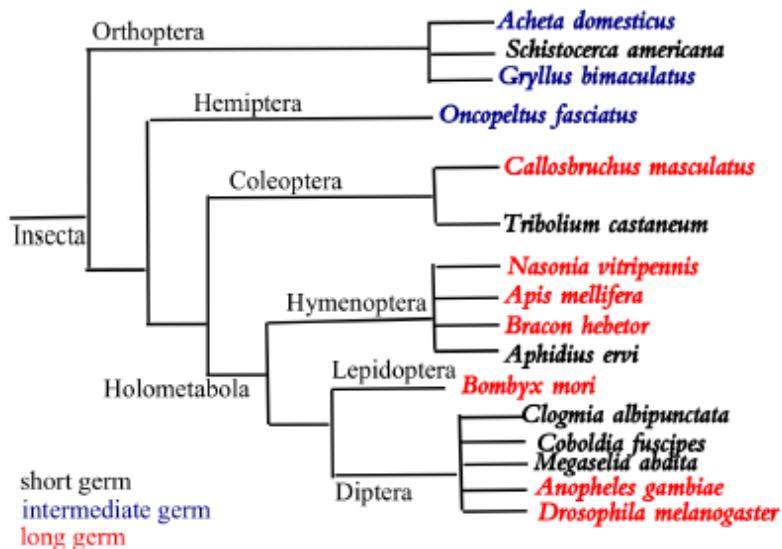
OR

*Side-effects of neutral drift*

# But what about Drosophila?

## 2 (3) mechanisms in insects short vs long germband (+intermediate)

B Long germ segmentation in the Insect tree



clock-wavefront (sequential) mechanism might be ancestral  
- reinvention of simultaneous mechanism long germband??