






# (Somatic) mutation inheritance in plants

RESEARCH ARTICLE

## The architecture of intra-organism mutation rate variation in plants

Long Wang<sup>1</sup>, Yilun Ji<sup>1</sup>, Yingwen Hu<sup>1</sup>, Huaying Hu<sup>1</sup>, Xianqin Jia<sup>1</sup>, Mengmeng Jiang<sup>1</sup>,  
Xiaohui Zhang<sup>1</sup>, Lina Zhao<sup>1</sup>, Yanchun Zhang<sup>1</sup>, Yanxiao Jia<sup>1</sup>, Chao Qin<sup>1</sup>, Luyao Yu<sup>1</sup>,  
Ju Huang<sup>1</sup>, Sihai Yang<sup>1\*</sup>, Laurence D. Hurst<sup>2\*</sup>, Dacheng Tian<sup>1\*</sup>

## Somatic Mutation Analysis in *Salix suchowensis* Reveals Early-Segregated Cell Lineages

Yifan Ren,<sup>†,1</sup> Zhen He,<sup>†,1</sup> Pingyu Liu,<sup>1</sup> Brian Traw,<sup>1</sup> Shucun Sun,<sup>2</sup> Dacheng Tian,<sup>1</sup> Sihai Yang,<sup>1</sup> Yanxiao Jia,<sup>\*,3</sup>  
and Long Wang ,<sup>\*,1</sup>

**The understanding of mutation processes is one of the most common conceptual difficulties for students in (evolutionary) biology** (e.g. Smith & Knight 2012 Genetics; Prevost et al. 2013 Proc. NARST)

**Why mutation rates are so important?**

- Genetic diversity within a population :

$$\theta = 4N_e\mu$$

(standing genetic variation)

- Divergence between species

Under neutral evolution, evolutionary rate = mutation rate

$$\text{rate of neutral substitutions} = 2N\mu * 1/2N = \mu$$

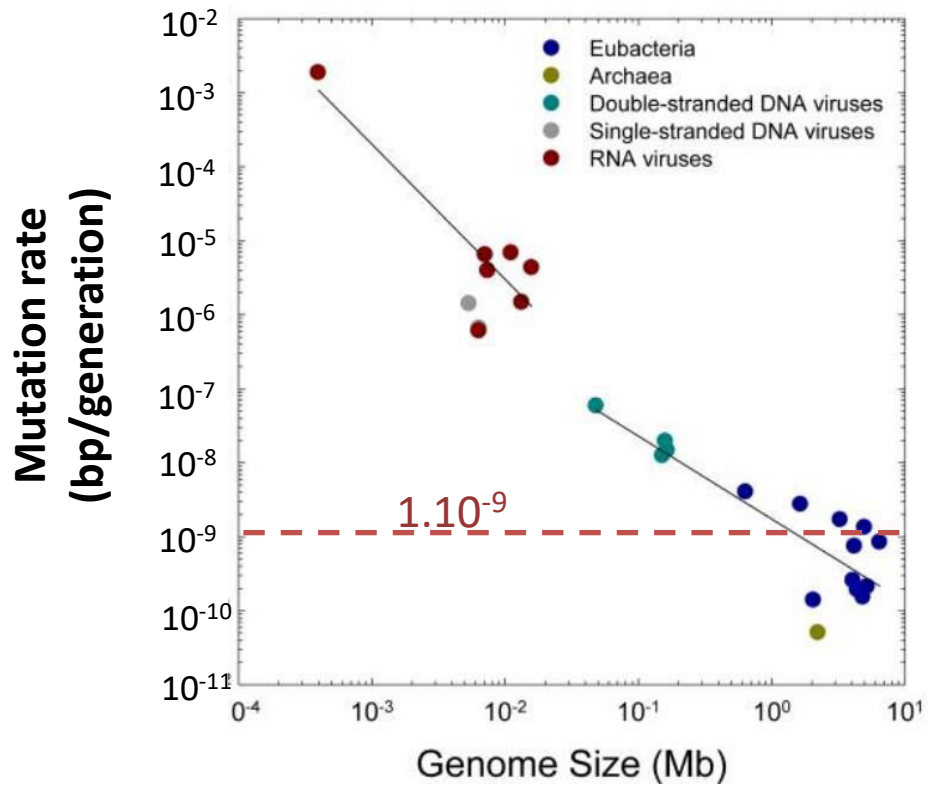
(with  $2N\mu$ : # new mutations per generation and  $1/2N$ : proba. to fix these new mutations)

i.e. Molecular clock (Kimura, 1968)

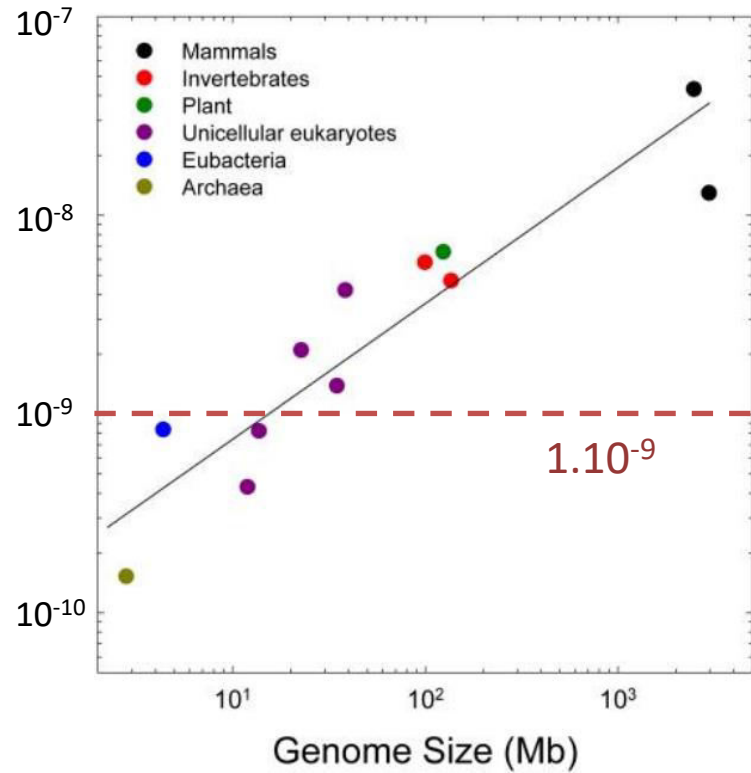
→ Expectation assuming neutrality, a lack of direct estimates of mutations rates



## Viruses & bacteria



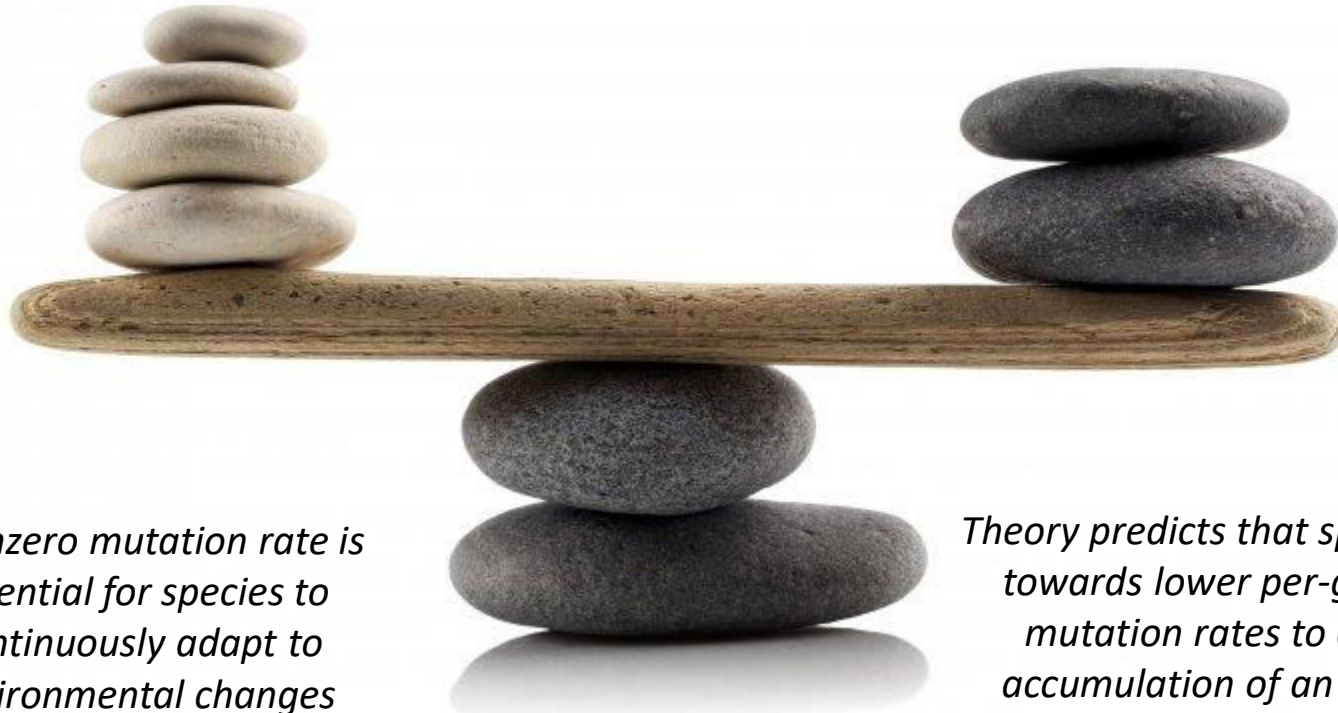
## (Bacteria &) Eukaryotes



## Lynch, 2010 Trends Genet

*Apparent simplicity but complex (selection-mutation) balance?*

**Evolvability  
(some new adaptive  
mutations)**



**Mutational burden  
(Deleterious mutations)**

*A nonzero mutation rate is  
essential for species to  
continuously adapt to  
environmental changes  
and perturbations*

*Theory predicts that species evolve  
towards lower per-generation  
mutation rates to avoid the  
accumulation of an increasing  
burden of deleterious variants (e.g.  
Kondrashov et al. 1988 nature)*

# Growth, ageing and heritable mutations



*Masao Matsumoto and Miyako Matsumoto  
(World's oldest living married couple in 2019)*



*« The Major Oak », Sherwood Forest, Nottinghamshire,  
England (800-1,000 years old, UK's most visited tree)*

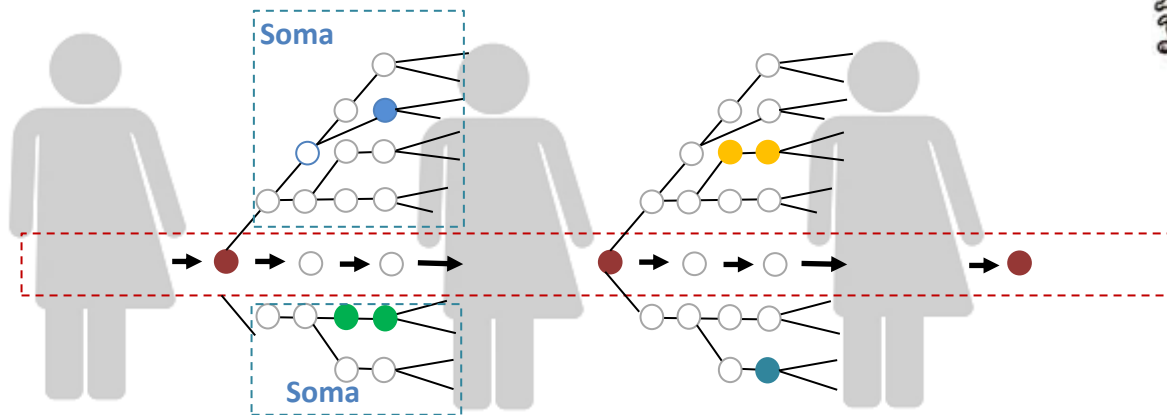


One of the greatest 19<sup>th</sup> century evolutionary biologist

One of the few early supporter of Darwin's theory of evolution ("On the Validity of the Darwinian Theory", Weismann, 1868)

He put a "final end" to the theory of Lamarck and the inheritance of acquired characteristics

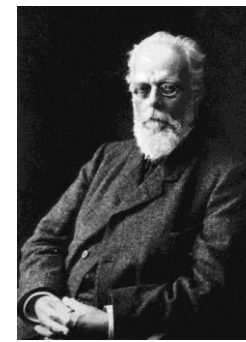
- Study of the inheritance of mutilations (Weismann, 1888)



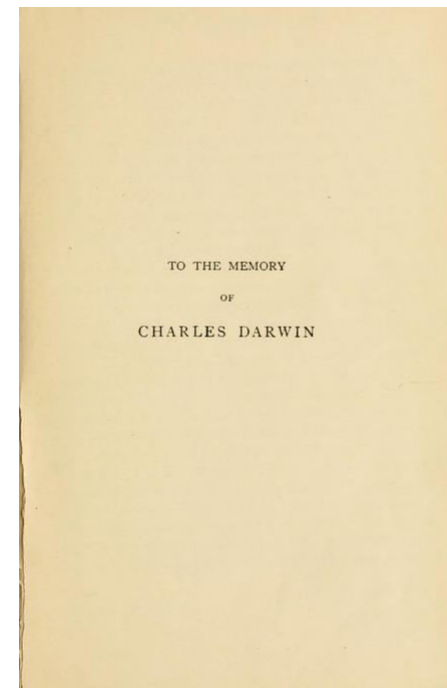
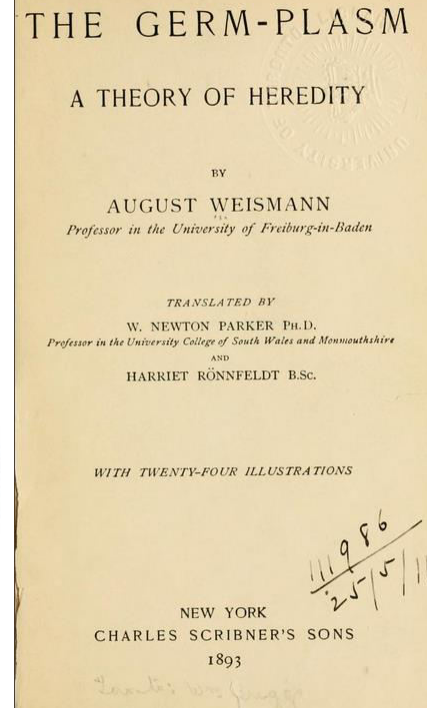
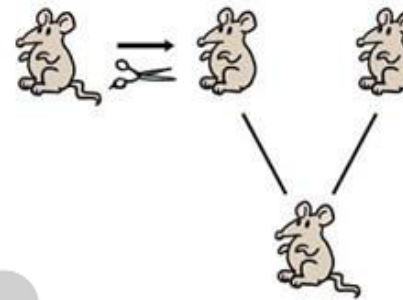
**Germline = immortal cell lineage**

**Somatic cell lineages = Mortal vessel**

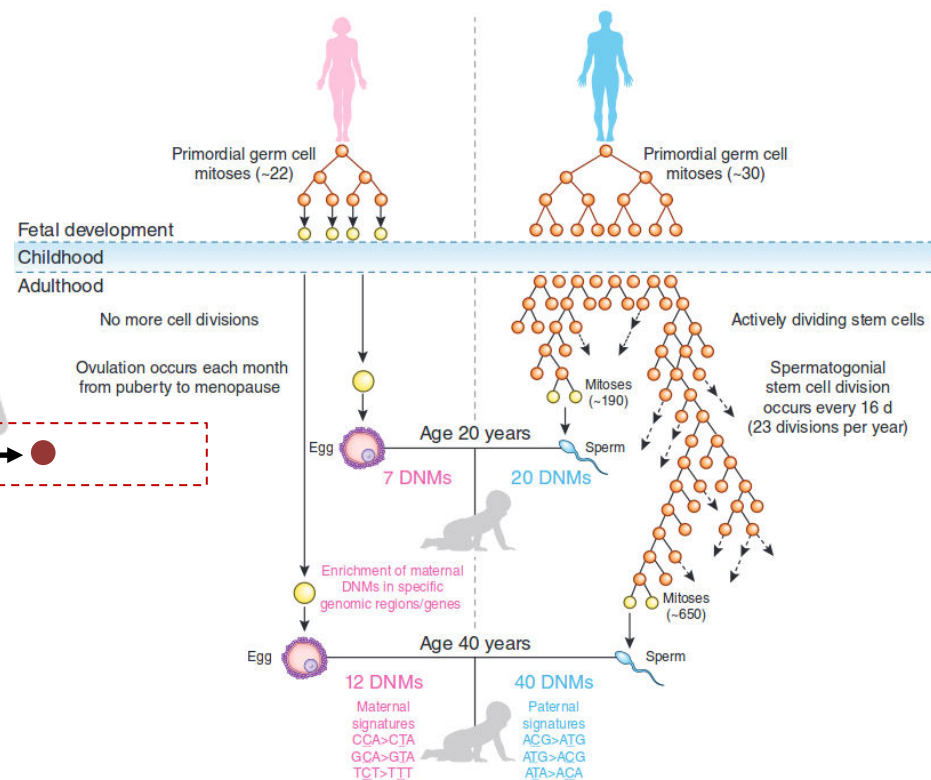
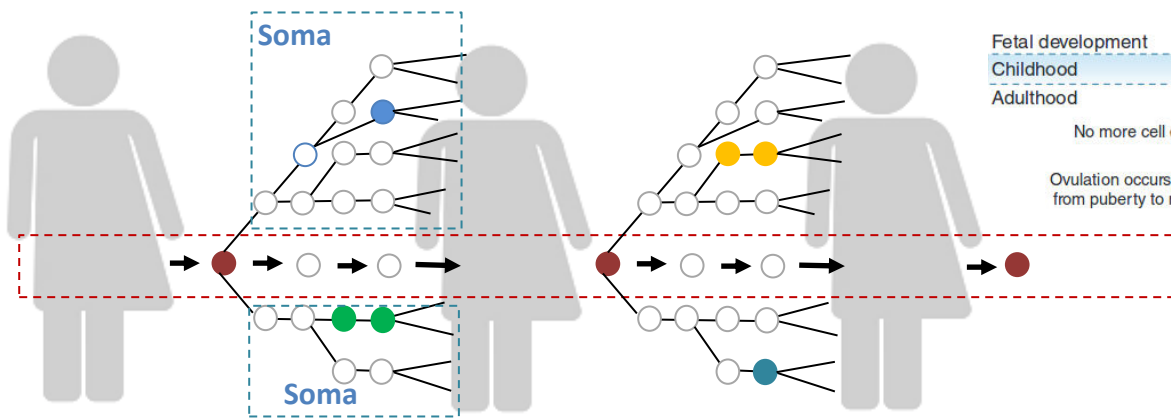
August Weismann's germ plasm theory: **hereditary information moves only from germline cells to somatic cells** (=somatic mutations are not inherited)



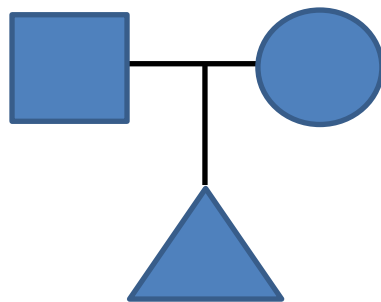
**August Weismann  
(1834-1914)**



# de novo mutations (DNMs) and ageing



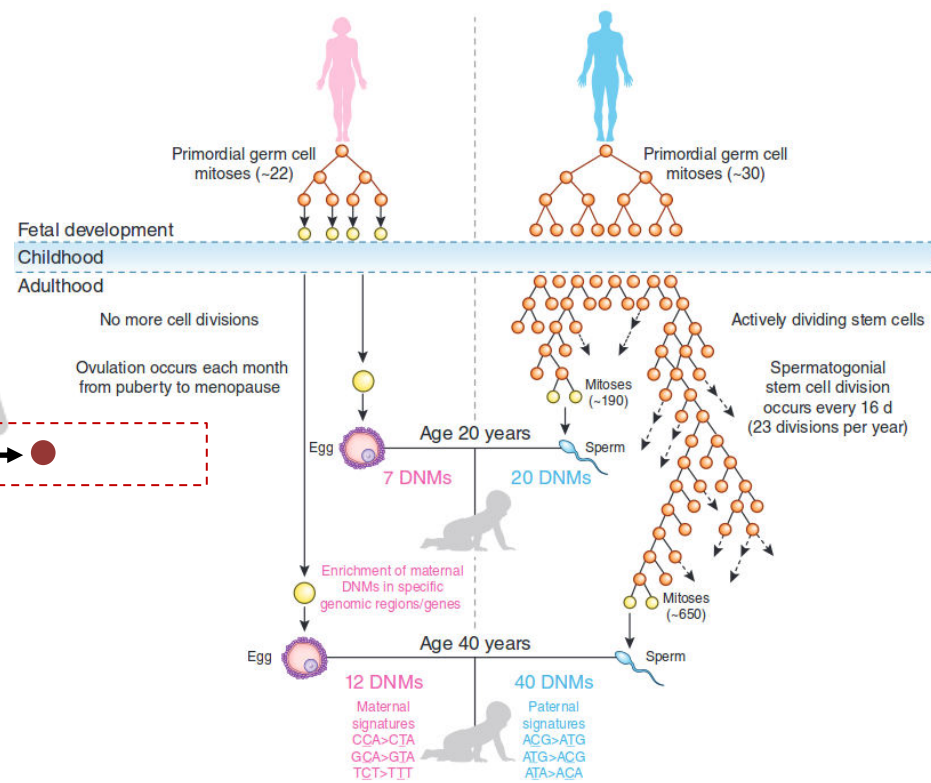
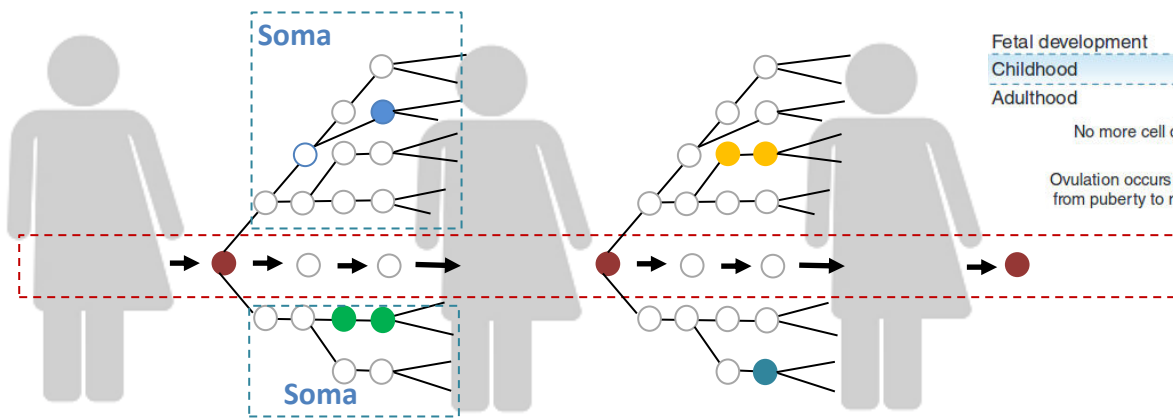
## « trio-based » sequencing approach



DNA sequencing of the two parents +  
one child (~50X) & detection of  
DNMs

*Goldmann et al. 2016 Nature Genetics*  
*Goriely 2016 Nature Genetics*

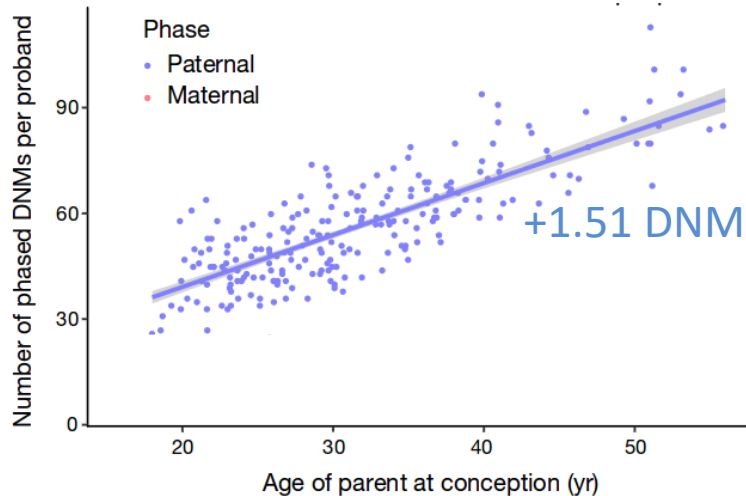
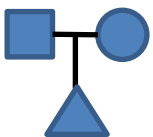
# de novo mutations (DNMs) and ageing



Goldmann et al. 2016 Nature Genetics  
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## « trio-based » sequencing approach

De novo mutations from 1,548 Icelanders families (« trios »)

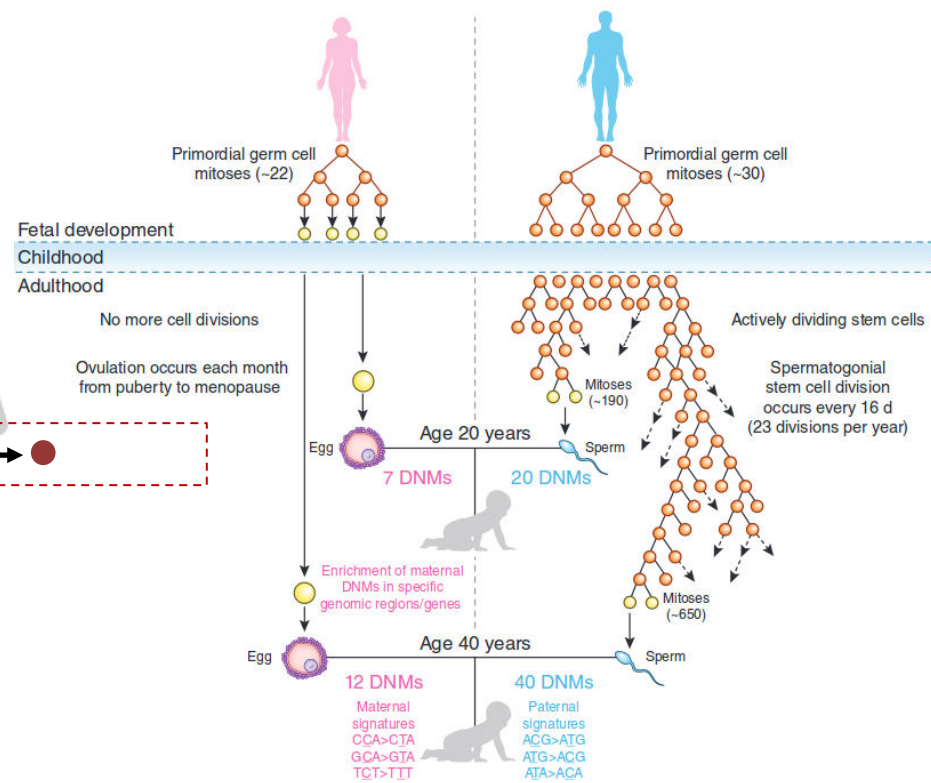
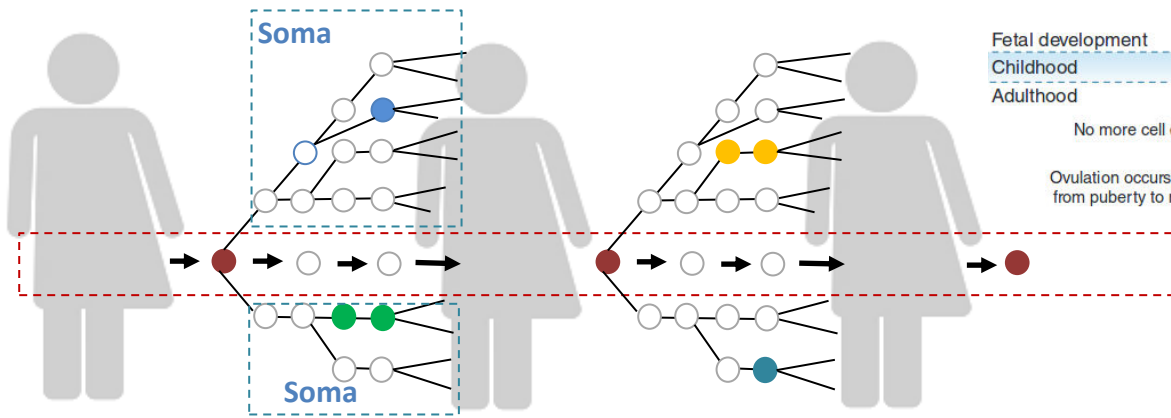


Germline mutations in humans =  
meiotic mutations +  
mitotic mutations on germinal  
cells

Jónsson et al. 2017 Nature (see also Kong et al. 2012 nature)



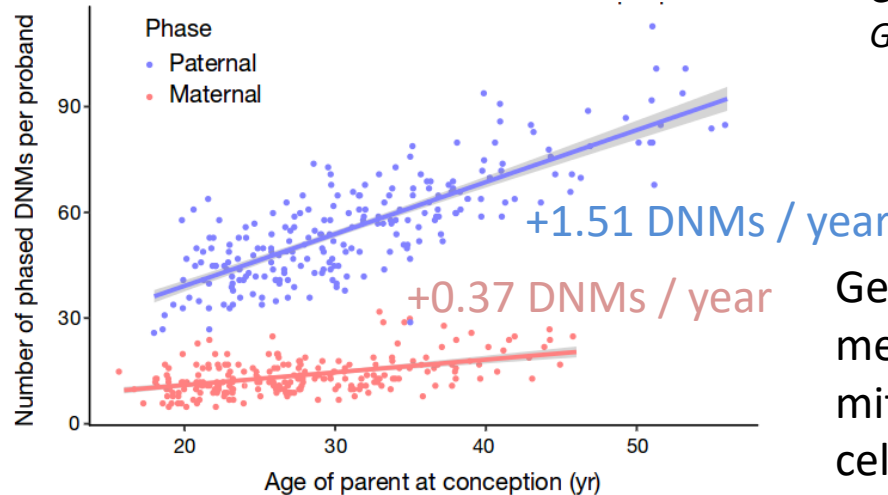
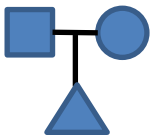
# de novo mutations (DNMs) and ageing



Goldmann et al. 2016 Nature Genetics  
Goriely 2016 Nature Genetics

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# Growth, ageing and heritable mutations



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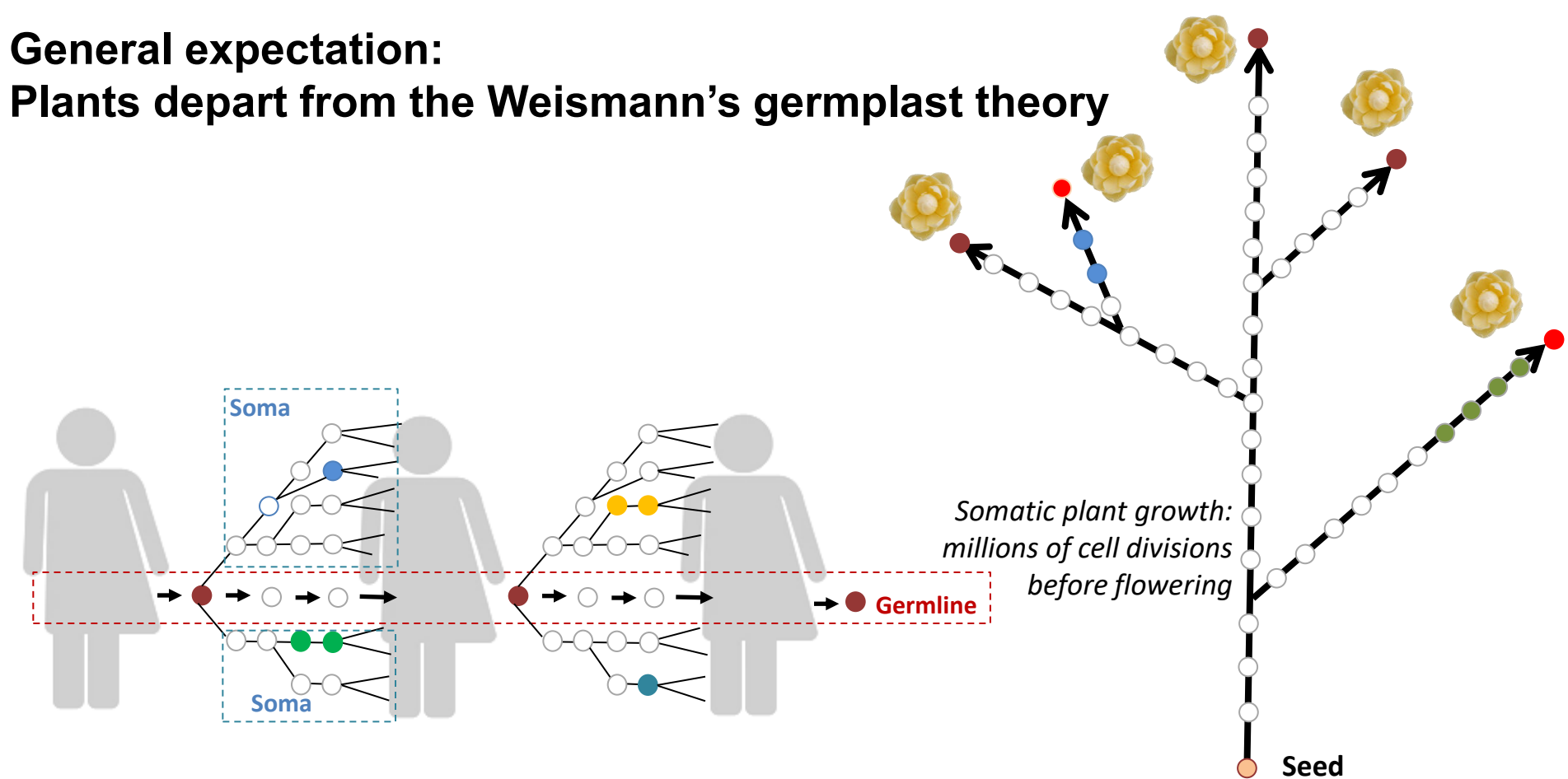


*« The Major Oak », Sherwood Forest, Nottinghamshire,  
England (800-1,000 years old, UK's most visited tree)*

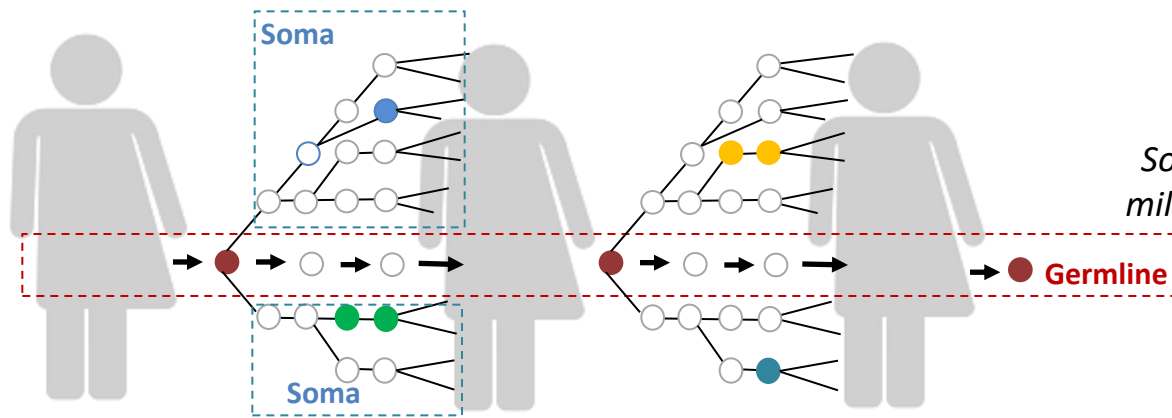
Heritable mutations in animals =  
germline mutations =  
meiotic mutations +  
mitotic mutations on germinal cells

?

# General expectation: Plants depart from the Weismann's germplasm theory



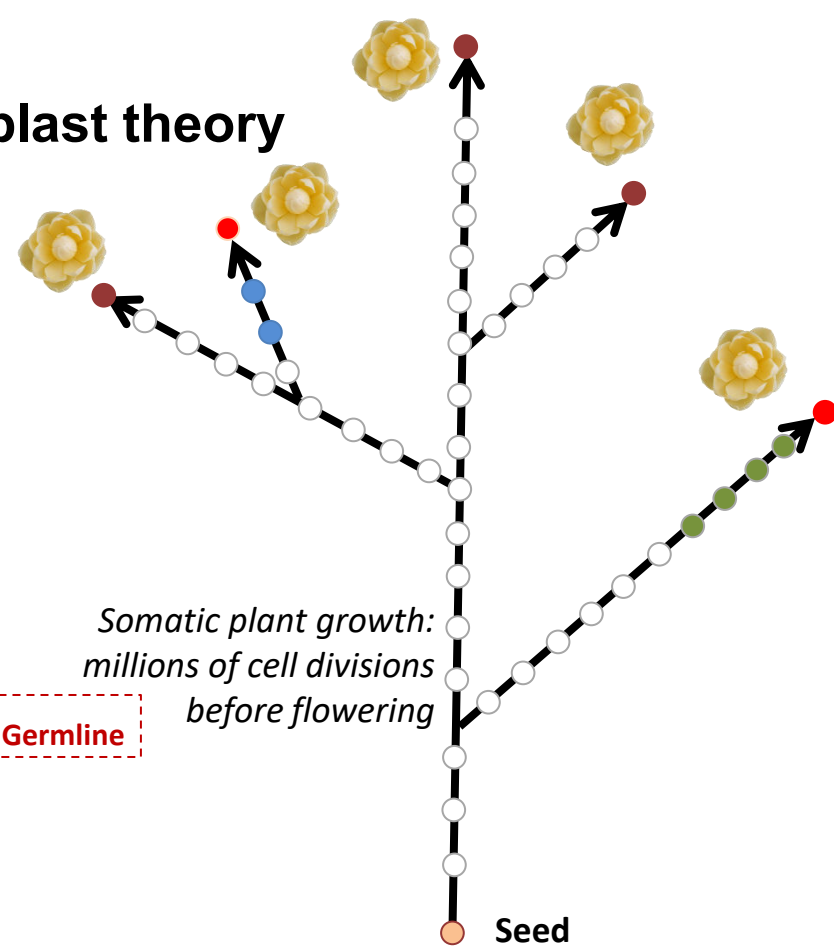
# General expectation: Plants depart from the Weismann's germplasm theory



Heritable mutations:  
- germline (mitosis and meiosis)

≠

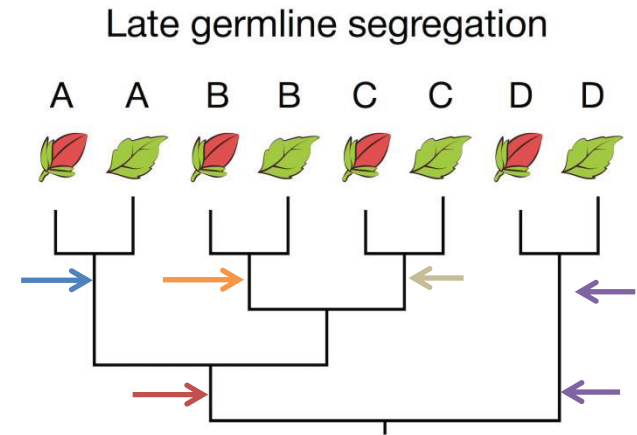
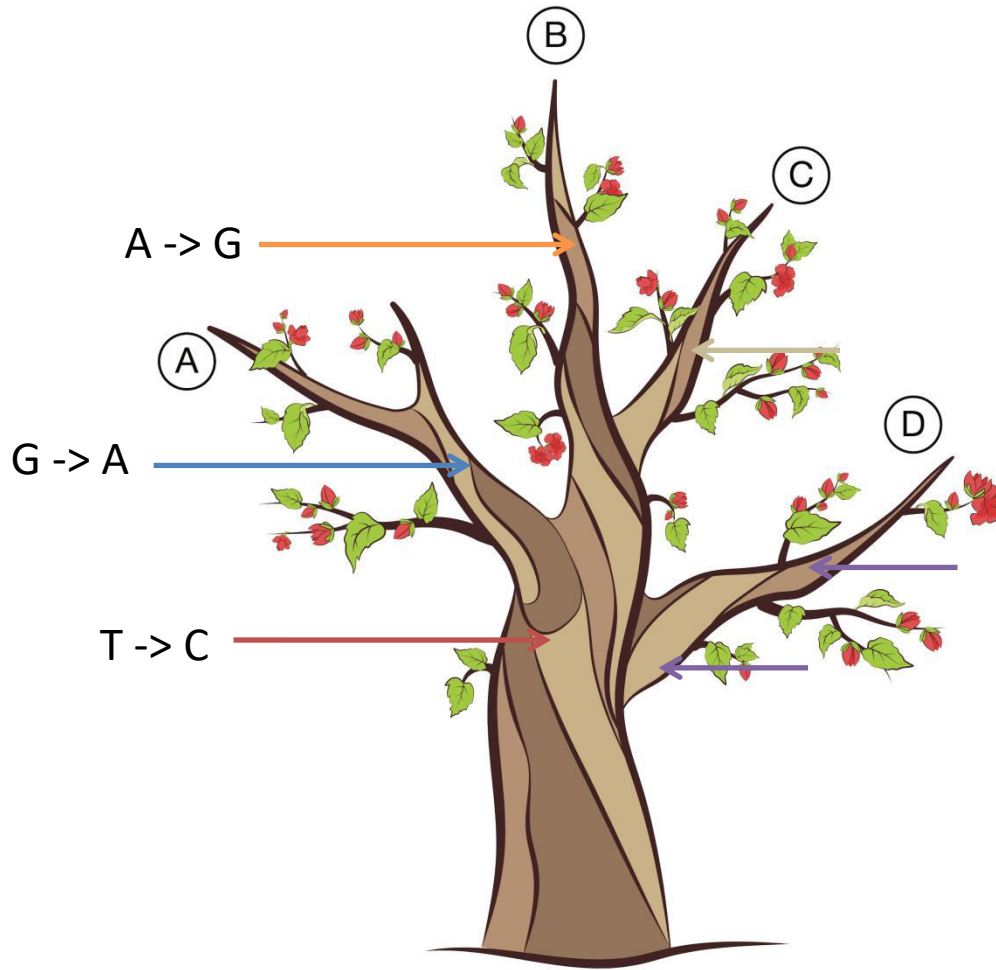
**Animal model:** no inheritance of somatic mutations



Heritable mutations:  
- germline (mitosis and meiosis)  
- somatic (mitosis)

**Plant model:** germline differentiation occurring late in development, inheritance of somatic mutations

Assuming « no » germline segregation for plants, mutations accumulate along growth axes







Following this view, a tree is also a ...  
phylogenetic tree!




## RESEARCH ARTICLE

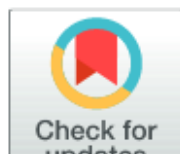
# The architecture of intra-organism mutation rate variation in plants

Long Wang<sup>1</sup>, Yilun Ji<sup>1</sup>, Yingwen Hu<sup>1</sup>, Huaying Hu<sup>1</sup>, Xianqin Jia<sup>1</sup>, Mengmeng Jiang<sup>1</sup>, Xiaohui Zhang<sup>1</sup>, Lina Zhao<sup>1</sup>, Yanchun Zhang<sup>1</sup>, Yanxiao Jia<sup>1</sup>, Chao Qin<sup>1</sup>, Luyao Yu<sup>1</sup>, Ju Huang<sup>1</sup>, Sihai Yang<sup>1\*</sup>, Laurence D. Hurst<sup>2\*</sup>, Dacheng Tian<sup>1\*</sup>

**1** State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, China, **2** The Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom

 These authors contributed equally to this work.

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## A huge quantity of sequencing data!

- Sequenced "754 genomes" from various tissues (e.g. 480 leaves, but also roots etc)  
"cleaned depth"  $\geq 40X$  per sample
- 22 individuals from 8 species:



*Prunus persica* (x7)



*Prunus mira* (x4)



*Prunus mume* (x2)



*Fragaria vesca* (x1)



*Oryza sativa* (x4)



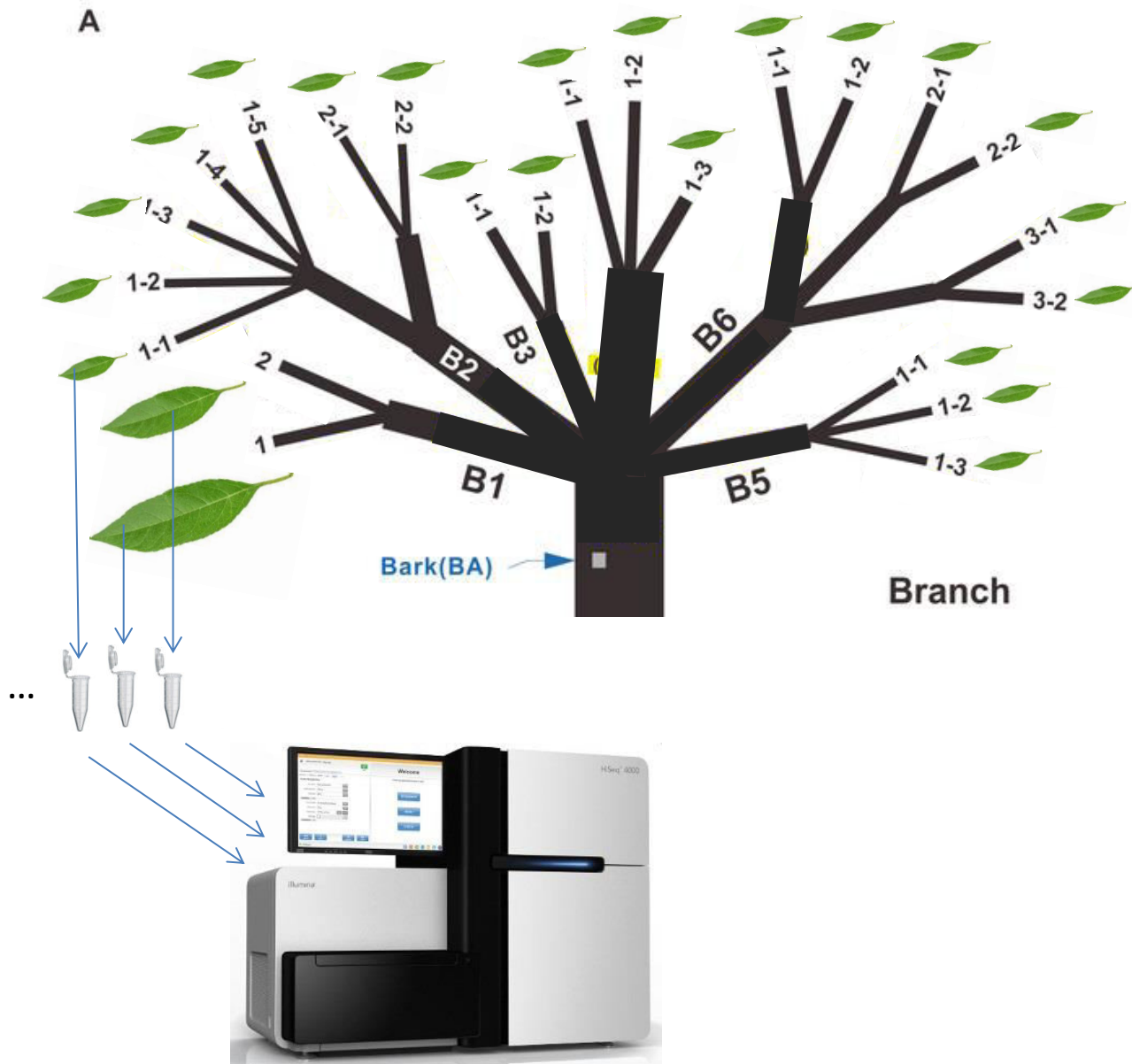
*Brachypodium distachyon* (x1)



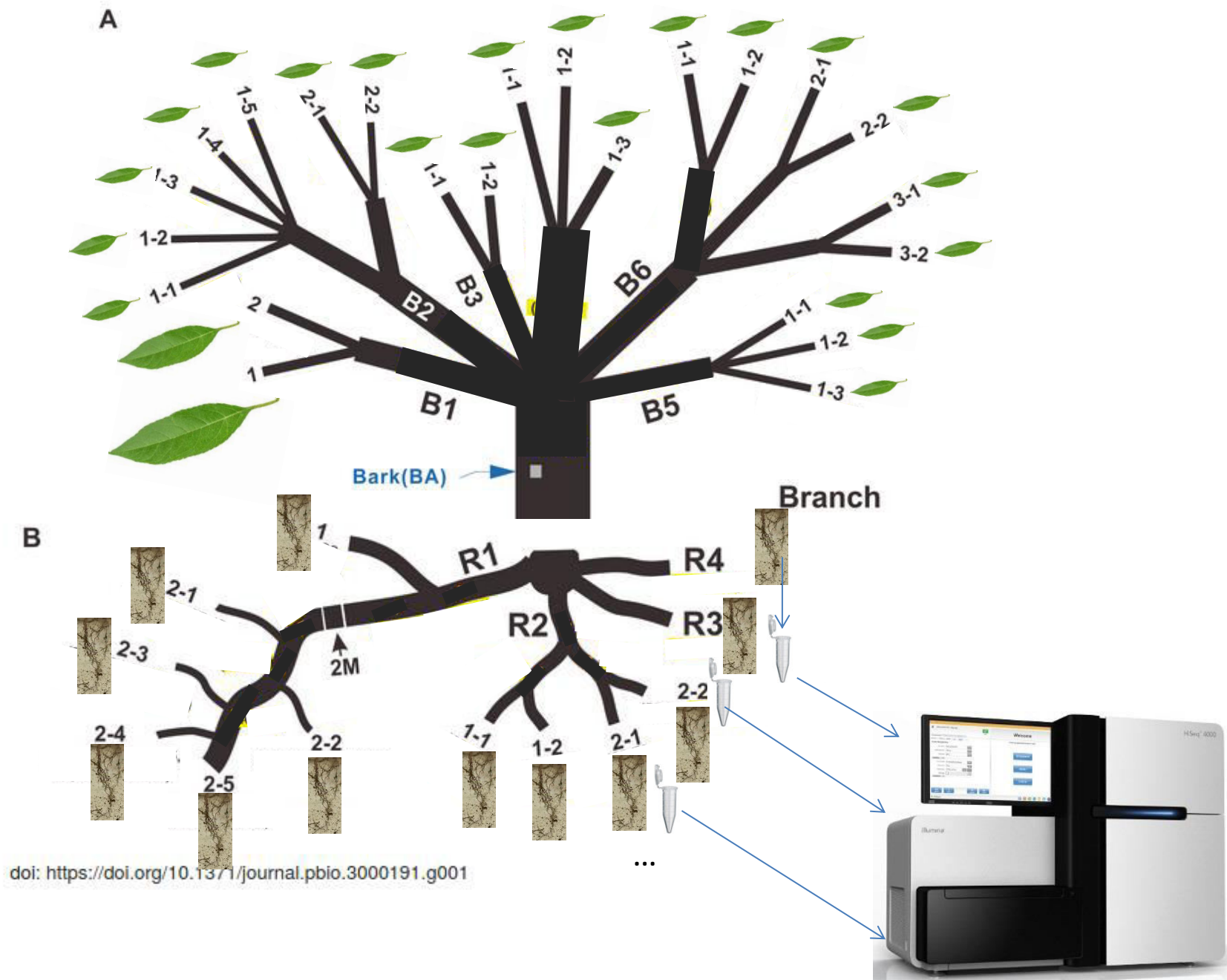
*Arabidopsis thaliana* (x2)

*Salix suchowensis* (x1)

# Crazy experimental design!



Very crazy experimental design!



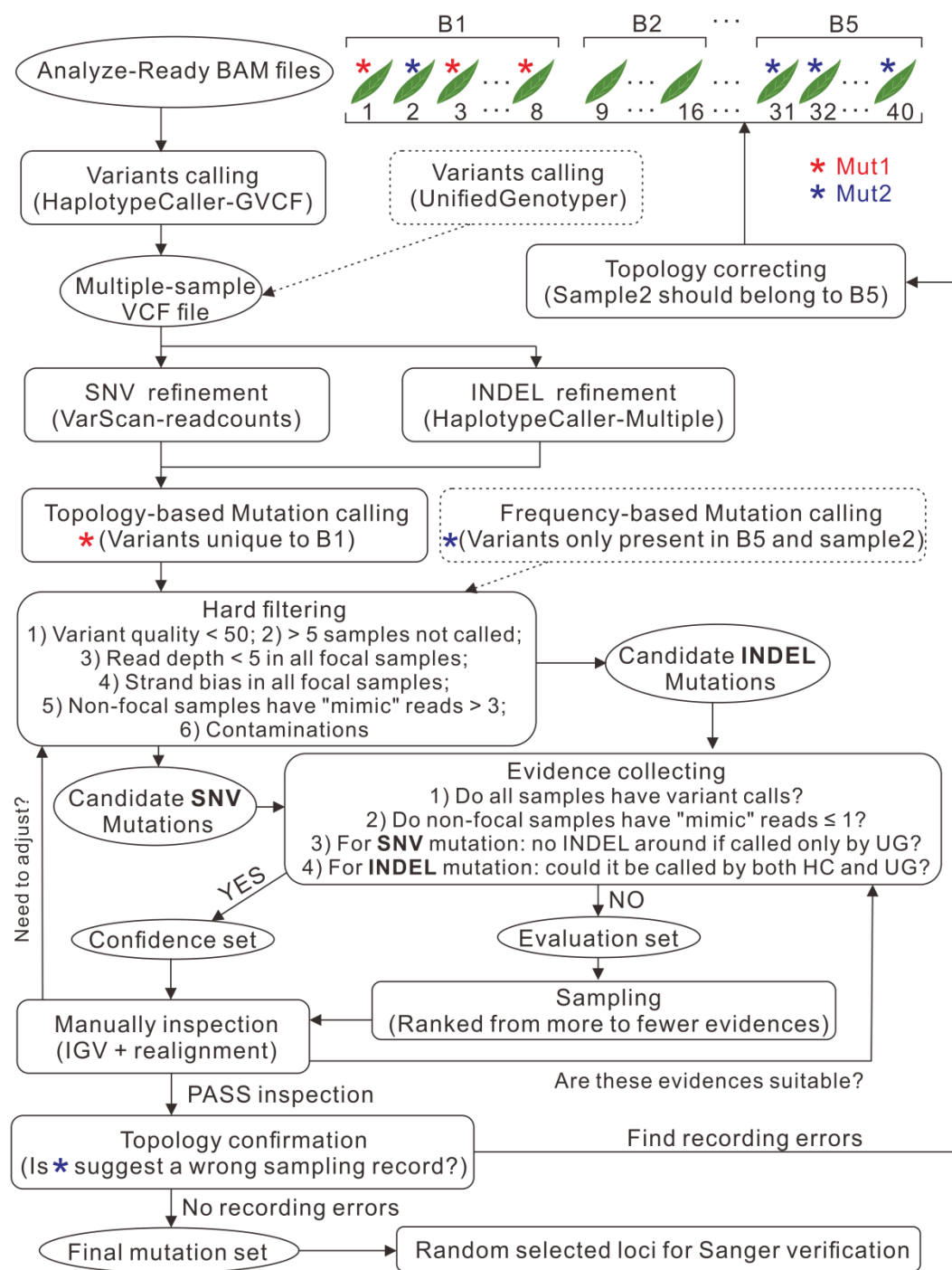


## Even more very crazy experimental design!

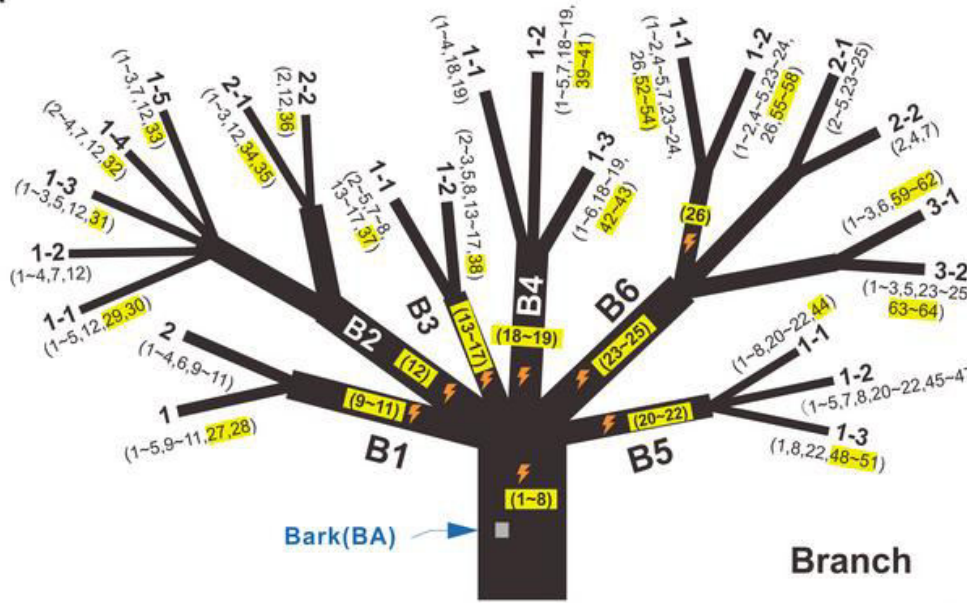
Species (reference genome size)	Samples	Diameter of the trunk (cm)	Estimated age (years)	DNA source	Sequenced samples
<i>P. mira</i> (225 Mb)	G1 <sup>b</sup>	207	600	Leaf	32
	G2	191	550	Leaf	12
	GL2	148	420	Leaf	23
	GZ	110	300	Leaf	9
<i>P. persica</i> (225 Mb)	PXL <sup>c</sup>	11.1	21	Leaf	23
				Root <sup>d</sup>	13
	HY2	14	25	Leaf	16
				Petal	13
	NJAU1	15.9	30	Leaf	26
	NJAU2	37.6	50	Leaf	8
	Maoping	12.8	40	Leaf	16
<i>P. mume</i> (220 Mb)	MHG1	17.5	20	Leaf	25
				Root <sup>d</sup>	32
	MHG2	10.2	8	Leaf	33
<i>S. suchowensis</i> (480 Mb)	YAF1	-	1	Leaf	19
				Root <sup>d</sup>	21
<i>B. distachyon</i> (272 Mb)	WD2	-	1	Leaf	29
				Root <sup>d</sup>	8
				Lemma	7
<i>Fragaria vesca</i> (210 Mb)	FH1	-	1	Leaf	45
				Stems <sup>e</sup>	4
<i>Arabidopsis thaliana</i> (119 Mb)	Col17+Col24	-	1	Leaf	64
<i>Oryza sativa</i> (373 Mb)	KA1+PA1+ DG1 +NIPB	-	1	Leaf (Tiller) <sup>f</sup>	29
				Leaf (Callus)	13

The two previous slides explain the experimental design for this individual !



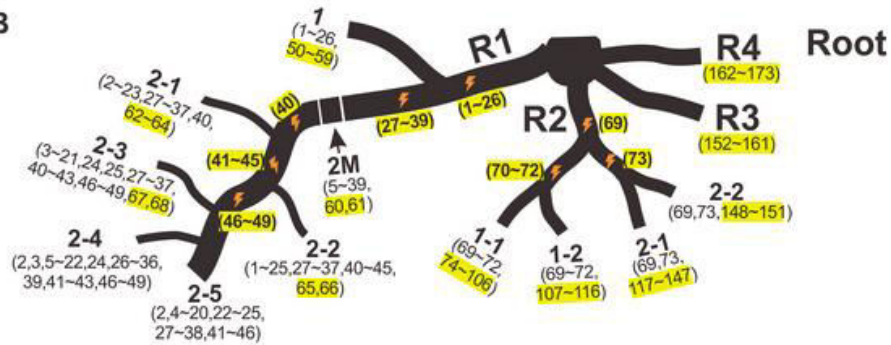


A

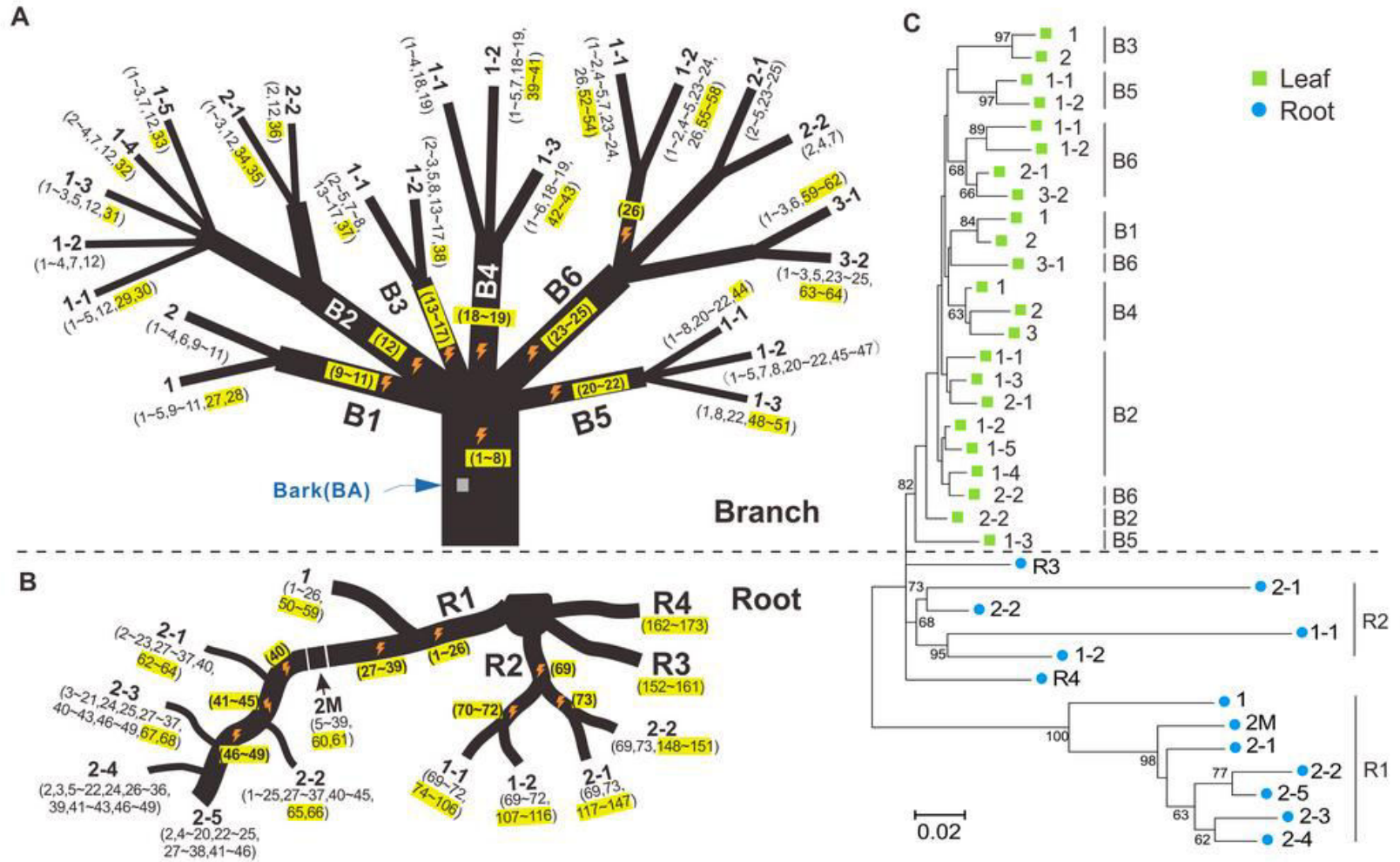


3.74  
mutations/sample

B



29.8  
mutations/sample



doi: <https://doi.org/10.1371/journal.pbio.3000191.g001>

**Table 1. Accumulated somatic mutations per sample in each terminal branch of all sequenced plant samples.**

Species (reference genome size)	Samples	Diameter of the trunk (cm)	Estimated age (years)	DNA source	Sequenced samples	Accumulated mutations	
						Average observed	Normalized rate ( $\times 10^{-9}$ per bp per year) <sup>a</sup>
<i>P. mira</i> (225 Mb)	G1 <sup>b</sup>	207	600	Leaf	32	12.7	0.08
	G2	191	550	Leaf	12	23.9	0.15
	GL2	148	420	Leaf	23	17.7	0.14
	GZ	110	300	Leaf	9	12.8	0.15
<i>P. persica</i> (225 Mb)	PXL <sup>c</sup>	11.1	21	Leaf	23	3.74	0.52
				Root <sup>d</sup>	13	29.8	4.06
	HY2	14	25	Leaf	16	6.19	0.62
				Petal	13	11.31	1.13
	NJAU1	15.9	30	Leaf	26	6.46	0.56
	NJAU2	37.6	50	Leaf	8	6.25	0.40
	Maoping	12.8	40	Leaf	16	3.56	0.26
	DHQ1	3.1	2	Leaf	75	1.97	2.54
<i>P. mume</i> (220 Mb)	MHG1	17.5	20	Leaf	25	12.9	2.17
				Root <sup>d</sup>	32	25.4	4.82
	MHG2	10.2	8	Leaf	33	5.7	2.38
<i>S. suchowensis</i> (480 Mb)	YAF1	-	1	Leaf	19	1.26	2.58
				Root <sup>d</sup>	21	2.86	6.60
<i>B. distachyon</i> (272 Mb)	WD2	-	1	Leaf	29	3.17	6.13
				Root <sup>d</sup>	8	4.75	8.97
				Lemma	7	2.57	4.97
<i>Fragaria vesca</i> (210 Mb)	FH1	-	1	Leaf	45	1.93	6.37
				Stems <sup>e</sup>	4	4.75	15.78
<i>Arabidopsis thaliana</i> (119 Mb)	Col17+Col24	-	1	Leaf	64	0.69	4.35
<i>Oryza sativa</i> (373 Mb)	KA1+PA1+ DG1 +NIPB	-	1	Leaf (Tiller) <sup>f</sup>	29	4.79	9.01
				Leaf (Callus)	13	194.8	287.1

x7.8

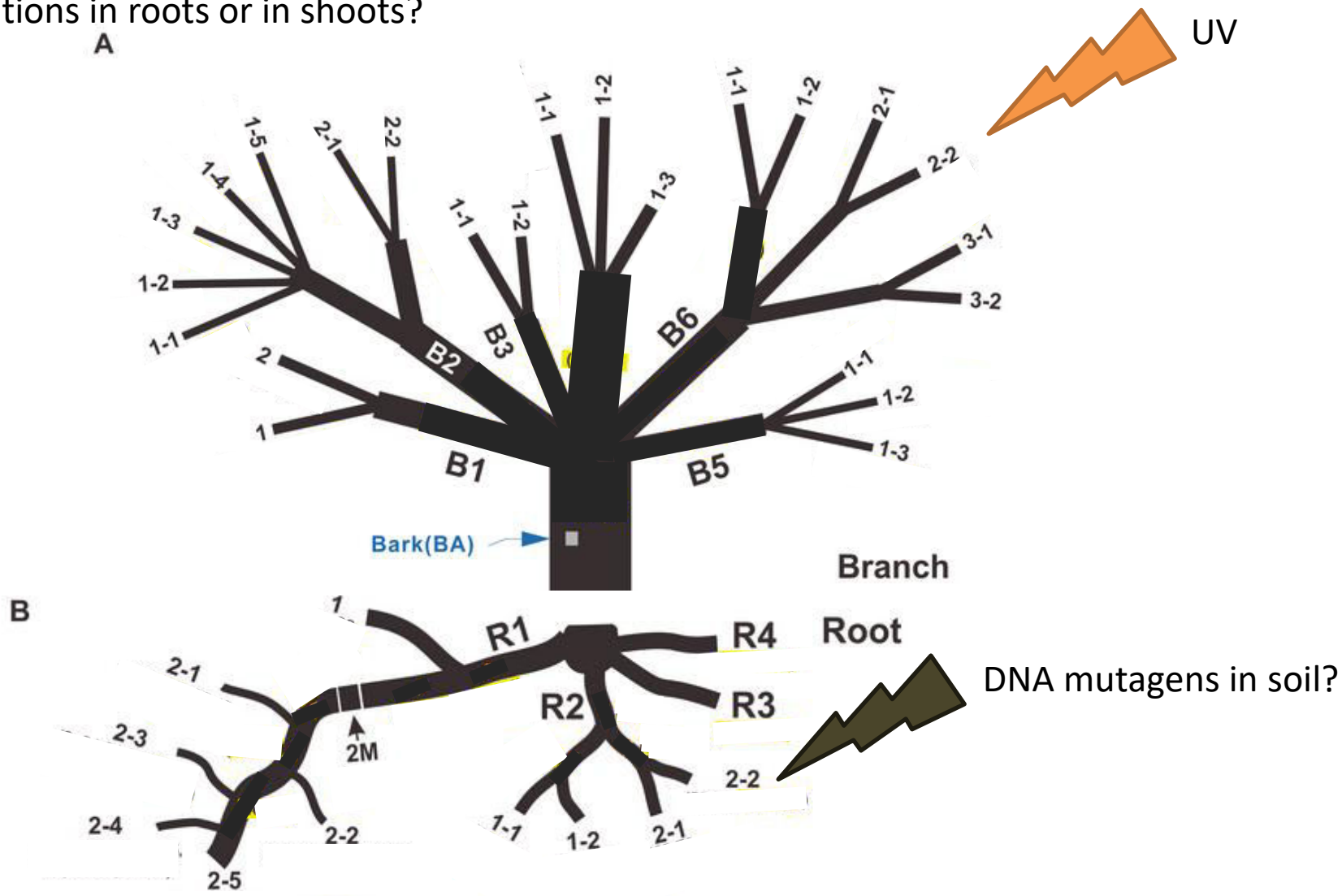
x2.2

x2.6

x1.5

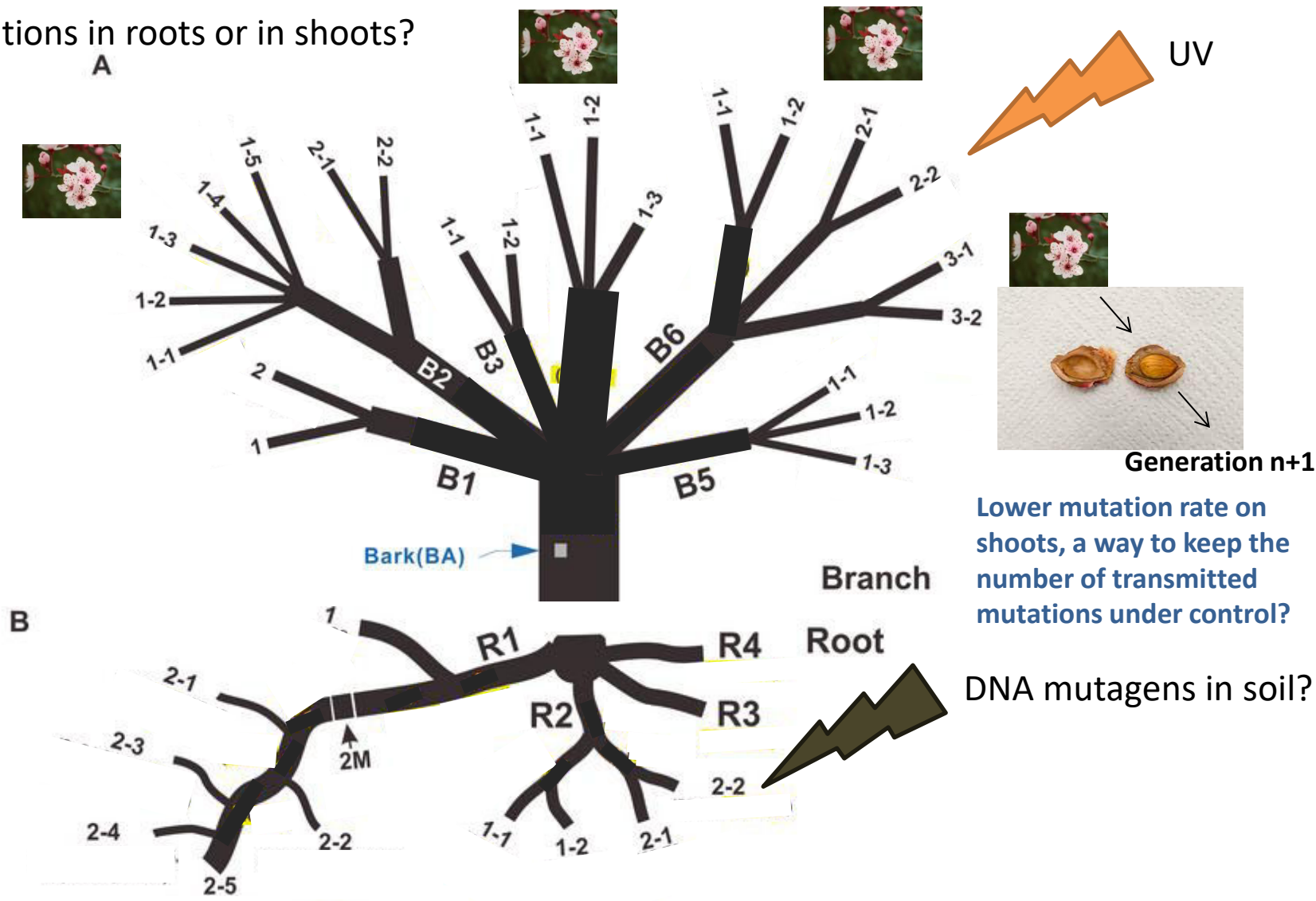
<sup>a</sup> Mutation rate was estimated for each sample as follows:

More mutations in roots or in shoots?

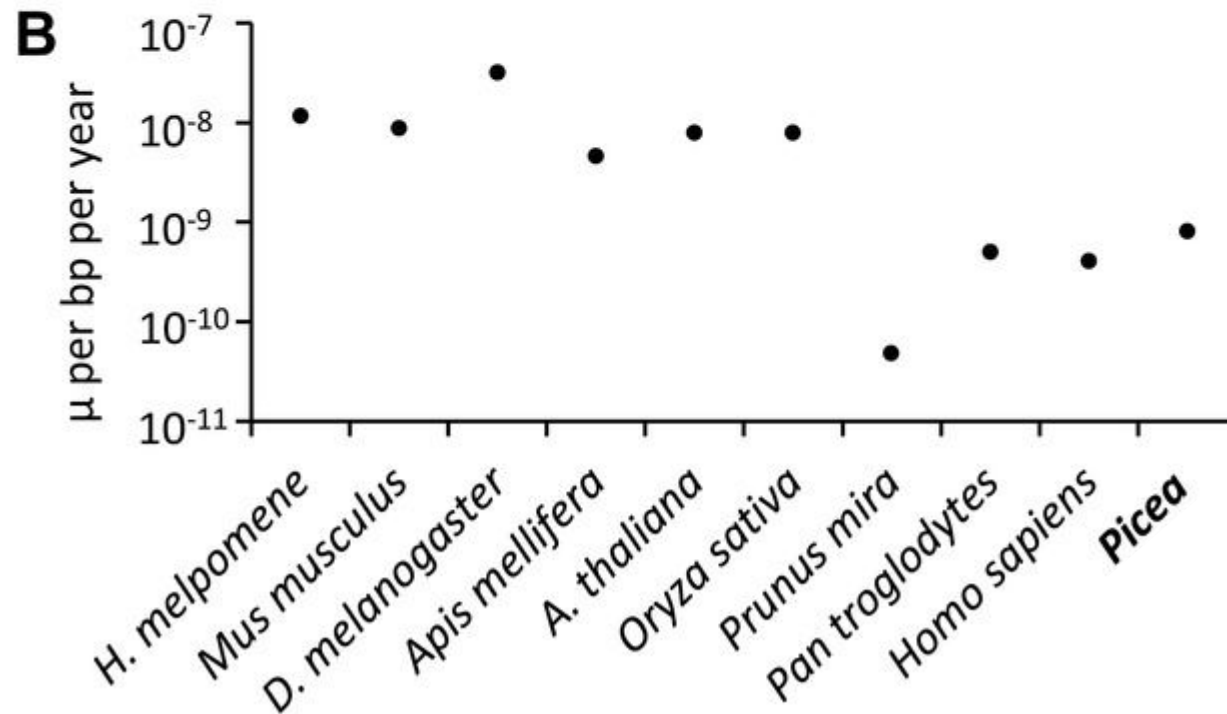
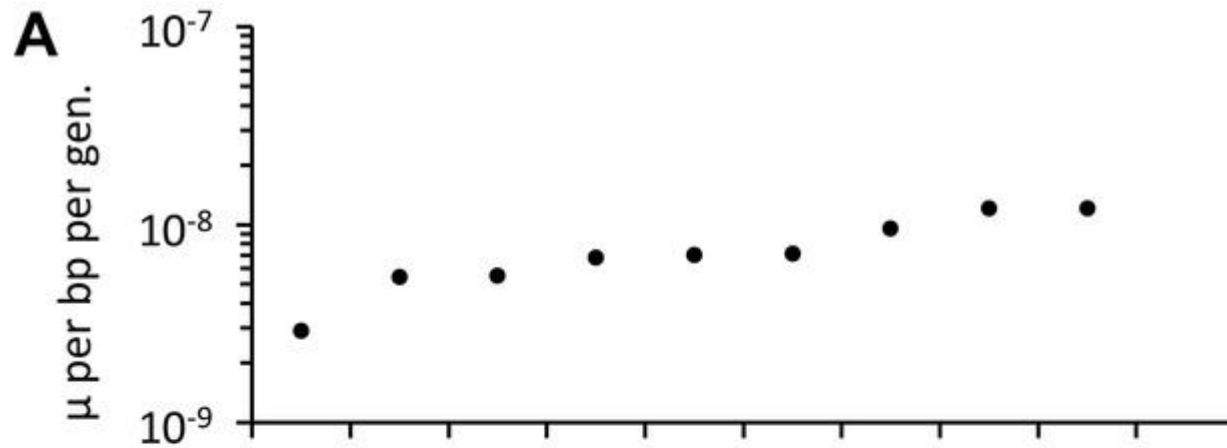




More mutations in roots or in shoots?



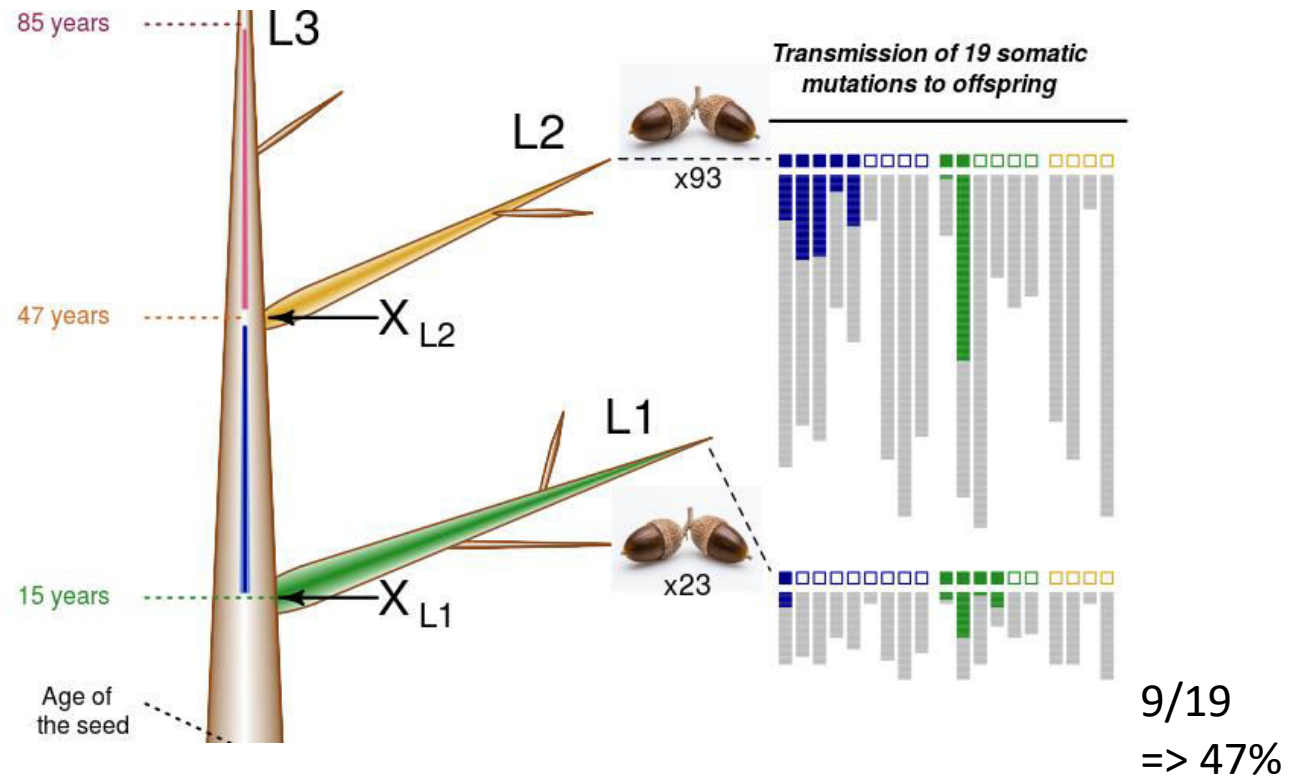
Long-lived species generally have lower mutation rates per unit of time than annuals



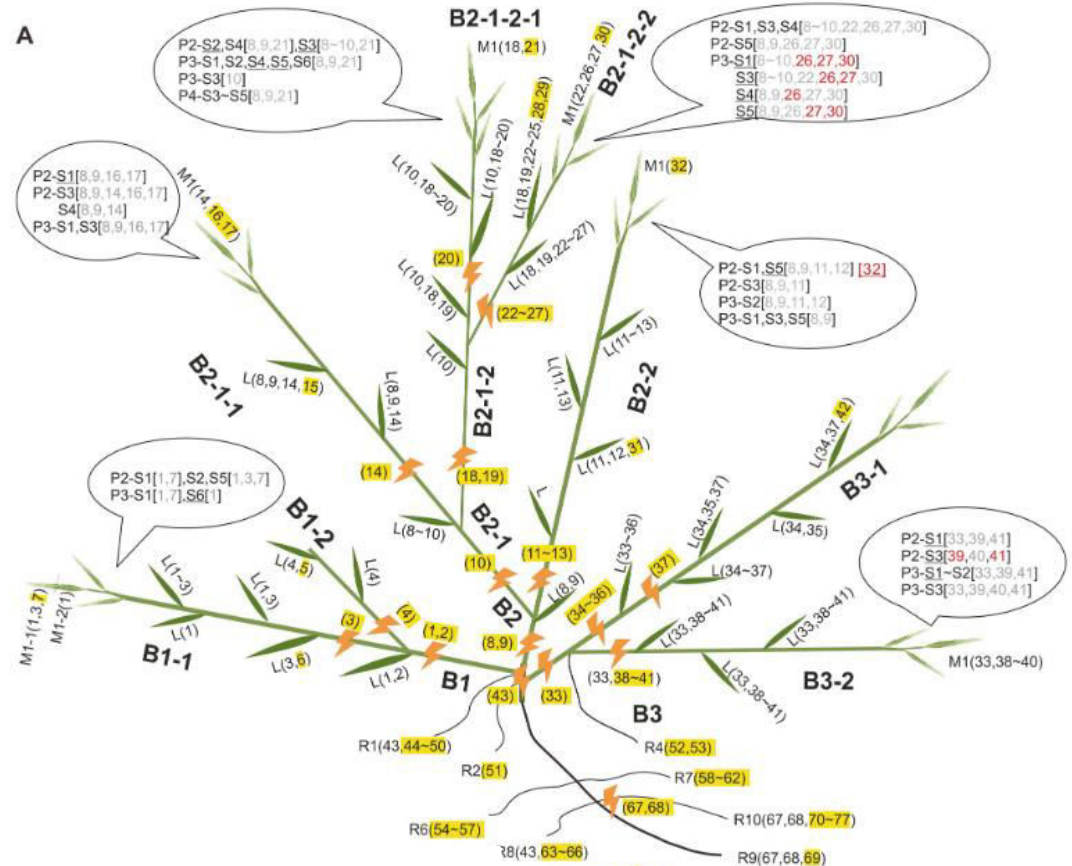
So-called  
« generation-time  
hypothesis »

Faster generation  
=> genomes more  
copied => more  
mutations  
(=> higher rates of  
molecular  
evolution)

Which proportion of somatic mutations will then be passed to the next generation?



**Ok roughly half of somatic mutations accumulated along tree growth are transmitted to the next generation**  
(so it means that oak really departs from the Weismann's germplasm theory)...









They also sequenced these 14 individuals!

=> Somatic mutations

=> New mutations (« not premeiotic », meiotic or mitotic near gametes)

Table 2. Mutations identified in 14 meiotic progeny of wild peach GL2.

Fruit ID	Inherited somatic mutations	Specific mutations in the progeny <sup>a</sup>	Proportion of the inherited mutations <sup>b</sup>
FR1 <sup>c</sup>	19	–	NA
FR2	18	7	18/25 (72%)
FR3	15	2	15/17 (88%)
FR4	17	5	17/22 (77%)
FR5	14	4	14/18 (78%)
FR6	15	4	15/19 (79%)
FR7	14	3	14/17 (82%)
FR8 <sup>c</sup>	5	–	NA
FR9	4	7	4/11 (36%)
FR10 <sup>c</sup>	5	–	NA
FR11	9	3	9/12 (75%)
FR12 <sup>d</sup>	8	6	8/14 (57%)
FR13	4	9	4/13 (31%)
FR14	7	0	7/7 (100%)
Mean	11.0	4.6	125/175 (71%)

—> 71% of new mutations have a somatic origin  
(66% for tree 'GZ')  
=> Somatic mutations are an important engine  
of genetic diversity in *Prunus*

## To summarize the findings up to now:

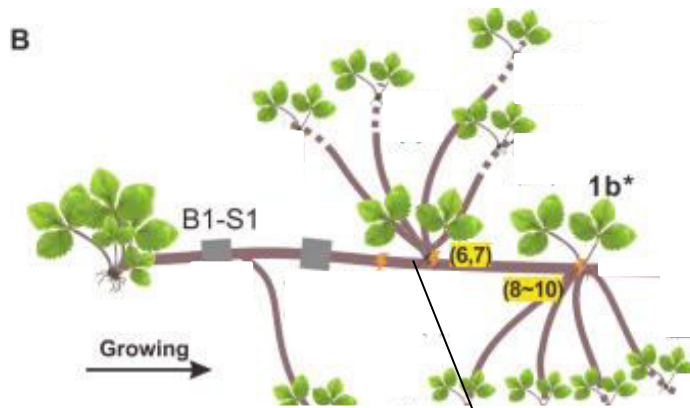
More mutations on roots than on shoots. Interesting patterns, why/how?

In *Prunus* or oaks, half of the somatic mutations are passed to the next generation, in such a way that somatic mutations rather than meiotic mutations explain the most important part (at least two thirds) of the number of new mutations from one generation to another (*Prunus* only)

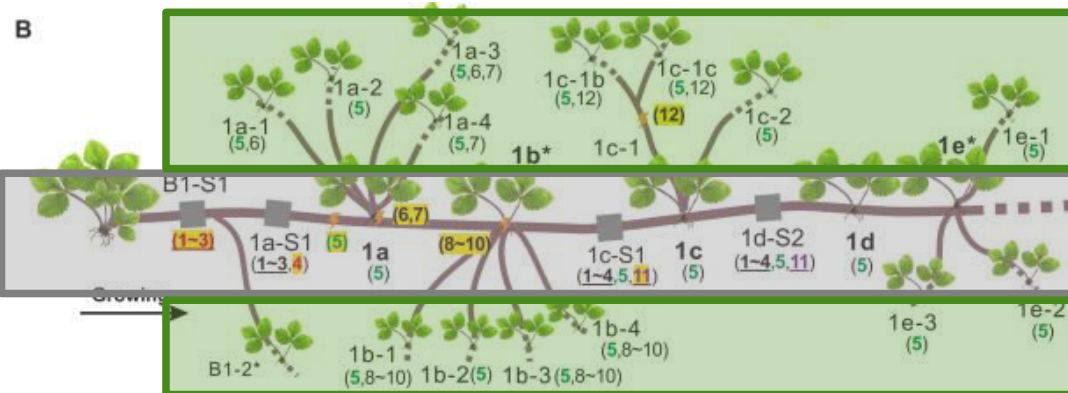
< 10% of the somatic mutations are passed in annuals.

⇒ Lower in annuals, Paradoxical result? Given that we observe higher mutation rates per year in annuals than in trees, we could have expected the opposite. Explained by a greater contribution of meiotic mutations in annuals?

Overall, for all species investigated up to now, at least some somatic mutations are transmitted to the next generation, as expected following the « plant » model (departing from the animal model, i.e. the Weismann's germline theory)



Runners (« stolons »)  
= Vegetative propagation




Mutations  
specific to  
descendent  
shoots and  
leaves

Mutations  
specific to  
runner

Two different cell lineages in runners? Some somatic mutations that are never transmitted? A way to keep mutations under control?

# Somatic Mutation Analysis in *Salix suchowensis* Reveals Early-Segregated Cell Lineages

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<sup>1</sup>State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, China

<sup>2</sup>Department of Ecology, School of Life Science, Nanjing University, Nanjing, China

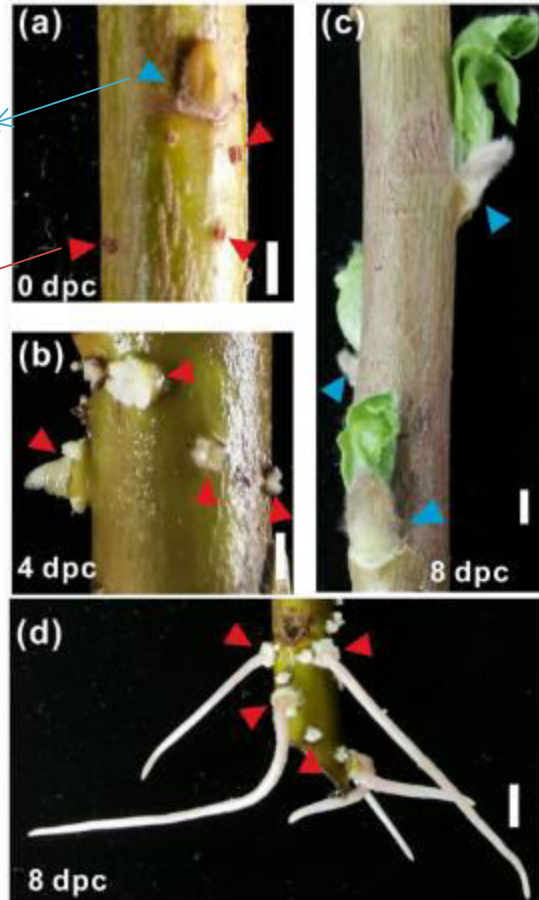
<sup>3</sup>State Key Laboratory for Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, China

<sup>†</sup>These authors contributed equally to this work.

**\*Corresponding authors:** E-mails: wanglong@nju.edu.cn; jiayanxiao@163.com.

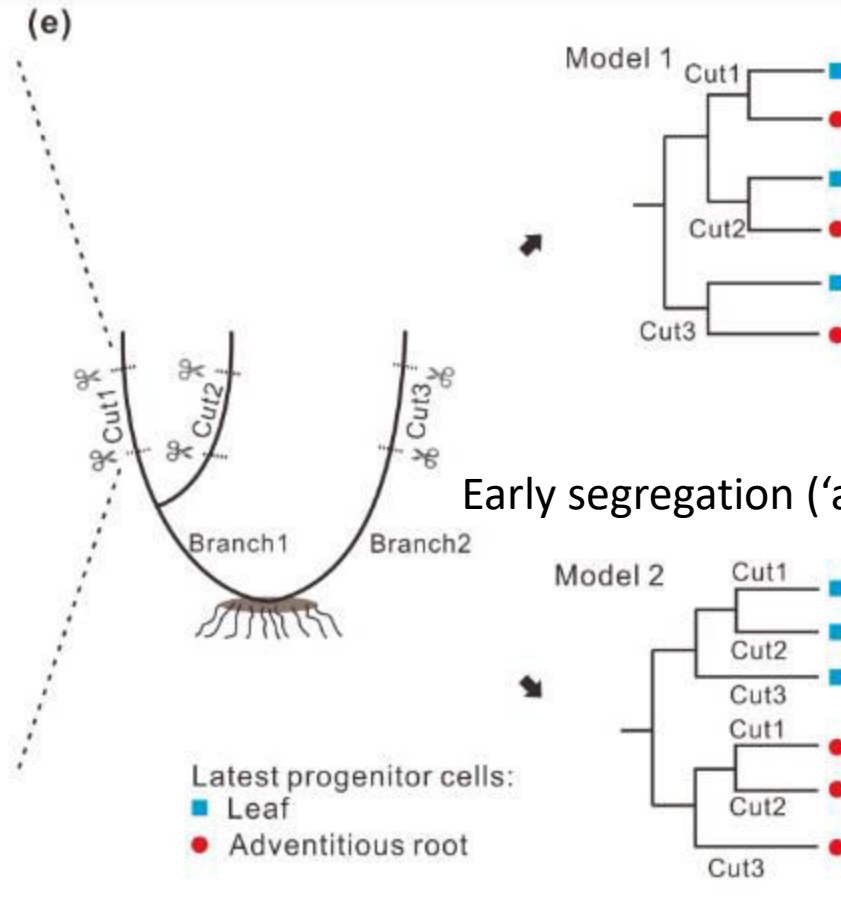
**Associate editor:** Gregory Wray

## Late segregation ('plant model')



Axillary merist.  
=> branching

Adventitious  
meristems  
=> resprouting



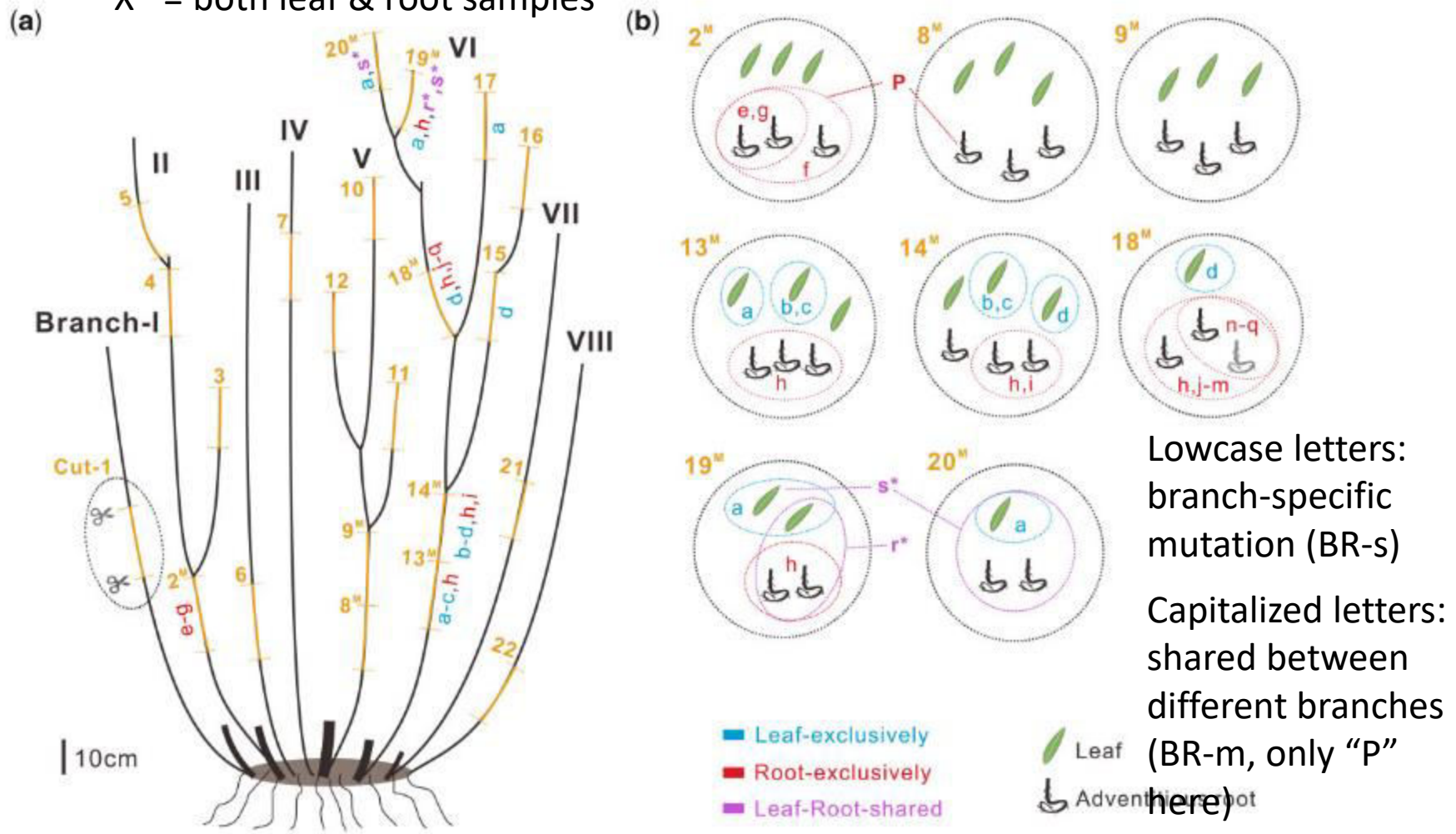
## Early segregation ('animal model')



# WGS of 33 leaves and 22 adventitious roots (>2000 X in total)

X = sample ID

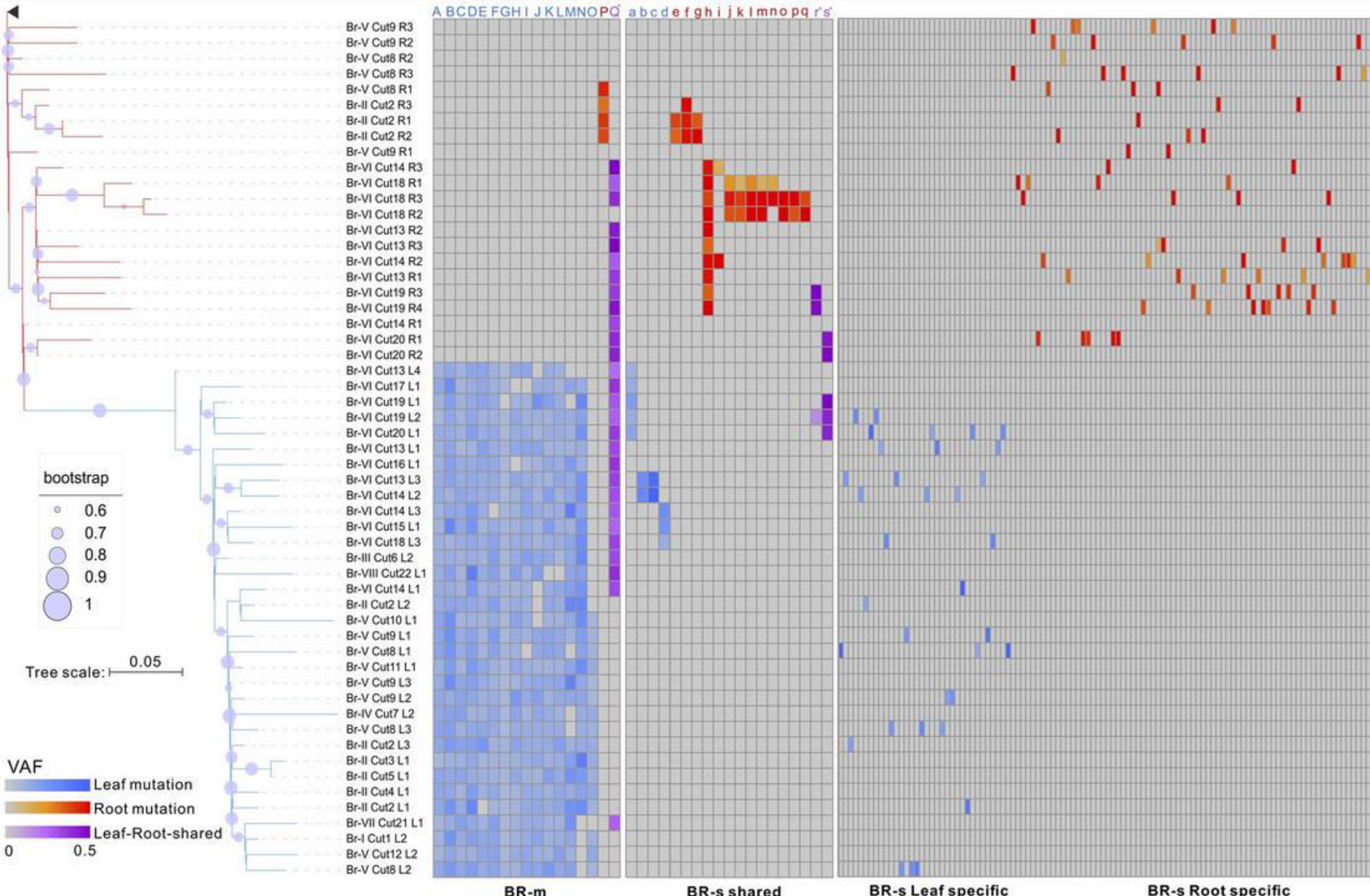
X<sup>M</sup> = both leaf & root samples



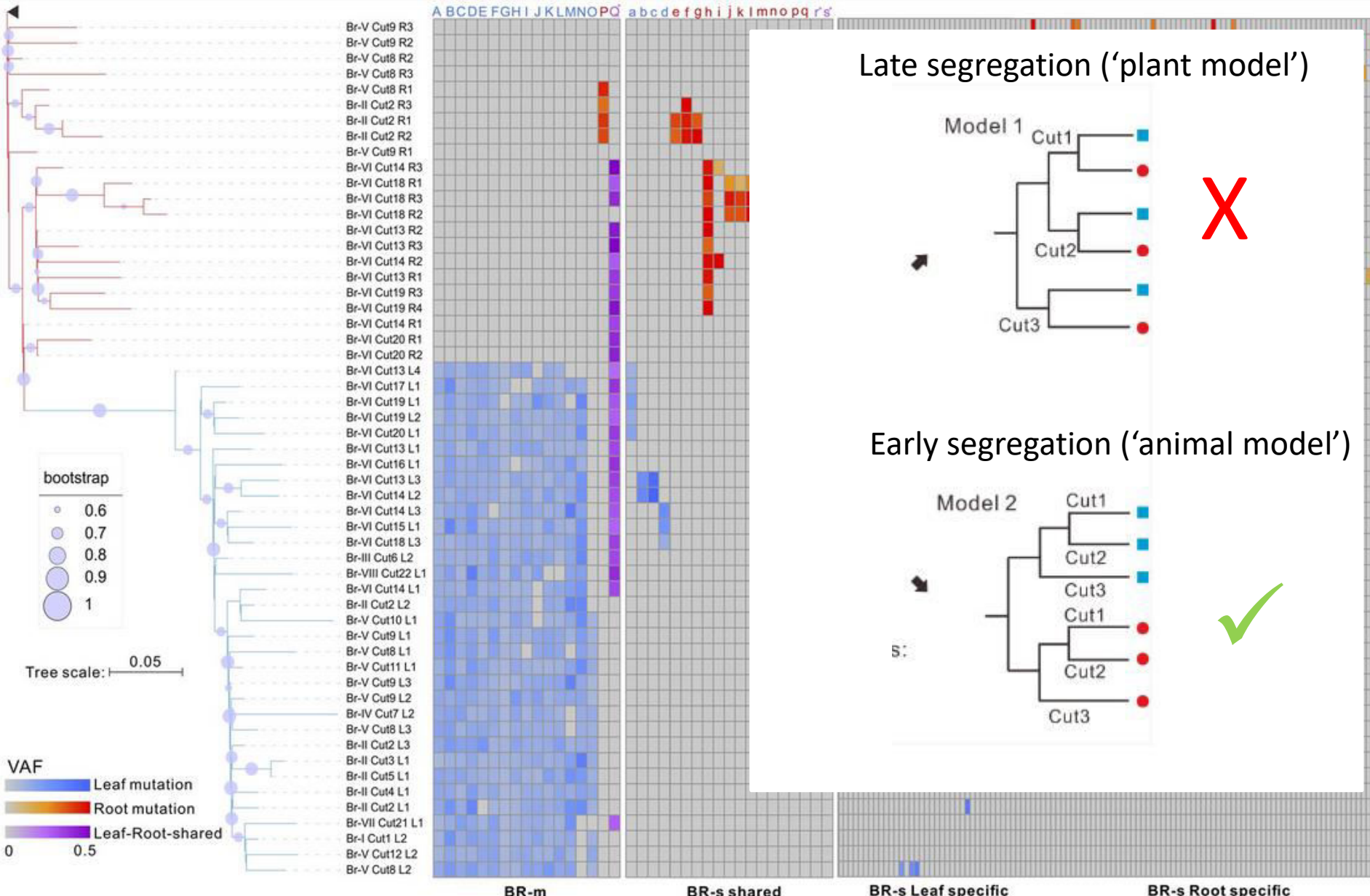
In total: 199 reliable somatic mutations: 182 BR-s and 17 BR-m

Among the 8 X<sup>M</sup>: 108 including 35 leaf mutations and 73 root mutations (again more on roots, but here a limit to conclude due to the callus formation step)

Tracking of mutations in all samples  
17 BR-m (“A~Q”), 19 BR-s shared (“a~s”), and 108 BR-s sample-specific mutations in each sequenced sample

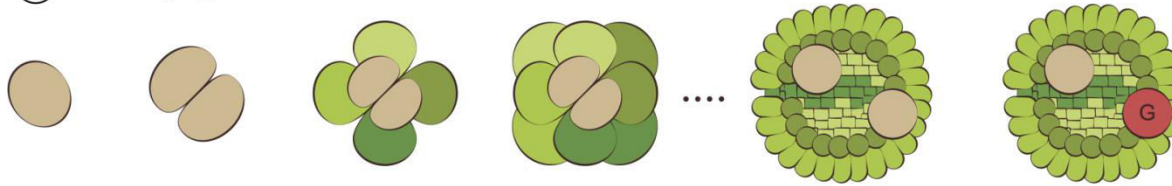


Tracking of mutations in all samples  
17 BR-m (“A~Q”), 19 BR-s shared (“a~s”), and 108 BR-s sample-specific mutations in each sequenced sample



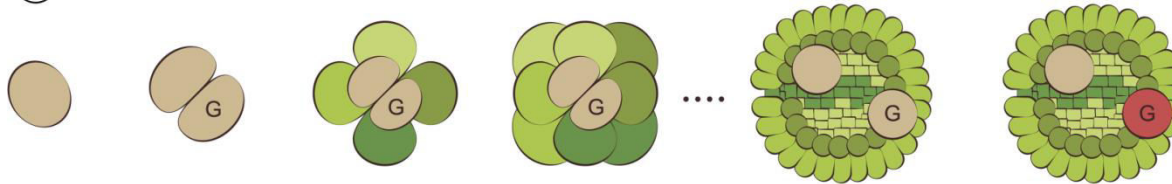


(A) Late segregation, late differentiation

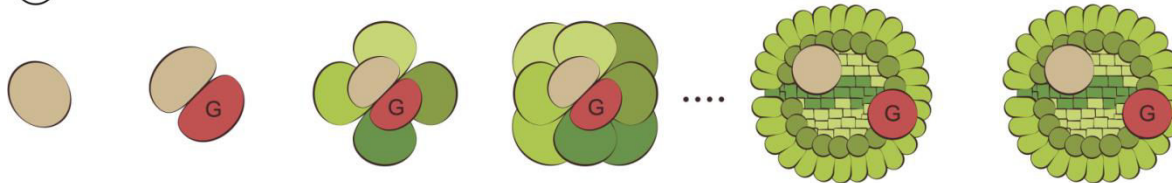


« plant model »

(B) Early segregation, late differentiation



(C) Early segregation, early differentiation



« animal model »

- G Segregated germline cell lineage
- Undifferentiated stem cell
- Differentiated somatic cell
- Differentiated germline cell

*“[...] recent studies have suggested that some, and possibly most, plants possess a nearly-segregating and slowly dividing germline cell lineage that bears a striking resemblance to the animal germline”*