Geometry of Epistasis in Developmental Patterning

With Francis Corson, Rockefeller

Discussions with MA Felix, ENS Paris,
Paul Francois McGill

Developmental Dynamics

Development (x,t): signaling, ... transcription⇒black box
Phenotypic (geometric) models, old idea: *Waddington wooliness*
Dialectics ⇒

System: Worm vulva (quaint?, pre-omics)

Results:

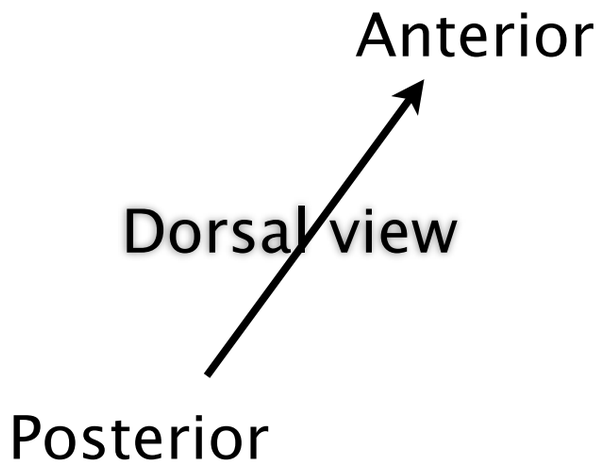
- Intrinsic definition of epistasis (its all a matter of variable choice),
- How many parameters needed for (x,t)?
- Are they 1:1 with experiments?
- Numerical predictions

Gastrulation of Xenopus

1.2mm egg

5 hrs fertilization to Movie0
4000+ cells

17hrs @23C Movie



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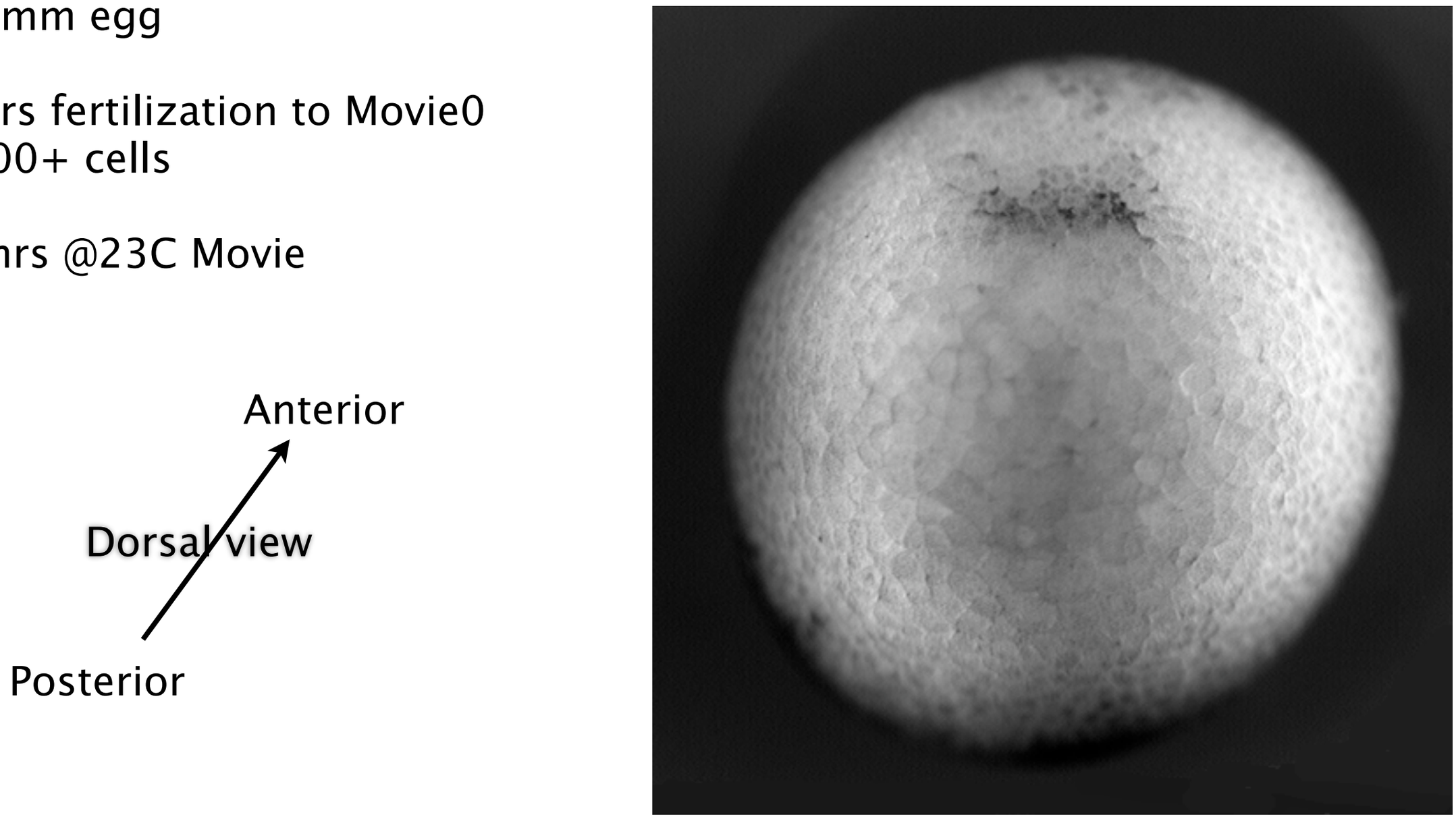
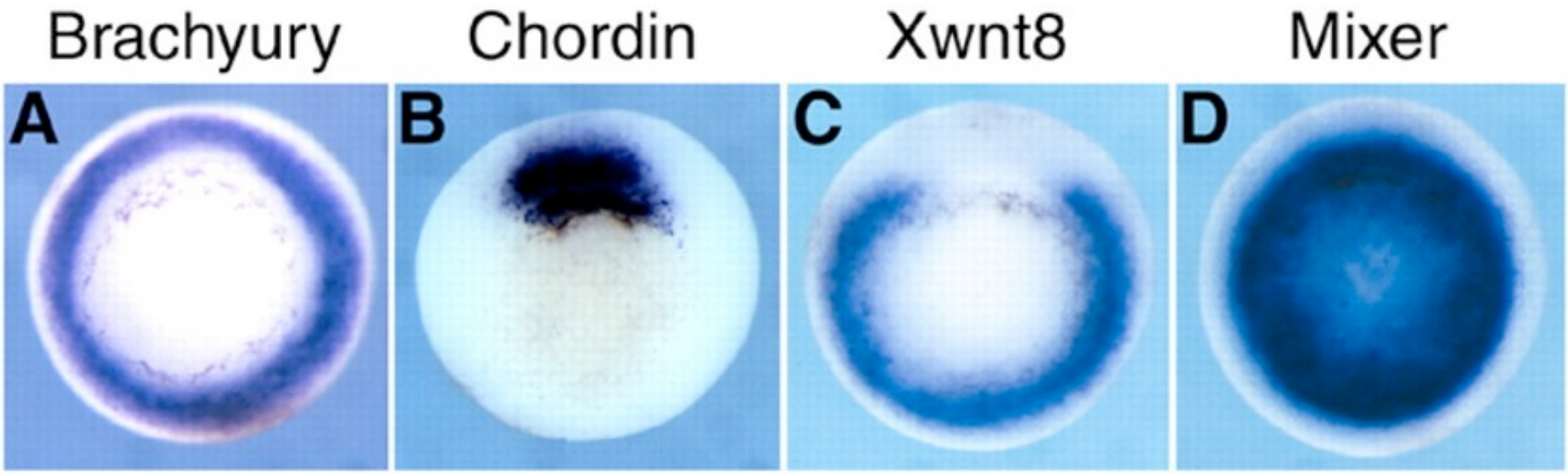


Gene expression delimits territories

(or why parameterize development)



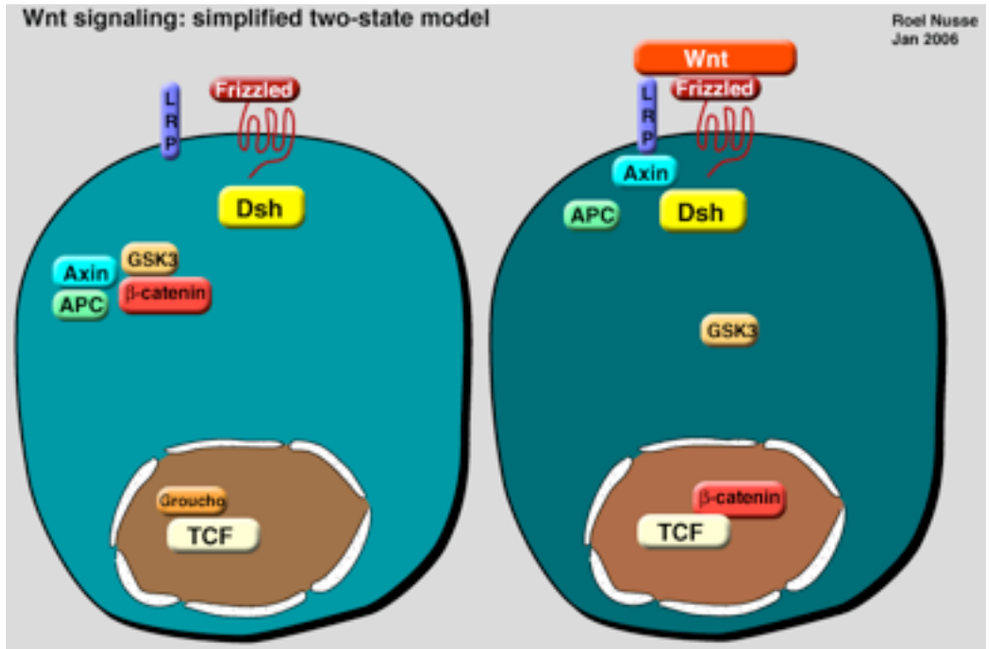
Steiner AB etal *Dev*. 2006. Stage 10.25 images

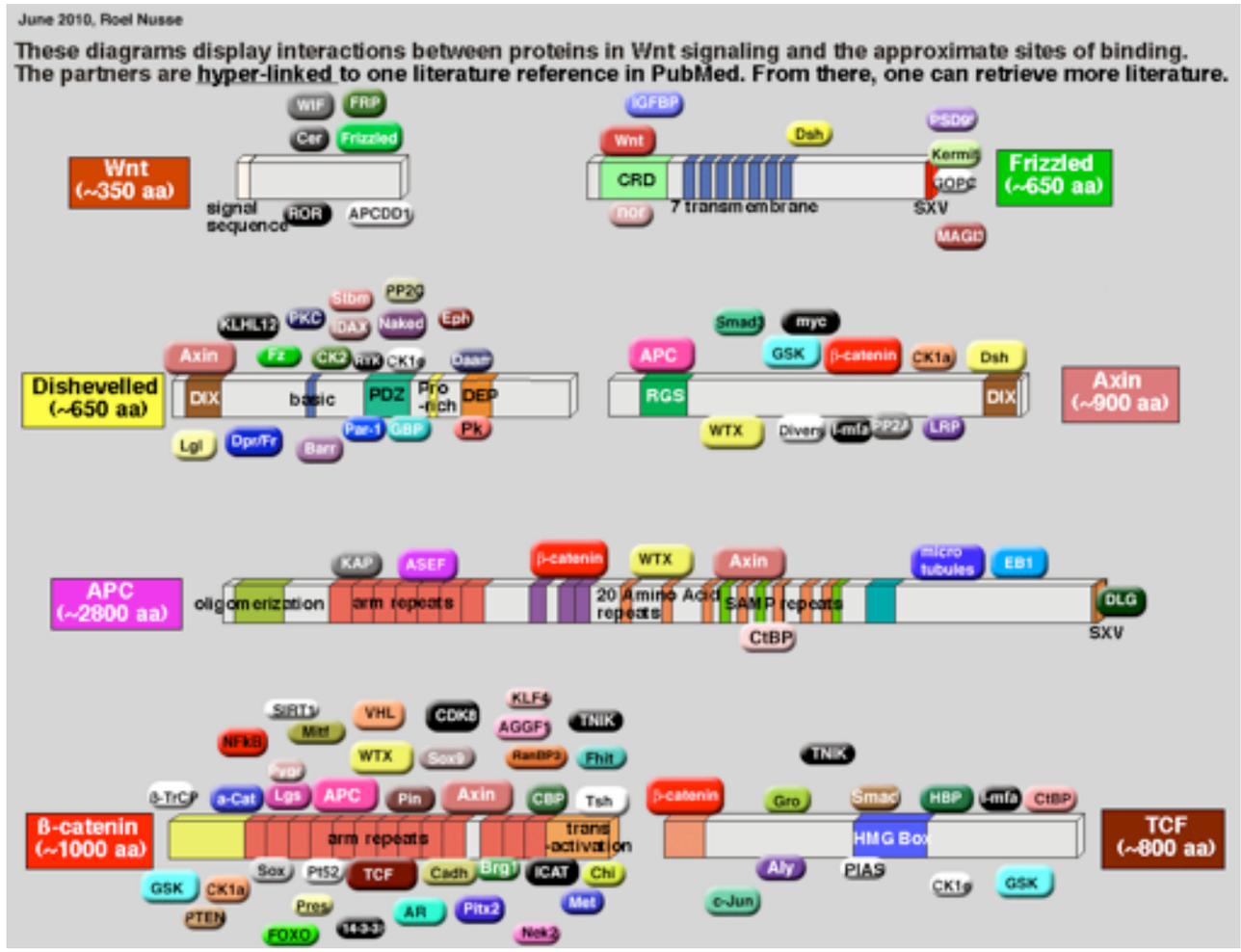
But many mutants active during gastrulation scored 0-10 on *belly-brain* axis (aka DAI) well after gastrulation.

Brivalnou lab

Signaling Pathways are Complex

Wnt's for dummies

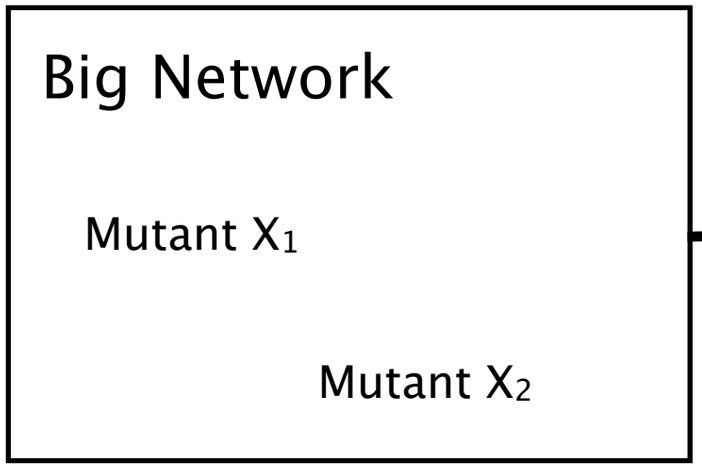




NB cell cycle frog ~ 20min, vs culture cells 12-24 hrs

Frog patterned w/o transcription

Epistasis depends on context



$$O = O_{wt} + O_1(\text{if mut 1}) + O_2(\text{if mut 2}) + O_{1,2}(\text{if mut 1 \& 2}) \text{ (epistasis)}$$

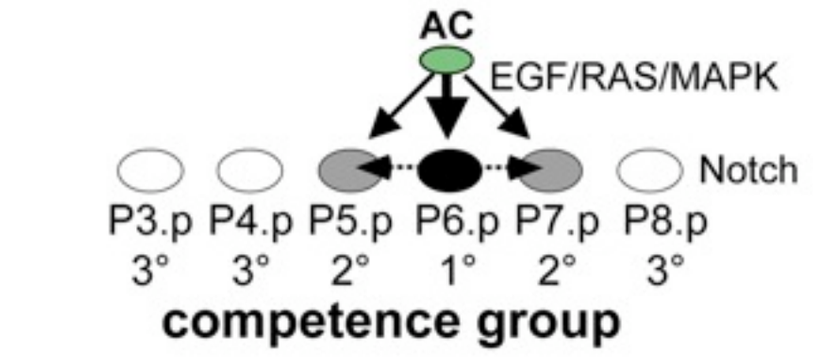
If mutations in ~ energy and O ~ probability of event, then

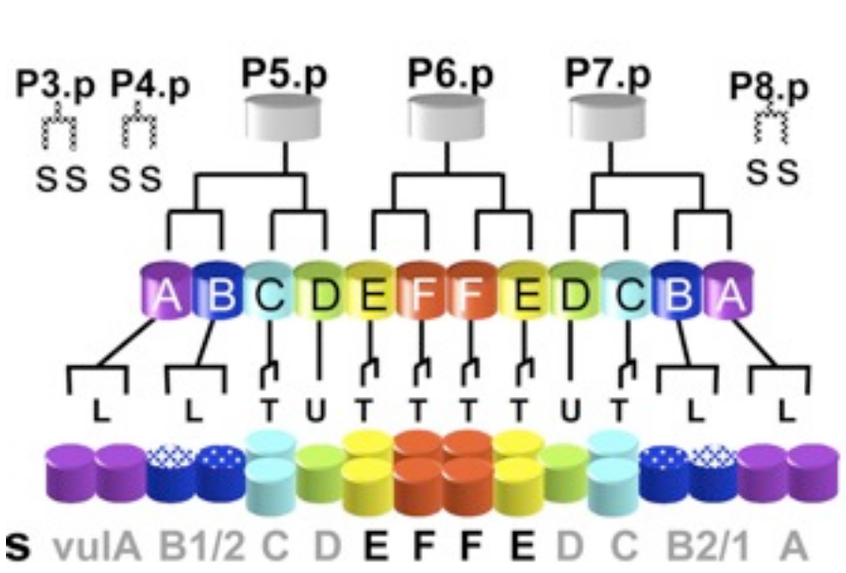
$$O = cst*\exp[- E_1(\text{if mut 1}) - E_2(\text{if mut 2}) - E_{1,2}(\text{if mut 1\&2})]$$

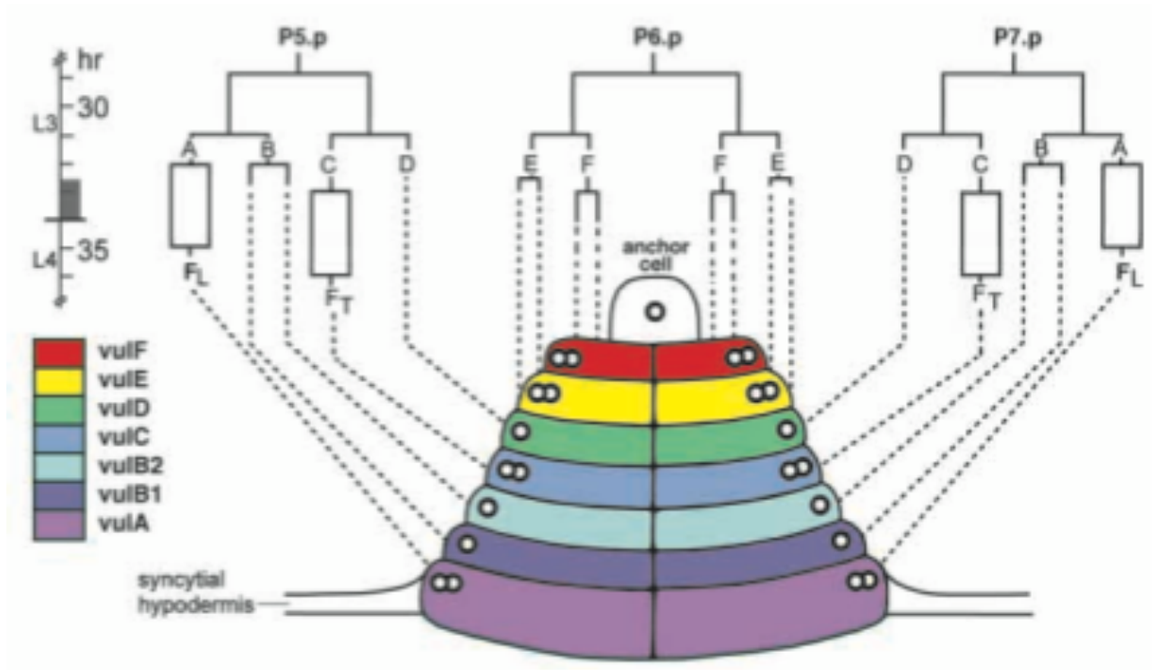
If O relates to (x,t) events during development how does one parameterize??

Worm Vulva

Score terminal fates

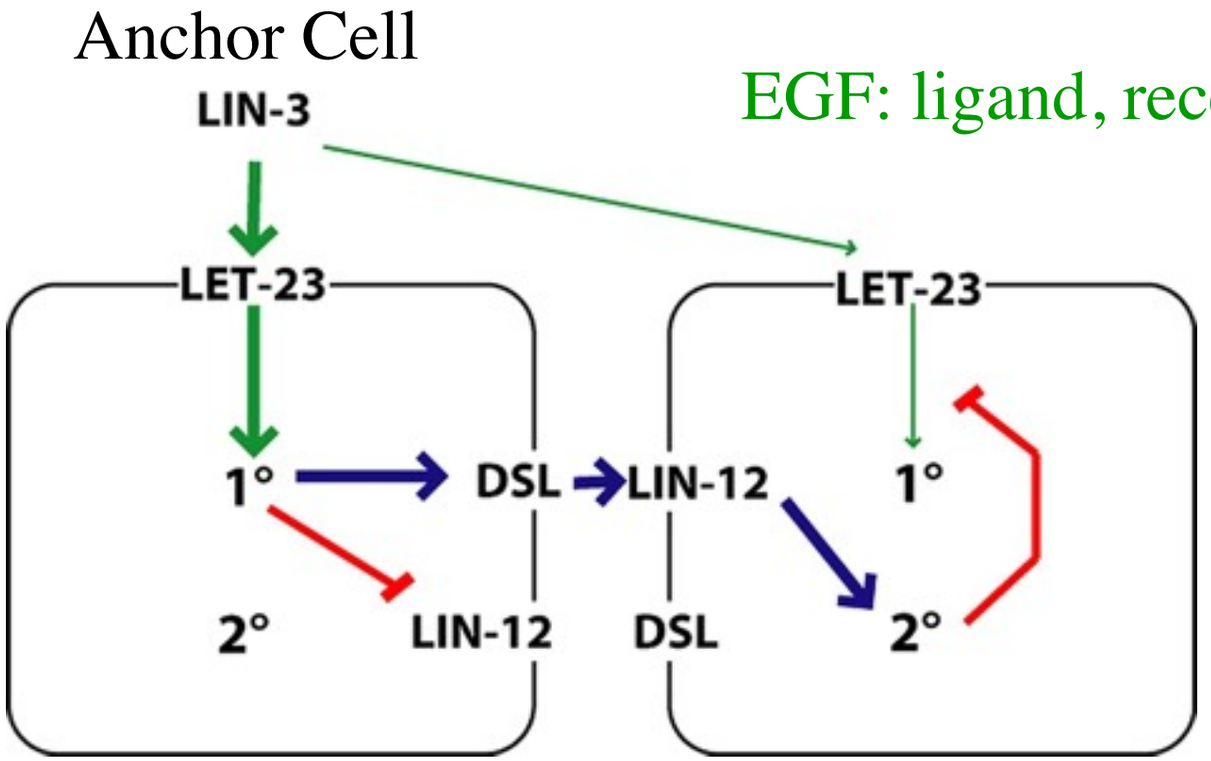






Klontke etal 2007
Sharma-Kishore etal 1999

EGF and Notch pathways define pattern



EGF: ligand, receptor, Ras MAPK

Many genes mutually inhibit

N ligands (diffusing & membrane) and receptor

EGF: graded *inductive* signal from anchor cell (AC)
Notch (N): *lateral* signal, necessary and sufficient for Fate 2

Sternberg *Wormbook*

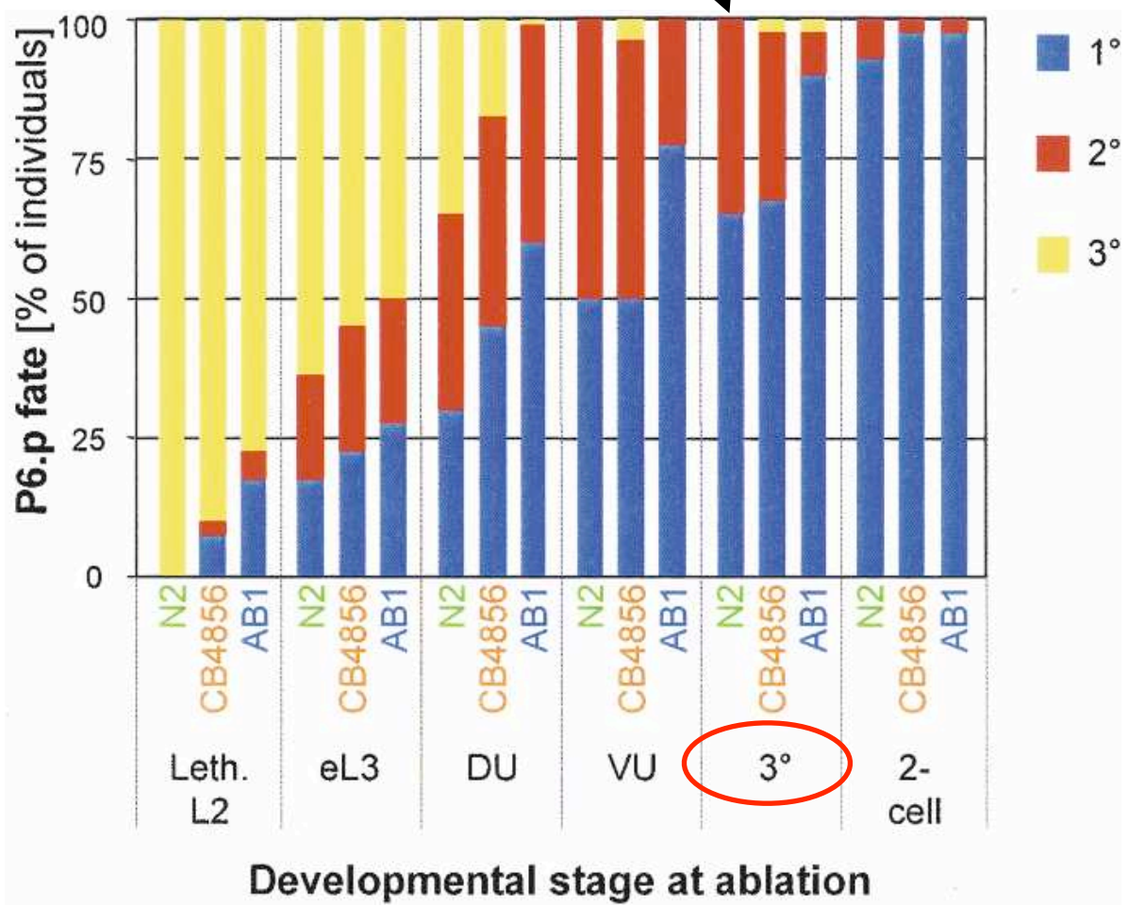
Typical data (Milloz 2008)

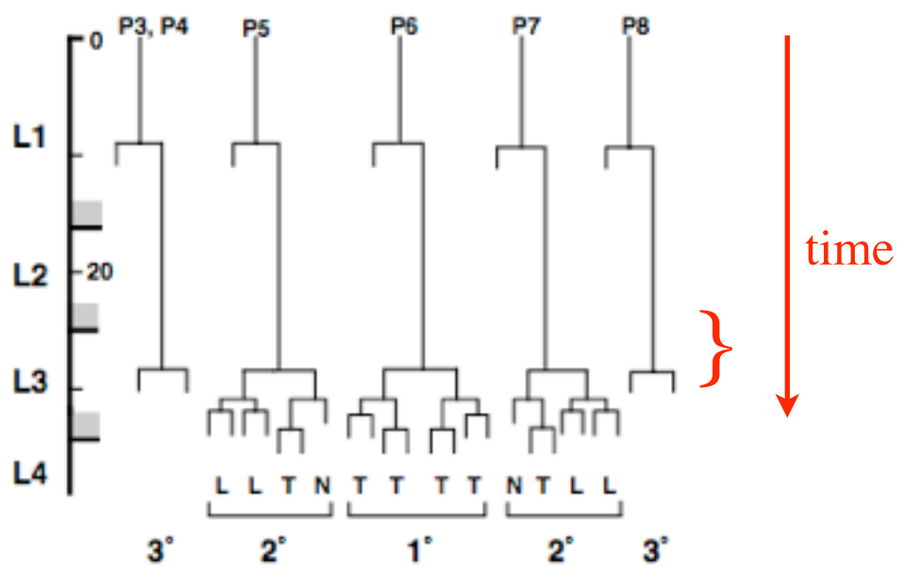
Cell ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	many
AC	3° divided	LLTU	TUUT	UTLL	**4/20**
		LLTU	TTUO	UTLL	**1/20**
		LLTU	TTUT	UTLL	**1/20**
		LLTU	TUTT	UTLL	**2/20**
		LLTU	TUTT	UOLL	**1/20**
		LLTU	TUDT	UTLL	**1/20**
		LLTT	TTTT	TLLL	**1/20**
		LLTU	TTTT	UDLL	**1/20**
		LLTU	TTTT	UTLL	**8/20**

Ablate Anchor Cell in strain N2 WT (for 3 vulva cells)

0.5 1°/0.5 2°

Orientations of Divisions
T=transverse
L=longitudional
O=Oblique
U=Undivided
S=fuse with Syncytium (3°)



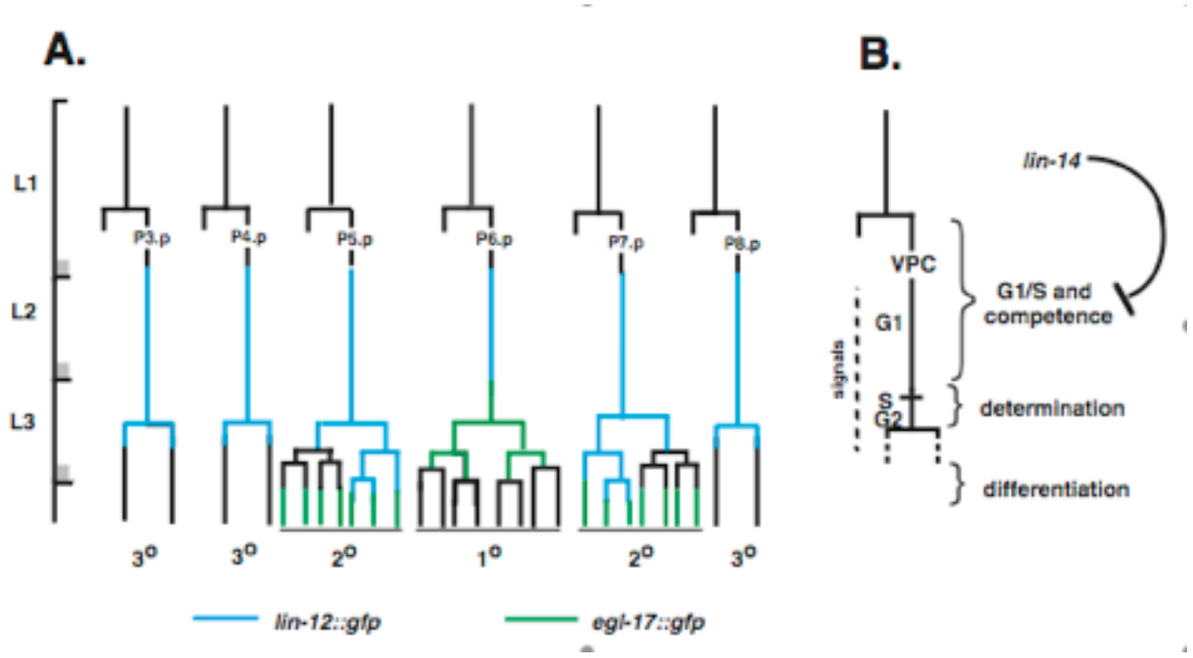


Embryological stages

Equivalence group: Set of cells able to assume a fate
Competence: ability to respond to signal
Specification (committed): Fate defined even if withdraw signal
Determination: Fate unchanged even if supply new signal
Differentiation: Changes in morphology, specific gene products.

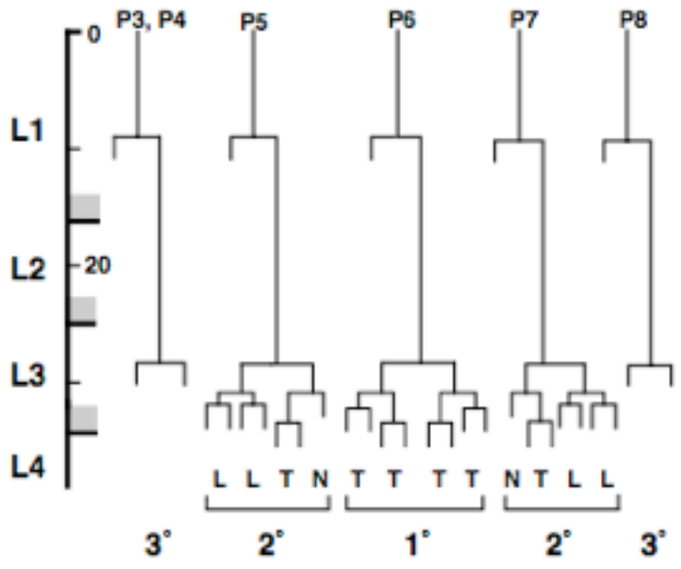
These concepts ~ math thus
Nature of evidence ??
Formulation of model

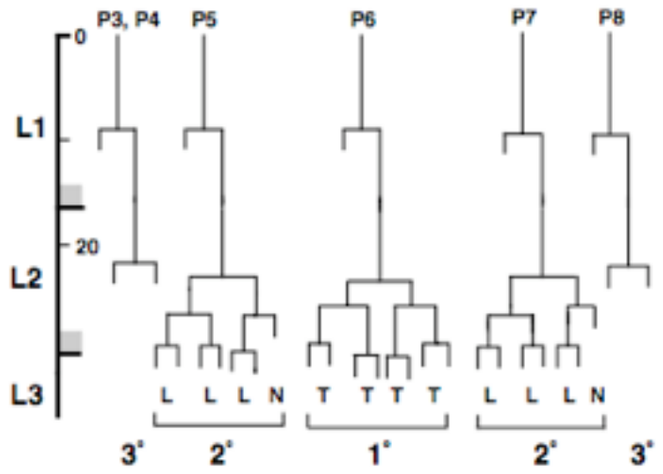
Specification & Determination tied to cell cycle



Larval stages and molts define land marks relative to divisions

Signal reception tied to cell cycle not clock time (HU & mutants).
(Euling 1996, Ambros 1999, Wang 1999, Li 2010)



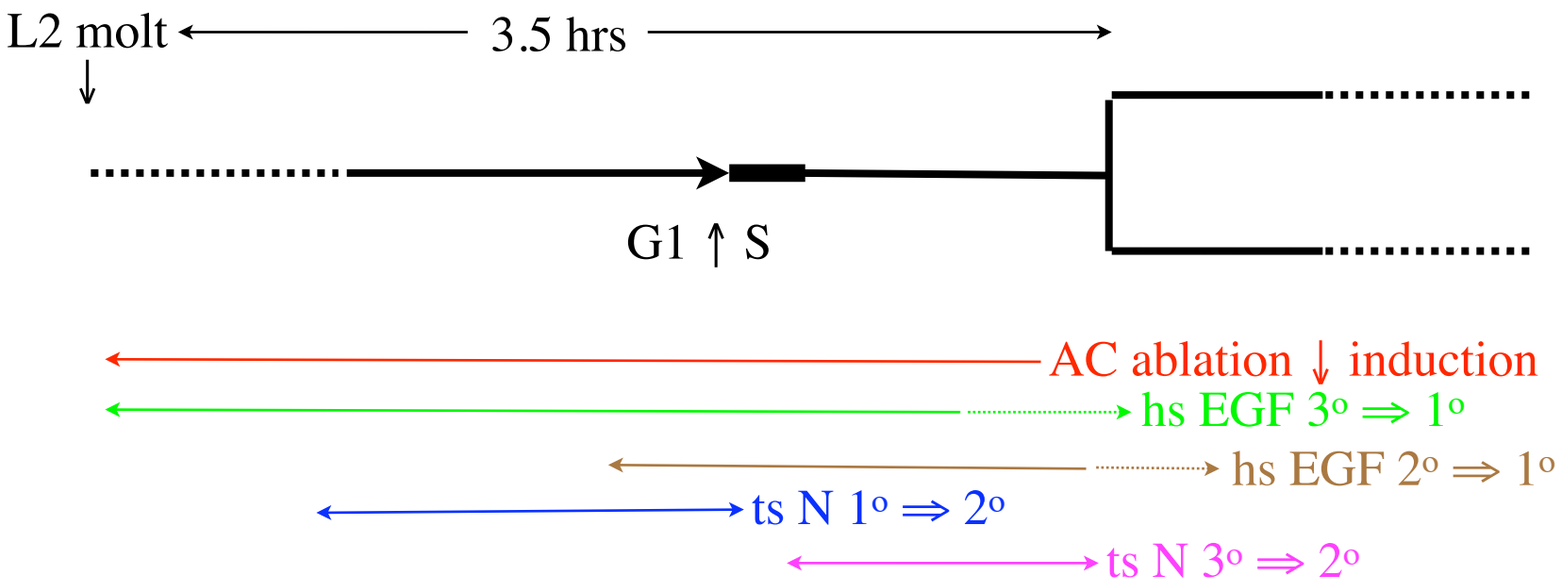


WT ⇒ lin14 mutant

Cell divisions and response to N, EGF? all move together

Specification & Determination (2)

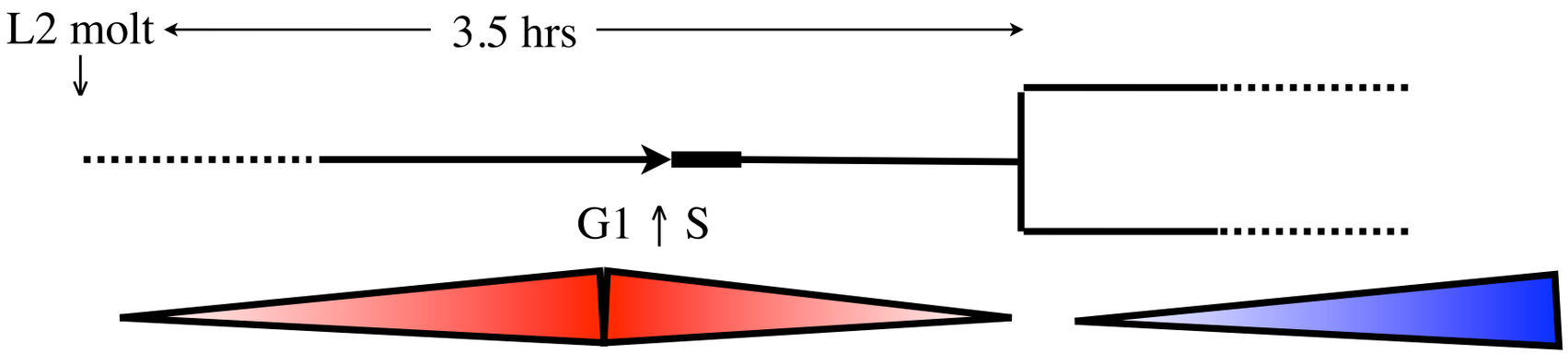
(+- ligands in sensitized backgrounds)



Wang etal 1999,
Ambros 1999

1. Ablate AC(time) removes EGF in WT & hypomorph record % induction.
2. EGF hypomorph (VPC ⇒3°): hs EGF ≤ 1st div. and % induce
3. N receptor (gf) (VPC⇒2°): hs EGF(time) induce % 1°
4. ts N receptor(gf): presumptive 1°⇒2° prior to S, 3°⇒2° until 1st div

Specification & Determination (3)



Specification: late G1-M gradual (AC ablation EGF ⇓, ts N)

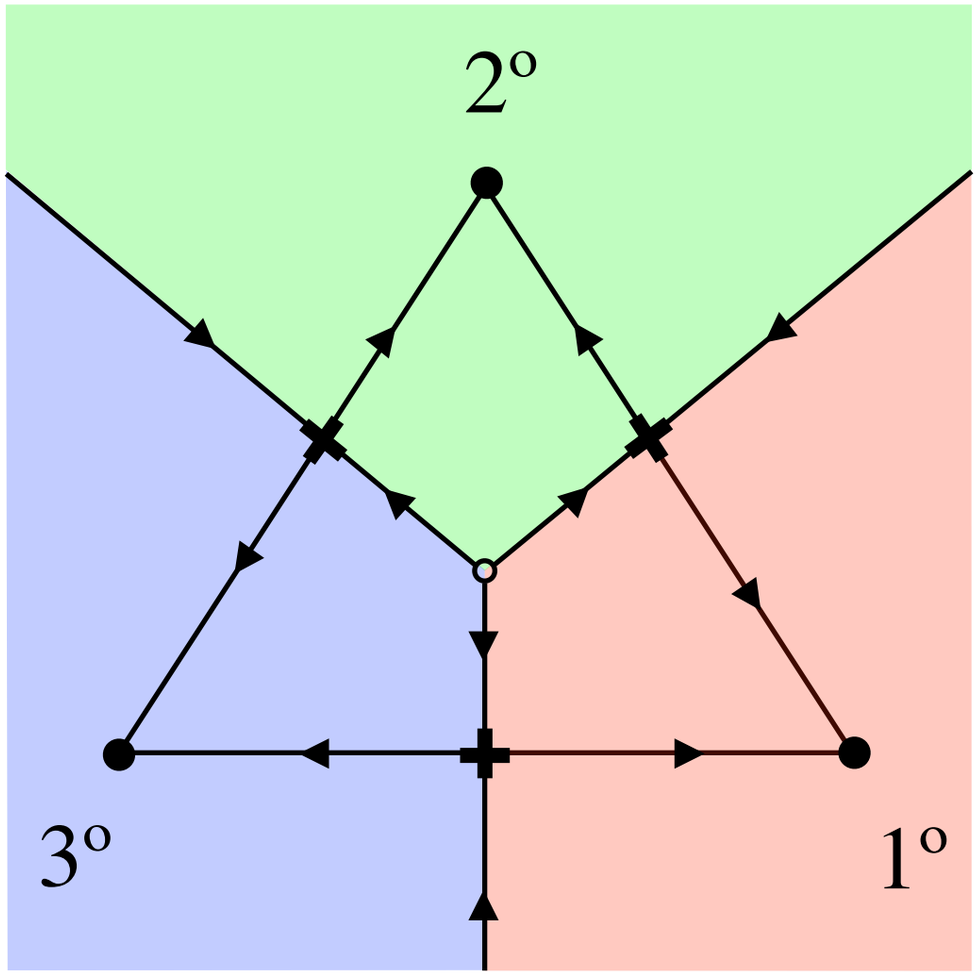
Determination: >~ 1st division, resistant to further signals, gradual ectopic ligands push cells around fate plane

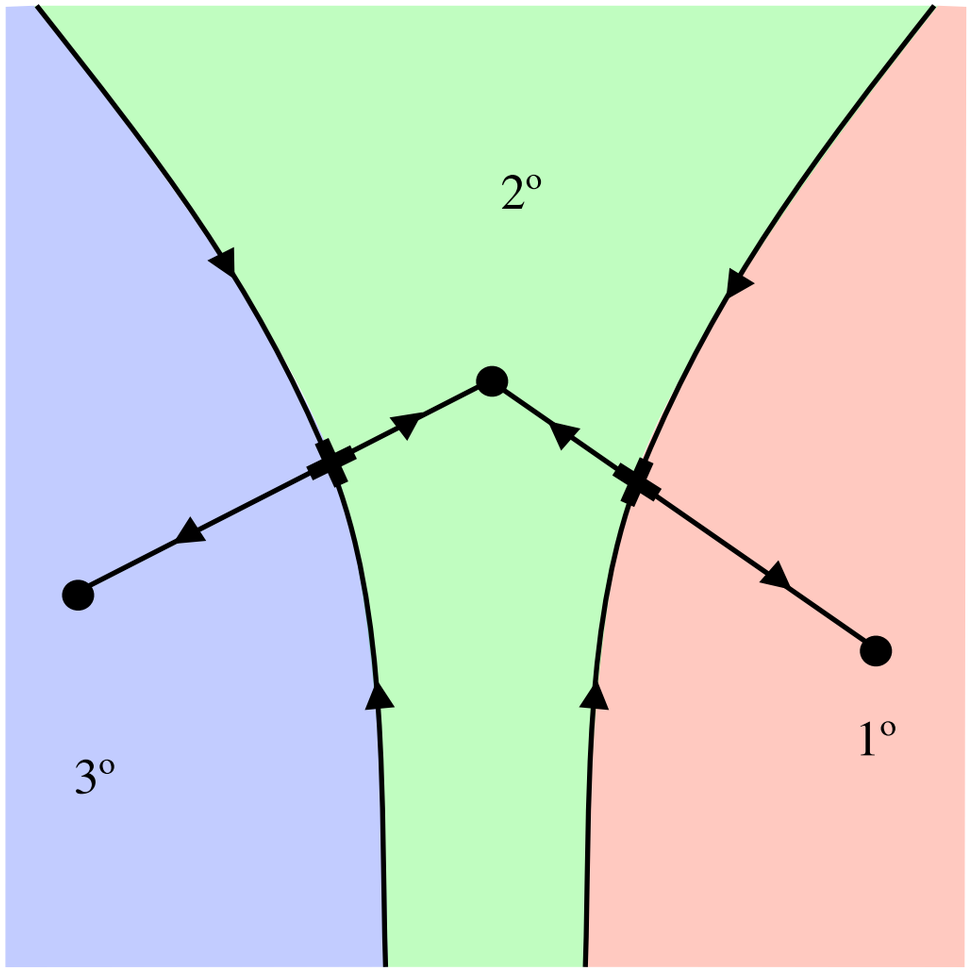
Embryology ⇒ Math

1. Equivalence group: Set of cells able to assume a fate
2. Competence: ability to respond to signal
3. Specification (committed): Fate defined even if withdraw signals
4. Determination: Fate unchanged even if supply new signal
5. Differentiation: Changes in morphology, specific gene products.

1. Direct product of phase space with 3 fixed points, (time=cell cycle)
2. Signaling pathway parameterized, tilts landscape
3. Cell in basin of attraction of fixed point (signal=0)
4. Signals ineffective near FP's or limits on signals, times
5. Ignored: FP ⇒ other 'dimensions'

Topology of phase plane: 1 VPC no signals





3 fixed points all basins meet

- ● fixed point (sink),
- ✚ saddle
- o source

To be ruled out by experiment

Chose coordinates to place fixed points at standard locations:
topological description,
Fit signaling pathways to these coordinates.

Flow with no signals

$$\frac{d\vec{r}}{dt} = k\left(\frac{\vec{f}(r)}{\sqrt{1+\vec{f}^2(\vec{r})}} - \vec{r}\right) \quad \text{+ noise}$$

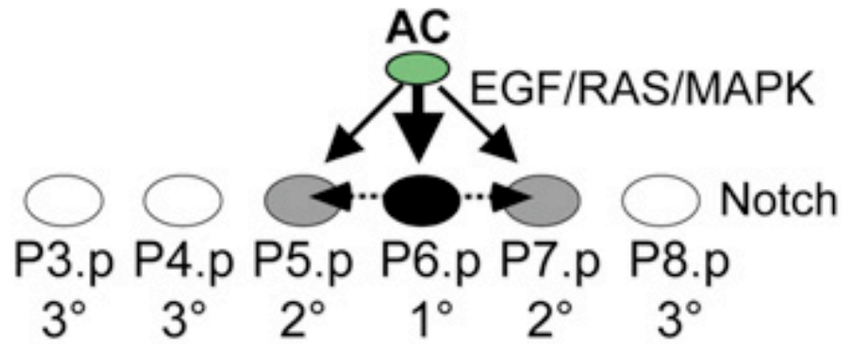
$$\vec{f}(\vec{r}) = \vec{f}_0(\vec{r}) = \vec{c}_0 + 2\vec{r} + c_2\left(-2xy, y^2 - x^2\right)$$

Choose coordinates to place fixed points on triangle
Flow limited to unit disk, small *f,* time scale of r defined by k

Need some form of saturation when ligands added to *f(r)*
Flow in from infinity

Add morphogens

$$\frac{d\vec{r}}{dt} = k\left(\frac{\vec{f}(r)}{\sqrt{1+\vec{f}^2(\vec{r})}} - \vec{r}\right)$$



$$\vec{f}(\vec{r}) = \vec{f}_0(\vec{r}) + l_1\vec{f}_1 + l_2\vec{f}_2$$

$l_1 = \{\gamma^2, \gamma, 1, \gamma, \gamma^2\} = EGF$ anchor cell signal in 5 cells P4p . . . P8p

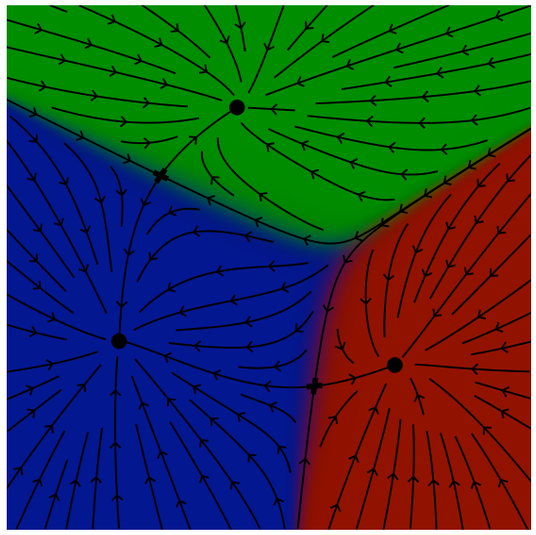
l_2 N signal in cell i due to itself (autocrine) and neighbors (paracrine)

2D vectors f_1 f_2 are to be fit. Intensity of signaling set by l_1 l_2 *in* [0,1]
Ignoring $f_{1,2}(r)$ ie reception of signal depends on cellular state,
Linear interpolation between ligand=0, max. Nothing more needed!
NB EGF-Ras pathway ⇒ one param!!

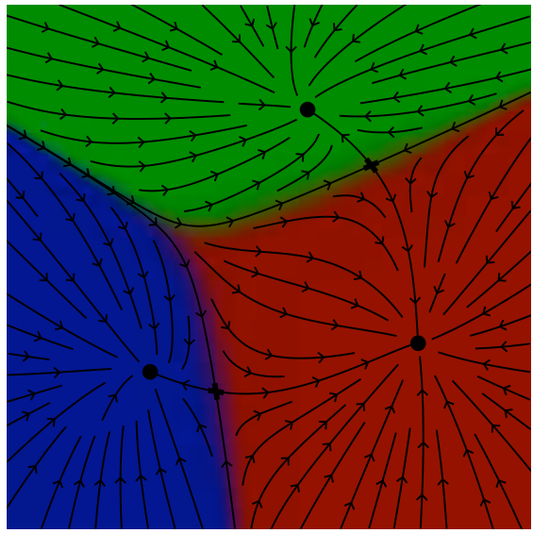
Phase plane (morphogens) 1°, 2°, 3°

(EGF -> 1° N -> 2°)

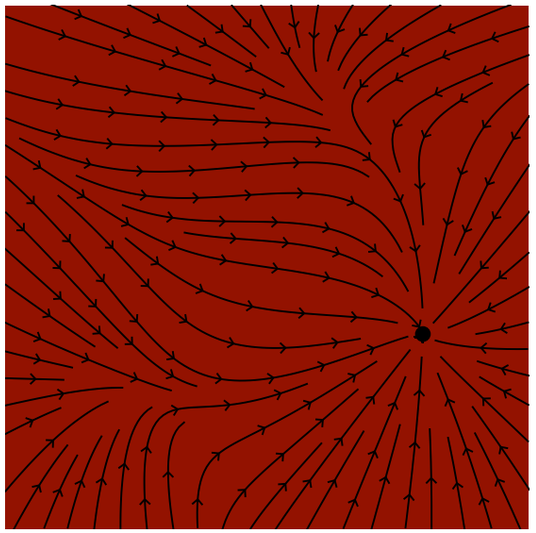
WT fit, 0 ligands



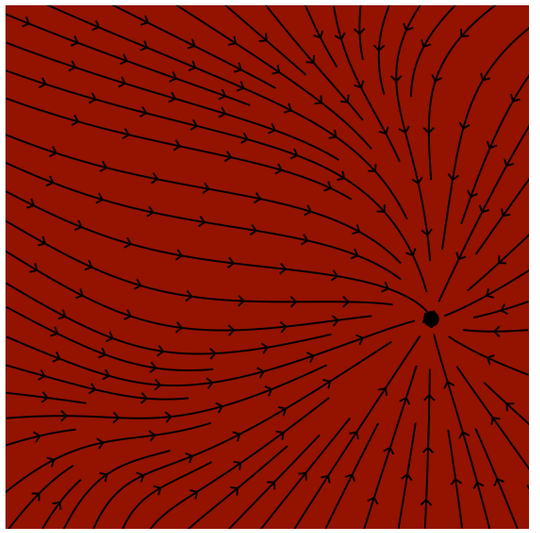
EGF=.3 WT P6



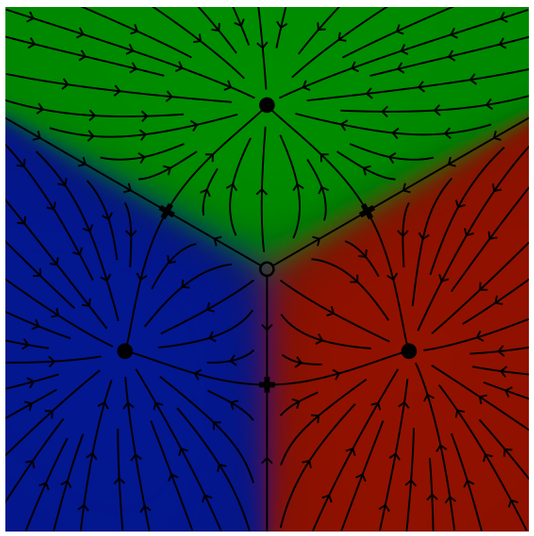
EGF=.5 WT P6



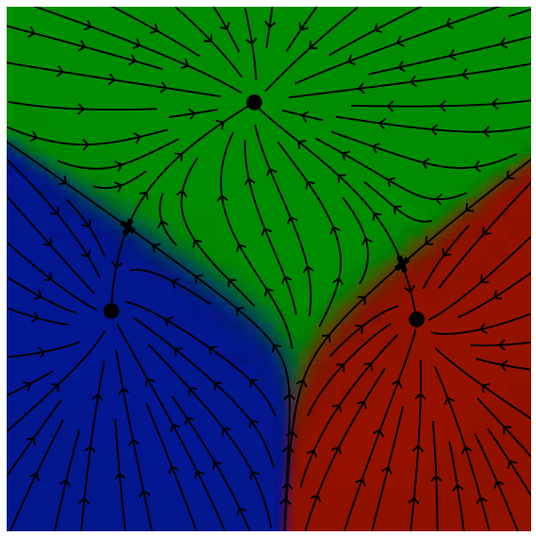
EGF=WT P6



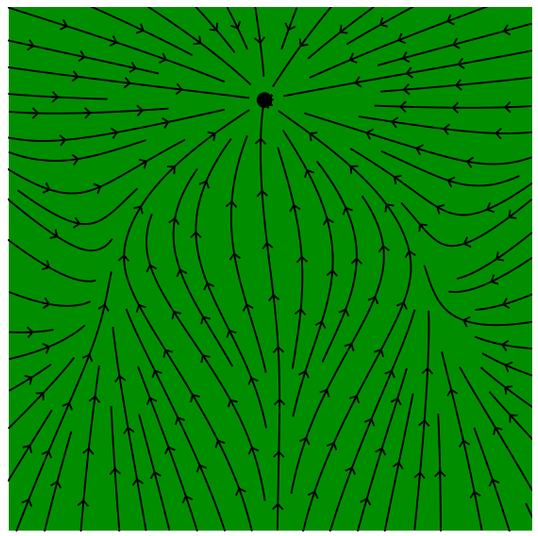
0 ligands $-\vec{c}_0$ towards 3°



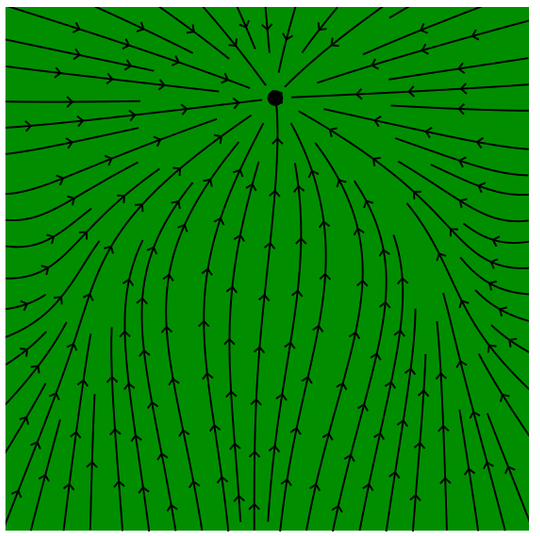
N~.3 WT P5/7



N~.5 WT P5/7



N~WT P5/7



Cell coupling via lateral signal (Delta)

$$L_2(x,y) = \sigma\left(L_{2,x}x + L_{2,y}y + L_{2,0}\right) \qquad \sigma(u) = \frac{1+\tanh 2u}{2}$$

Lateral signal L_2 from cell k depends on state (x,y) via a vector and offset.
Sigmoid keeps it [0,1]

L_2 decomposed into diffusing fraction α (<1) & membrane bound
Diffusing fraction goes to self and neighbors

$$l_2(k) = \frac{\alpha}{1+n_k} L_2(k) + \left(\frac{1-\alpha}{2} + \frac{\alpha}{1+n_{k-1}}\right) L_2(k-1) + \left(\frac{1-\alpha}{2} + \frac{\alpha}{1+n_{k+1}}\right) L_2(k+1)$$

l_2 = N signal in cell k = autocrine + paracrine from neighbors
($\vec{f}(\vec{r}) = \vec{f}_0(\vec{r}) + l_1\vec{f}_1 + l_2\vec{f}_2$, n_k = #neighbors cell k)

Parameter count = 14 ⇒ 10

2:1 time scale, k, nonlinearity base flow $c_2 \Rightarrow 1$

3:2 EGF vector (points to 1°) + exp decay of signal from AC

2:1 N vector (points to 2°)

4:2 Lateral signal as fn of (x,y) (⊥ 2-1,width<<1) + diffusing ratio

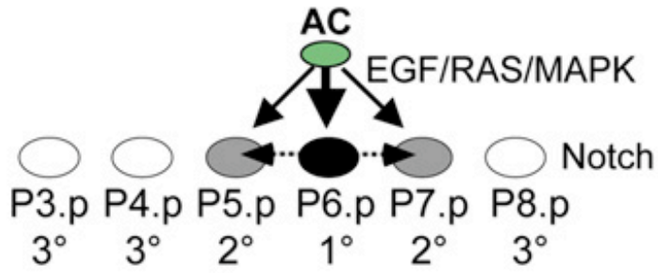
3 : Initial condition (x,y) in phase plane + noise

(+ 1 param each non WT allele)

Ignoring N |--| EGF ie reception of signal depends on cell state..
BUT assuming bistability between 1° and 2° which suffices to fit

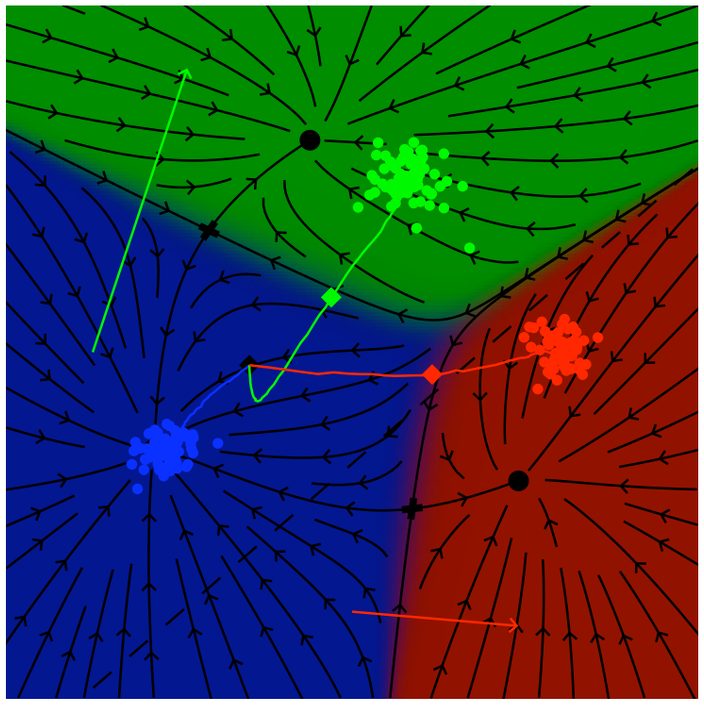
5 cells (AC EGF symmetric) *r(t)* 10 dimensions (6 dims with sym)

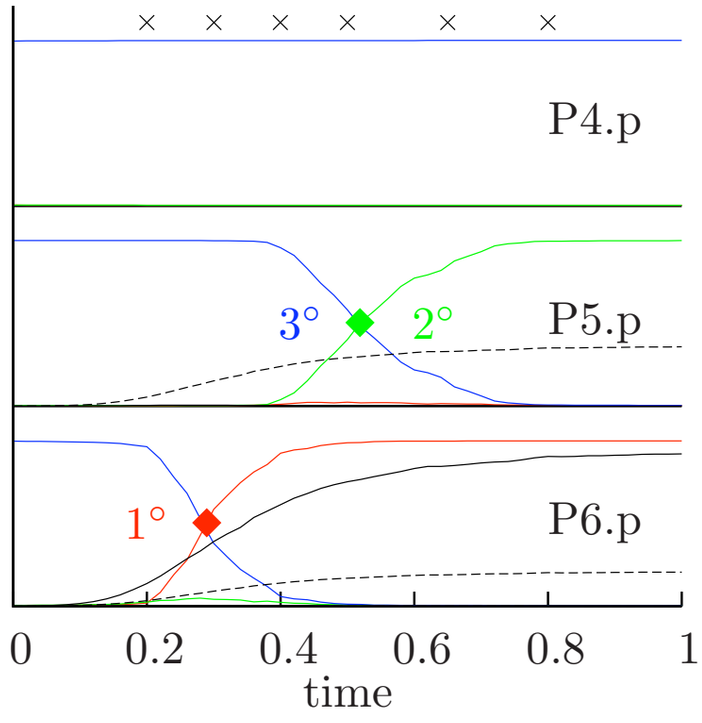
Visualizing flows in 6 dimensions with 10 params



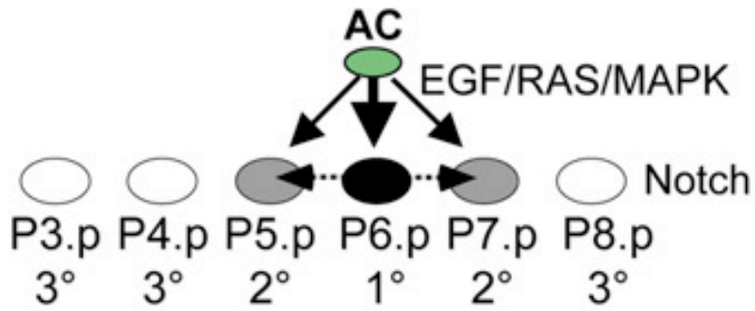
0 ligand phase plane WT
basins of 3 fates 1° 2° 3°
trajectories of P6, P5, P4 + noise
Notch vector
EGF vector
secretion of lateral signal

Fraction of 1° 2° 3° fated cells(time)
x x ablation times
——— Secreted lateral signal
- - - - - - N signaling (autocrine P6)
◆ ◆ cross 3-2, 3-1 boundaries

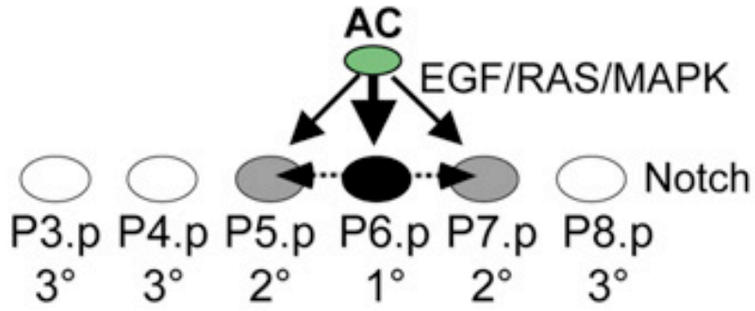


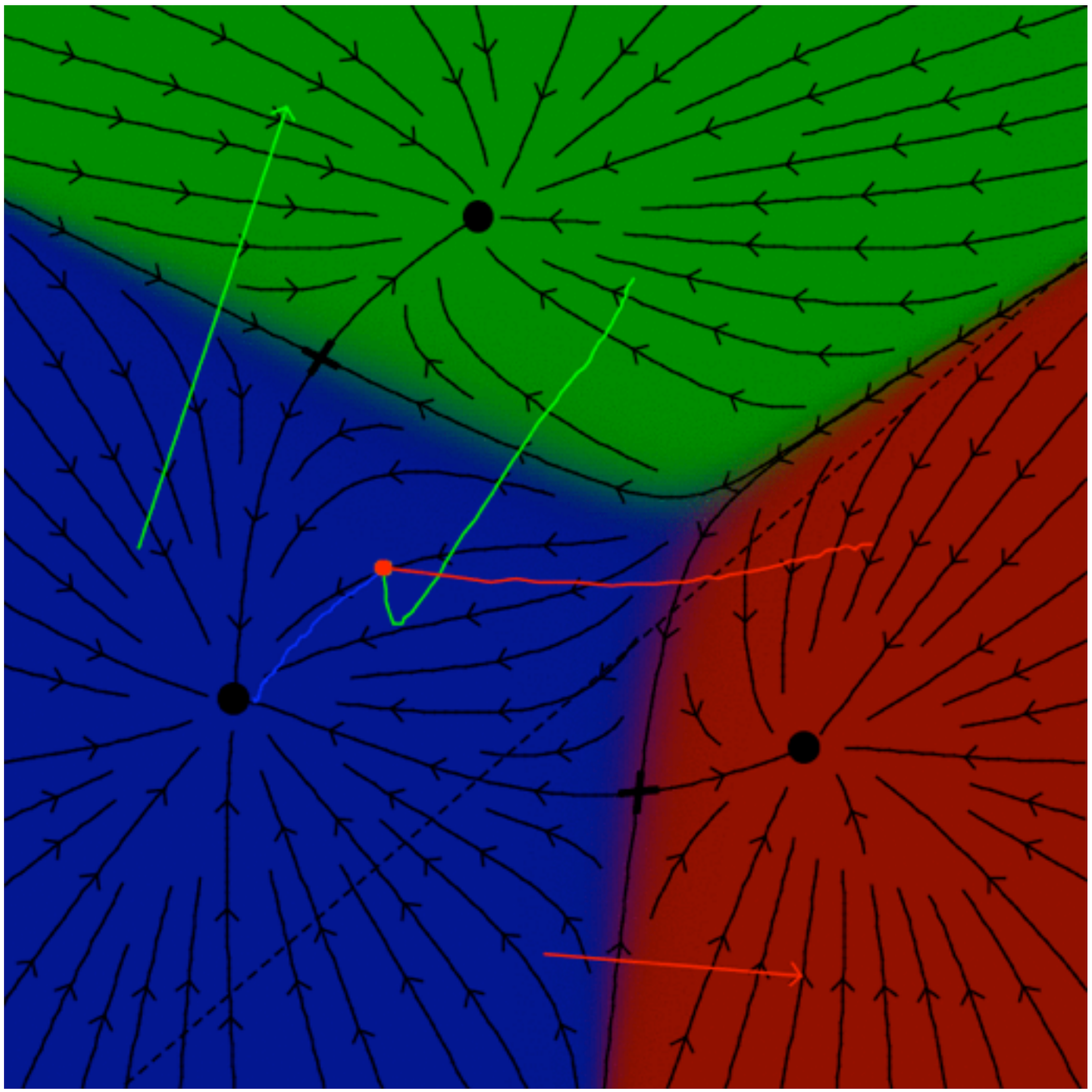


..and as a movie



..and as a movie





What do we fit

Data = % fates for cells P6p, P7p, P8p,
(NB partially penetrant phenotypes most informative ⇒ boundaries)
Fitting conditions...

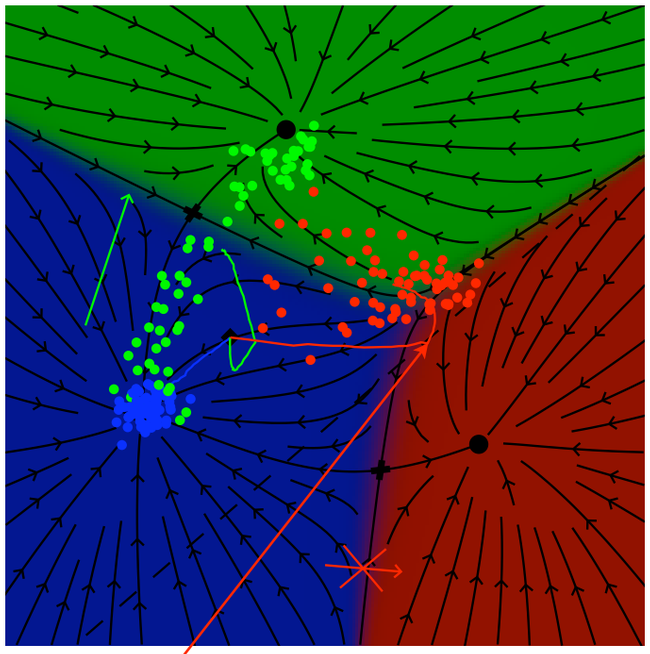
WT, single copy EGF, N-receptor; EGFR mosaics
Anchor cell ablation (time)
EGF over expression from AC, global (lin-15)
(ignore fluor pathway markers (time) slowly varying over time window)

For selected EGF, N hypo/hypermorphs fit single mutants predict double
Predict isolated cells
Predict matrix of all single condition experiments used in fit....

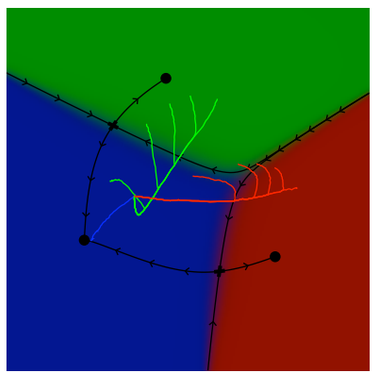
(Fitting parameters to (data - model)^2: Levenberg-Marquardt ie simple)

Anchor cell ablations ≠ EGF hypomorph

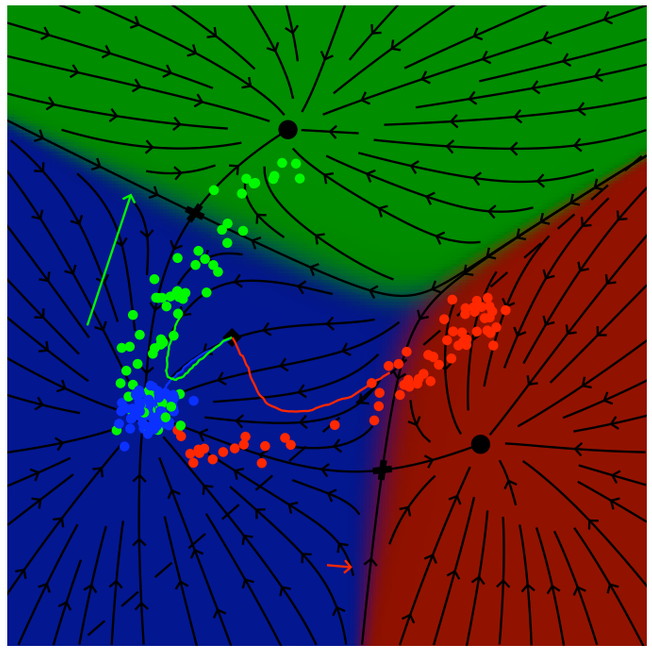
(both fits reduce EGF: ablation fixes autocrine, mobile Delta)



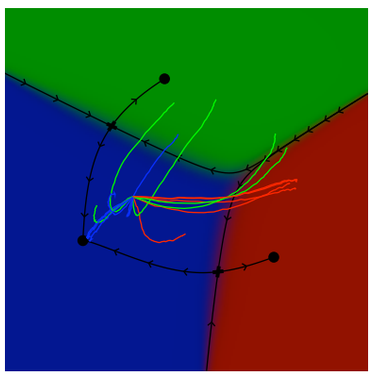
Orbits at various ablation times



Ablation at time P6p induction of Delta is half max. Autocrine pushes P6p to 2^{o}, $\Rightarrow$ fit single cell. Most of Delta is mobile.
32223 19%, 33333 16%, 33233 14%, 32123 14%



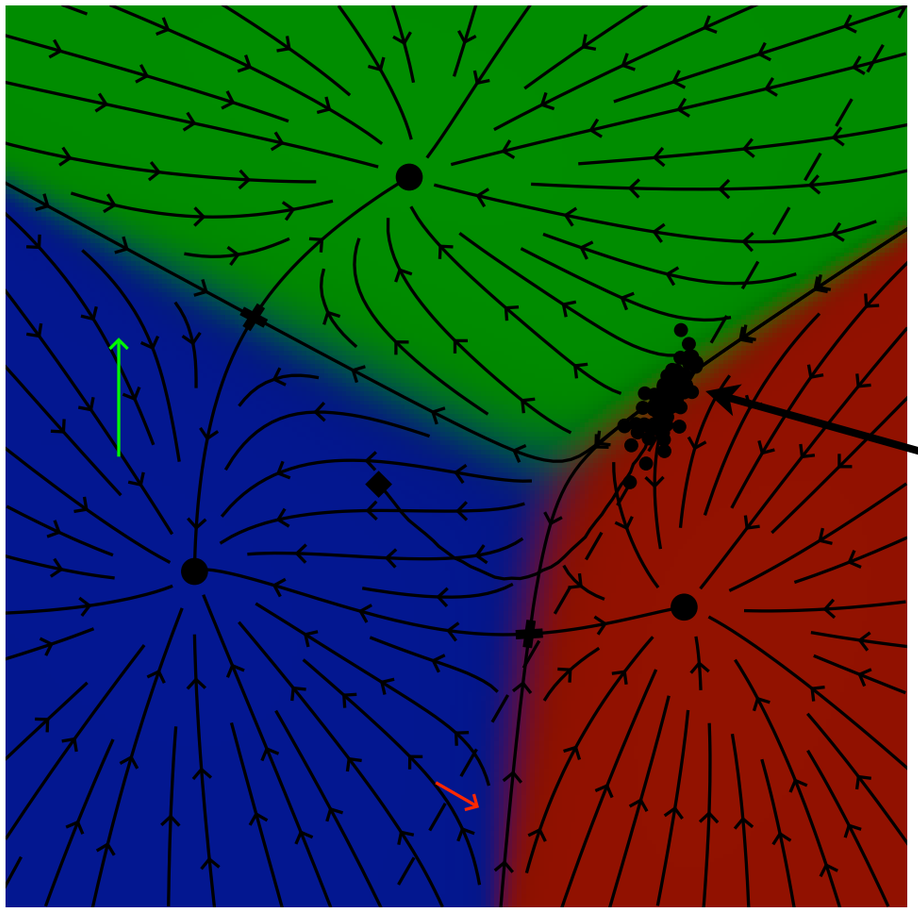
Orbits for EGF .3, .5,1,2,3 x WT



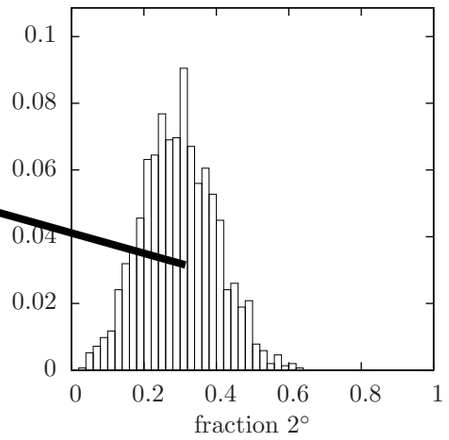
Weak EGF signal from AC makes P6p ->1^{o} late, less Delta.
33133 34%, 33333 33%, 32123 14%..

Figs for comparable induction (ie 1^{o}+2^{o})

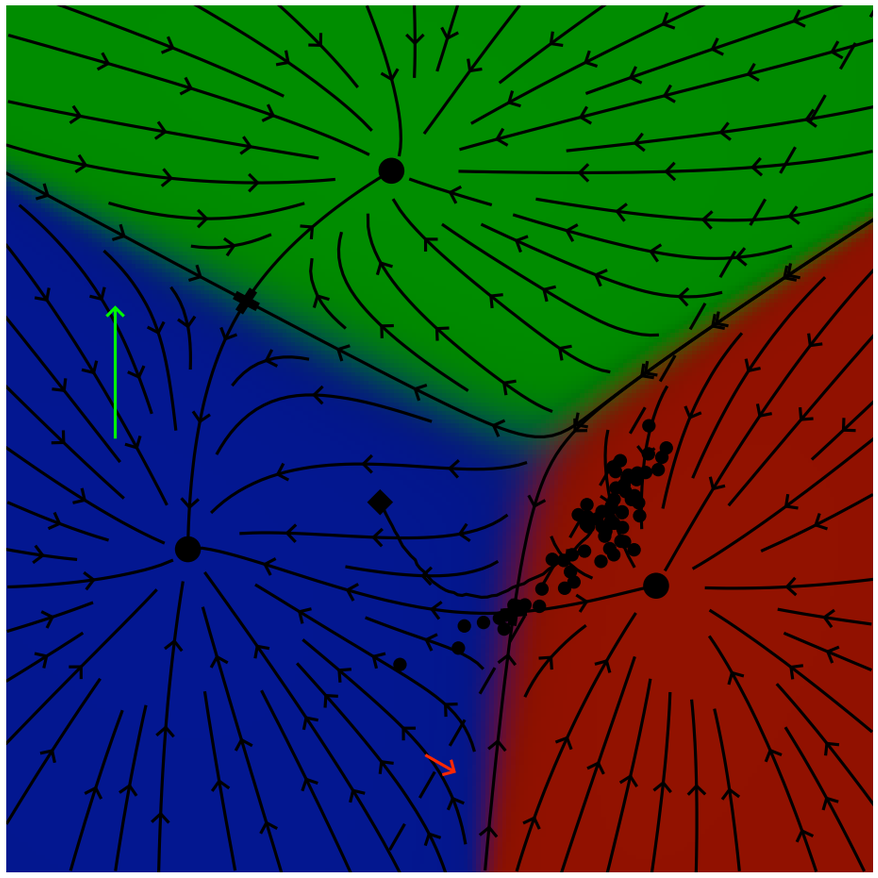
Predictions (1) Single cell and autocrine signals



Isolated VPC chose EGF level to get max induction of 2°. Requires all secreted Delta -> isolated cell. Autocrine signaling fit by ablations.



% 2° fates, sample parameter space. Experiment ~ 0.4 Hoyos 2011



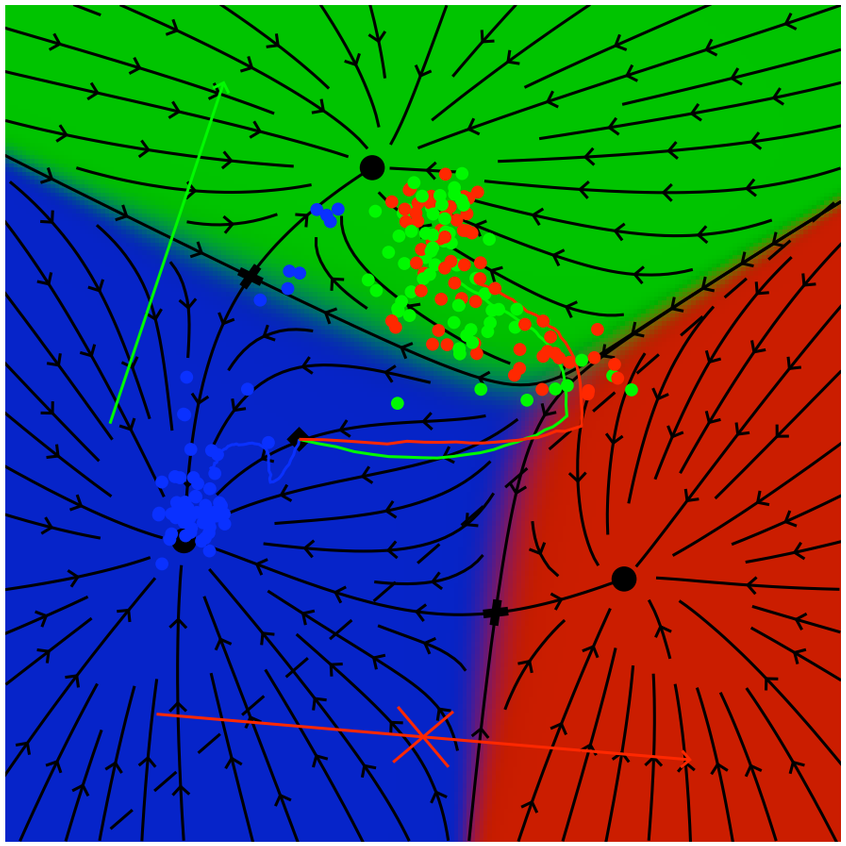
Isolated VPC getting 1/3 secreted Delta can not make 2°

Temporally delayed specification in isolated cells??

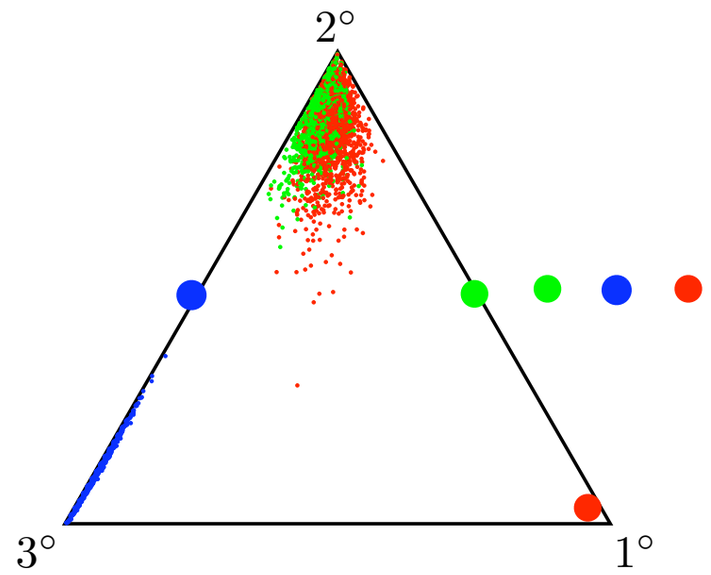
Predictions (2)

Fit EGF (⇑AC Hoyos 2011) predict AC ablations(time)

Fit EGF⇑ to 3/2, 2/1, 1, 2/1, 3/2 predict fates(ablation time) e.g., max 2°



Sample parameter space and record fates of VPC

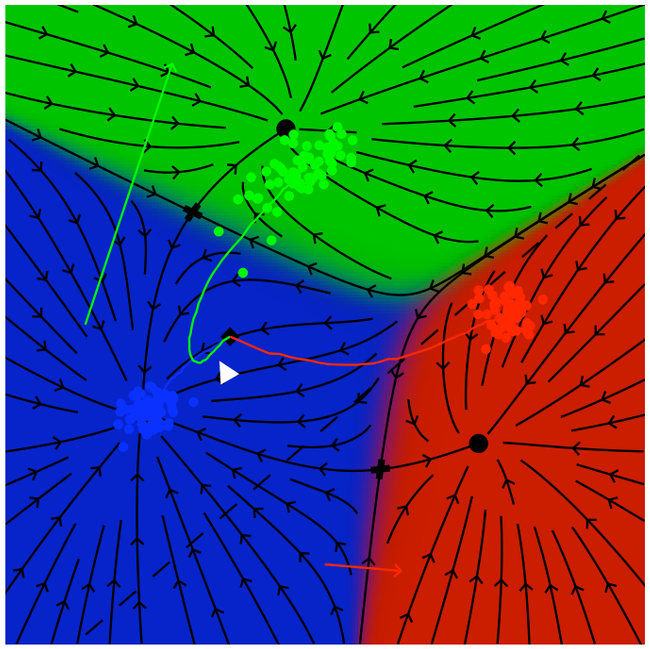


Fit data
P6->1°
P5->half 1°2°
P4->half 2°3°

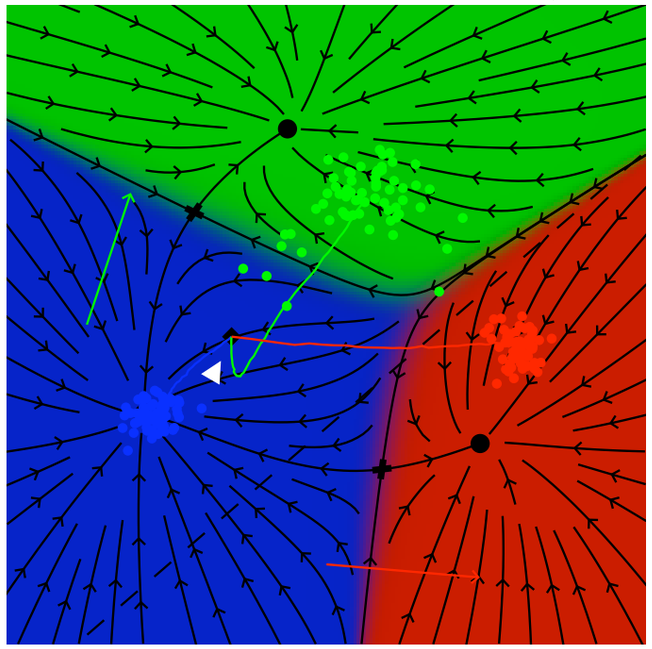
32223 53%, 32323 8%, 32123 7%
32233 4%.... actual fates

WT × WT = phenotype
(Epistasis from geometry)

Fit ‘half’ dose EGF,

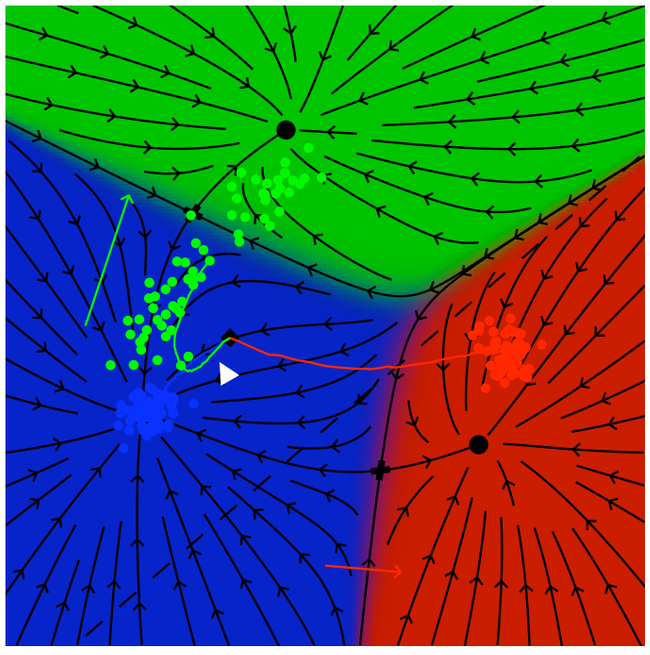


‘half’ dose N as WT,

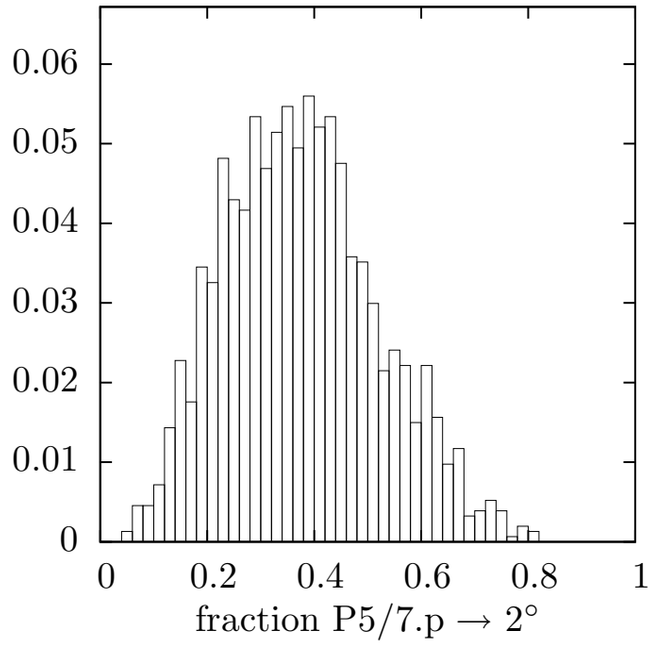


Bayesian fit ⇒ marginal

cross has phenotype



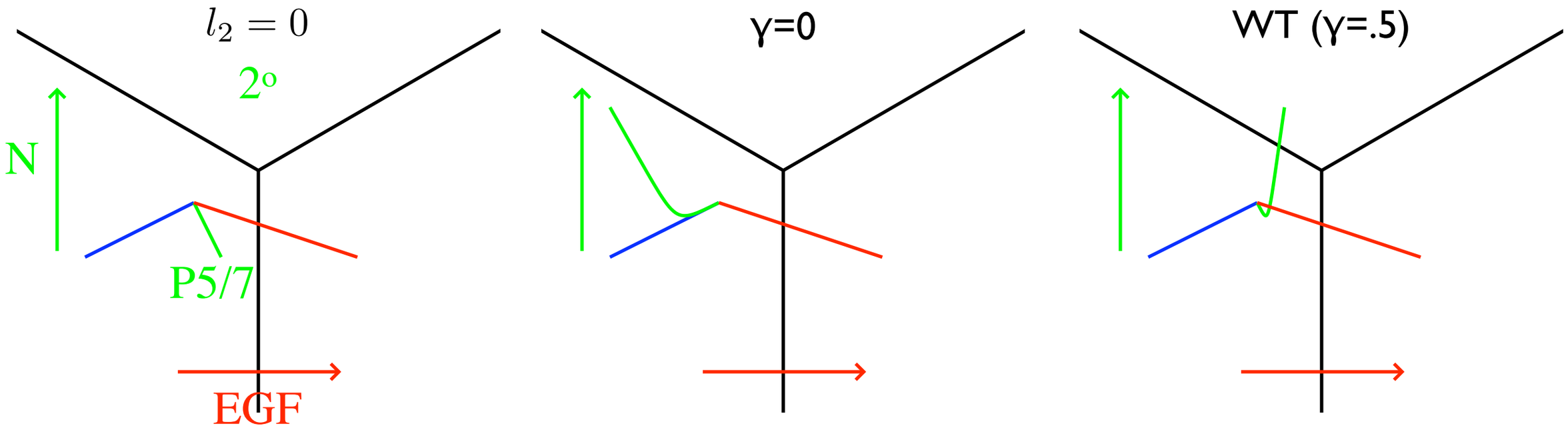
Robust to parameter variation via MC



Epistasis from linear dynamics in fate plane

(Specification of P5/7 by EGF and N (from P6))

$$\frac{dx}{dt} = x + \gamma \qquad \frac{dy}{dt} = y + l_2$$



0 Notch (WT EGF) **cross** 0 direct EGF (WT N from P6) ⇒ P5/7 -> 2°

P5/7 trajectory in cross is vector sum of two alleles,
boundary in fate plane -> epistatis

Cross multiple EGF-Ras-MAPK mutations
(epistasis from geometry)

lip-1 phosphatase -| EGF (N target, part of N |–| EGF), assume (lf) marginal, lin15 (adds uniform EGF, *sensitized* bckgnd for N signaling) data:Predict X

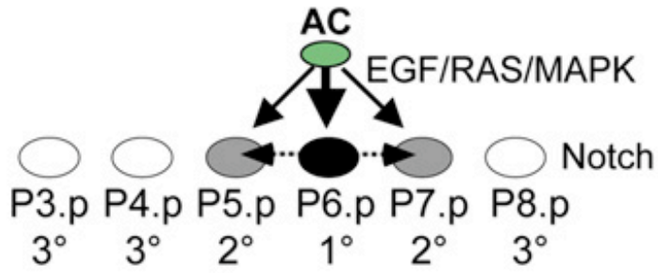
Experi: Berset etal *Science* 2001
(Egl-17-GFP used to score 1°)
concluded lip1 --| MAPK in P5/7

Mutant	% 1° fate				
	P4.p	P5.p	P6.p	P7.p	P8.p
lip-1(lf)	0 ± 0	3 ± 4	99 ± 2	3 ± 4	0 ± 0
	0	0	100	0	0
lin-15(rf)	87 ± 8	9 ± 6	98 ± 2	10 ± 6	87 ± 9
	71	0	100	4	79
lin-15(rf);lip-1(lf)	93 ± 5	25 ± 16	99 ± 1	25 ± 16	93 ± 5
	60	40	100	30	87
let-60(gf)	2 ± 7	6 ± 5	97 ± 3	6 ± 5	2 ± 5
	6	0	100	0	3
let-60(gf);lip-1(lf)	40 ± 20	20 ± 14	96 ± 5	20 ± 13	40 ± 20
	45	53	100	45	62

But a model with no pathway interaction can reproduce phenotype of double mutant hence

Can not conclude from genetics that N->down regulation of MAPK in P5/7p.

Extrinsic vs Intrinsic Noise: Correl in fates of P5/7



This quantifies extrinsic var. induced in P5/7 by variable lateral signal from P6 vs intrinsic variation in P5 & P7 due to AC and internal dynamics

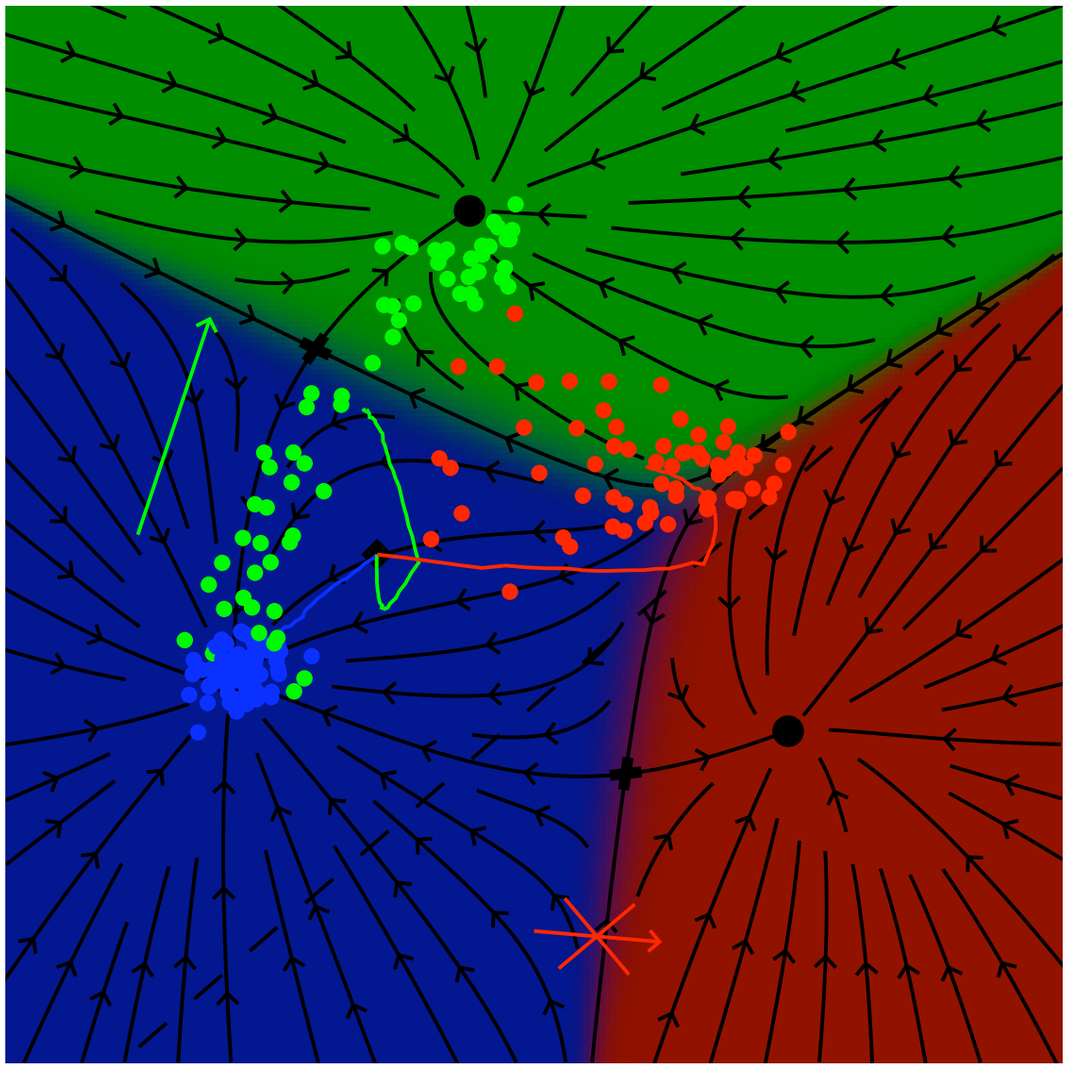
	EGF(rf)	Exp.	EGF(rf)/Notch(rf)		Notch(d)/AC$^-$	
33333	47	40				
33133	31	37	33133	73	-3-3-	77
32123	8	7	**32123**	3	**-2-2-**	2
32133	5	7	**32133**	11	**-2-3-**	11
33123	5	6	**33123**	10	**-3-2-**	9

Uncorrel model fates:
212/213/312: 2/8/8%

Uncorrel model:
2-2/2-3/3-2: 1.5/10.5/10.5%

Adjust EGF gene dosage to get same average 2^o as experiments in red (Felix)
EGF(rf) gives greatest correl, Notch(gf) least correl

Extrinsic-Intrinsic noise (2)



AC ablations
from prev. slide

Fit data for Prob (1^o, 2^o, 3^o) in single VPC, but can *predict* correlations vs exper

32223 19%, 33333 16%, 33233 14%, 32123 14%

-222- 23%, -333- 18%, -323- 10%, -212- 19%... -213- 2% Milloz 2009 (20+ animals)

ie symmetric configs >> asym

(picking ablation time to get same %P6 induction)

Remaining parameter degeneracies

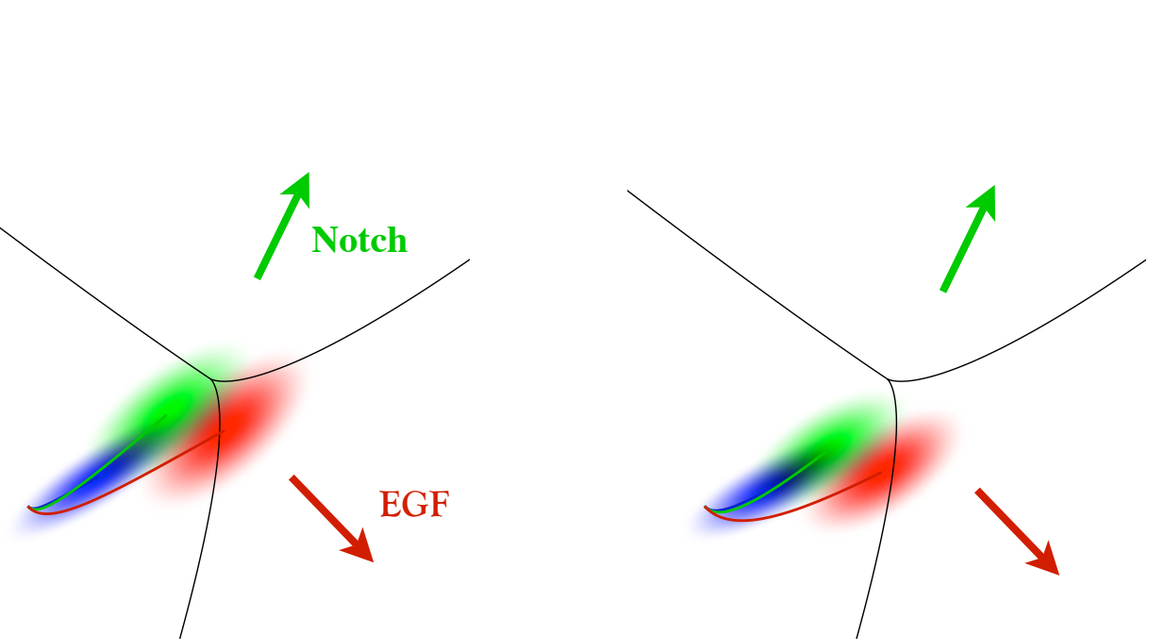
Two constraints on the 5 parameters γ, l_1^(hypo, hyper), $lm_{1,2}l$ ie need some absolute scale of EGF under/over expression in units of WT, ie what is the band of EGF levels that yields WT pattern.

f_o plays against r_o ie *cst* force vs initial position, no obvious experiment, coordinate choice.

A failure

EGF hypomorph with P6p 50/50% 1°/3° × N receptor (rf but WT)

⇒ P6p 34/66% 1°/3°



EGF(rf) fit

EGF(rf) × N(rf)
predicted

Prior linear model worked for this ×

Prior Models

2 most recent ODE models:

- Model signaling only, not multistable, define regions EGF-N space ⇒ fates
- Select model by volume in parameter space,
- Do not fit or predict partial penetrance, deterministic models
- No dynamics fit

1. Giurumescu..Sternberg 2009, 2 variables/cell (EGF, N), 9 dim'less params, vol. in p-space computed over range 10^5 ⇒ 500/4^6 fate assignments allowed

2. Hoyos..Felix 2011, 10 variables/cell, ~40 params, sampled ~100x range

Summary features of Geometric models

Few master variables control many slaves, or fast variables follow slow
(~1/signaling pathway, exp. tests of dimension)

Signaling and specification one dynamical system

Cell fates ~ fixed points, clearest when terminal fates
(intermediate cell types in hematopoiesis stable??)

Ligands change topology of flow (saddles and fixed points)

Mutants that land near basin boundaries take longer to specify??
(time ~ degree of penetrance??, VPC daughters inherit maternal state?)

Models not literal representation of competence window, differentiation

Lessons

Signaling more enmeshed in cell biology than transcription, thus phenotypic model more useful.

Abundant evidence that relative strength of pathways changes, WT pattern fixed. Thus no reason to measure all the molecular *bricolage*

Outcomes, times for specification etc highly variable, ignored in deterministic ODE models. extr/intr noise in cell lineage tree in worm??

Developed interpolation scheme, null model, like linear correl, but illuminates epistasis: gene interacions

Crude predictions for many properties (2 bit theory)

'Geometric' model not obvious, e.g.,

vulva ~ 3 way culture cell choice

(eg C2C12 hi TGFβ proliferate, lo TGFβ muscle, hi BMP bone)

short ⇒ long germ band insects HOX patterns (Francois EDS)

DV patterning neural tube.

fly leg, AP, DV, PD via boundary model.

The end