Robustness and evolution of the *Caenorhabditis* vulva signaling network

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When considering any biological process:

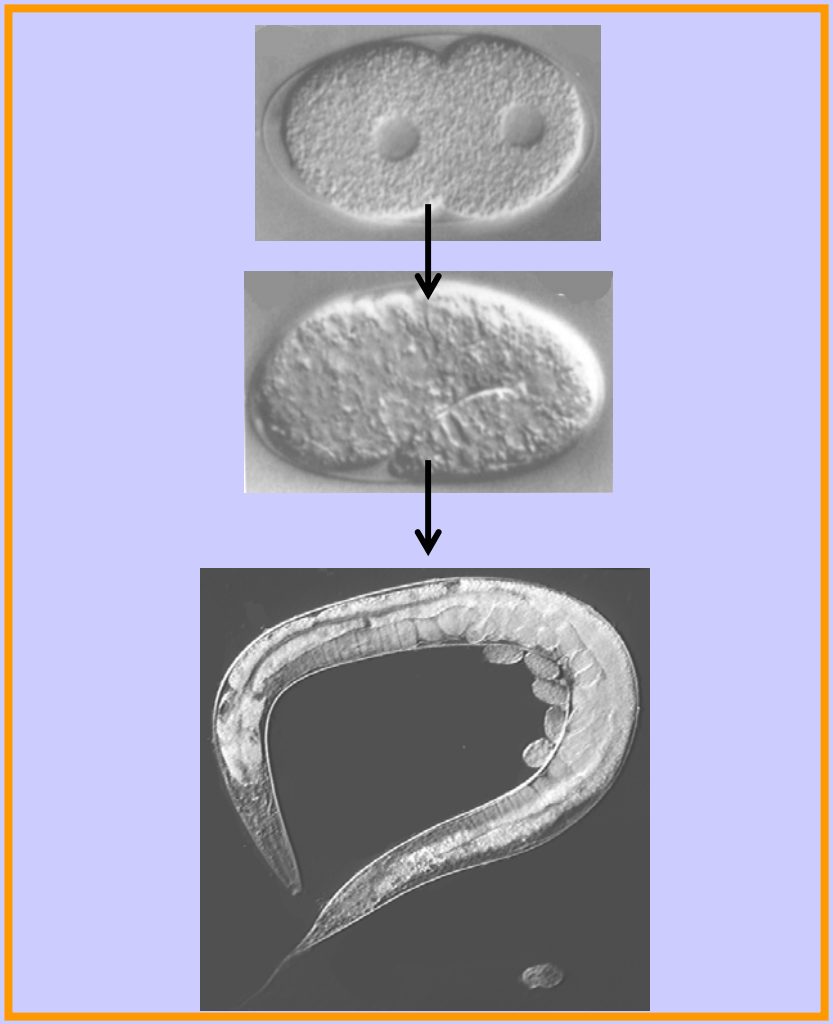
- What are the ecological/environmental conditions it has to face?

- How robust/sensitive is the system output to environmental variations?

- How does it evolve?
  genotype-environment / phenotypes

Evolution of a developmental system

in evolution:
random
genetic
variations



developmental
variations

environmental
variations

selection

phenotypic
variations
or stasis
in adult
morphology

Facing variation

Genetic variations

Environmental variations

stochastic noise, external variations

Biological system → output phenotype

Plasticity vs. Robustness

Sensitive to internal noise or an environmental variation

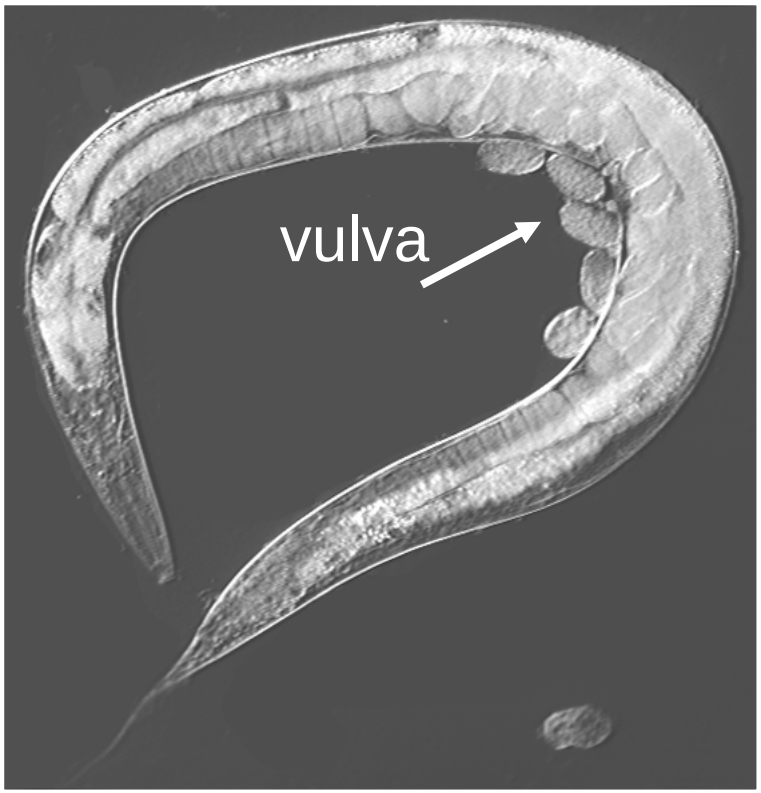
Insensitive to internal noise or an environmental variation

Genotypic space

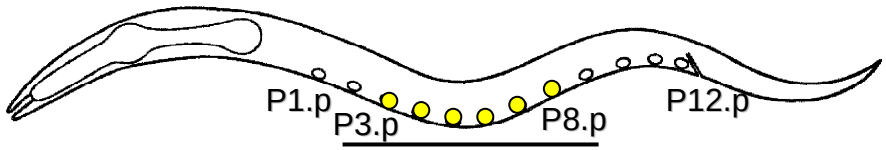
developmental system

Phenotypic space
System output

The Experimental System:
Vulva development in *Caenorhabditis elegans*



L1 larva



6 cells

anchor cell of the uterus

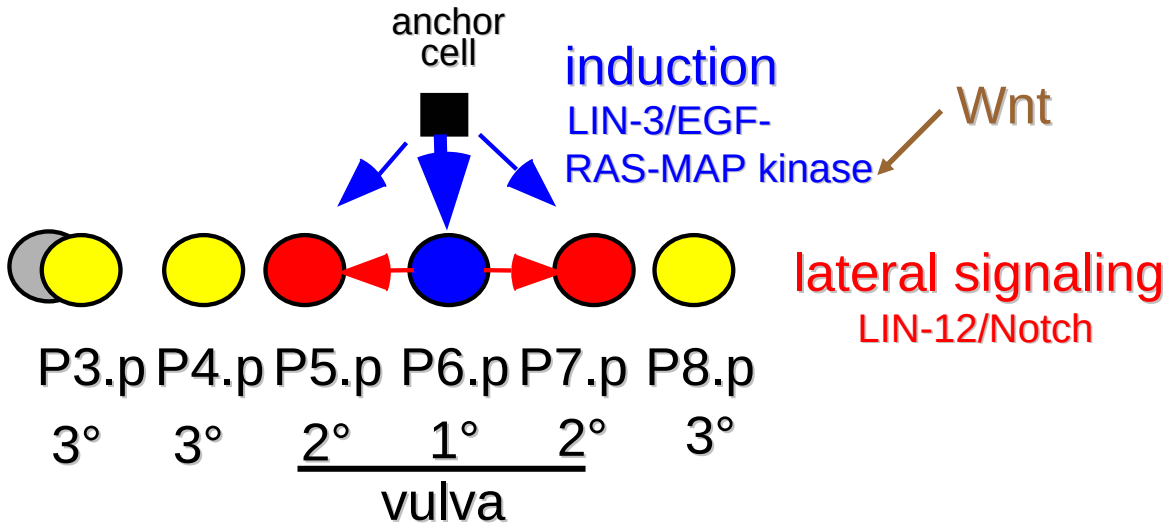
vulva precursor cell

necessary for egg-laying
& copulation with males

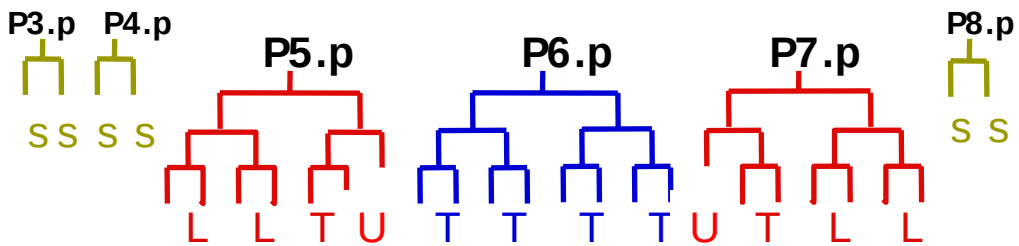
quasi-invariance of cell fates
self-fertilizing hermaphrodite: isogenic strains

C. elegans vulval precursor fate patterning

Early-mid L3 stage
Vulva cell fate patterning

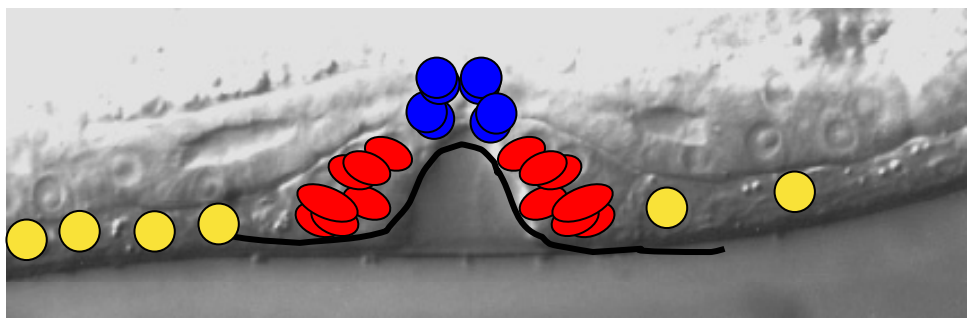


Late L3-early L4 stage
Vulva divisions

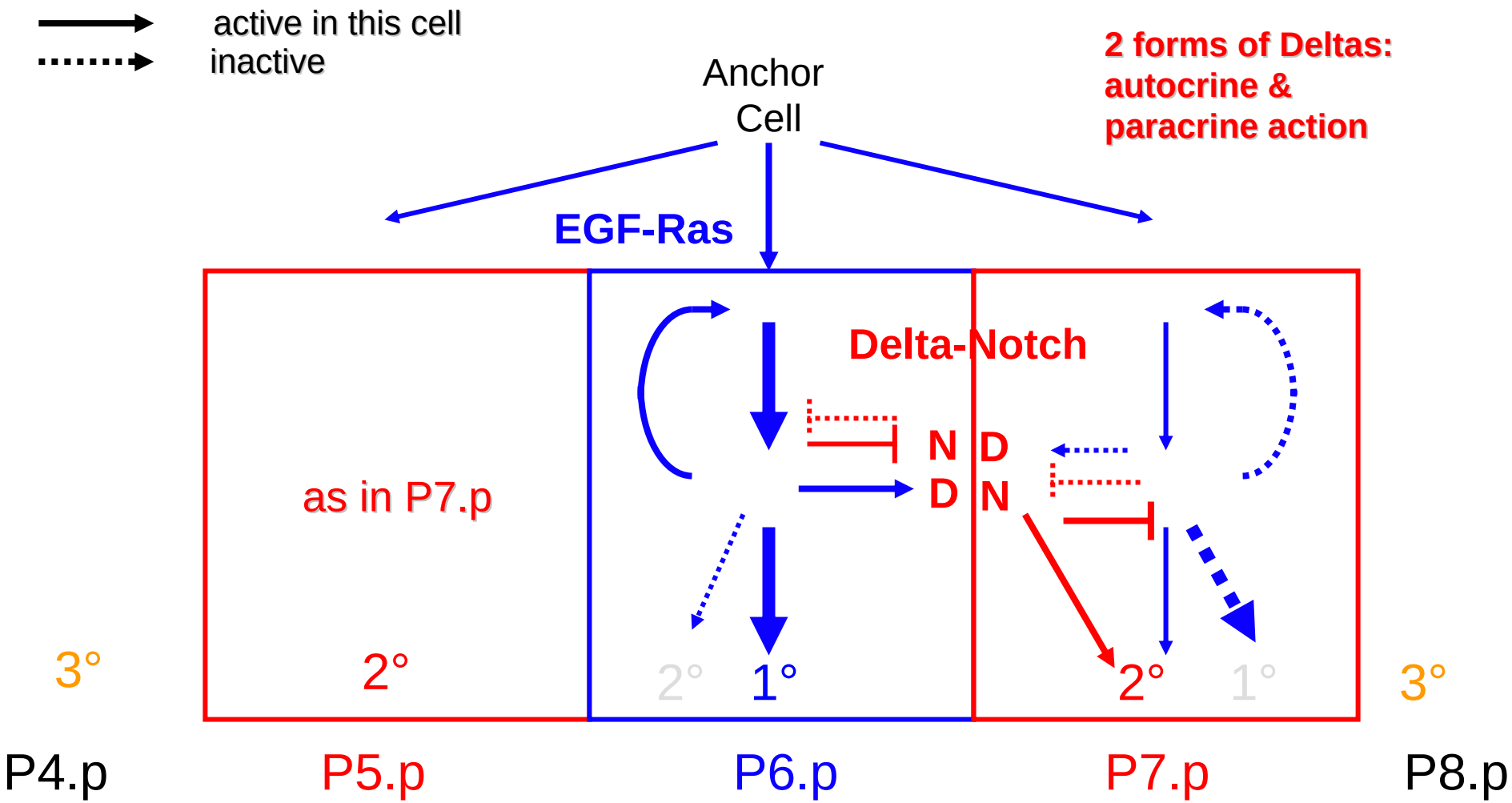


L = Longitudinal division (A/P); T = Transverse (L/R); U = Undivided
S = non-vulval, fusion to hyp7 syncytium

L4 stage
Vulva morphogenesis



Vulva signaling network architecture



- Positive feedback loops
- Crosstalk between Ras and Notch pathways

C. elegans vulva development

I.
Sensitivity to **noise and environmenta**l change
'Variant/error' type and rates
Cryptic variation in different environments

II.
Cryptic evolution in *C. elegans* and the *Caenorhabditis* genus

III.
Sensitivity to **random mutational** change
Bias/constraints

- Measure precision of the system in different environments

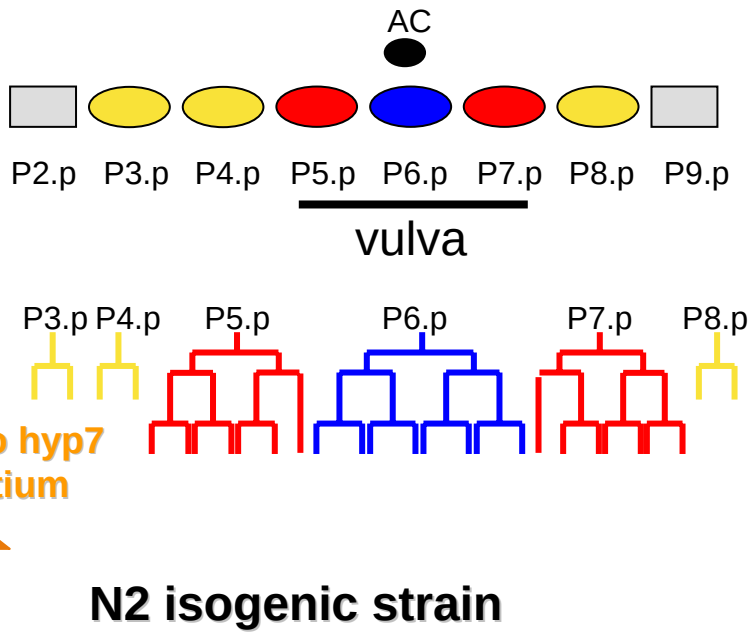
- Type and rate of variants / errors?

I. Vulva Development in Different Environments

- 6 standardized environments - tested in parallel:

called 15°C, 20°C, 25°C, liquid, L2 starvation, dauer

- Scoring vulva development after Pn.p divisions in the L4 stage:



N2 isogenic strain

divides once in 50% of the individuals
does not divide in 50%, fuses to hyp7 in L2

A Phenotype Sensitive to Stochastic Noise: P3.p Division Frequency

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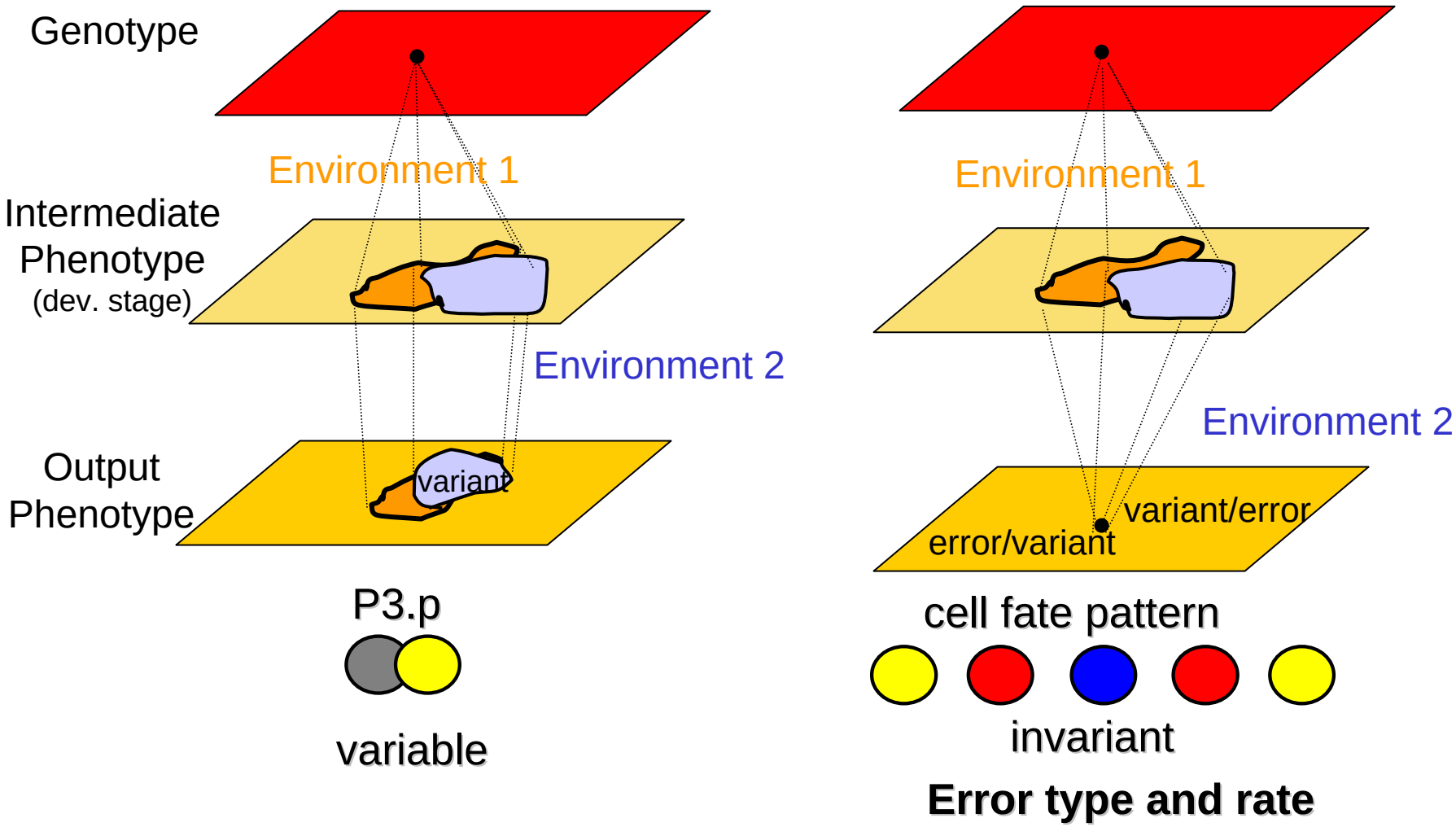
Sensitivity to Noise and Environmental Variations

Sensitive / non-robust

Insensitive / robust

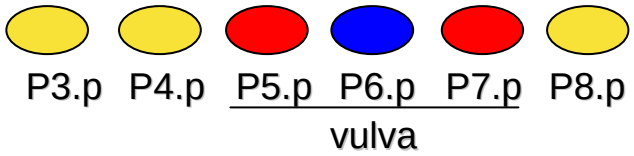
against internal or environmental noise



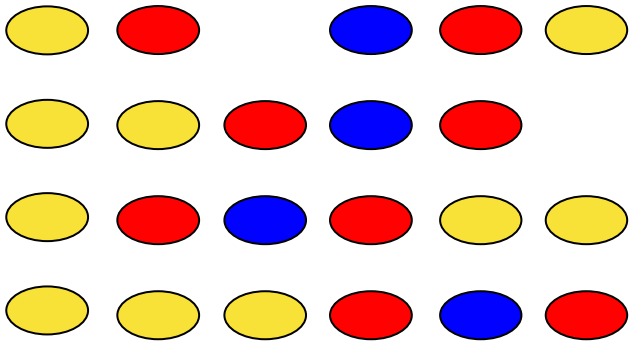
Error type and rate

Vulva Development: Minor Variants / 'Errors'

Canonical pattern



Variants corrected by cell redundancy in the competence group



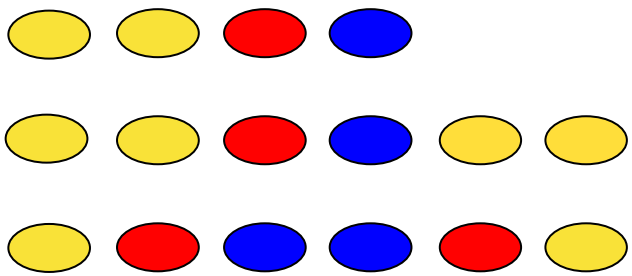
anterior Pn.p missing

posterior Pn.p missing

centering on P5.p

centering on P7.p

Variants that are not corrected: abnormal vulva ('errors')



2 Pn.p missing

hypoinduction

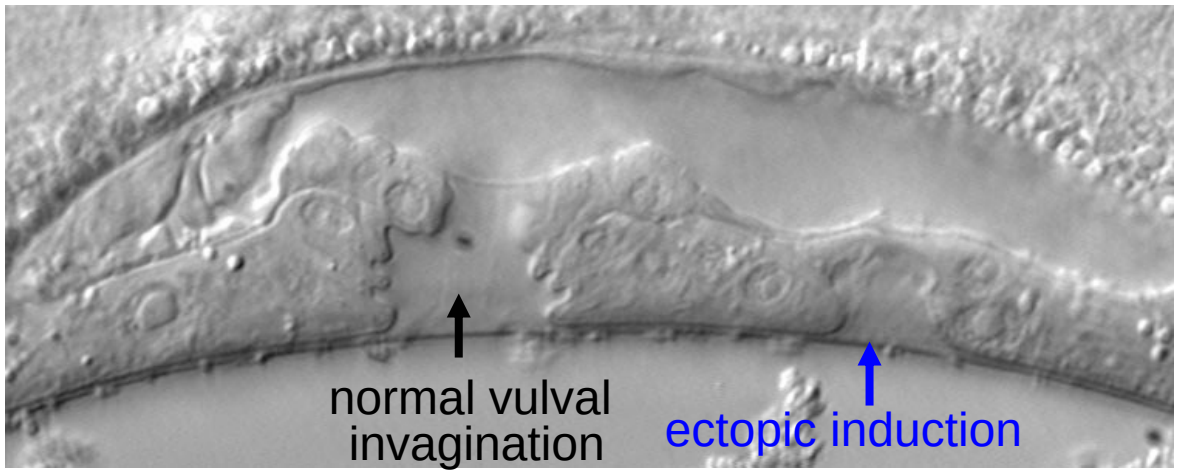
<3-cell induction

hyperinduction

>3-cell induction

Vulva development 'errors'

an 'error' at 20°C



induction index = 4

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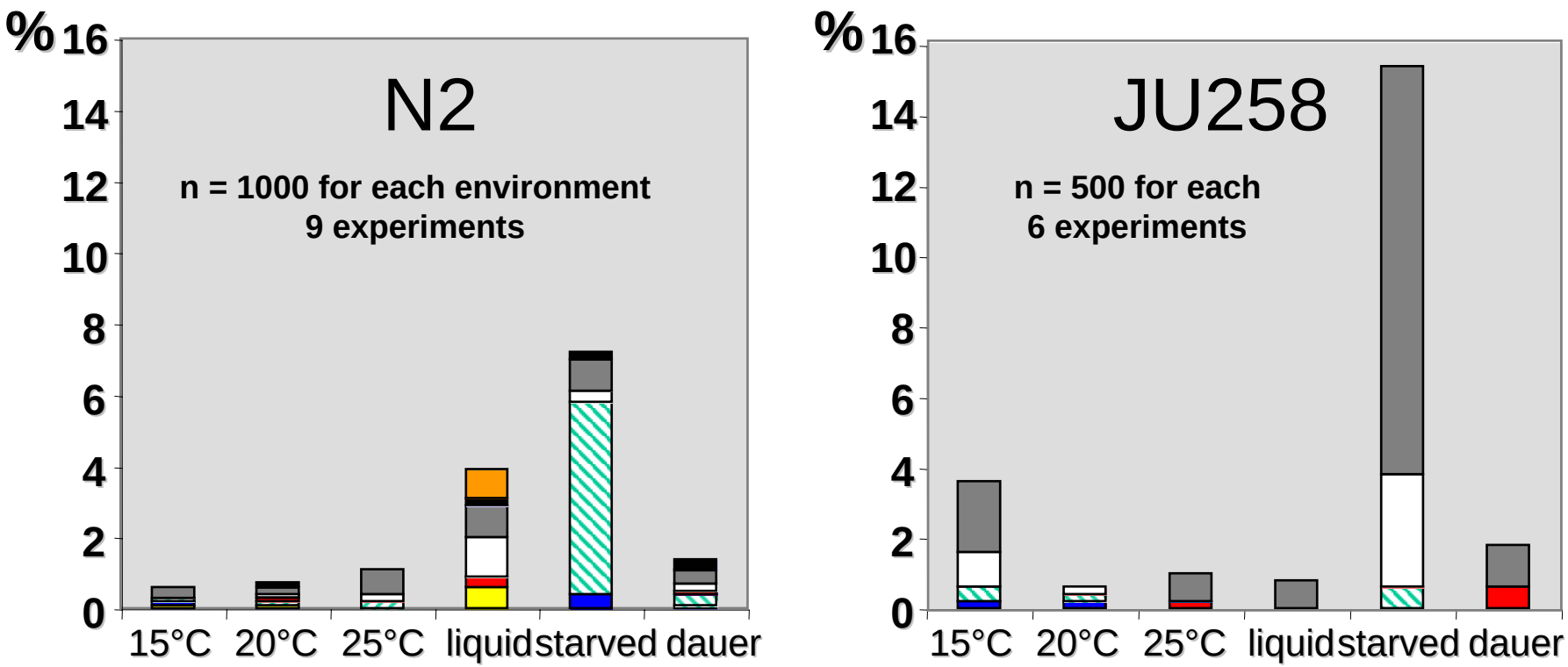
Vulva variants in **six different environments** for **two *C. elegans* genotypes**



hypoinduction
hyperinduction
other fate error
centered on P5.p
centered on P7.p
morphogenesis
3° Pn.p missing
P4/8.p undivided
P4/8.p >1 division

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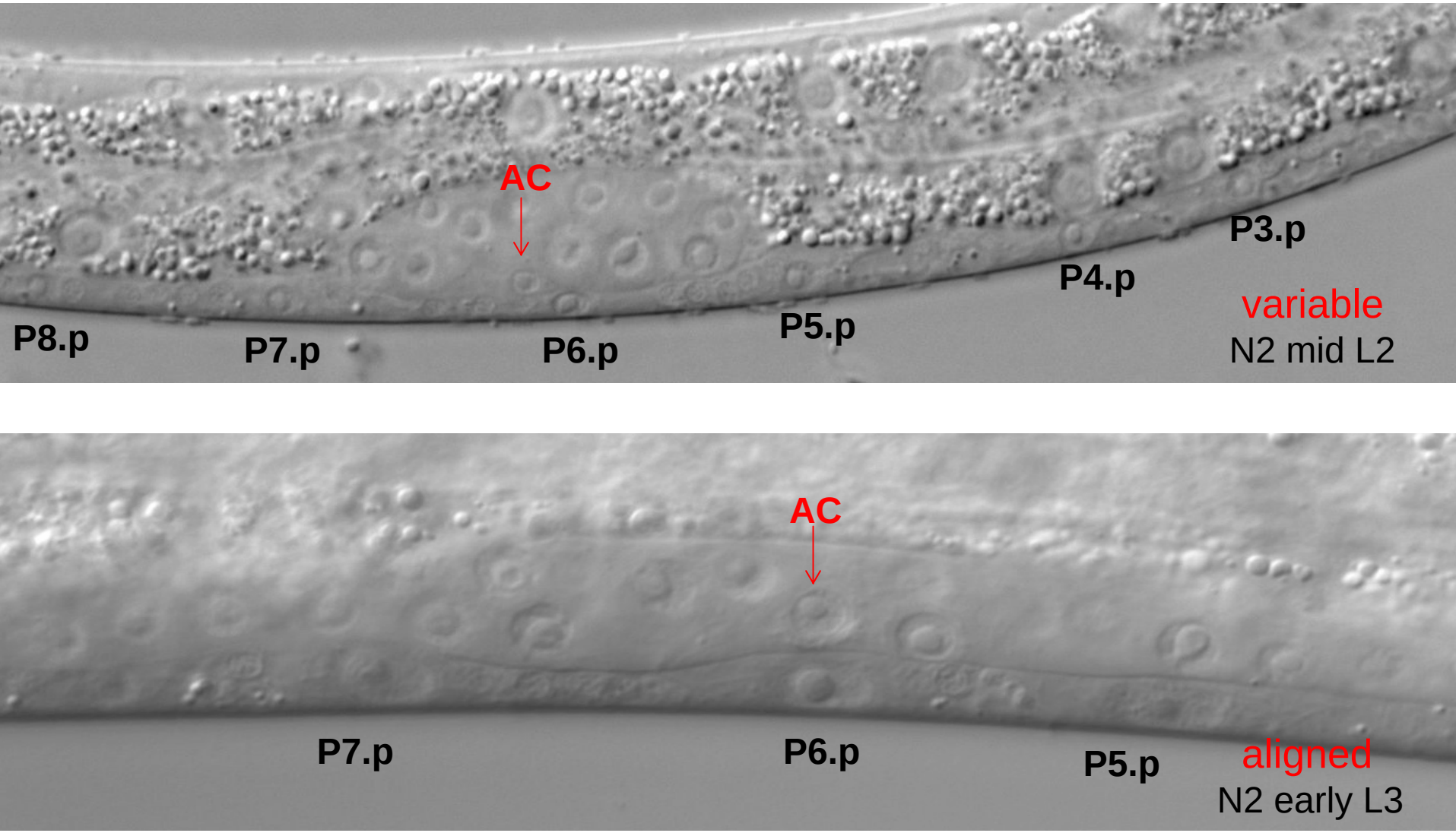
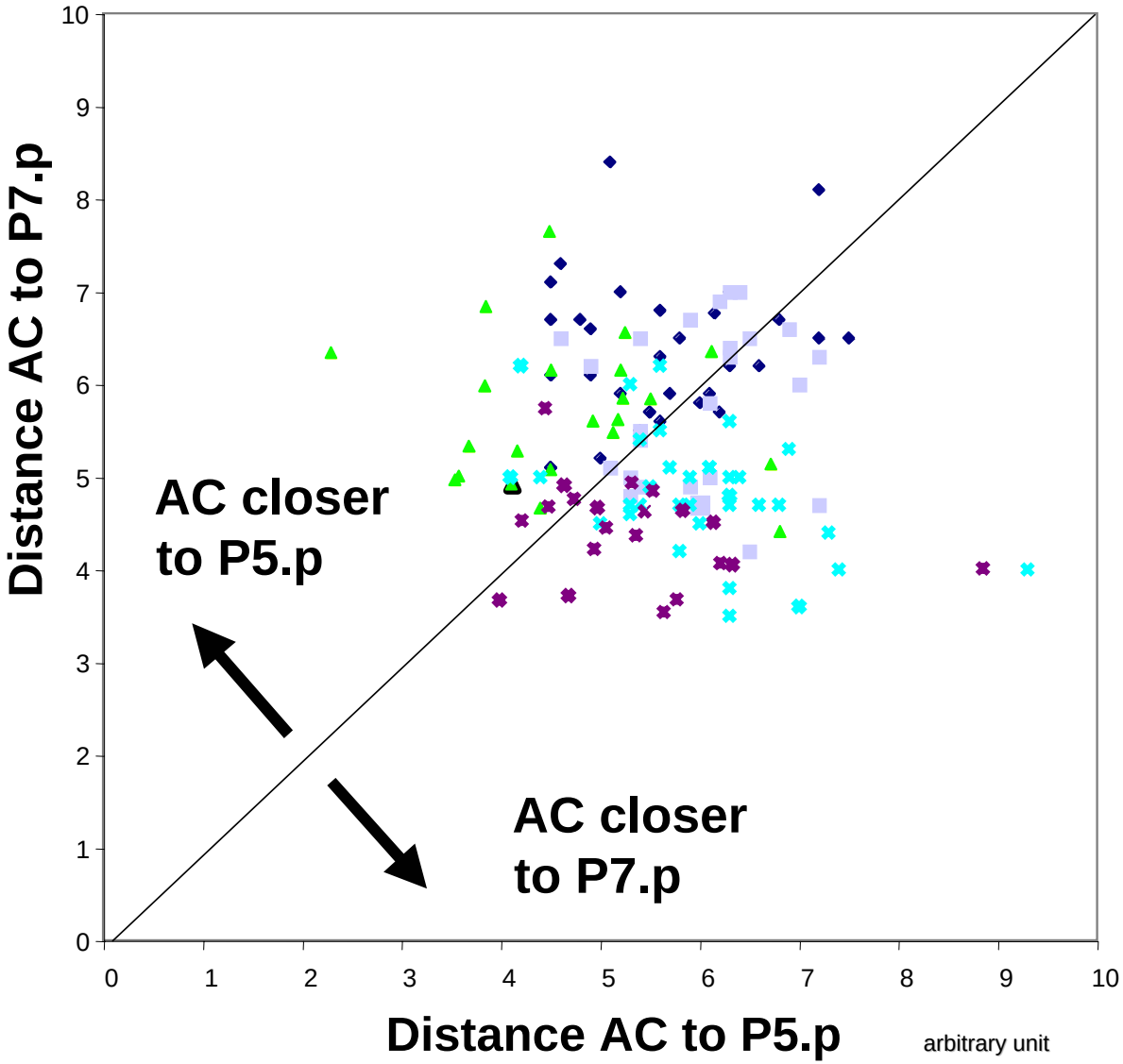
Anchor cell - P6.p alignment during the L2 stage



Stochastic and Genetic variation in Anchor Cell position during the L2 stage



C. elegans → P5.p

- N2 (ancestral)
- N2 (standard)
- JU258

C. briggsae → P7.p

- AF16
- HK105

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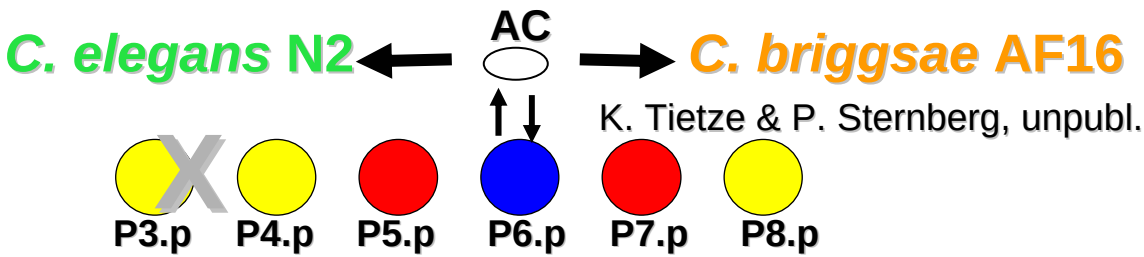
Evolution of centering errors



***C. elegans* N2**

P5.p centering is frequent

on P7.p very rare

P3.p: competent

***C. briggsae* AF16**

P7.p centering is frequent

on P5.p never observed

not competent (Delattre & Félix, 2001)

⇒ **'Errors' indicate a selection pressure on the competence group and reveal evolution in robustness mechanisms**

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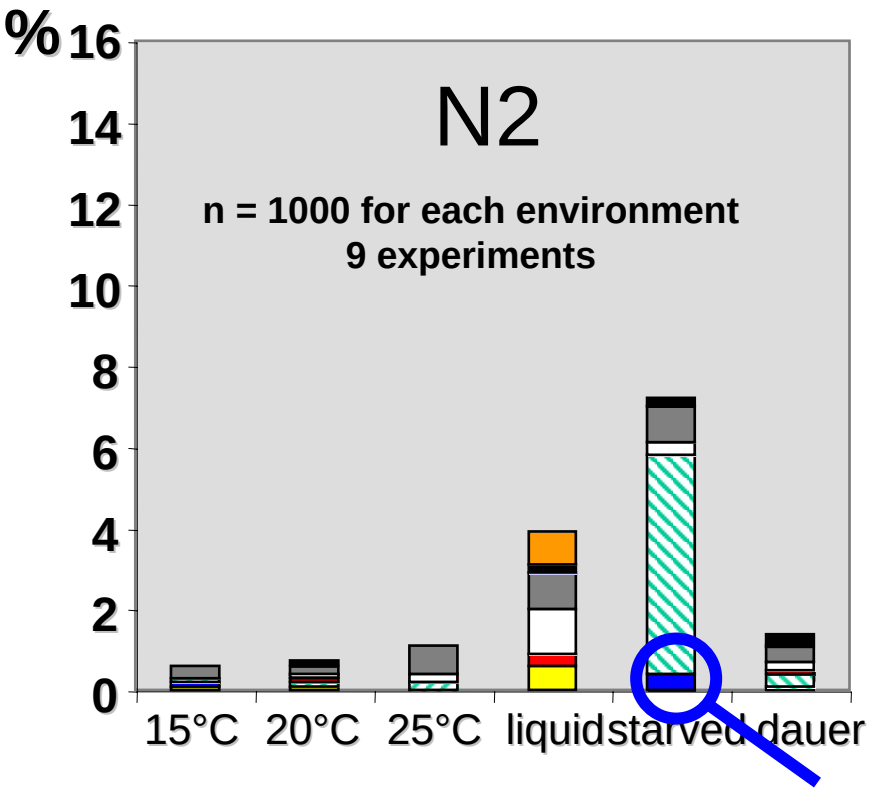
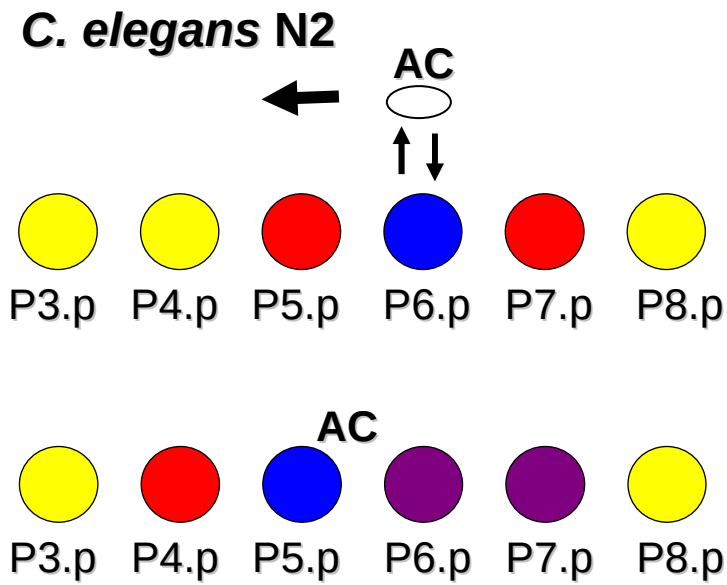
Loss of precision of centering may result in hyperinduction





hyperinduction

centered on P5.p

All but one of the **hyperinduced** errors in N2 in starvation conditions are also miscentered on P5.p

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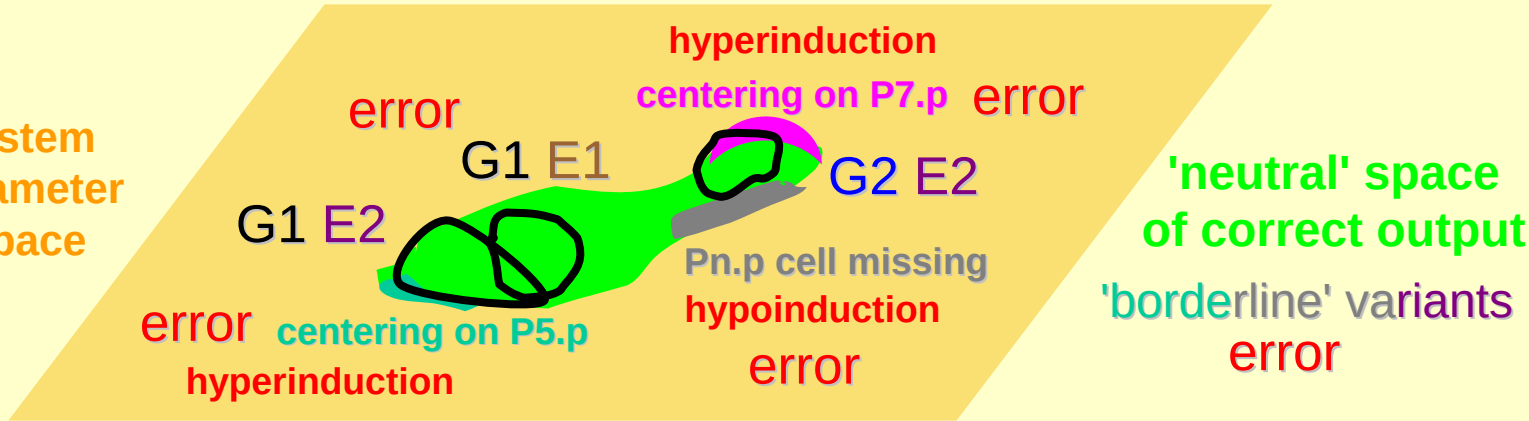
Conclusion (Ia.)
Vulva Development Precision

- Variants with defective vulvae occur at a rate of < 1% in tested environments

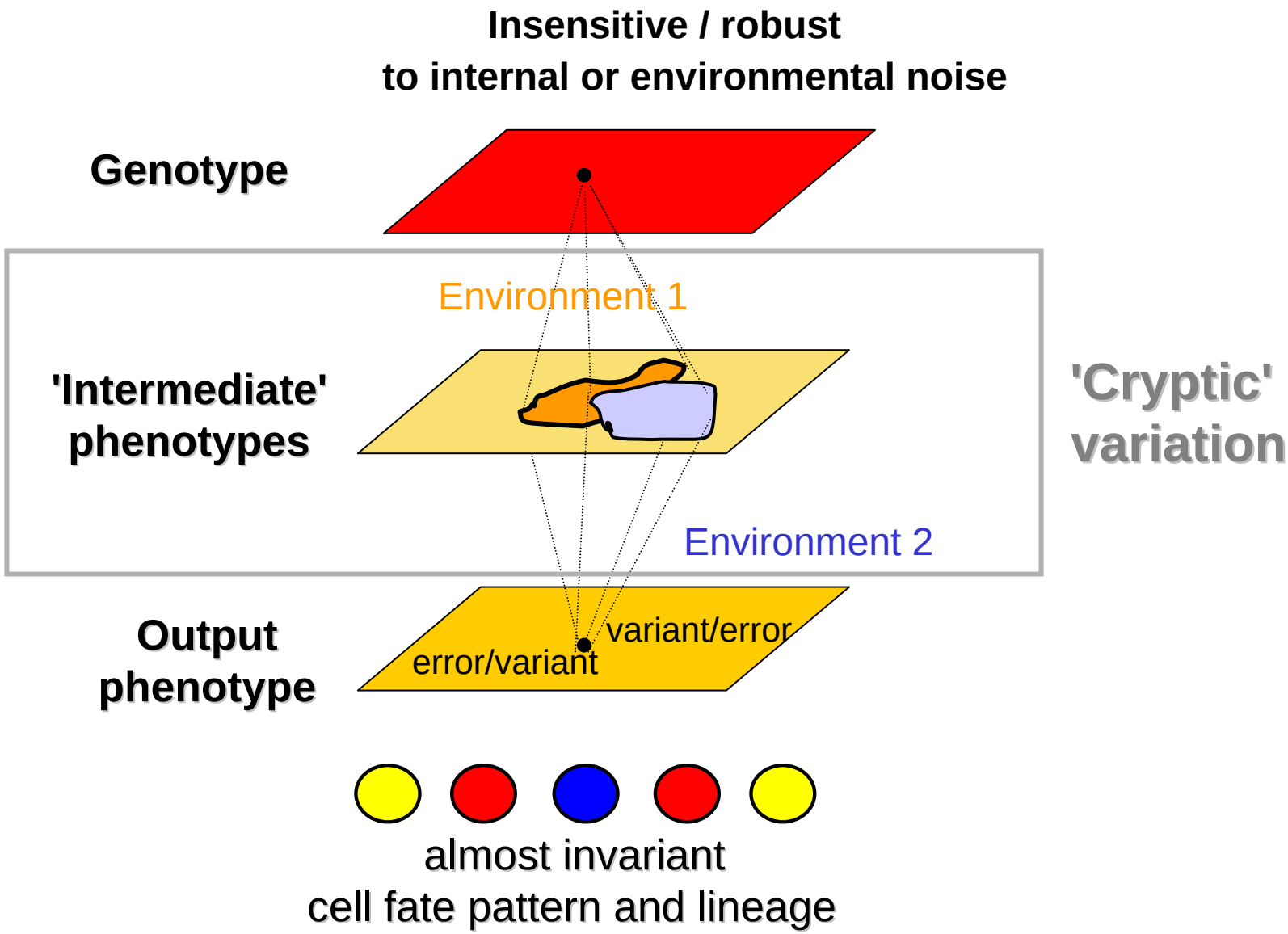
- The type and rate of variants / errors vary with Environment and Genotype and indicate constraints on the system



=> The molecular mechanisms in the vulva development process are likely to differ among **environments** and among **wild isolate**s.

Ib. II.

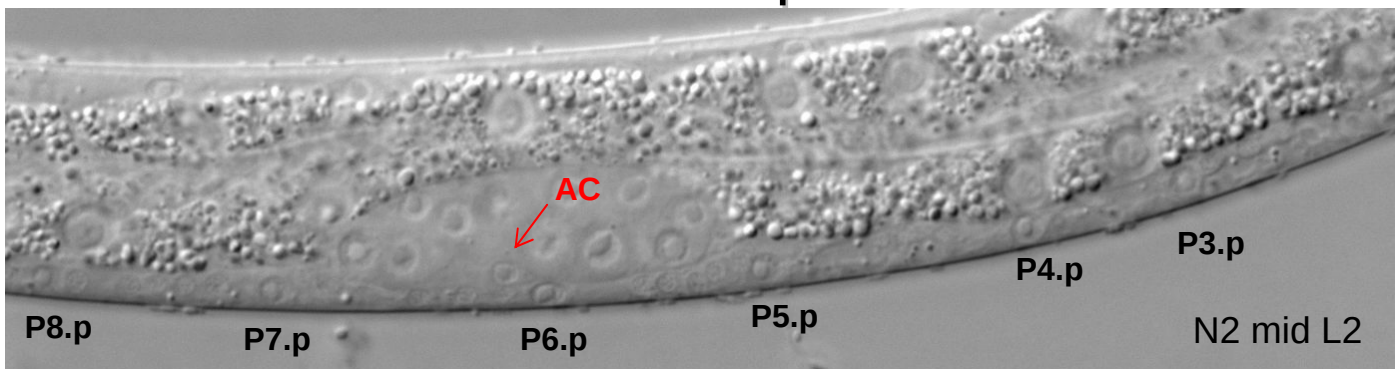
Sensitivity to Noise and Environmental Variations



Revealing cryptic variation in the vulva network

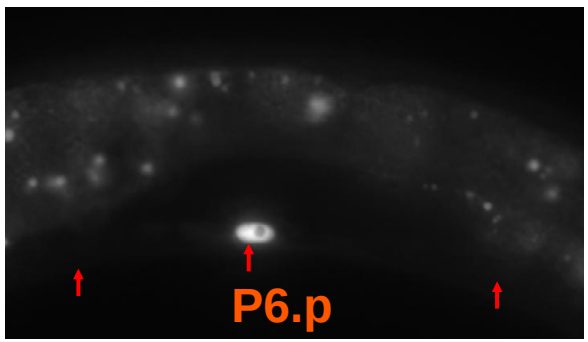
Variant 'Intermediate' Phenotypes

Anchor cell position

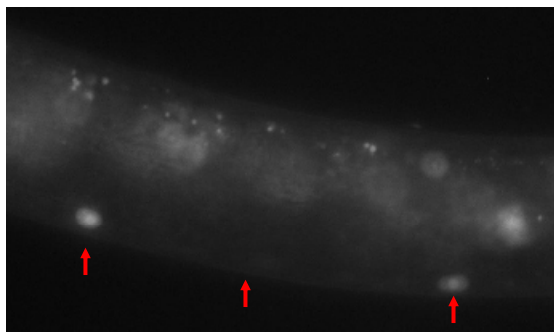


Ras and Notch pathway activities

Ras pathway reporter
*egl-17::*CFP

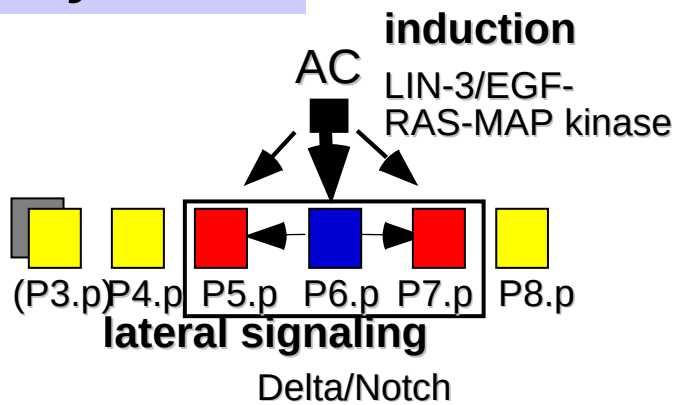


Notch pathway reporter
*lip-1::*YFP



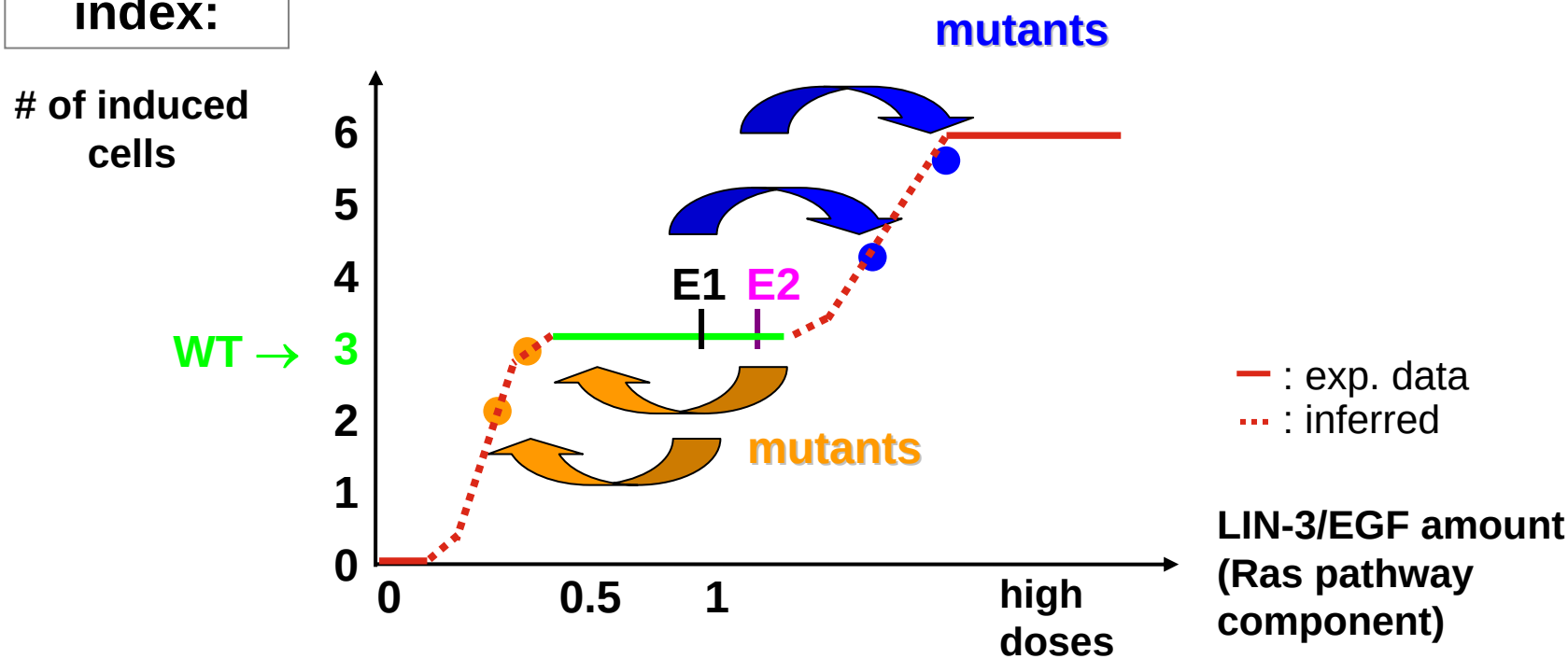
Revealing cryptic variation in the vulva network

Perturbation of the system



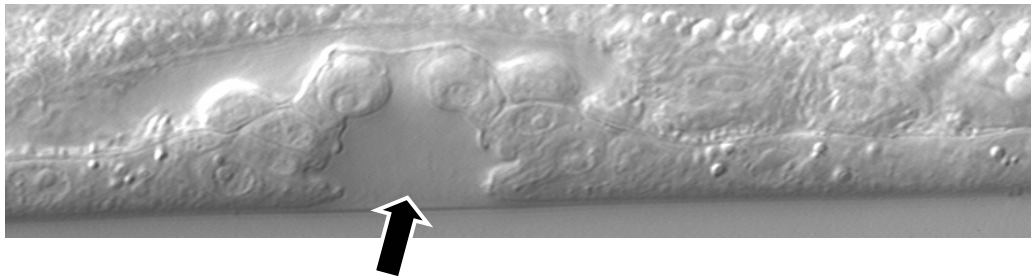
normal output:
3 induced cells

Induction index:

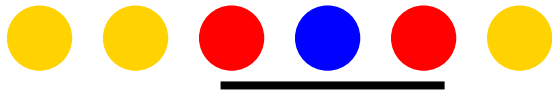


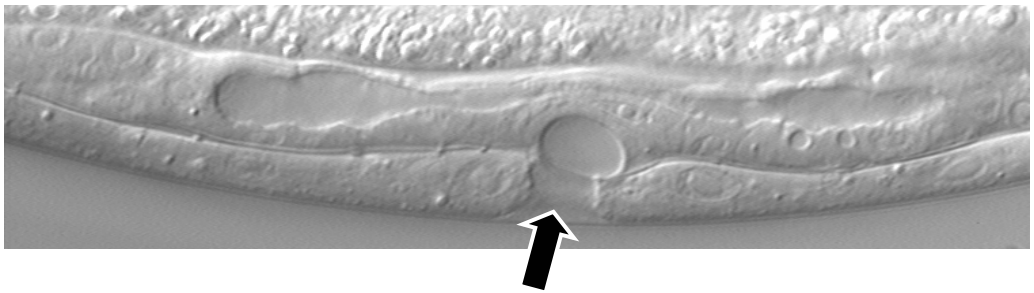
Ib. Environmental effects on the vulva developmental process

Quantification of vulva mutant phenotypes
(sensitizing the system)



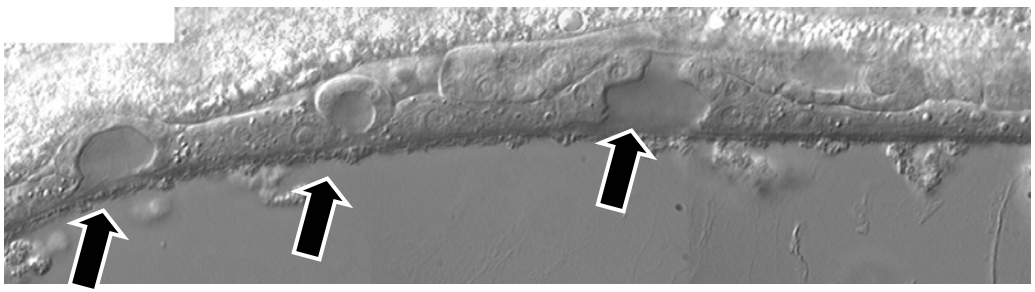
WT: **Induction = 3**



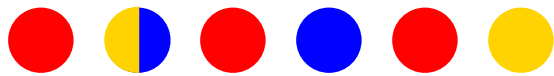


Vulvaless: **Induction < 3**

Induction index = 1



Multivulva: **Induction > 3**

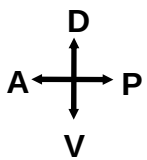


Induction index = 4.5

1°: vulval

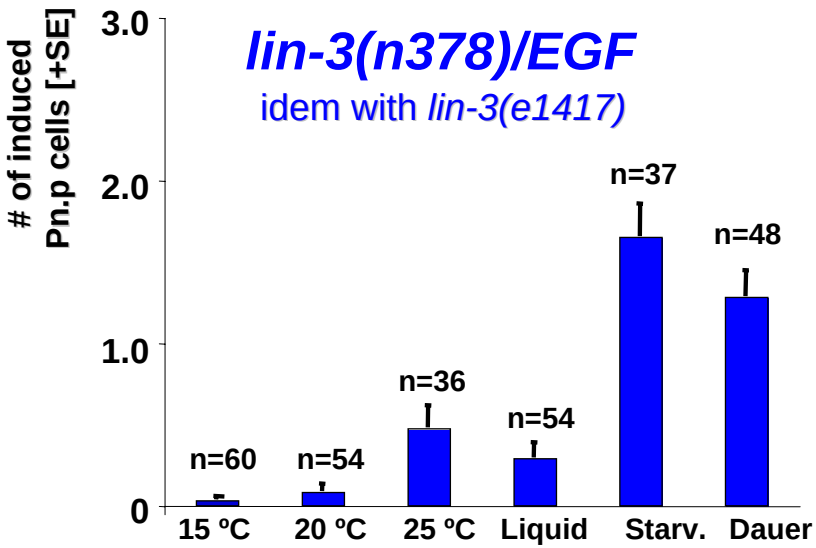
2°: vulval

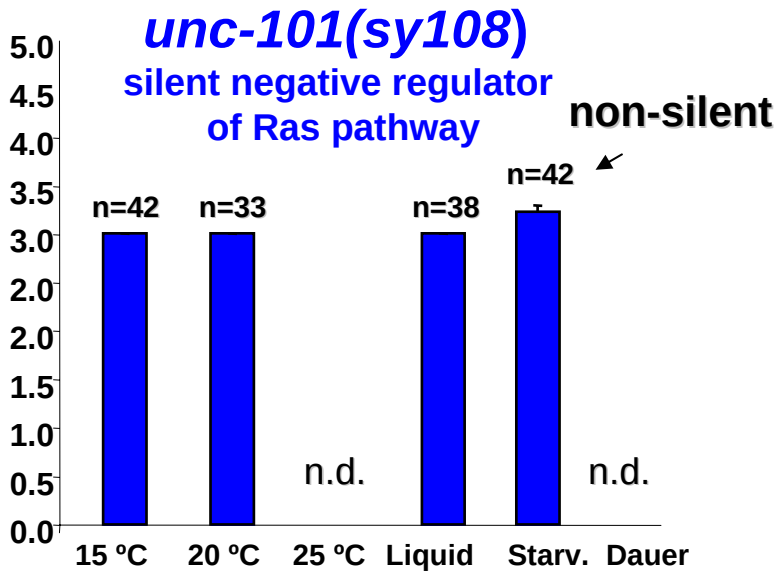
3°: non-vulval

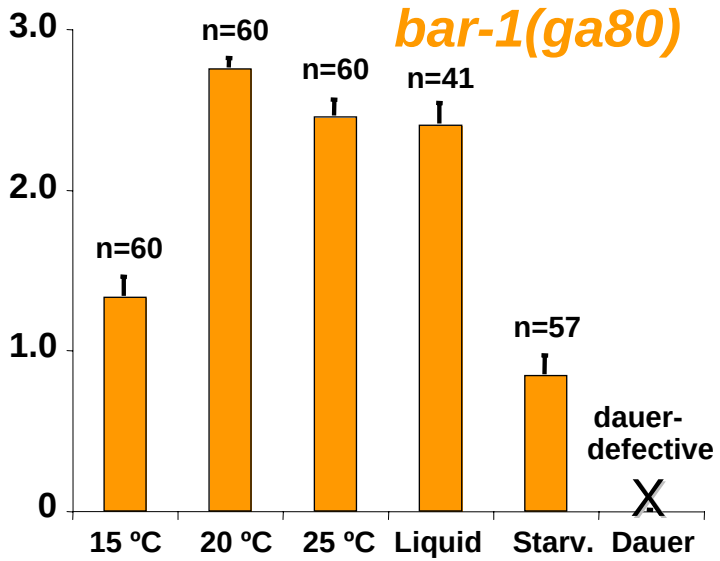


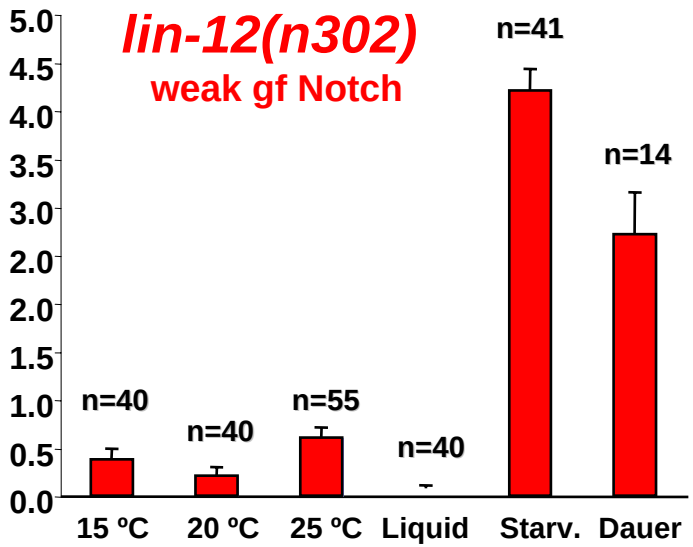
L4 stage. Nomarski pictures.

Ras, Wnt, Notch pathway mutants in different environments









***lin-3; bar-1*, starved: very low induction index**

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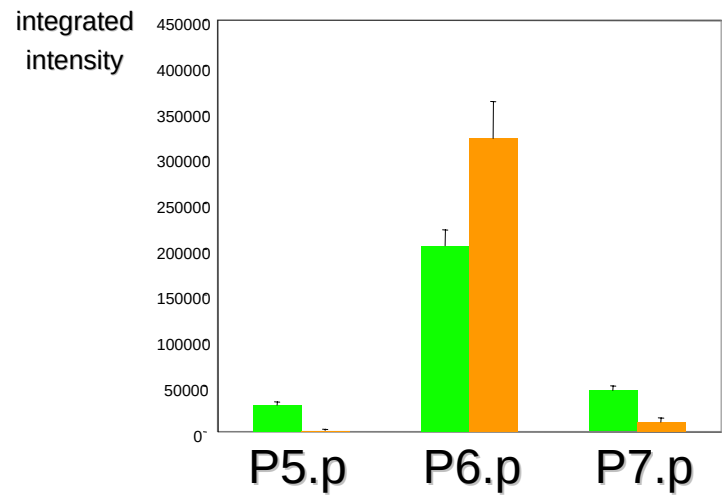
Quantification of signaling pathway activities

Transcriptional reporters

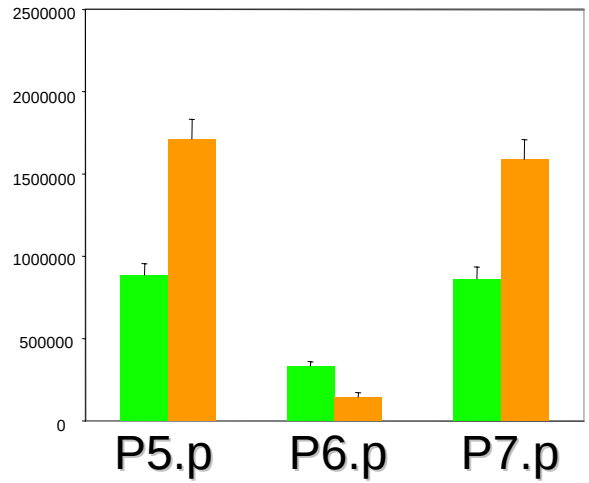
Ras pathway
(*egl-17*::CFP)



Food (N=33)
Starvation (N=17)

L2 lethargus

Notch pathway
(*lip-1*::GFP)



Food (N=40)
Starvation (N=42)

Earlier pattern formation and higher induction levels upon starvation

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Conclusion:
Environmental Effects on Vulva Development

- **The vulva mutant screens may have given a different result had they been performed in a different environment**

- **The environment may affect the developmental signaling pathways (intermediate phenotype) without variation in the final output**

C. elegans vulva development

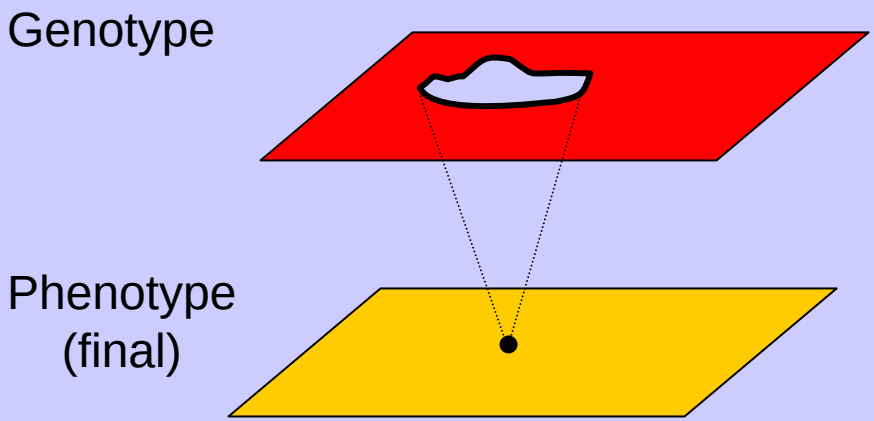
I.
Sensitivity to **noise and environmental** change
'Error' type and rates
Cryptic variation in different environments

II.
Cryptic evolution in *C. elegans* and the *Caenorhabditis* genus

III.
Sensitivity to **random mutational** change
Bias/constraints

II. **Cryptic** Genetic Evolution

System that is robust to <u>environmental</u> variations
is likely to be robust to a set of <u>genetic</u> variations

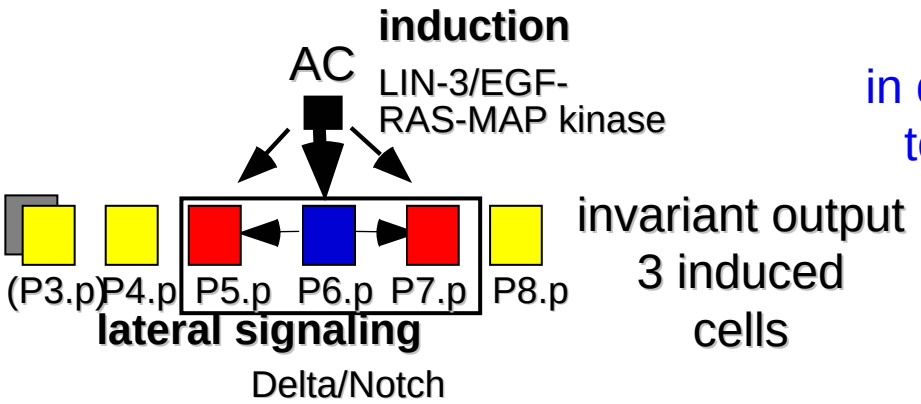


insensitive/robust/buffered/canalized
against some genetic variations

Robust output
in wild isolates:
3 induced cells: P(5-7).p
with '2°1°2°' pattern

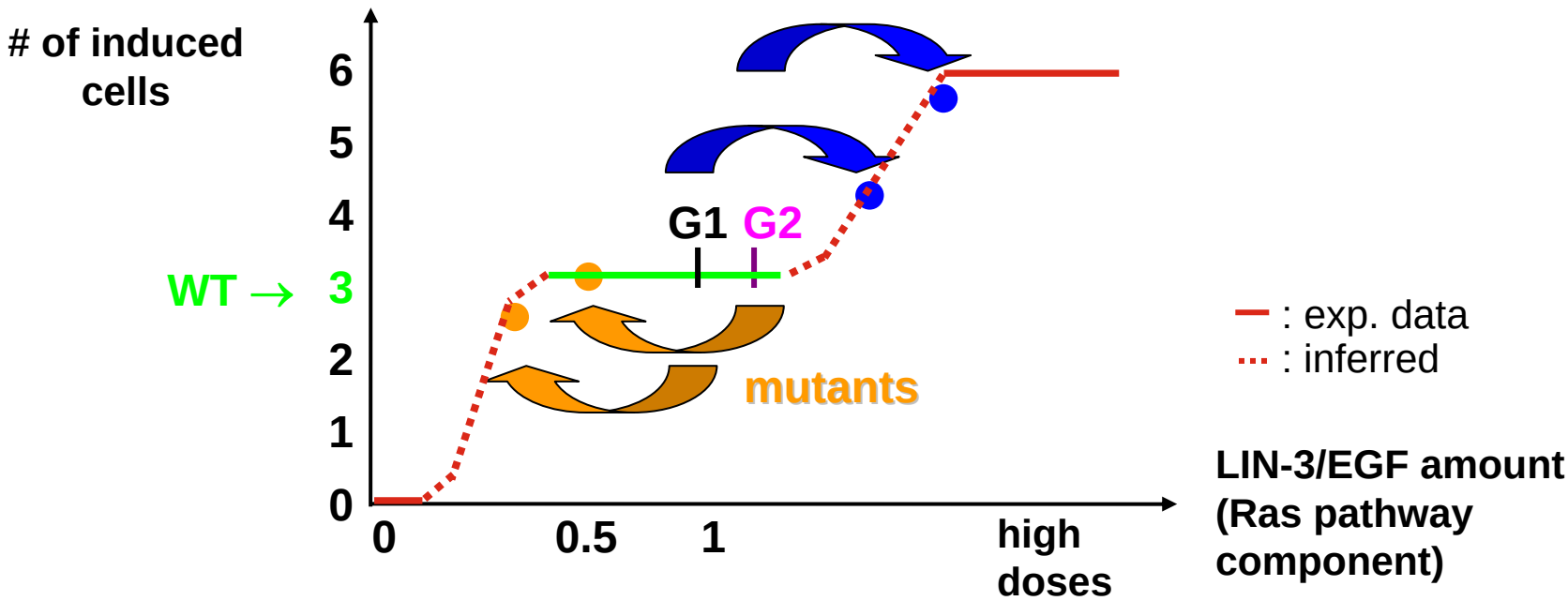
Allows Cryptic Evolution
of the System Without Change
in the <u>Final</u> Phenotype

Revealing cryptic variation in the vulva network



Strategy: Perturb the system
in different "wild" genetic backgrounds
to unravel hidden genetic variation

Induction index:



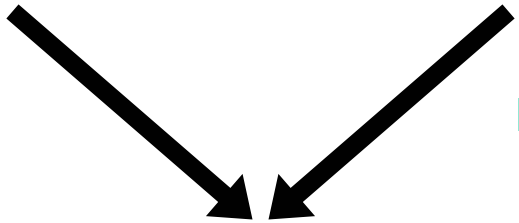
IIa. Cryptic Genetic Variation within *C. elegans*:

Introduction of Mutations in Different Wild Genetic Backgrounds

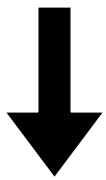
Mutations
(**EGF**, **Notch** & **Wnt** pathways)

7 wild isogenic
genetic backgrounds

N2, CB4856, JU258,
PB303, PB306, PS2025, AB1



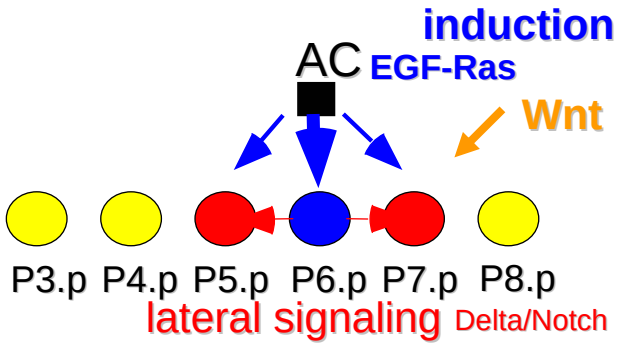
Introduction of the mutations in the different backgrounds by repeated out-crosses



Vulva index

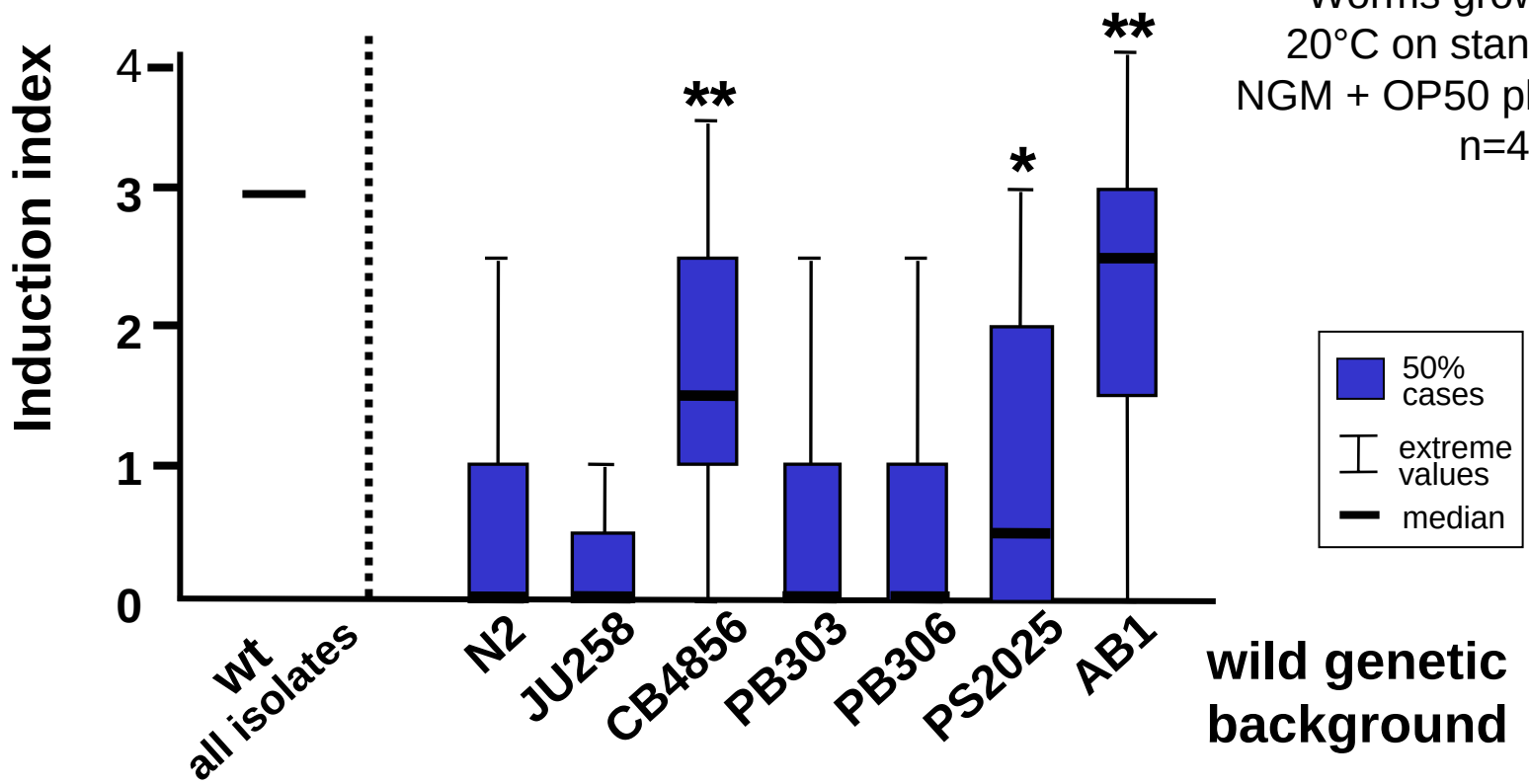
J. Milloz and I. Nuez

Different wild isolates: Mutation of the Ras pathway



let-23(sy1) EGF receptor

Worms grown at
20°C on standard
NGM + OP50 plates
n=49-70

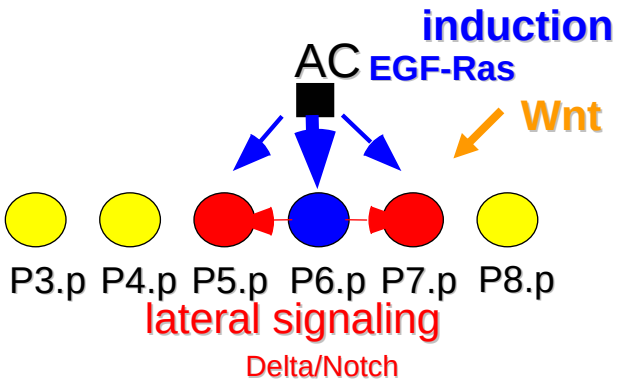


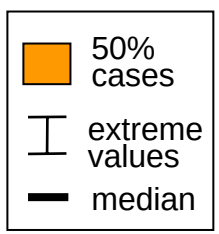
Kruskal Wallis F-test, $p<0.0001$.
Paired Mann-Whitney U test with N2, *: $p=0.01$; **: $p<10^{-7}$

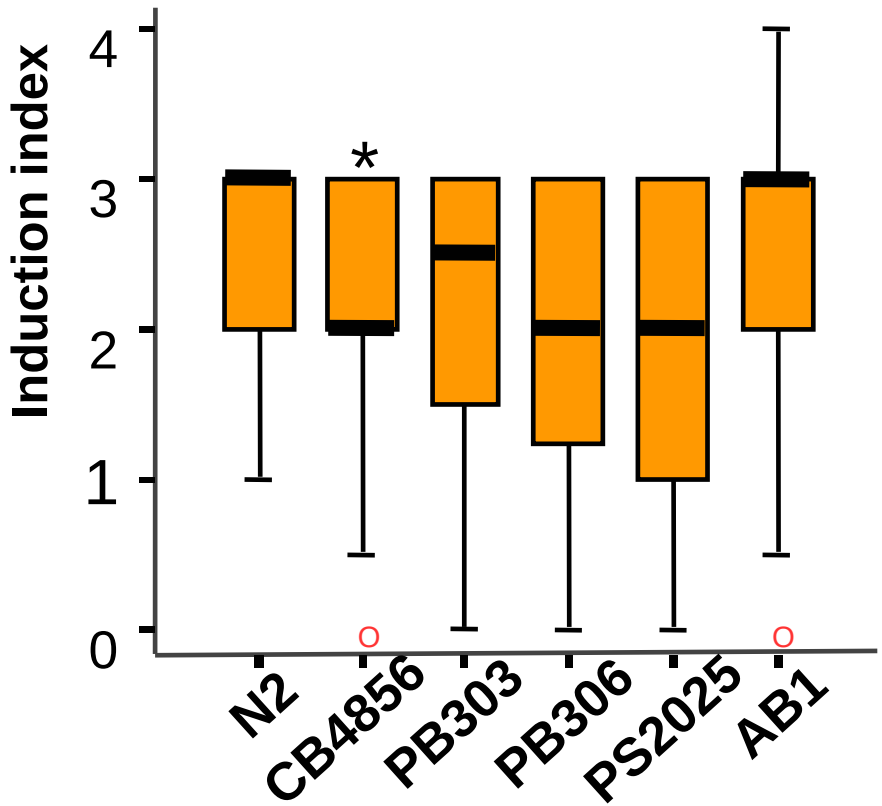
Josselin Milloz

Different wild isolates: Mutation of the Wnt pathway

bar-1(ga80) β-catenin (null)







=> The induction index of Wnt pathway reduction-of-function mutations is lower in other backgrounds.

Josselin Milloz

The vulva mutant screens would have given a different result had they been performed:

- **in a different environment**

or

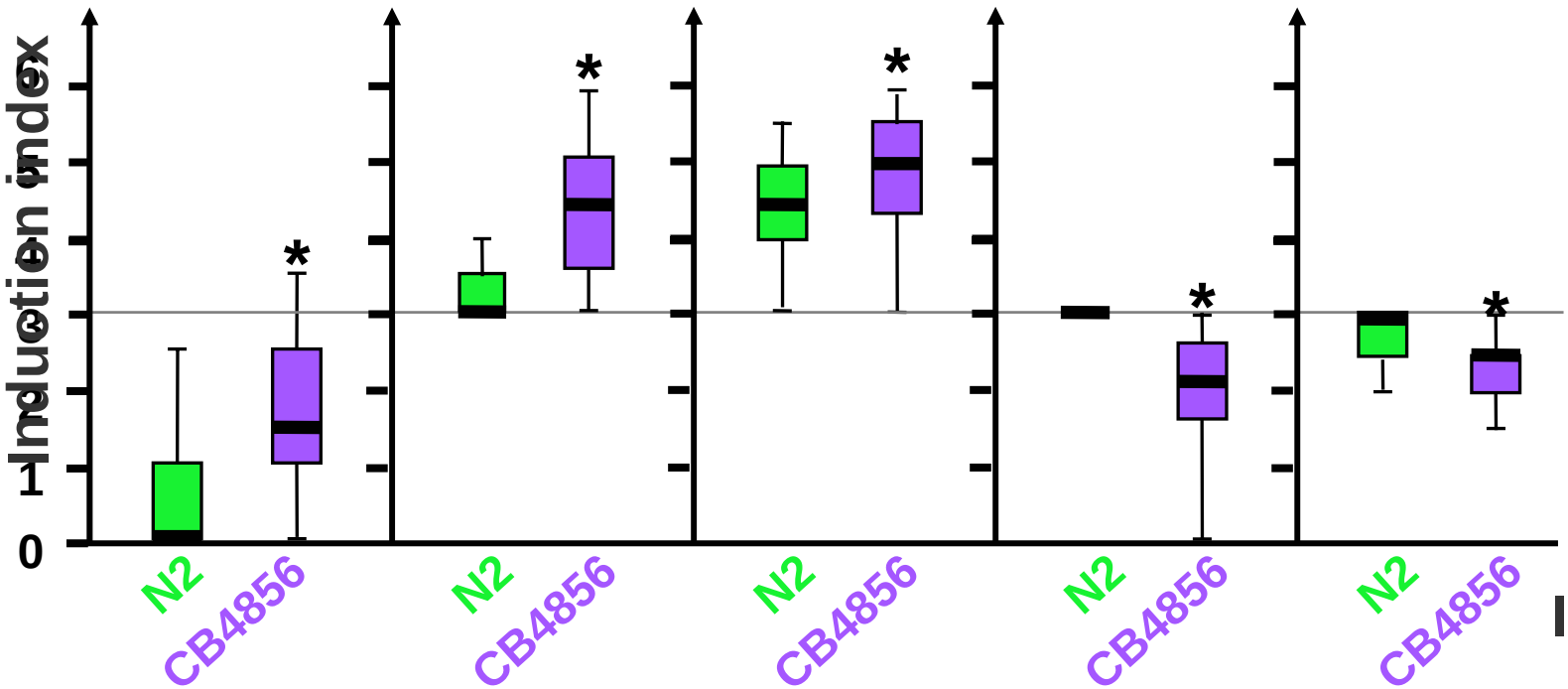
- **in a different *C. elegans* wild genetic background**

Environmental variation and genetic variation may act on the same system's parameters

Effects of mutations along the Ras pathway

N2 vs. CB4856

let-23(rf) | *ark-1(lf); gap-1(lf)[a]* | *let-60(gf)* | *lin-45(rf)* | *mpk-1(rf)*



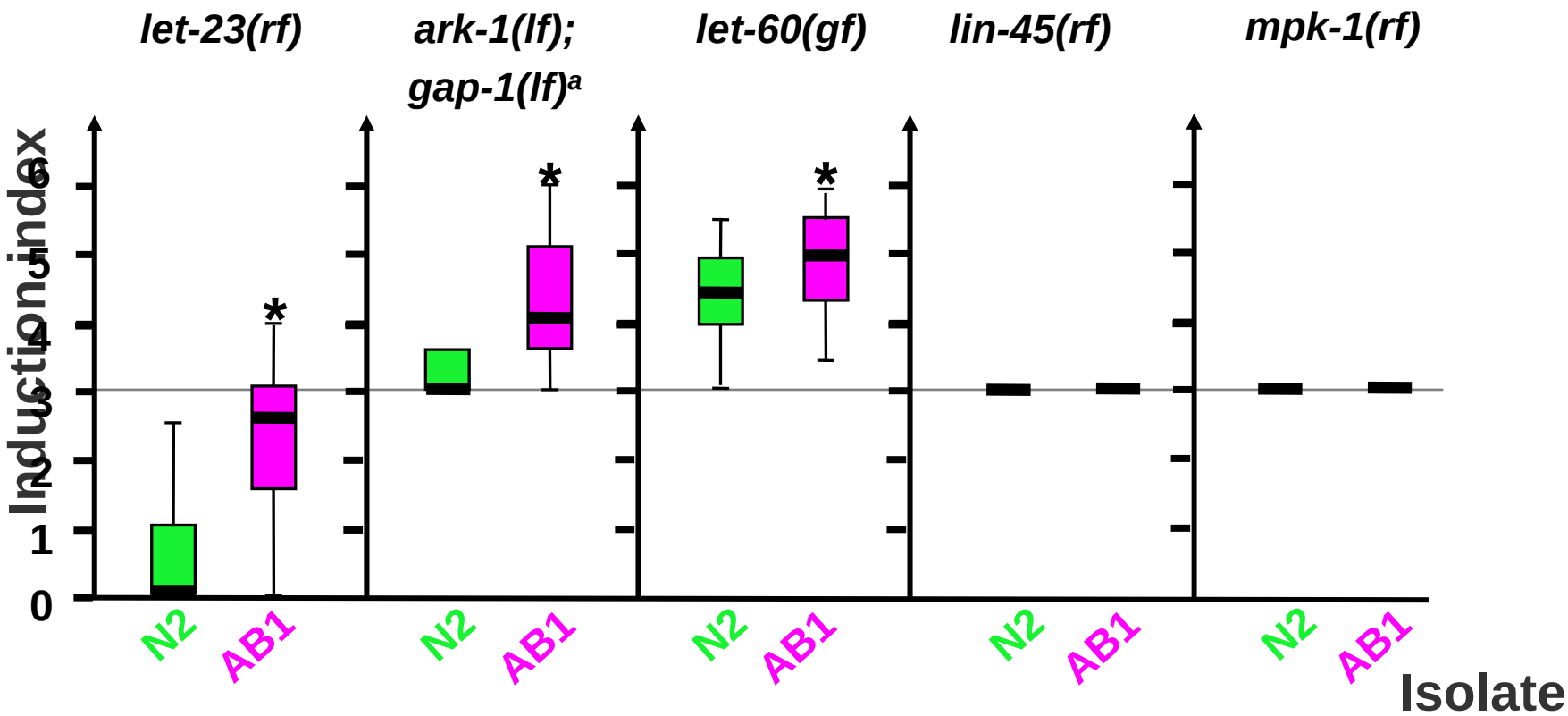
=> Compensatory evolution along the pathway?

*: Paired Mann-Whitney U-test with N2, p<0.0001, n>60. @ 20°C, except *a* @ 23°C

Josselin Milloz

Effects of mutations along the Ras pathway

N2 vs. AB1



*: Paired Mann-Whitney U-test with N2, p<0.0001, n>60. @ 20°C, except *a* @ 23°C

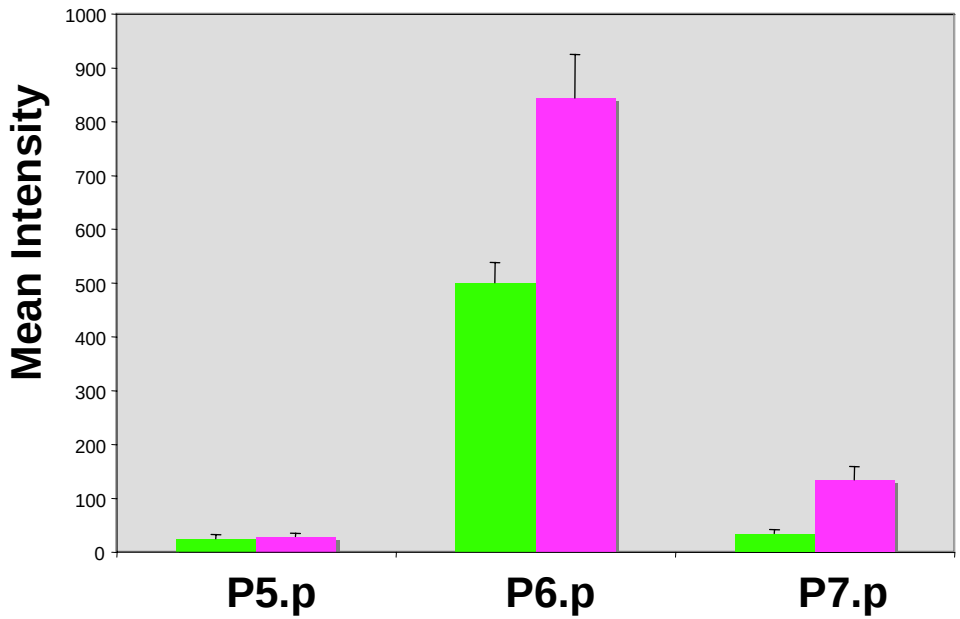
Josselin Milloz

Quantification of signaling pathway activities

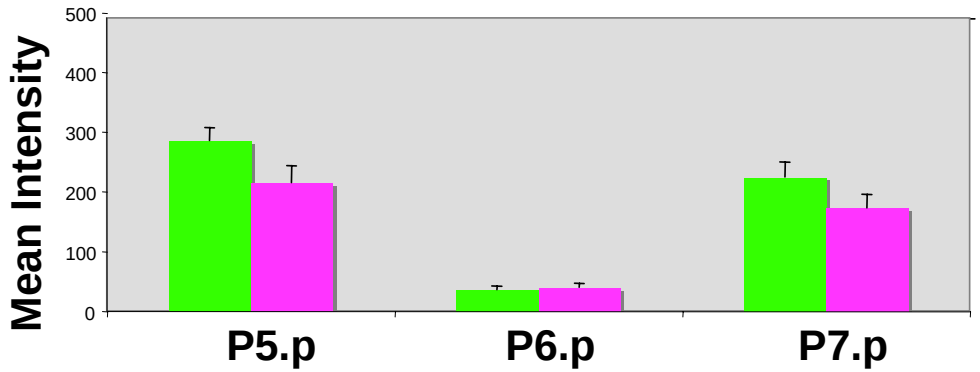
N2 vs. AB1

Transcriptional reporters

Ras pathway
(*egl-17*::CFP)



Notch pathway
(*lip-1*::YFP)



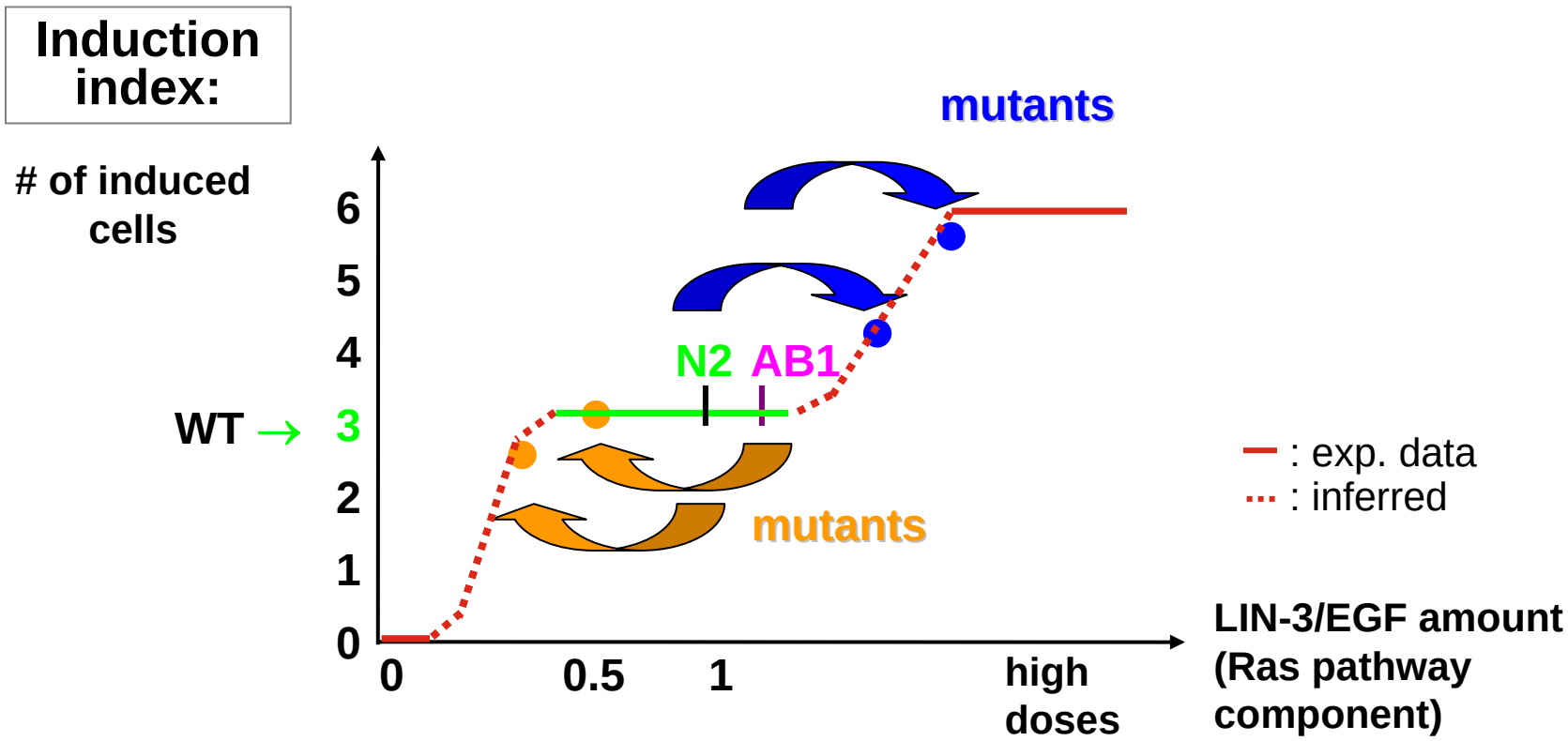
Mean + S.E.M.
n = 20
Lethargus L2

⇒ **The Ras pathway appears more active in AB1 compared to N2**

Josselin Milloz

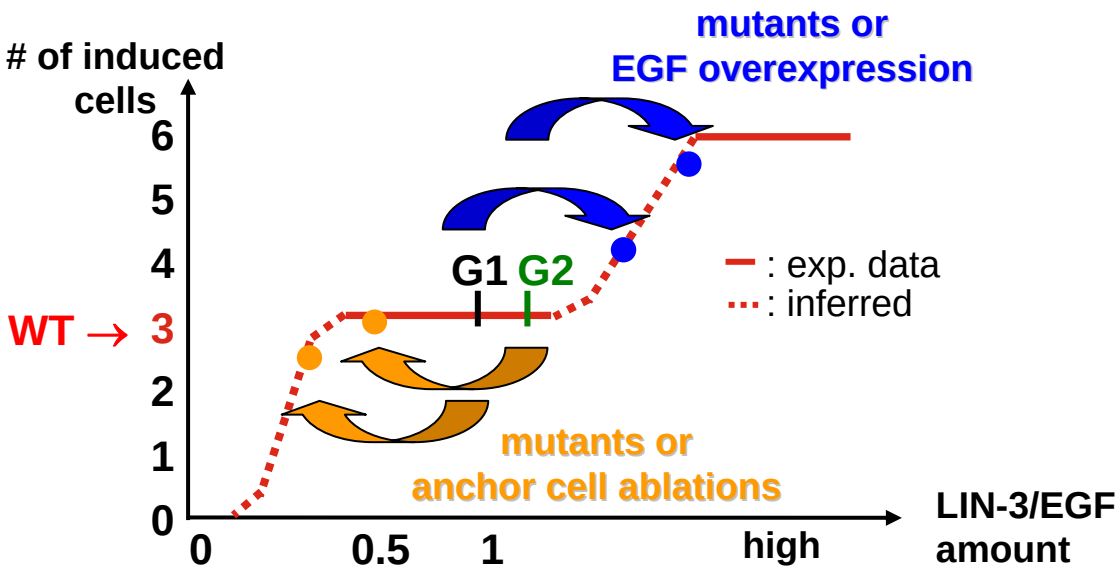
The Ras pathway appears more active in wild isolate AB1 compared to N2

(in standard laboratory environment)

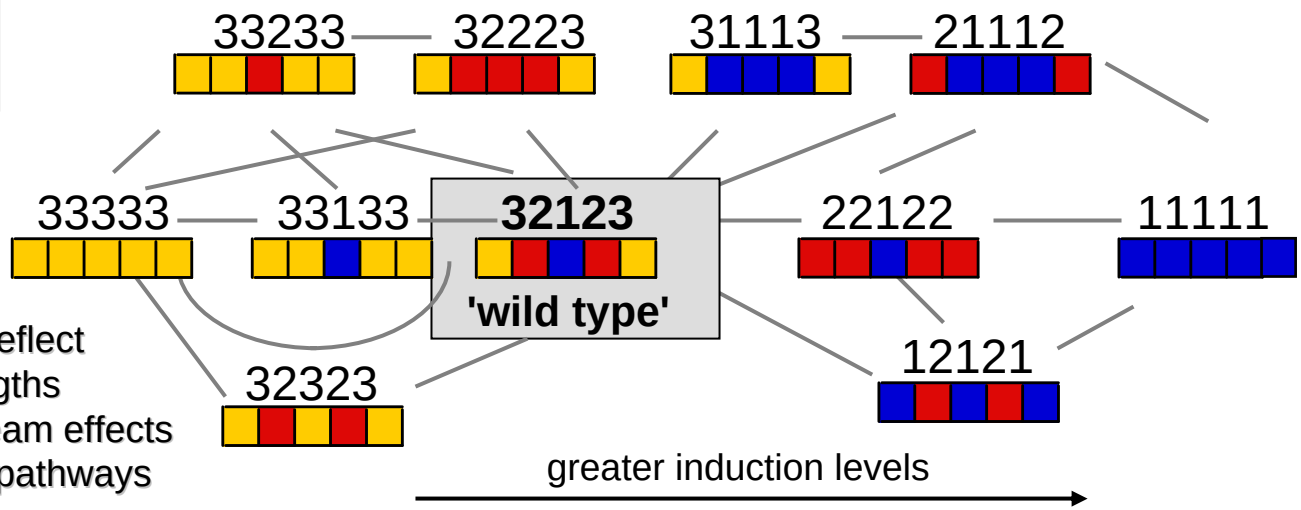


Revealing cryptic variation in the vulva network

Induction index:



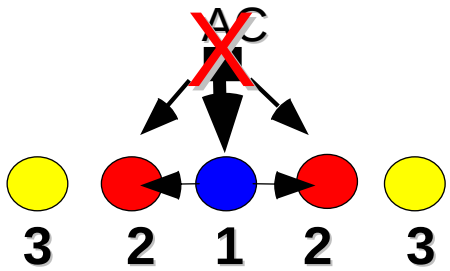
Cell fate patterns:

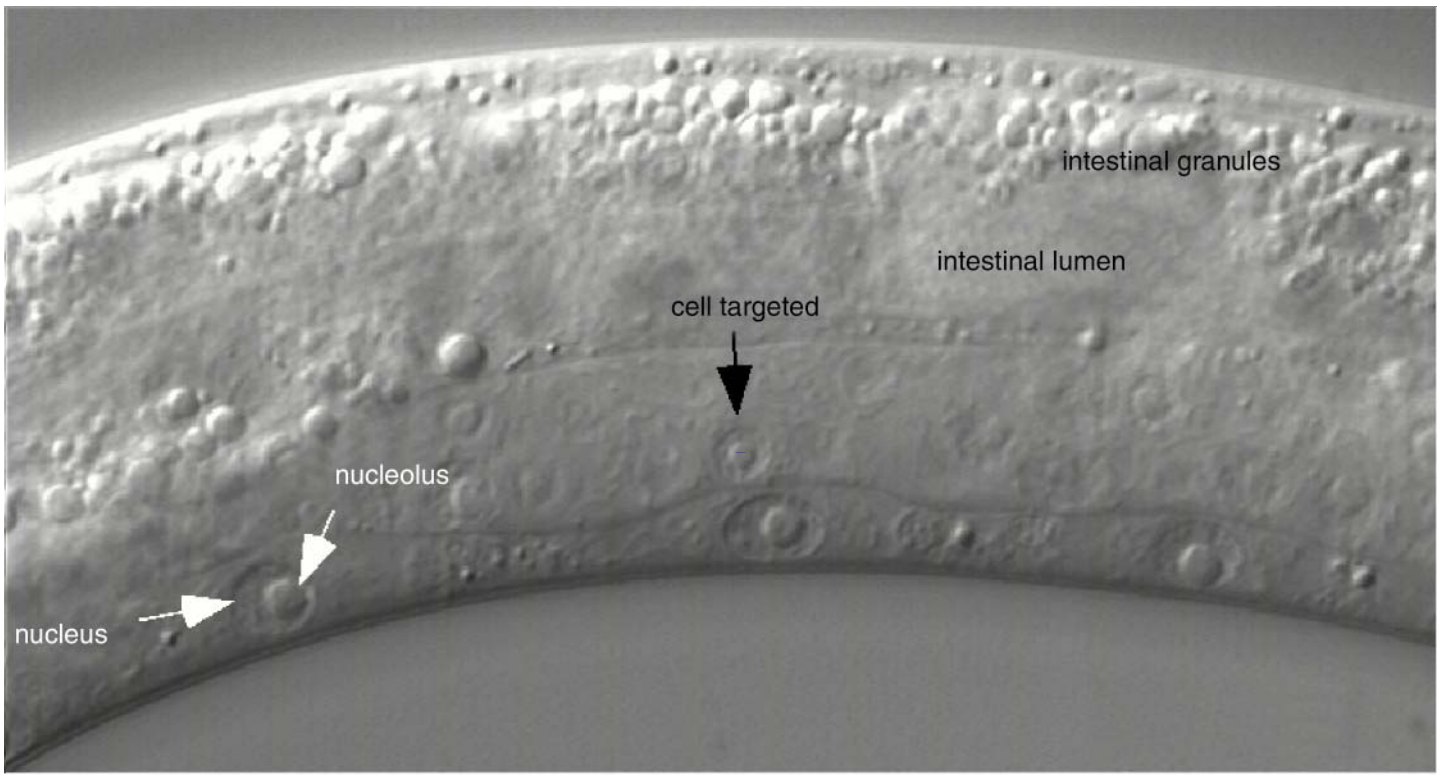


The fate patterns reflect the relative strengths of the different downstream effects of the Ras and Notch pathways

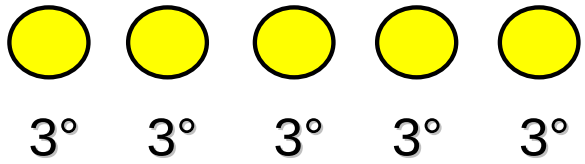
IIb. Cryptic Genetic Variation among *Caenorhabditis* Species:

'Intermediate' States Revealed by Anchor Cell Ablations



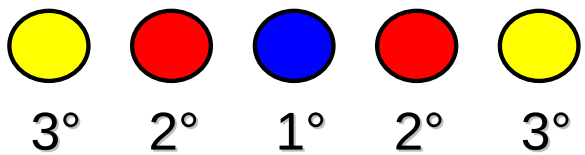


Anchor Cell Ablations
Caenorhabditis japonica DF5079



Ablation time in L3

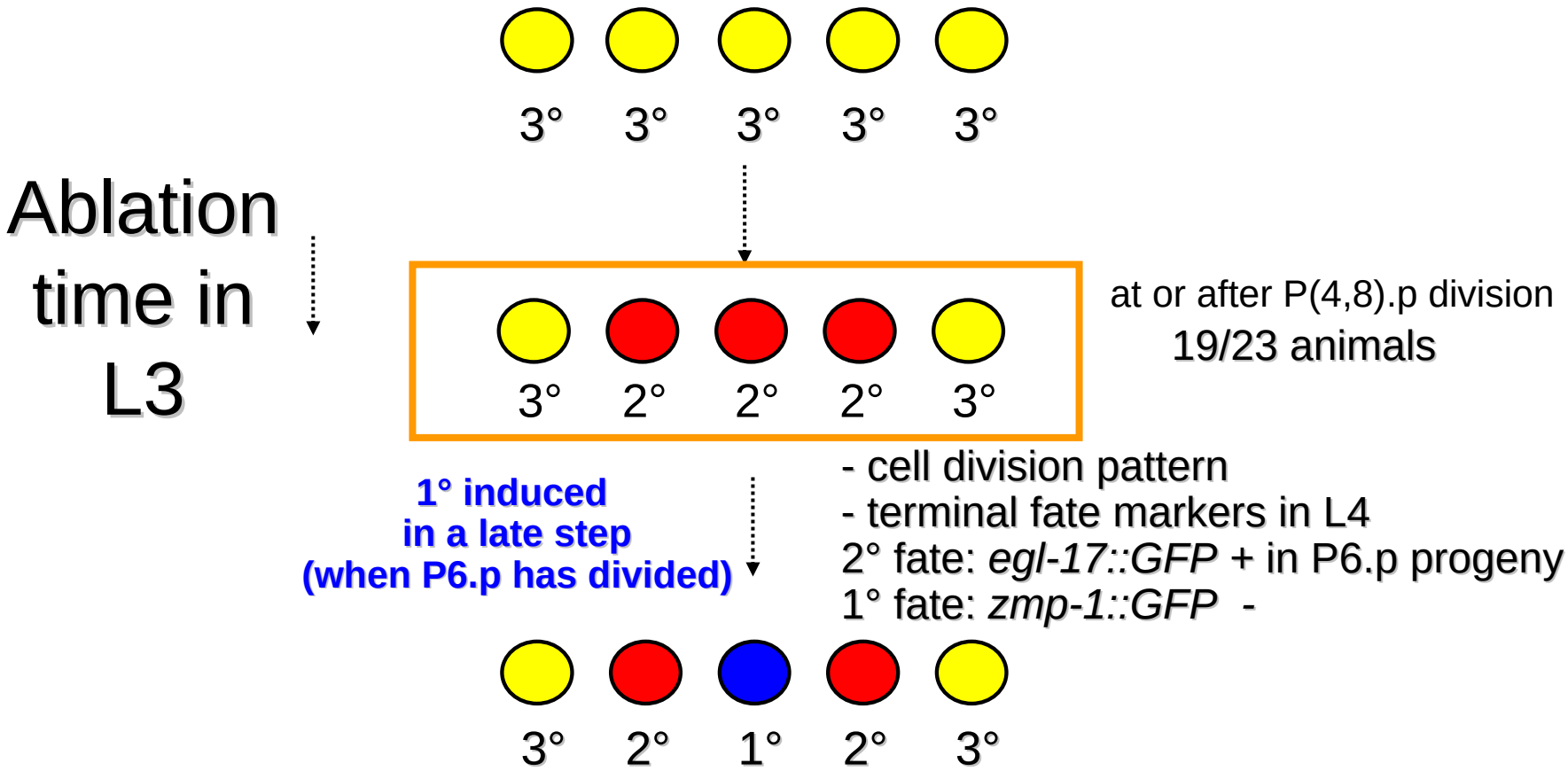
direct induction of the final pattern



28/32 animals

ablated before P6.p division

Anchor Cell Ablations
Caenorhabditis briggsae AF16



Anchor Cell Ablations
Caenorhabditis remanei PB4641

Anchor Cell Ablations in the Mid-L3 Stage

QuickTime™ et un
décompresseur TIFF (LZW)
sont requis pour visionner cette image.

P5.p

2°

P6.p

3°

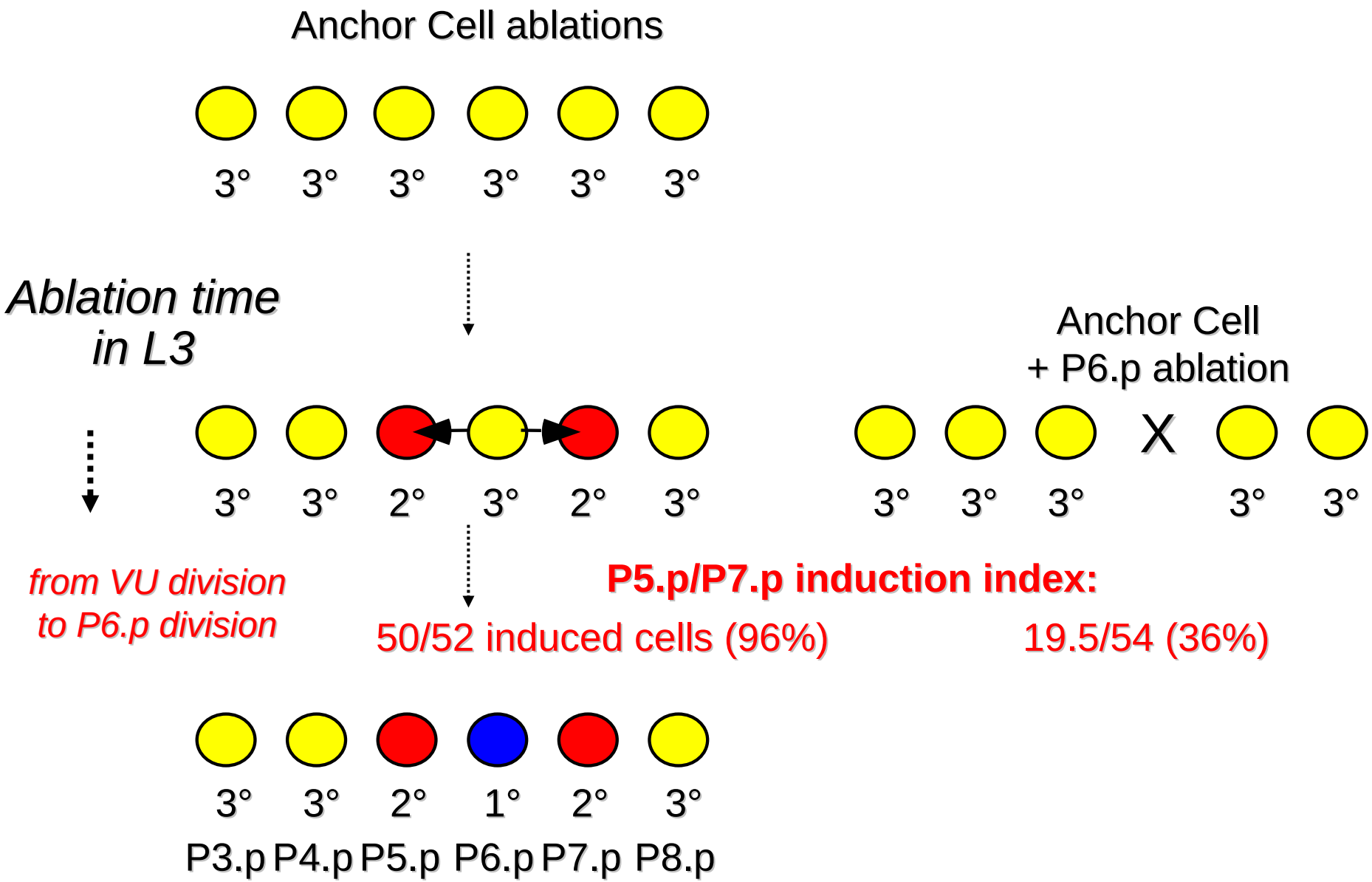
P7.p

2°

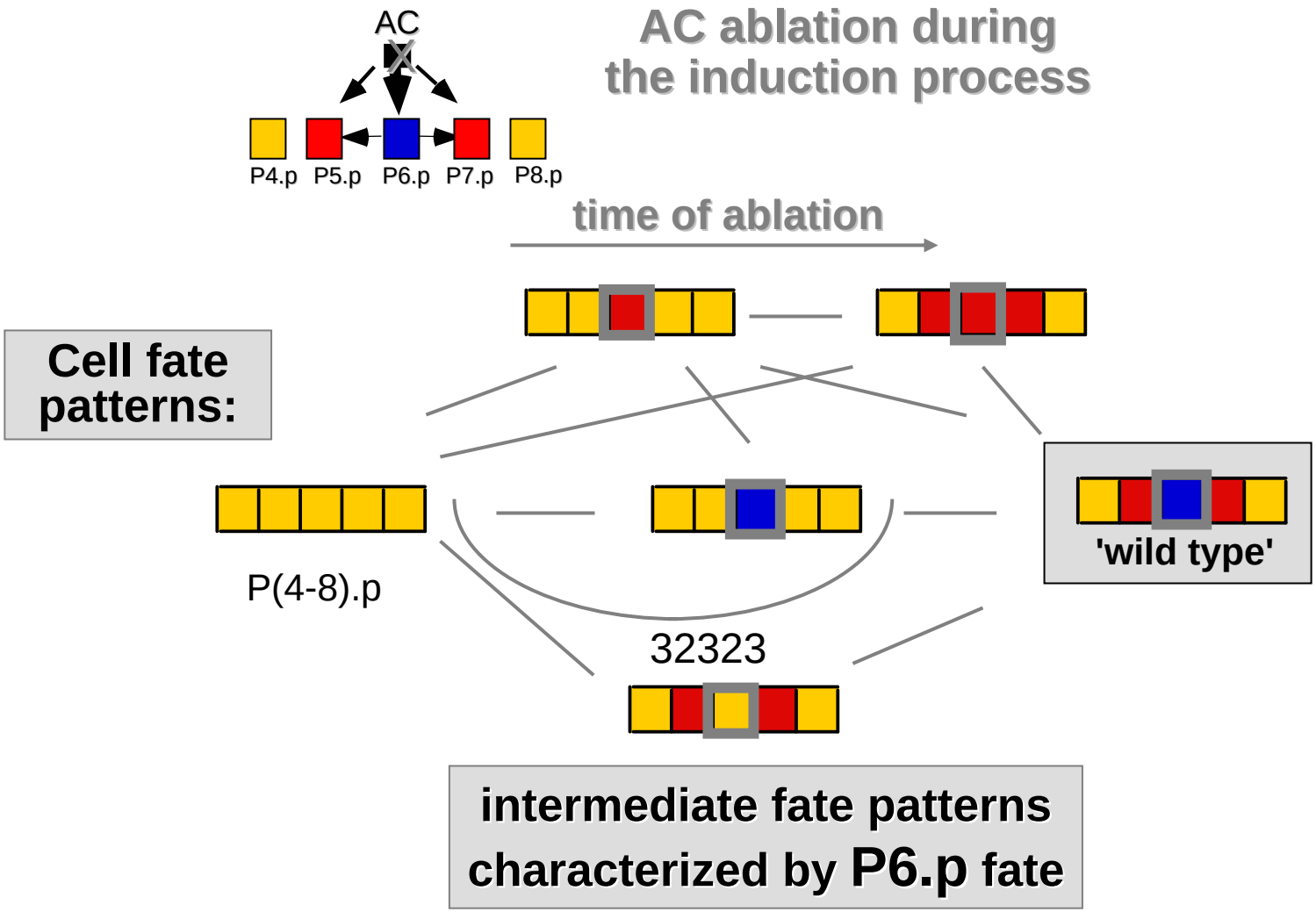
at or after P(4,8).p division

12/17 ablated animals

Caenorhabditis remanei PB4641



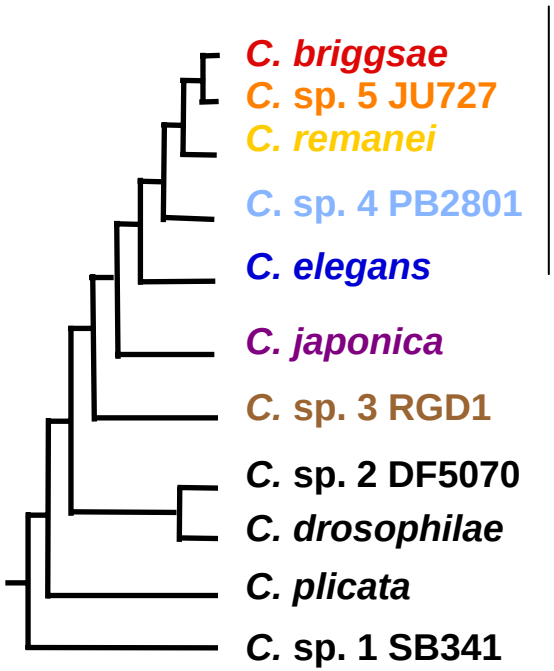
Cryptic Genetic Variation among *Caenorhabditis* Species:
'Intermediate' States Revealed by Anchor Cell Ablations



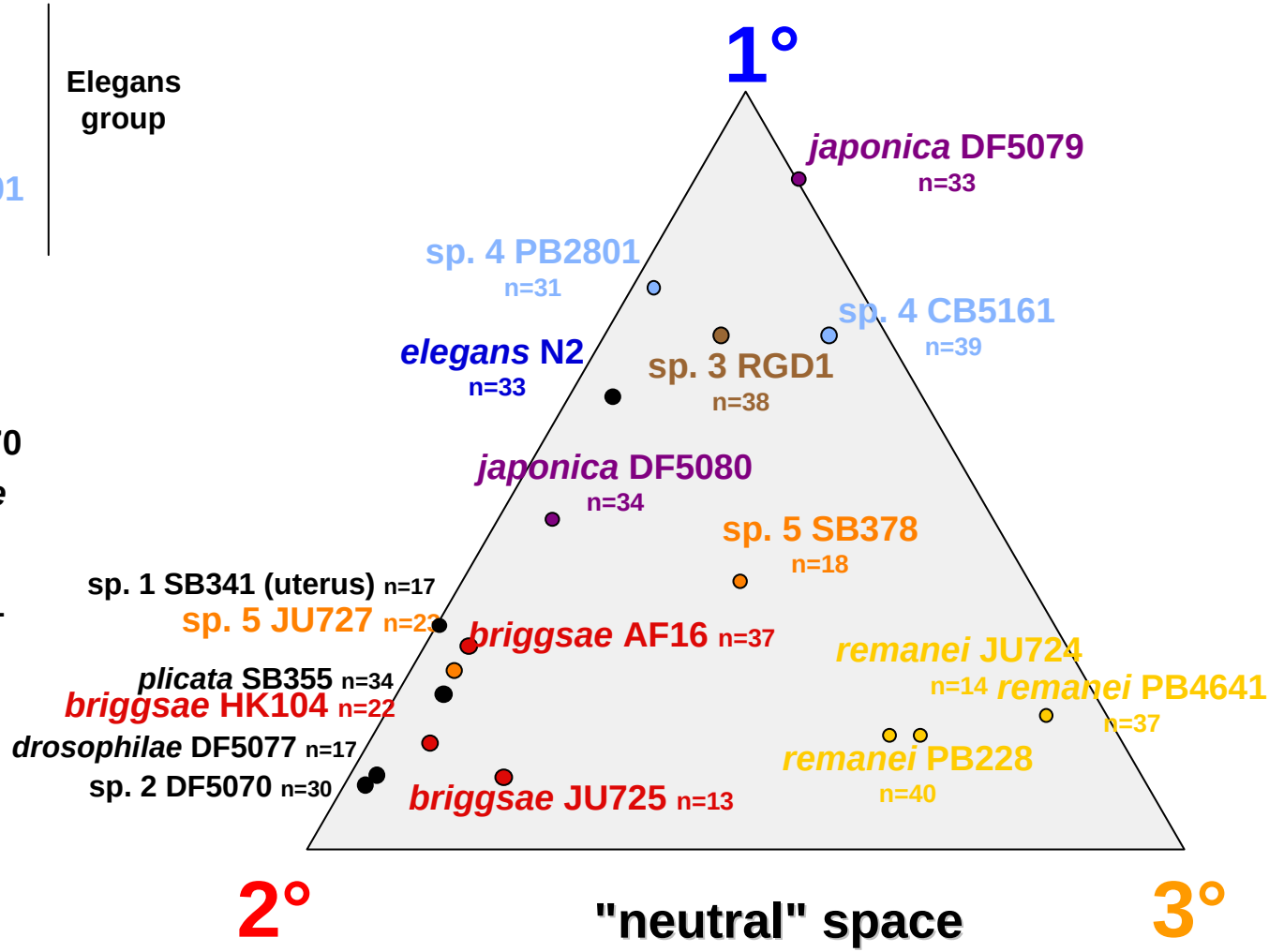
Cryptic evolution in the *Caenorhabditis* genus

Anchor Cell Ablations in *Caenorhabditis*:

Quantification of the Proportion of 1°/2°/3° Fates Adopted by P6.p
- animals with at least one Pn.p induced
- anchor cell ablations before P6.p division



Kiontke and Fitch



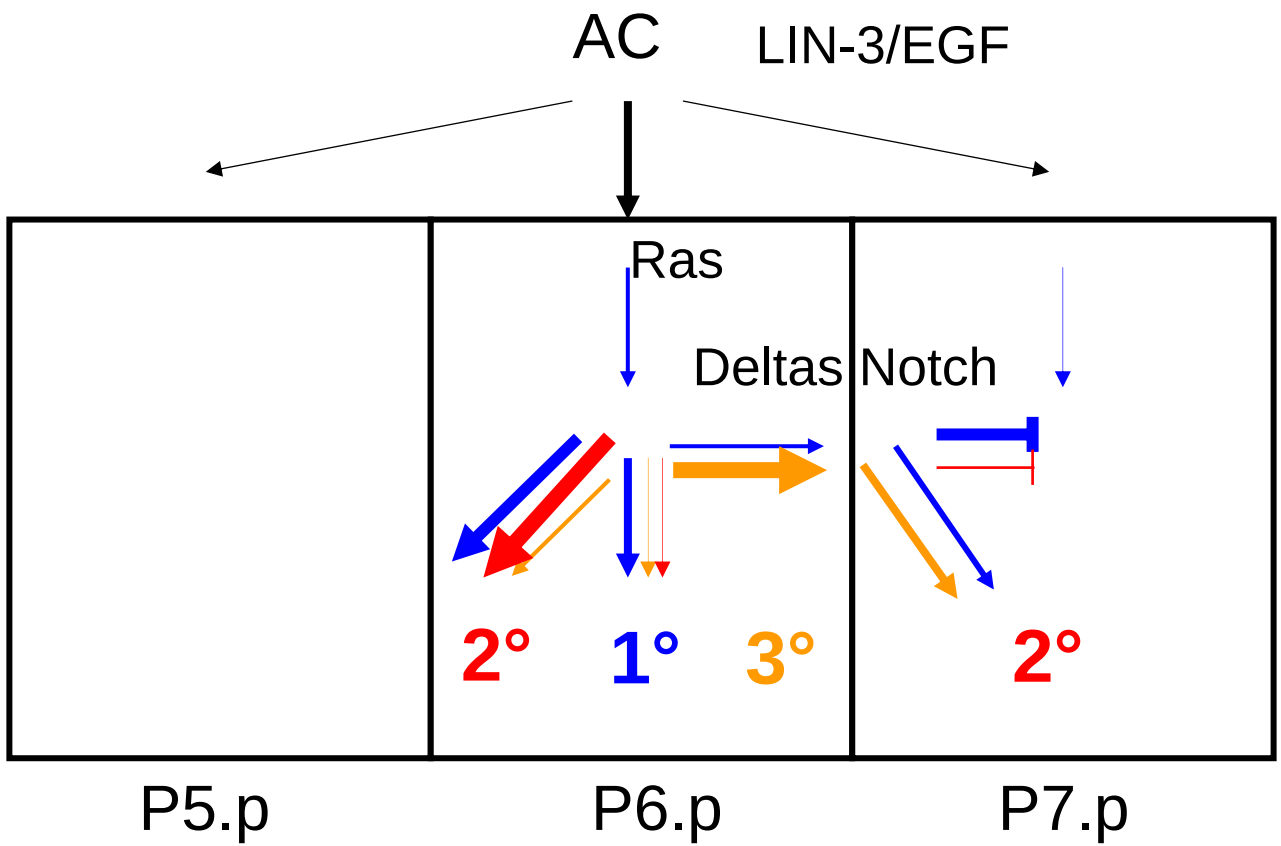
Curr. Biol. Jan 2007

Evolution of the Relative Activities of Signaling Pathways?

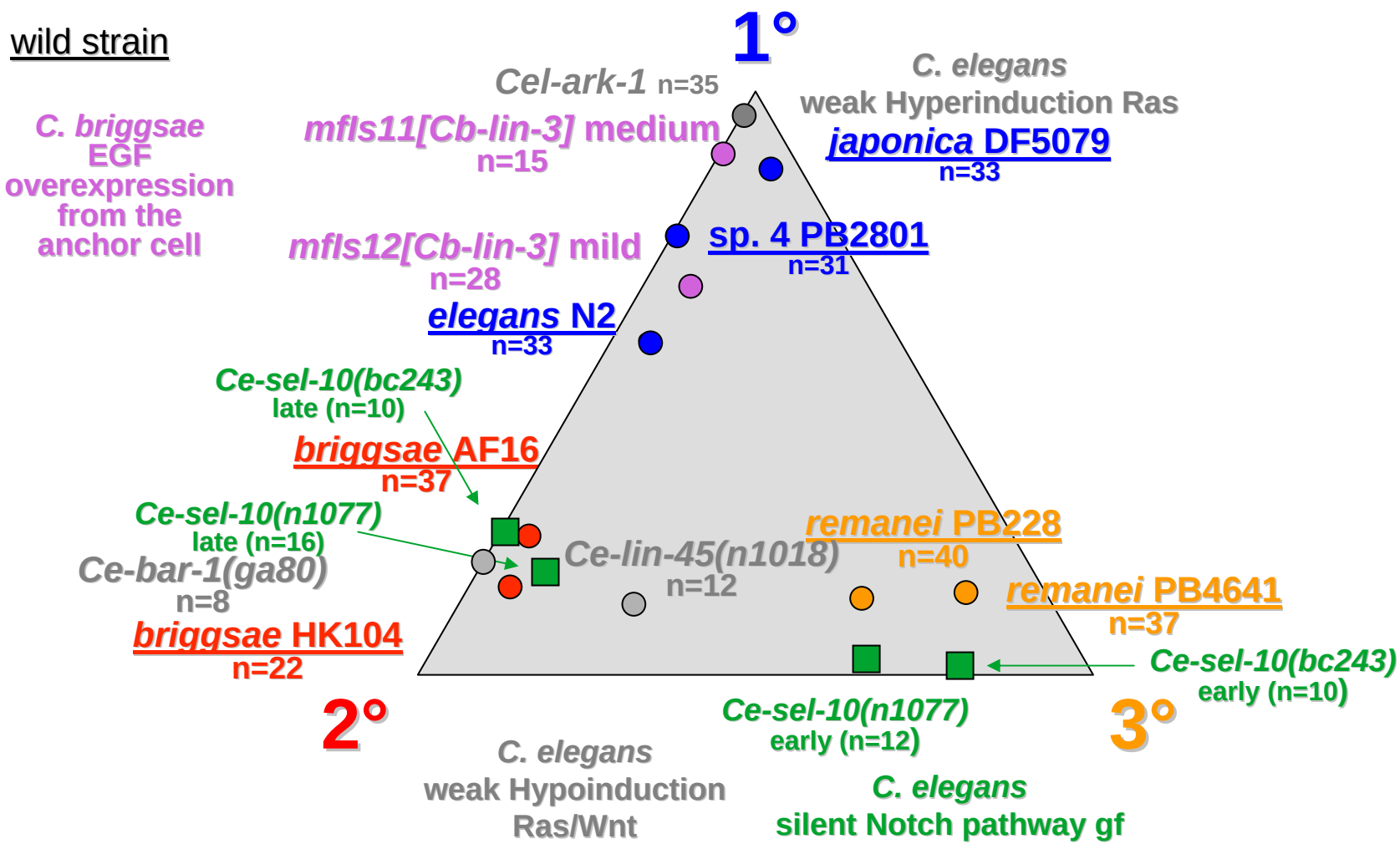
Caenorhabditis elegans: **212**

Caenorhabditis briggsae: **222**

Caenorhabditis remanei: **232**



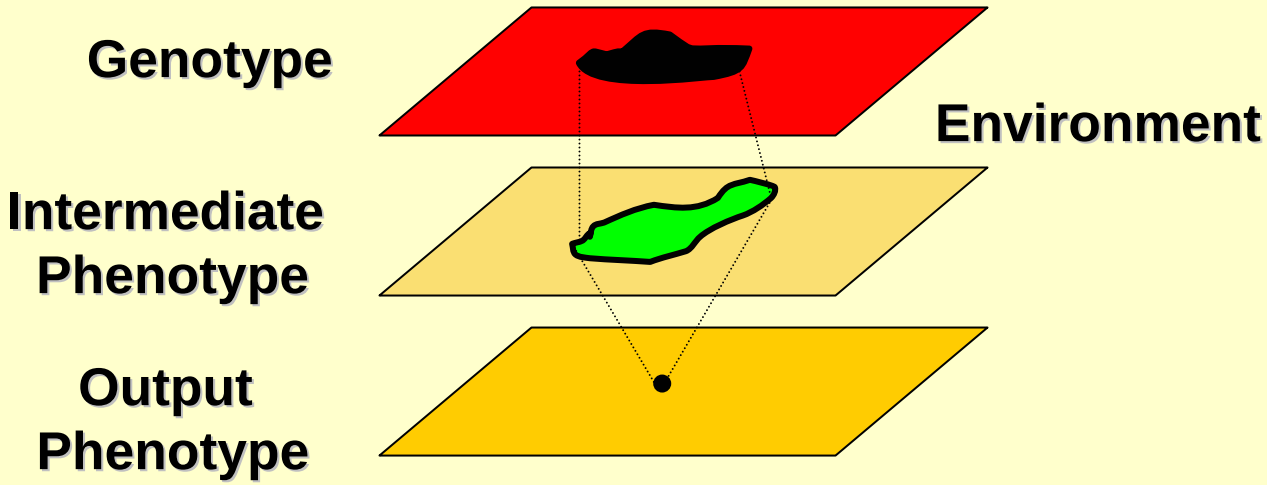
Experimental alterations in Ras and Notch pathway activities in *C. elegans* and *C. briggsae*:



The cryptic difference between *C. elegans* and *C. briggsae* can be explained by evolution within the signaling network

Conclusion

Whereas the final vulval pattern is invariant, we can uncover cryptic variations in the vulva patterning network



neutral evolution?
selection against rare errors?
variations in environmental conditions?
pleiotropic gene action?

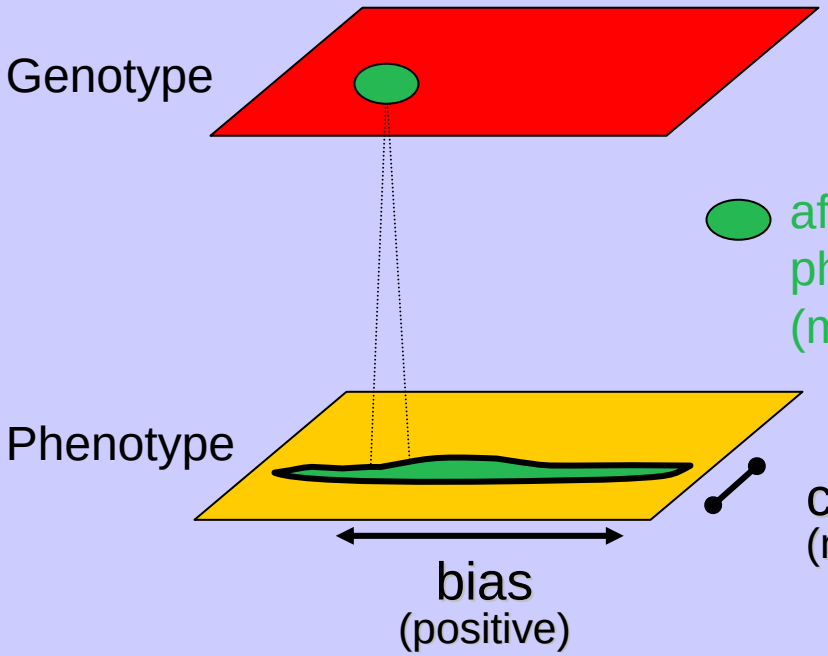
C. elegans vulva development

I.
Sensitivity to **noise and environmental** change
'Error' type and rates
Cryptic variation in different environments

II.
Cryptic evolution in *C. elegans* and the *Caenorhabditis* genus

III.
Sensitivity to **random mutational** change
Bias/constraints

III. Effect of **Random Genotype Variation** on the Phenotype



non-isotropic

Relative Roles in Phenotypic Evolution:

Developmental Constraints/Bias

Natural Selection

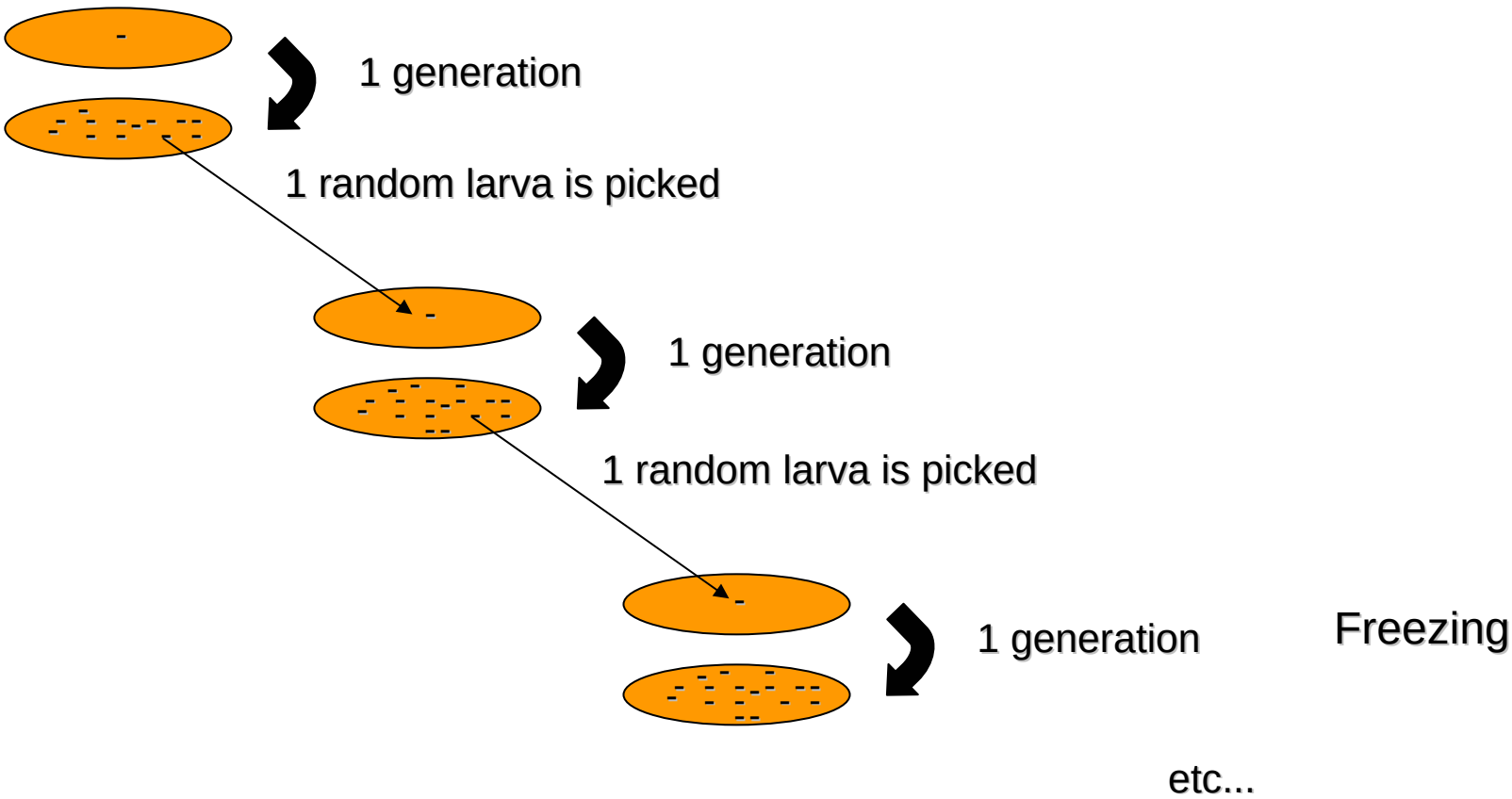
Environment

Stochastic effects ?

Mutation Accumulation Lines

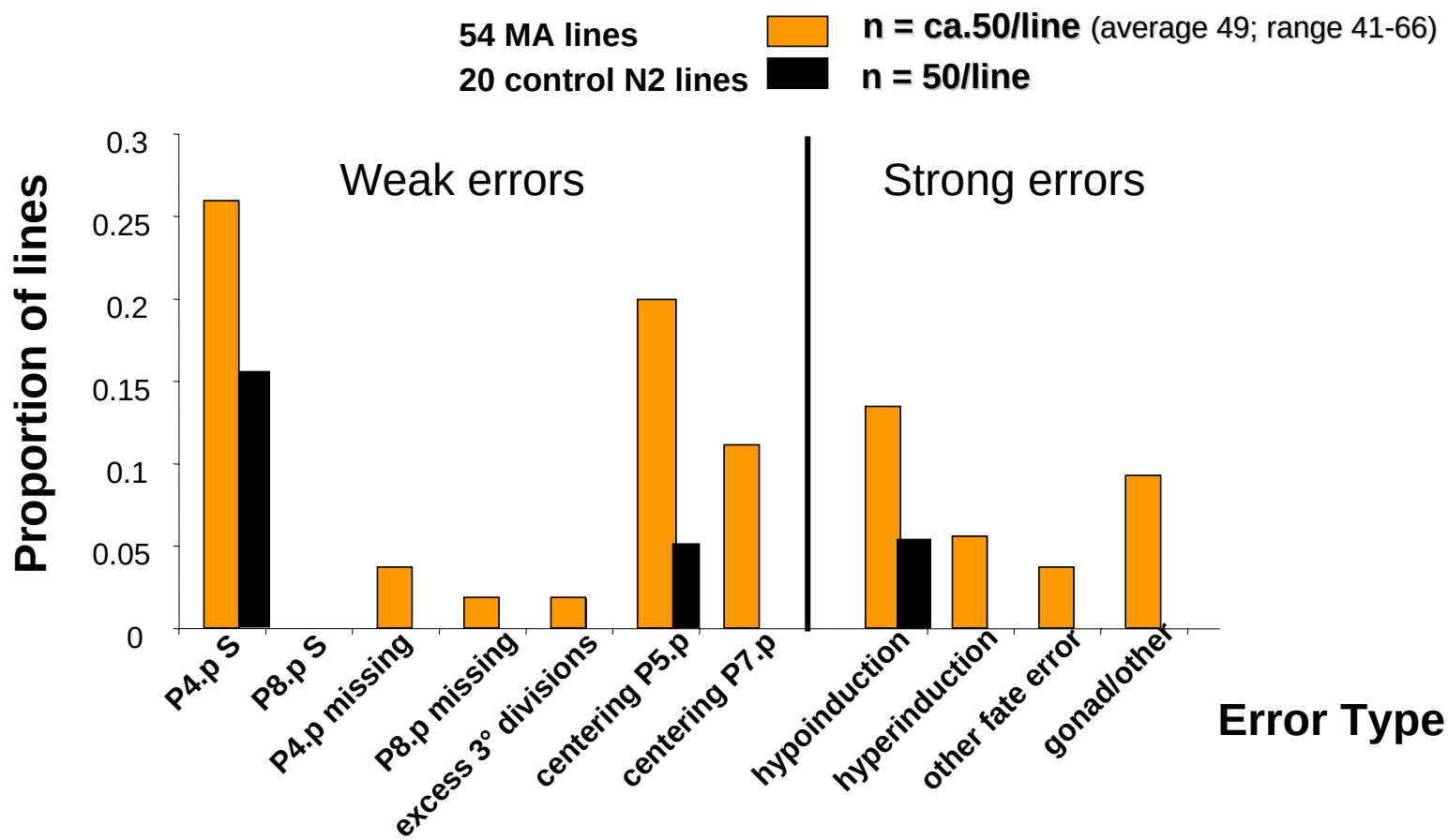
Transfer of one individual per generation for many generations:
no selection (except that some lines die out)

L. Vassilieva & M. Lynch
approx. 400 generations starting from *C. elegans* N2 strain



Proportion of Mutation Accumulation Lines with vulva 'errors'

MA lines from L. Vassilieva & M. Lynch
approx. 400 generations



⇒ **Mutational Degradation of Vulva Development**

Error rates not found in natural isolates: action of natural selection

Evolvability of P3.p vs. P4.p in *C. elegans*

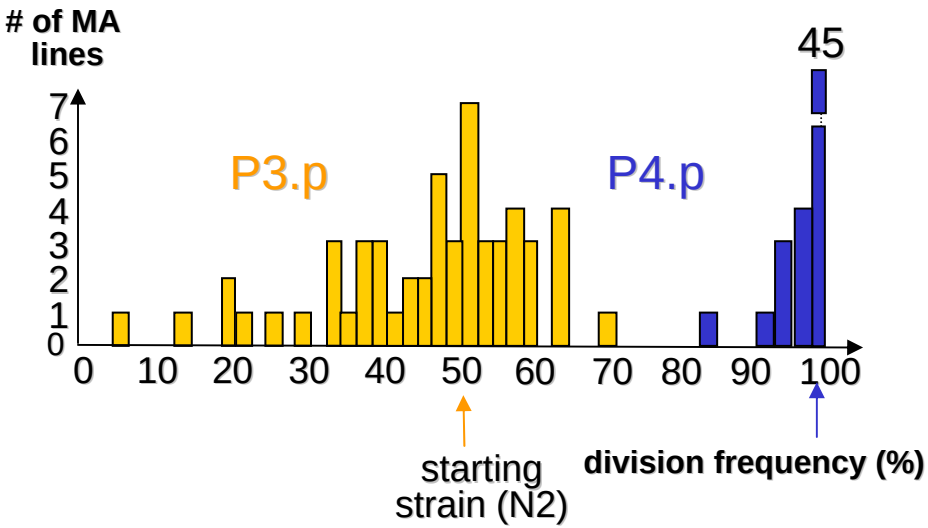
N2 isogenic strain

P3.p or P4.p

stochastic noise (non-robust)
division frequency: 49%

invariant
100%

mutability in *C. elegans*
54 mutation accumulation lines



from Vassilieva & Lynch, 2000
average
411 generations

49 animals/line
(range 41-66)
20 control lines
(P3.p: ANOVA, p<0.01)

- P3.p division frequency is an easily mutable character - with an asymmetry towards lower frequencies
- P4.p division frequency does not vary as much

Mutability vs. Evolution of P3.p and P4.p

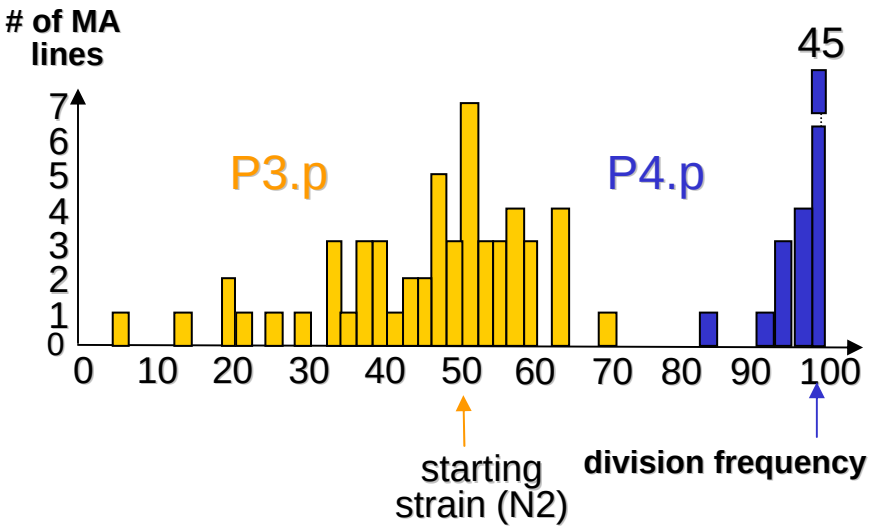
N2 isogenic strain

P3.p or P4.p

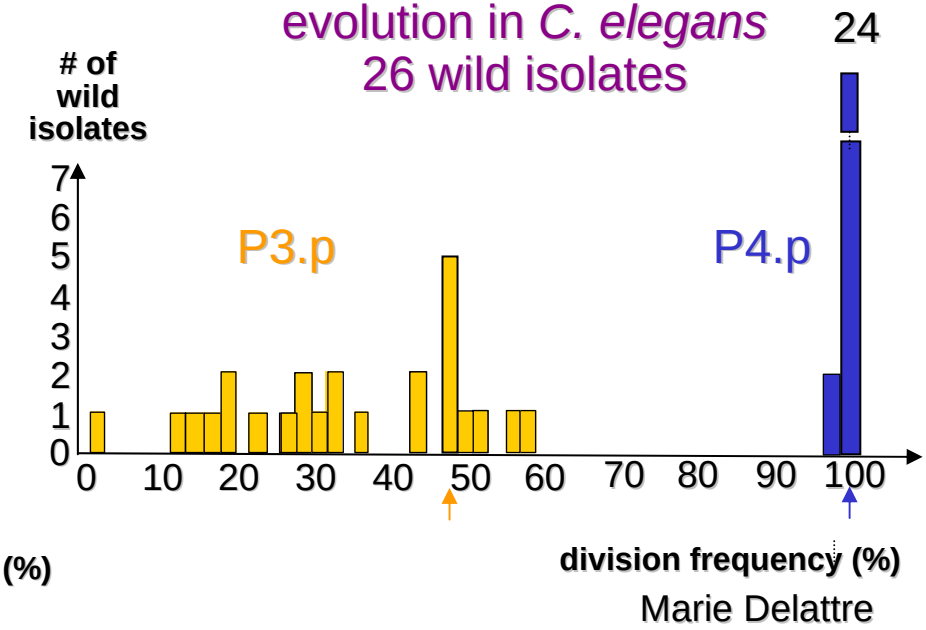
stochastic noise (non-robust)
division frequency: 49%

invariant
100%

mutability in *C. elegans*
54 mutation accumulation lines



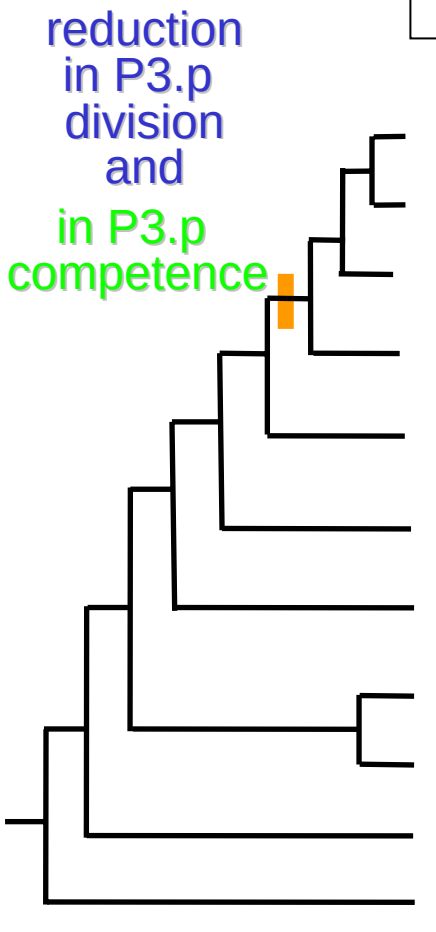
evolution in *C. elegans*
26 wild isolates



Marie Delattre

Mutational variance correlates with P3.p evolution in *C. elegans* and reduction of competence group size in *Caenorhabditis* genus...

P3.p in *Caenorhabditis* spp.: decrease in division frequency and competence



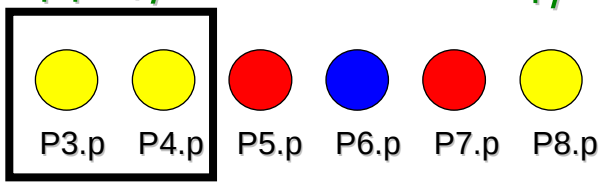
Phylogeny
Kiontke et al. PNAS 04

Species	Isolate	P3.p division frequency (%)	P3.p competence
		mean [range]	
C. sp. 5 JU727	several of each species	8 [0-16]	low
C. briggsae		13 [0-52]	no/low
C. remanei		25 [10-35]	low
C. sp. 4 CB5161		20 [5-62]	low
C. elegans		35 [5-60]	yes
C. japonica	SB339	92 (n=2)	8/18 yes
C. sp. 3	RGD1	97	
	PS1010	100	7/12 yes
C. drosophilae	DF5077	98	
C. sp. 2	DF5070	100	1/11 low
C. plicata	SB355	N.A.	
C. sp. 1	SB341	N.A.	4/9 yes

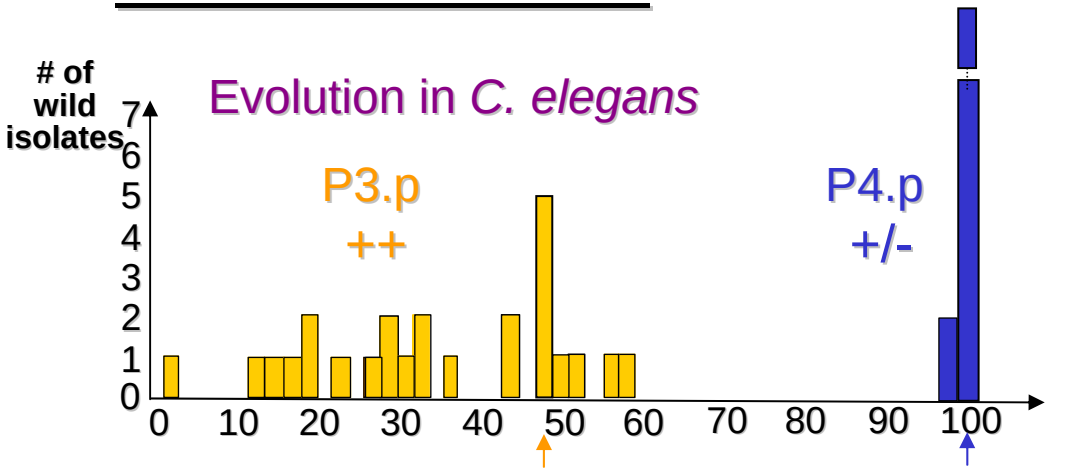
Marie Delattre, Laure Bonnaud, MAF

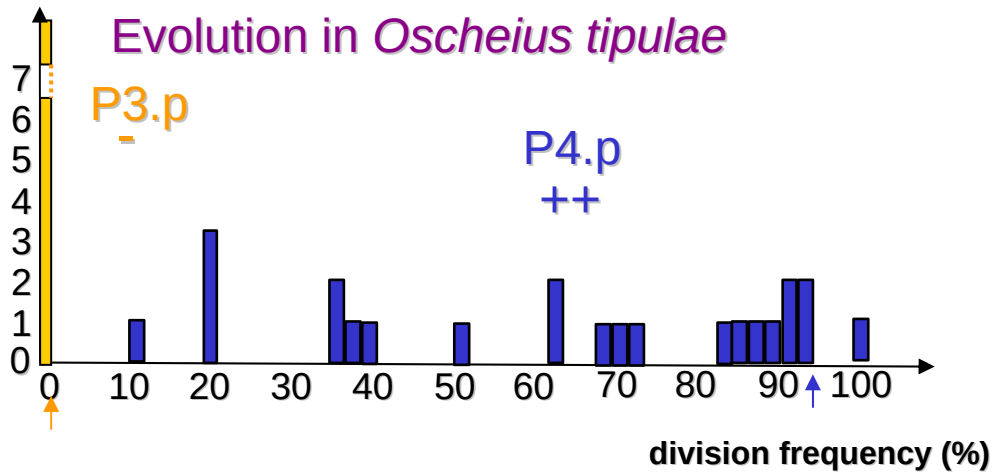
Mutability and Evolution of Phenotypes: Comparison Between 2 Genera

Mutability
in *C. elegans*



Mutability
in *Oscheius tipulae*
EMS screen with M.-L. Dichtel





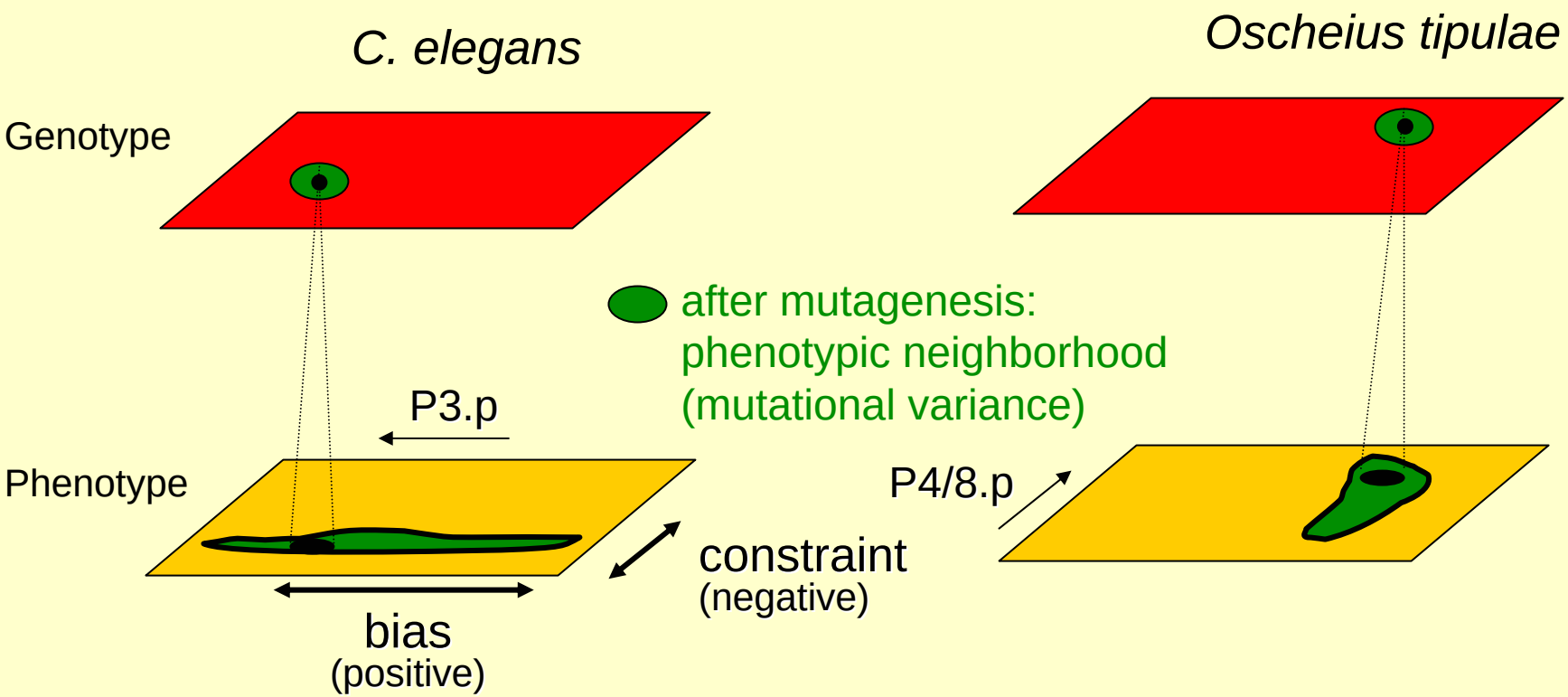
Comparison between Phenotypic Distributions after Mutation vs. in Natural Isolates
Reveals the (direct or indirect) Action of **Selection**

Divergence in the Same Characters within Each Genus

=> The mutability bias can result in differences in evolutionary tendencies between different groups

Bias / Constraints
Revealed by Phenotypic Mutability

Evolution
of the Phenotypic Neighborhood:



Relevant to Phenotypic Evolution in Each Genus

1. The determination of the mutational variance
for phenotypic traits
and the comparison with natural variants
help to evaluate the respective roles
of **constraints or biases due to genetic architecture**
and of **natural selection**

2. The mutability bias can cause differences
in **evolutionary tendencies** between different groups

Thanks to:

Neil Hopper
Paul Sternberg
CGC

Karin Kiontke
Walter Sudhaus
for strains
and unpubl. results

INSTITUT JACQUES MONOD

Marie DELATTRE
P3.p polymorphism

Isabelle NUEZ

Josselin MILLOZ
vulva genetics in *C. elegans* wild isolates

Christian BRAENDLE
vulva precision
environmental effects

Antoine BARRIERE
C. elegans population genetics