16th ASEICA
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European Research Council

.cancer evolution

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Universidade Vigo
CINBIO

aseica
Asociación Española de Investigación sobre el Cáncer

- cancer is **somatic evolution**

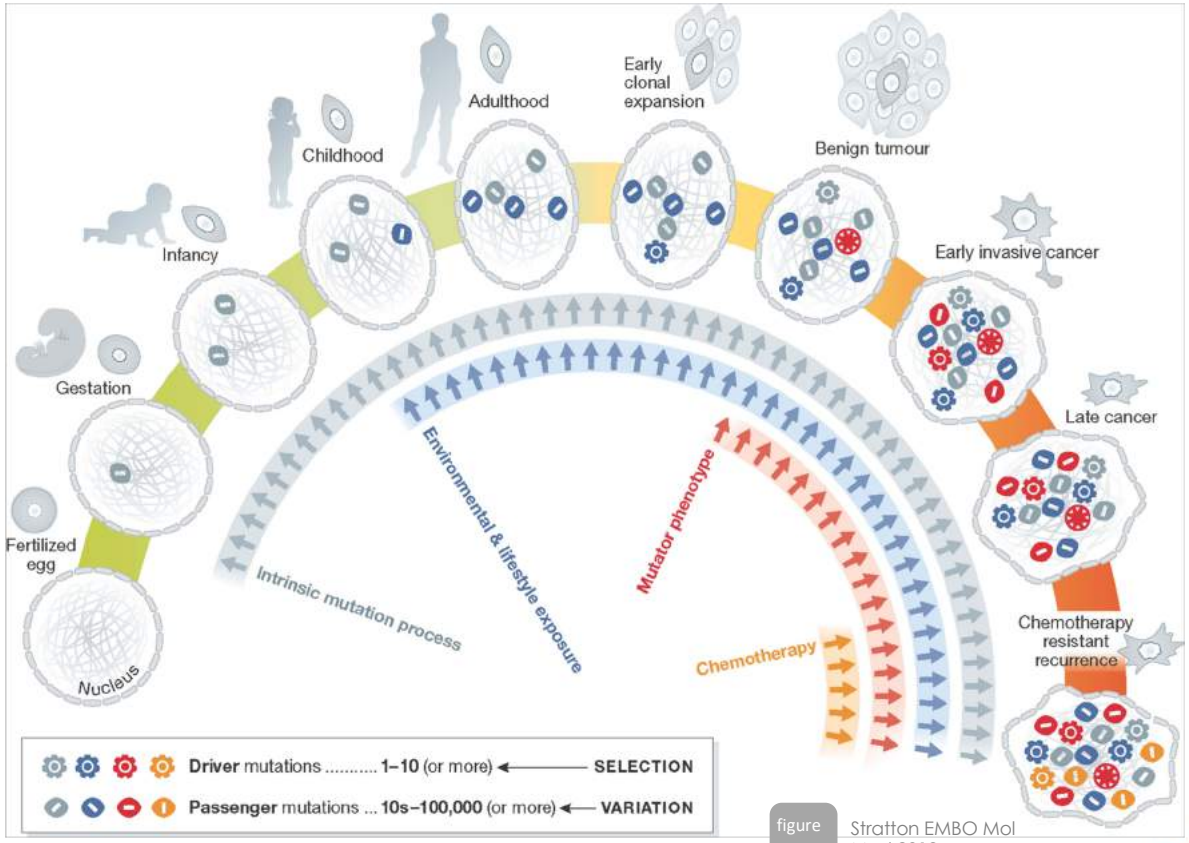
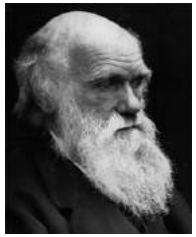


figure from Stratton EMBO Mol Med 2013

- cancer as **adaptive evolution**



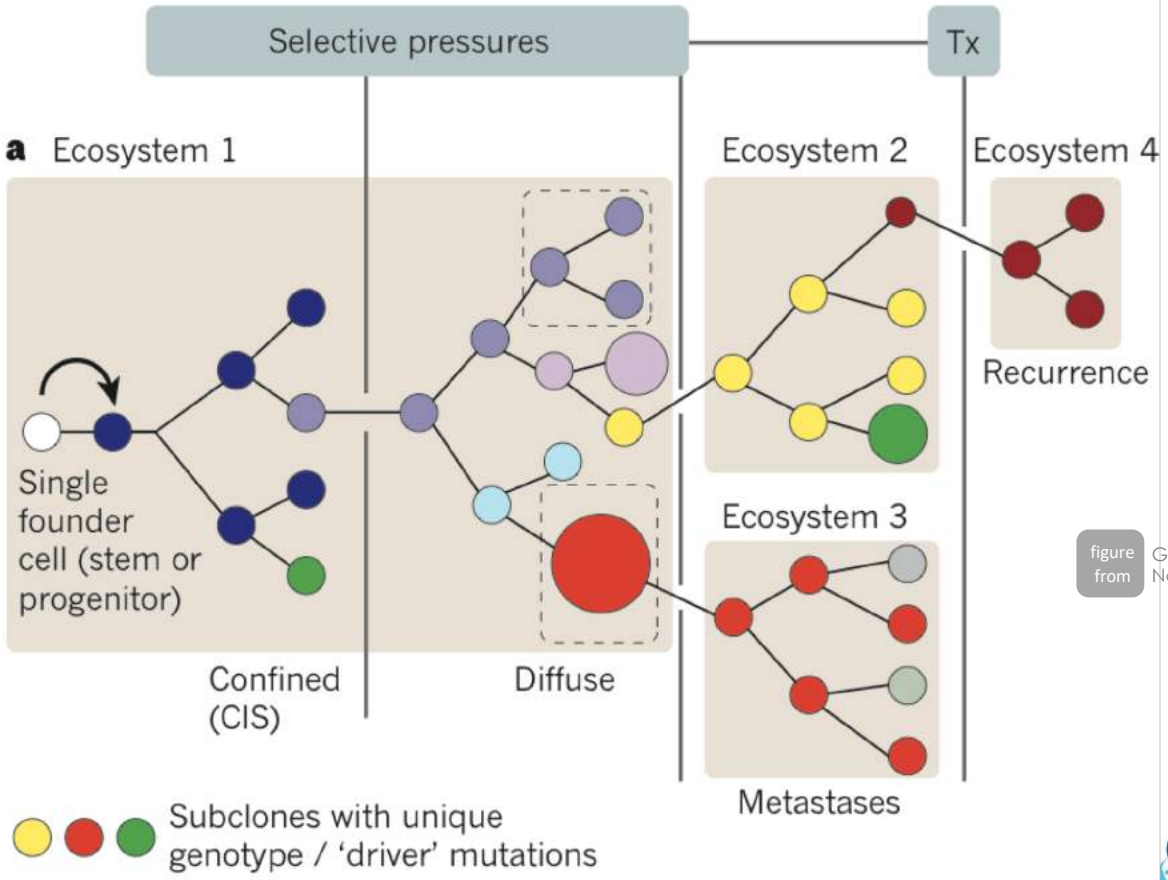


figure from Greaves and Maley Nature 2012

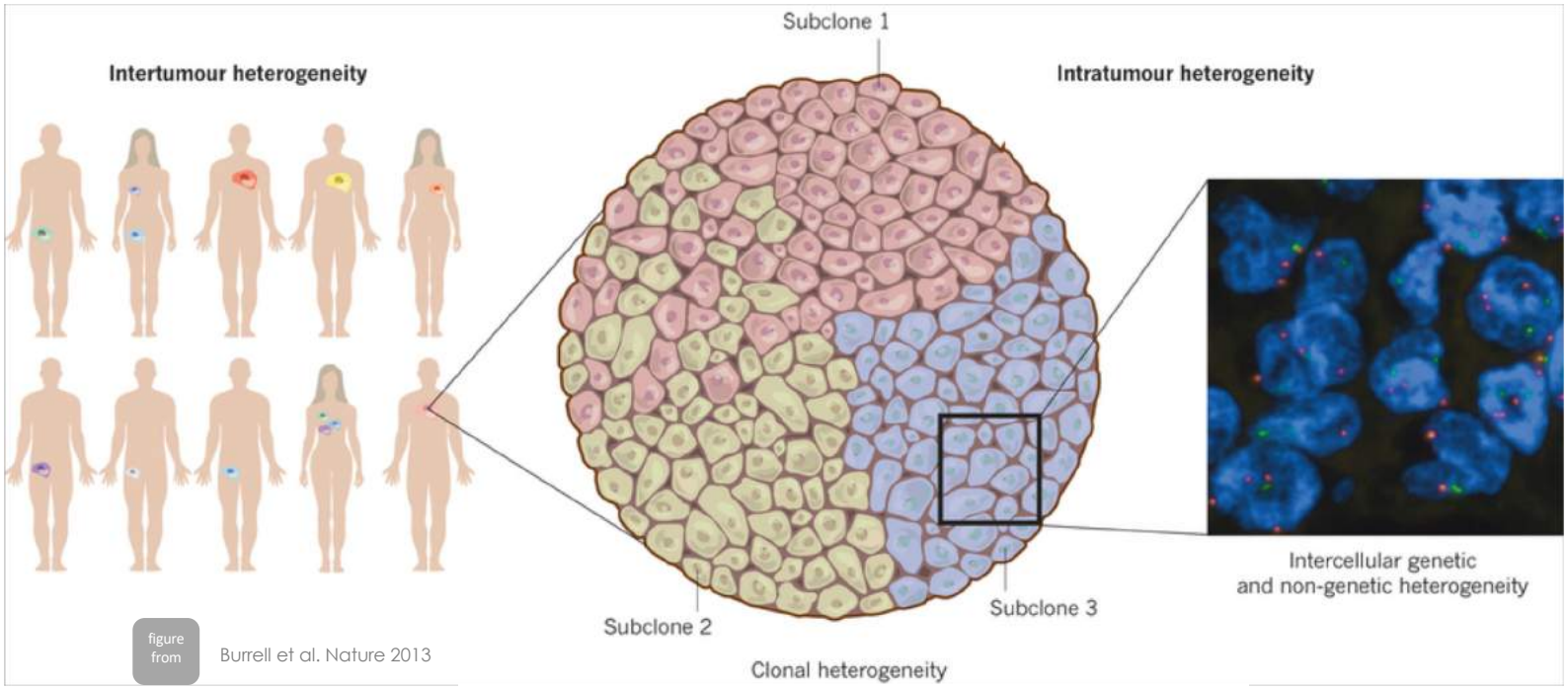
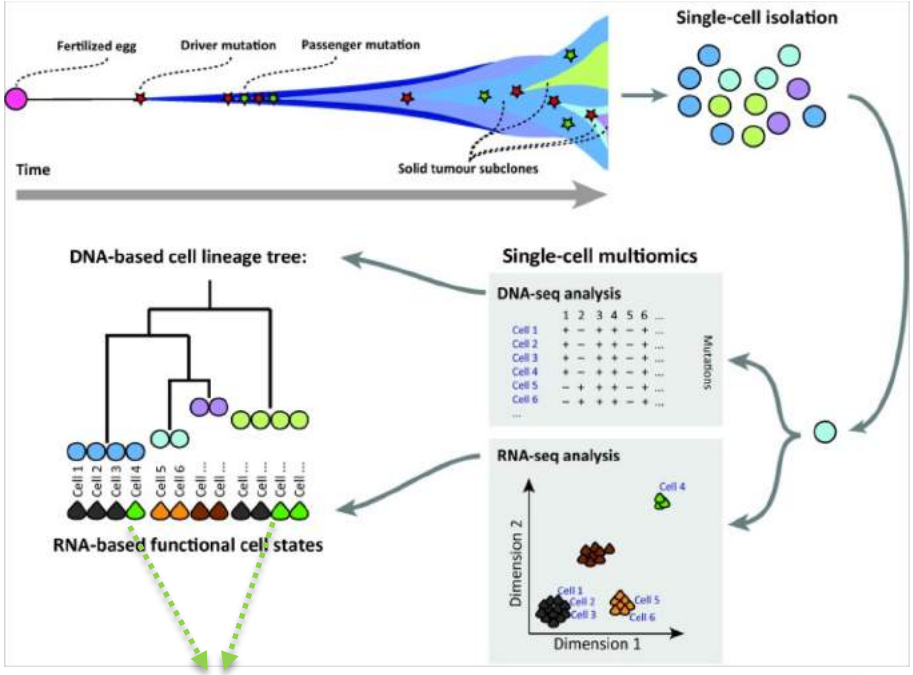


figure from Burrell et al. Nature 2013

intratumor heterogeneity (ITH)

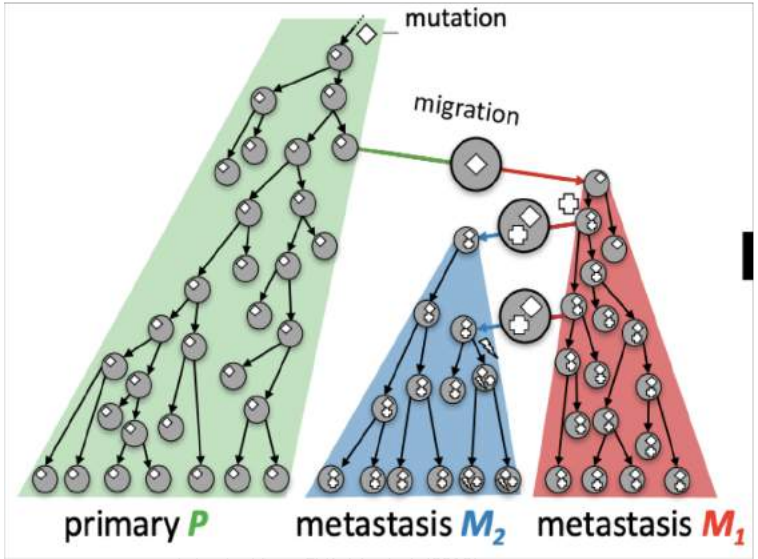
- understanding somatic/tumor evolution is understanding cancer
  - parallel changes
  - adaptation
  - migration
  - growth rate
  - migration patterns



green function was acquired twice

figure from Macaulay et al. TIG 2017

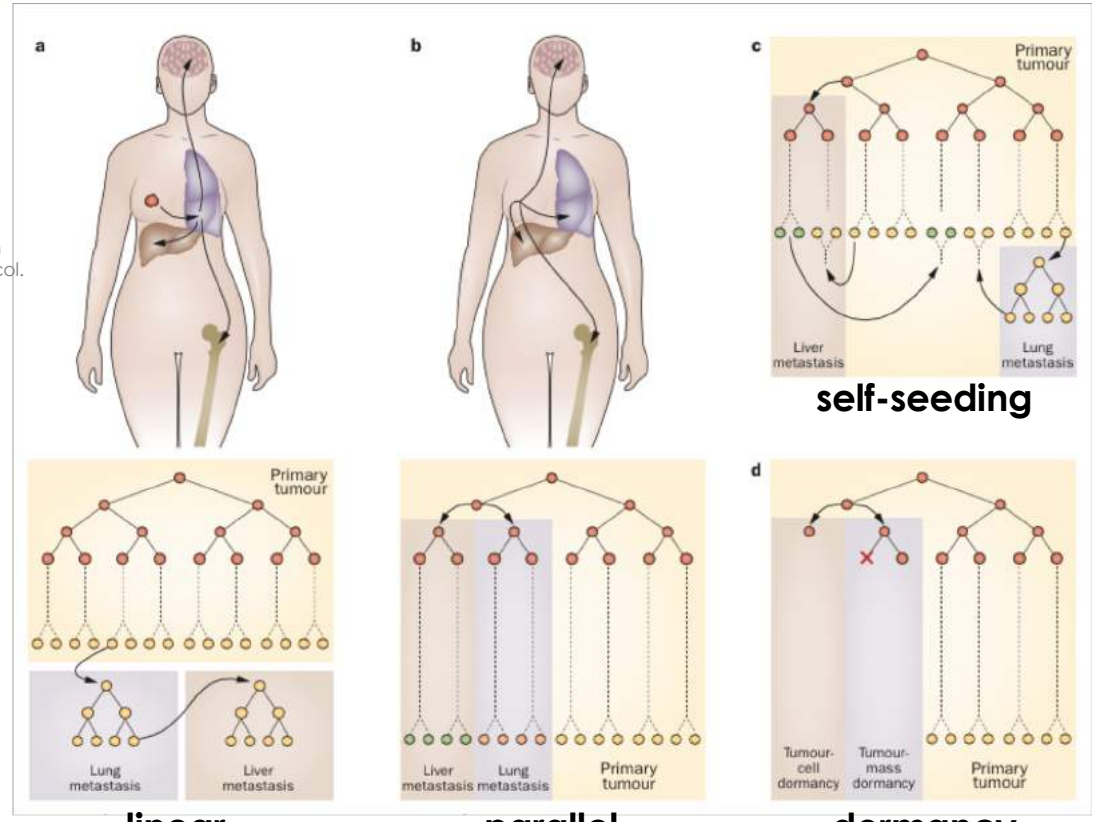
- metastatic patterns



Adapted from El-Kebir et al. (2018)

figure from

Naxerova & Jain
Nat.Rev.Cln.Oncol.
2015



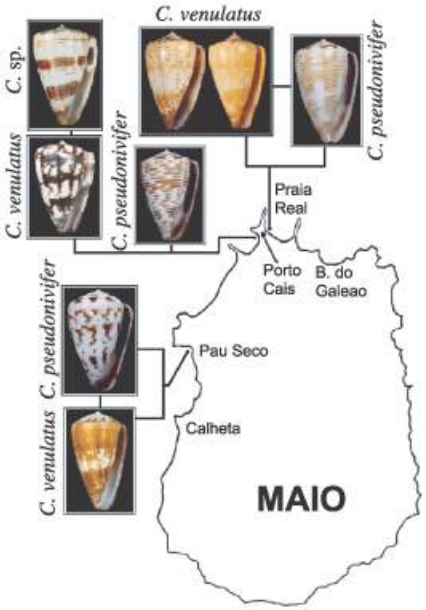


figure from Cunha et al. Mol Ecol 2008

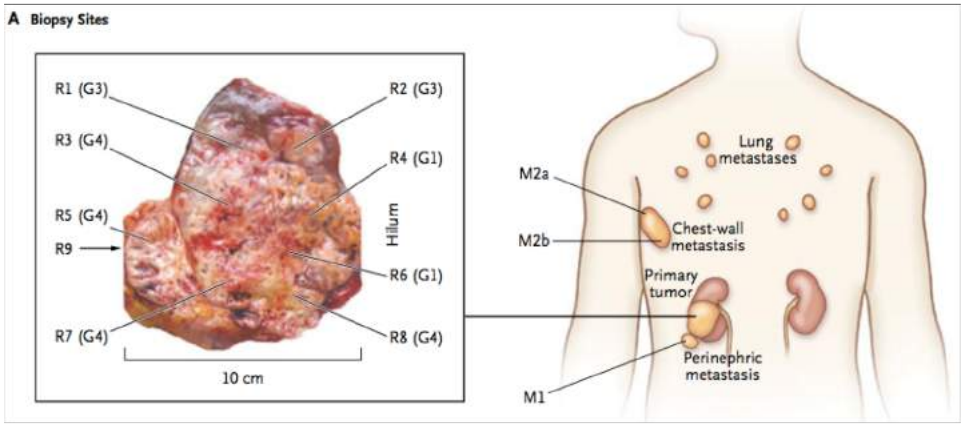
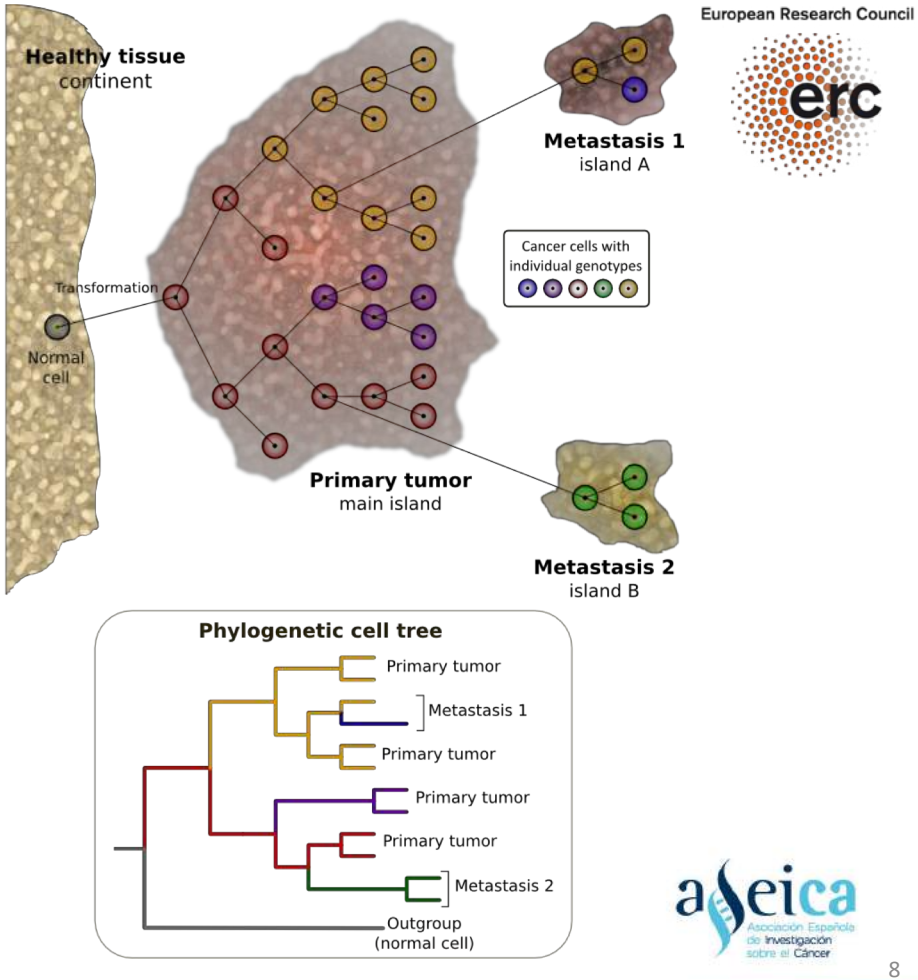


figure from Gerlinger et al. NEJM 2012

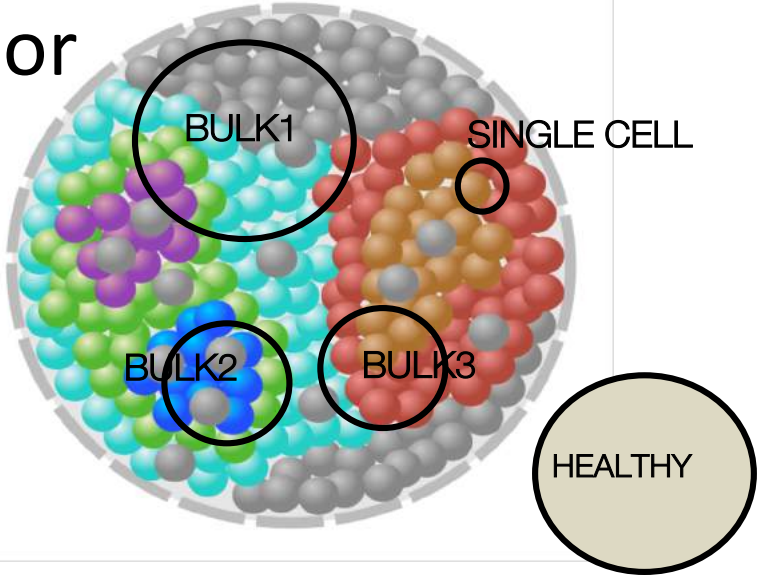
.organisms <> cancer

- **cancer phylogeography**
  - single tumor cells / clones = individuals
  - groups of tumor cells = populations
  - tumors = islands/regions
  - body = archipelago/continent



.sampling tumor ITH

- **bulk-sequencing** of mixed DNA from a pool of cells
- **multiregional sequencing** of several bulk samples from the same patient/tumor
- **single-cell sequencing**



.multiregional/sample trees

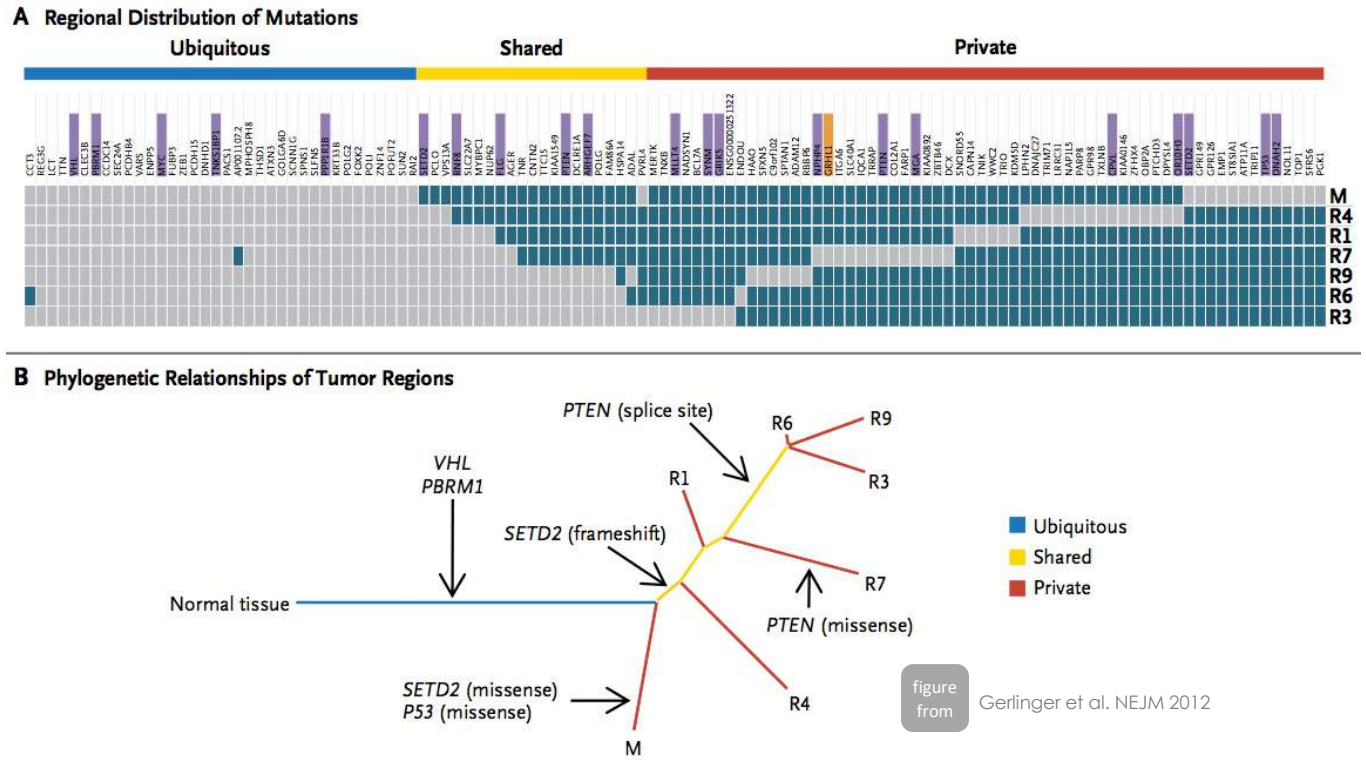
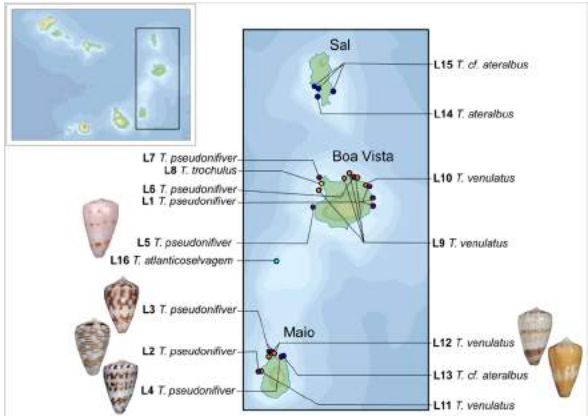
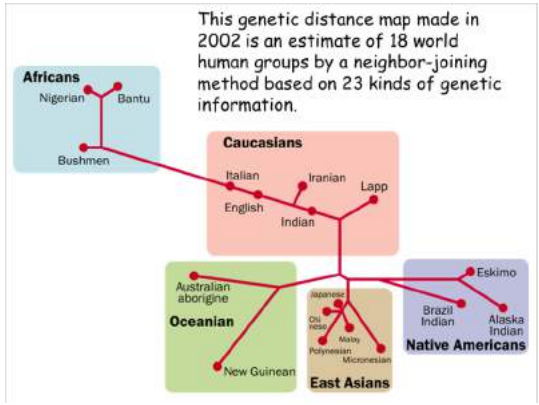


figure from Gerlinger et al. NEJM 2012

- bulk sample = **pool-sequencing of mixed DNA** from multiple individuals (cells)
- (primary) bulk **tumor samples are not populations**
  - "population" implies some type of well-defined isolated group with a more or less clear common origin
  - recognizable geographical, environmental or morphological features (despite ILS, gene flow or admixture)





- sample trees are not phylogenies

- biases
  - **apparent homoplasies**
  - wrong branching orders
  - wrong branch lengths

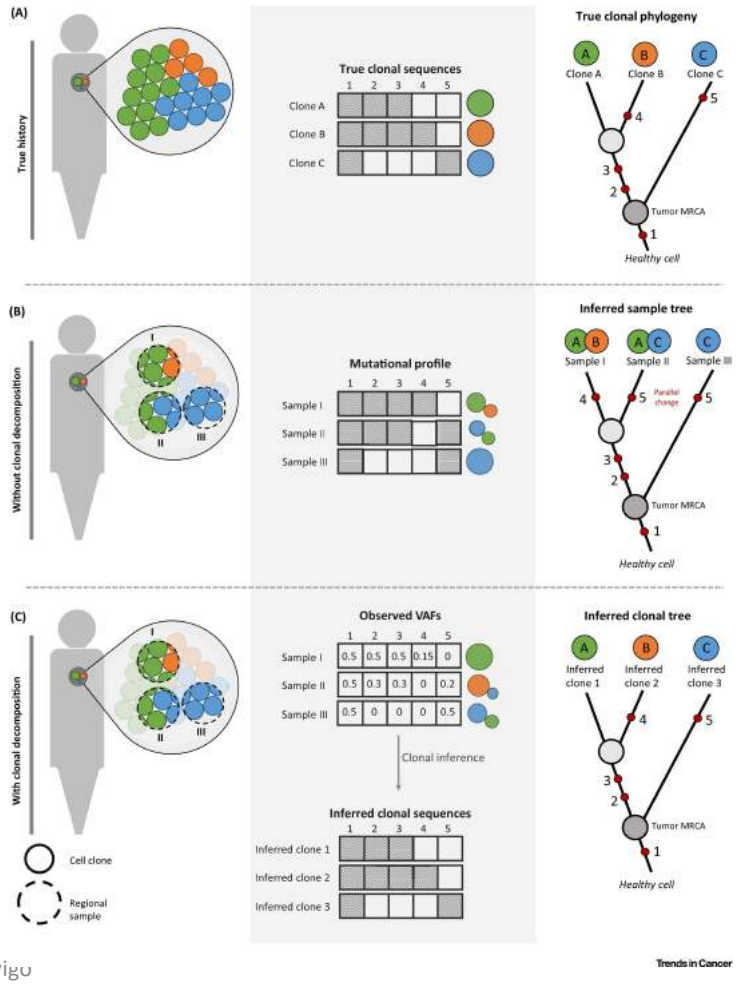


figure from Alves et al. Trends in Cancer 2017

- sample trees are not phylogenies

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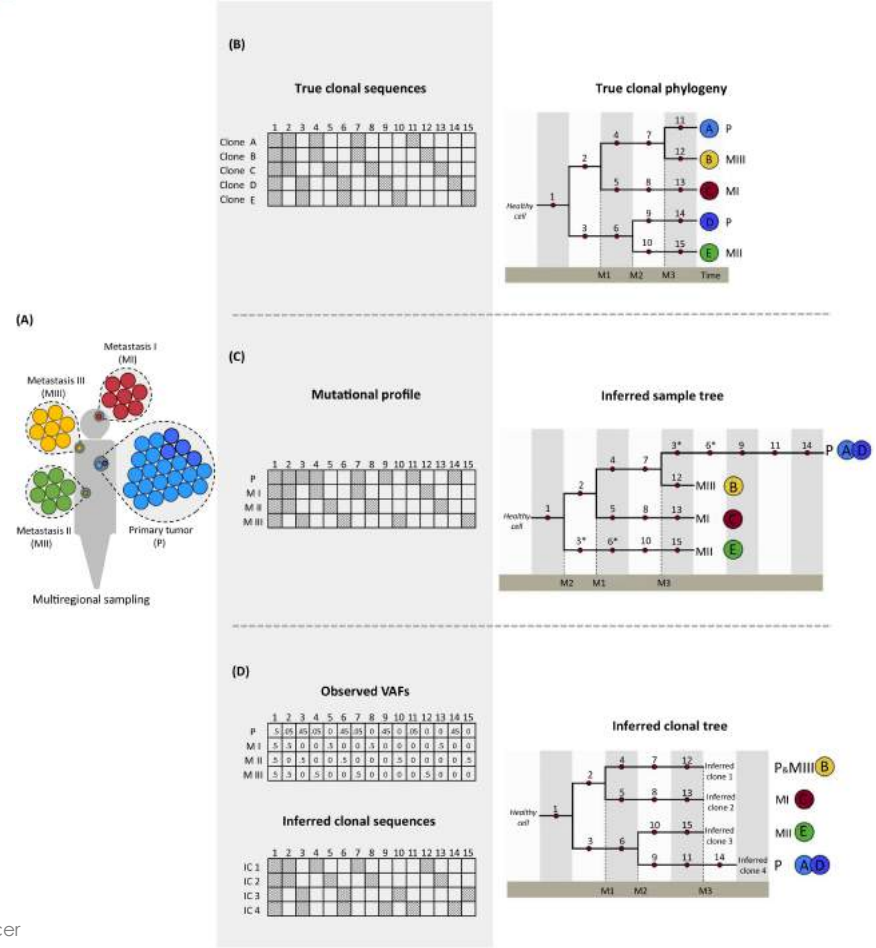
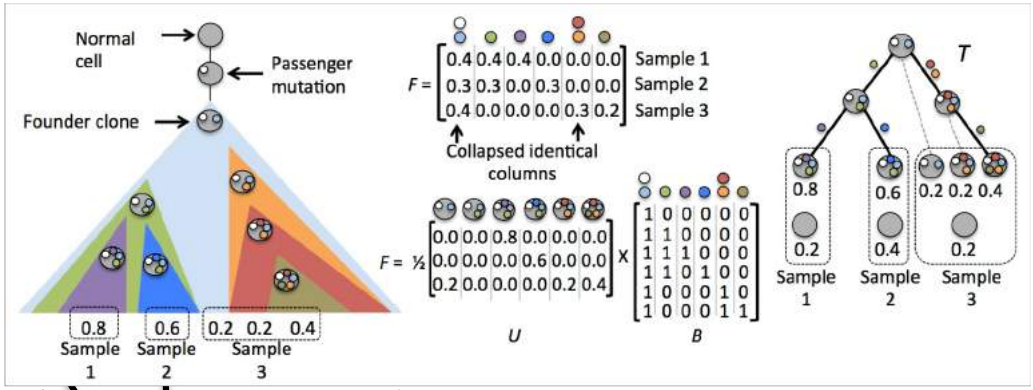


figure from Alves et al. Trends in Cancer 2017

- **clonal deconvolution** from bulk samples



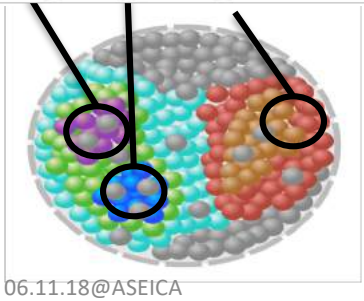


figure from http://compbio.cs.brown.edu/projects/ancestree

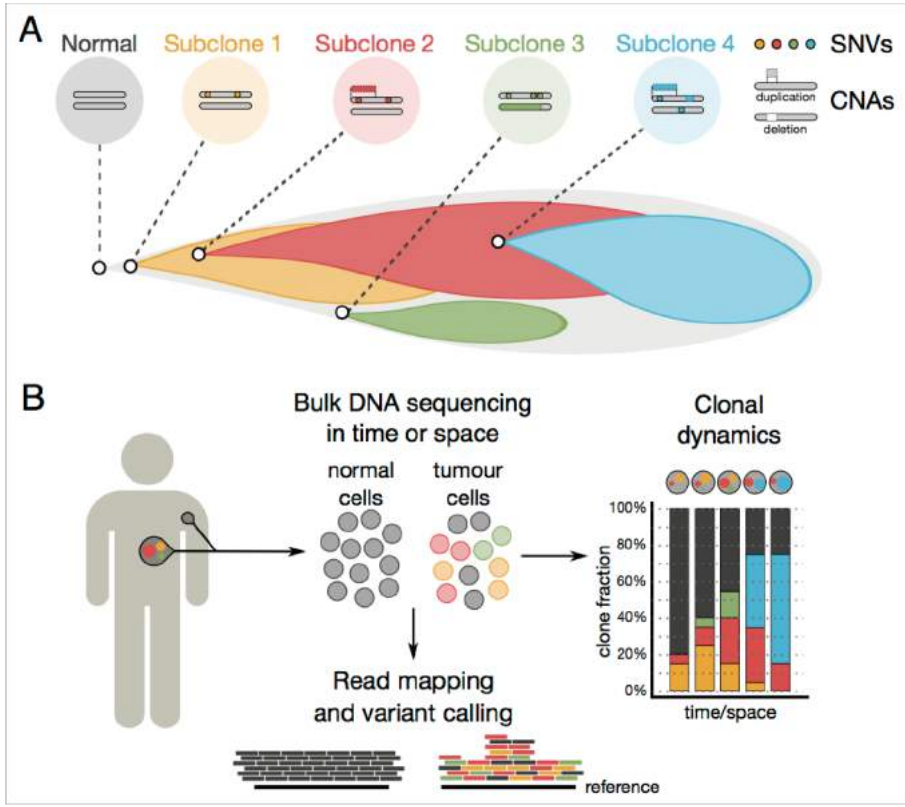
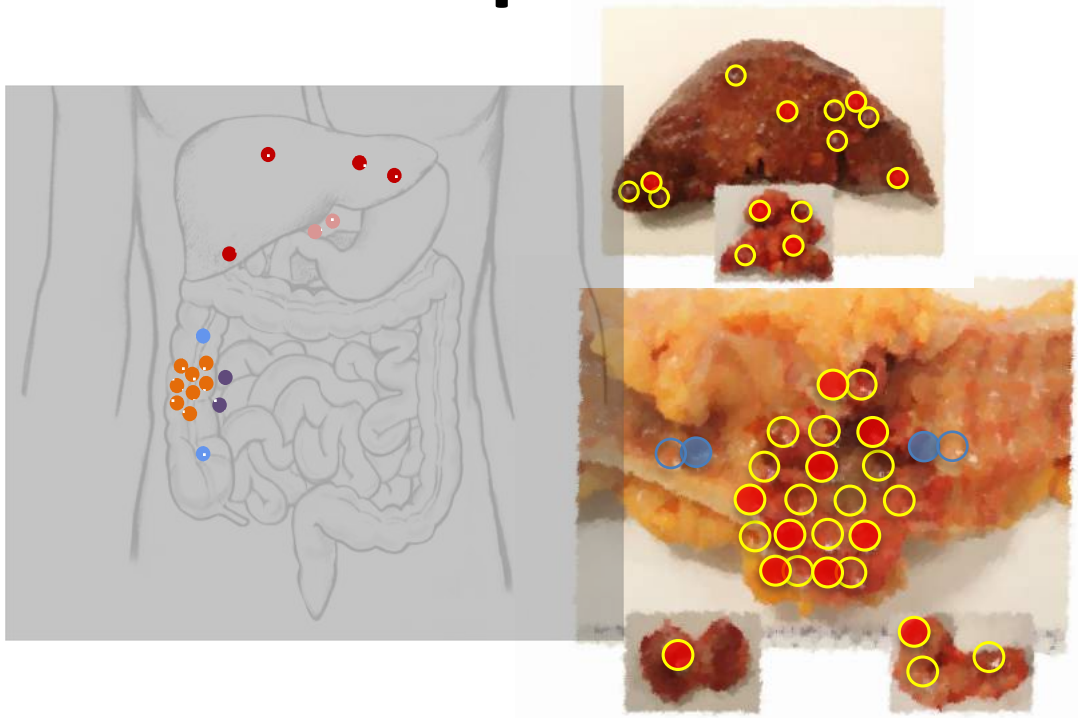


figure from Fischer et al. Cell Reports 2014

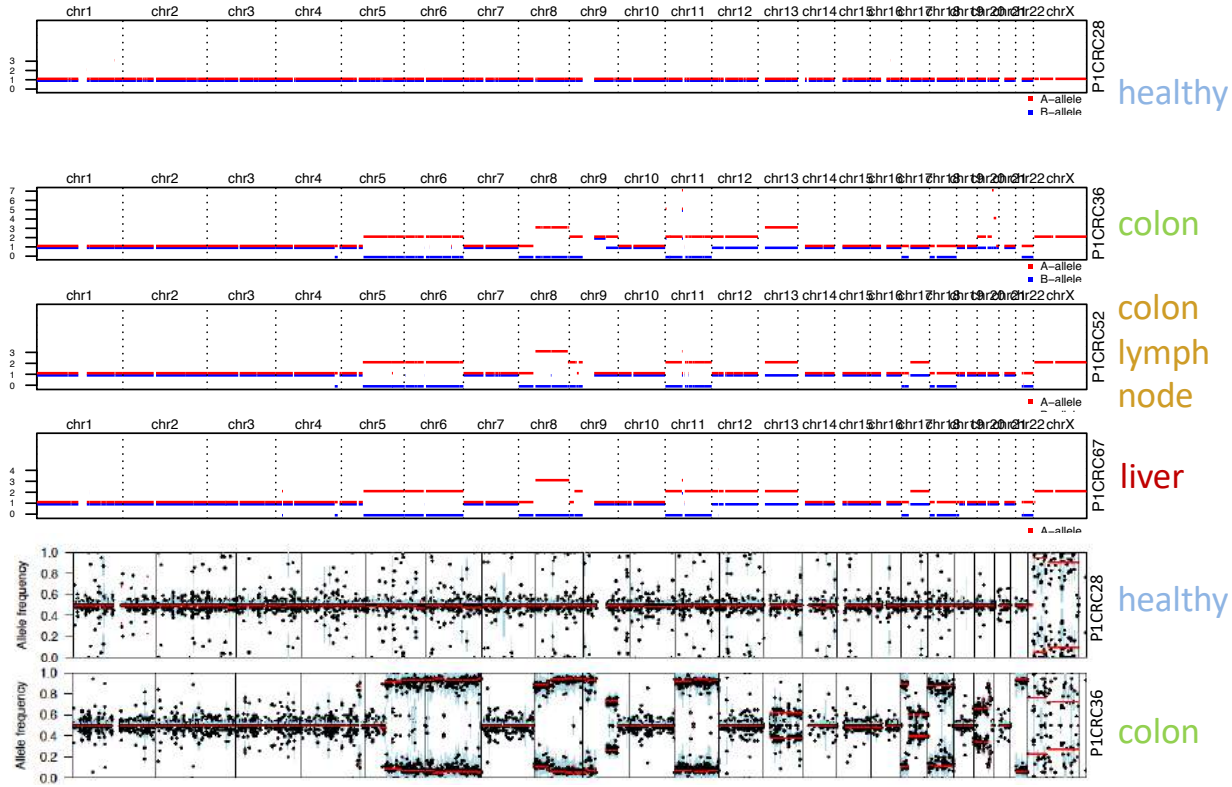
.CRC evolution in time and space

- CRC01: 20 locations
  - control: colon mucosa (2)
  - primary tumor: colon (8)
  - metastases:
    - colon lymph nodes (2)
    - hepatic lymph nodes (2)
    - liver (4)
- WES Ion Torrent >60X

Joao Alves

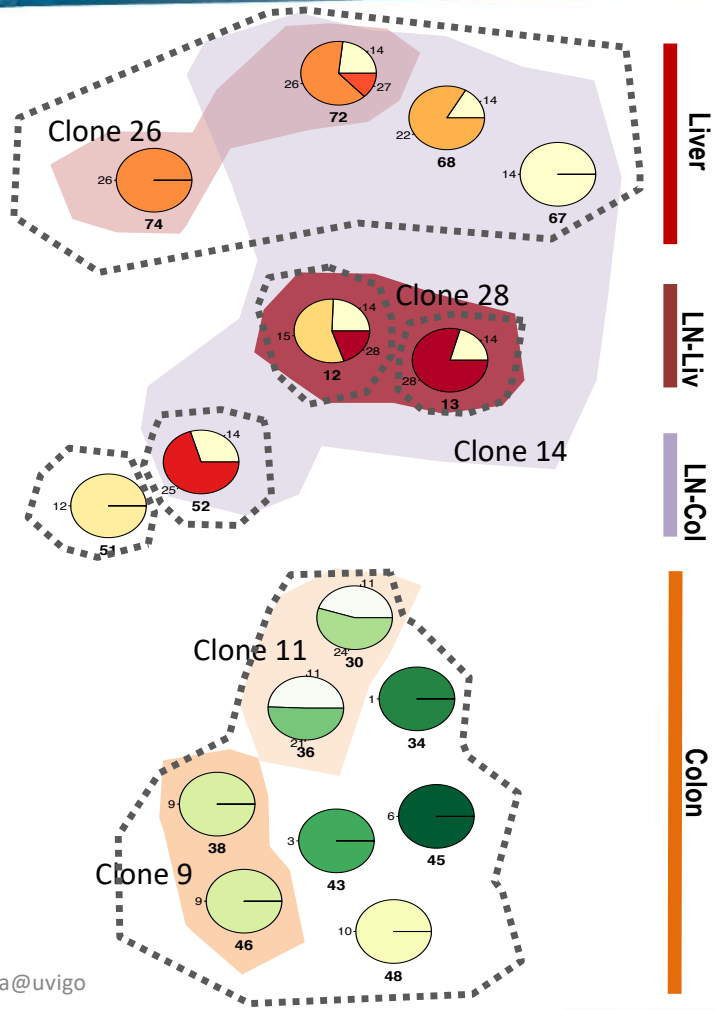


- clonal copy-neutral LOH event of **5q** (***APC***), large deletion of **17p** (***TP53***)…



CN-profiles obtained with **Sequenza**.

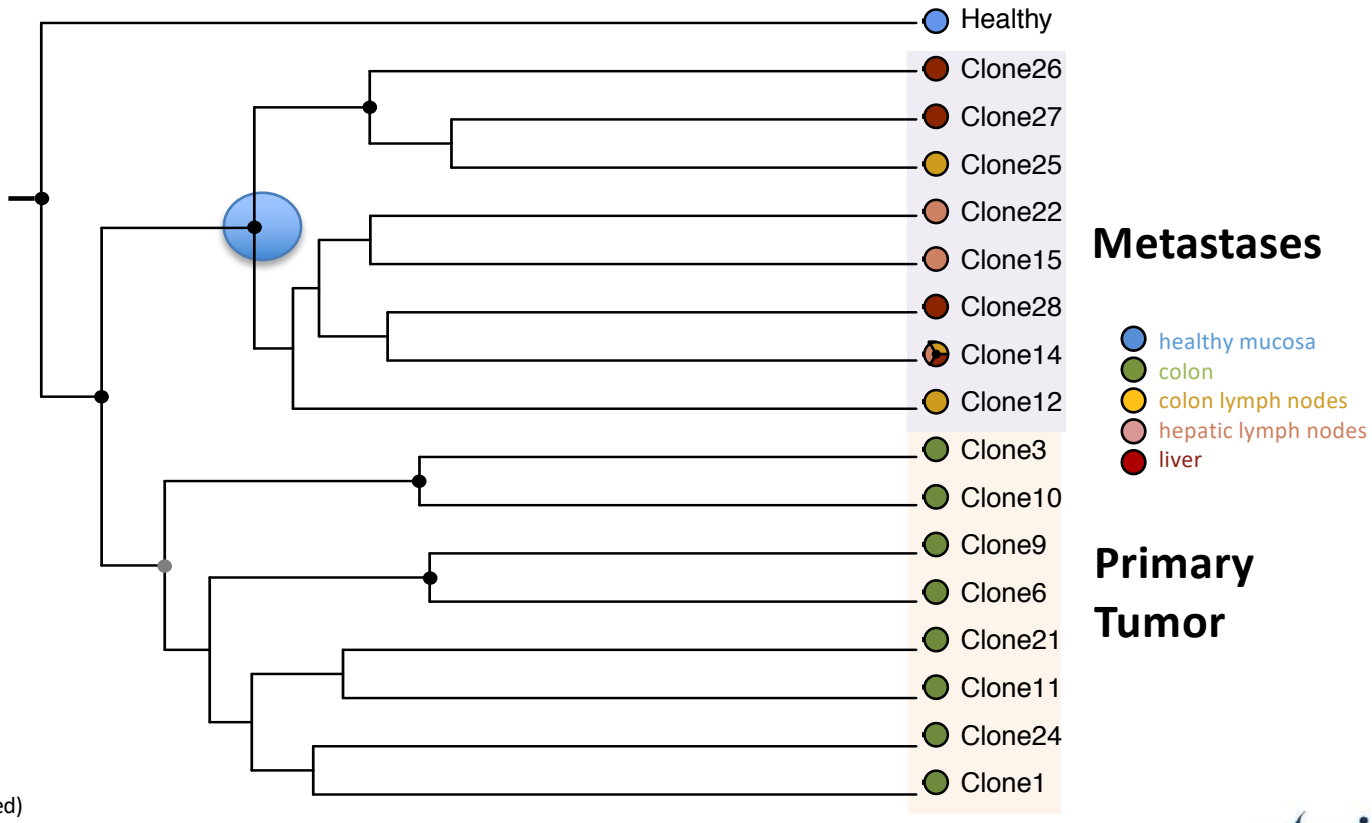
- TVC – 5650 potential SNVs (min VAF 0.02)
- => 16 clones
  - with CloneFinder
  - VAF filter [0.1,0.7]
  - 299 mutations



Clones highlighted (displaying physical movement):

- Clone 14: 6 samples (3 locations)
- Clone 9: 2 samples (1 location)
- Clone 11: 2 samples (1 location)
- Clone 26: 2 samples (1 location)
- Clone 28: 2 samples (1 location)

- Bayesian relaxed clock analysis
- **single origin for all metastases**



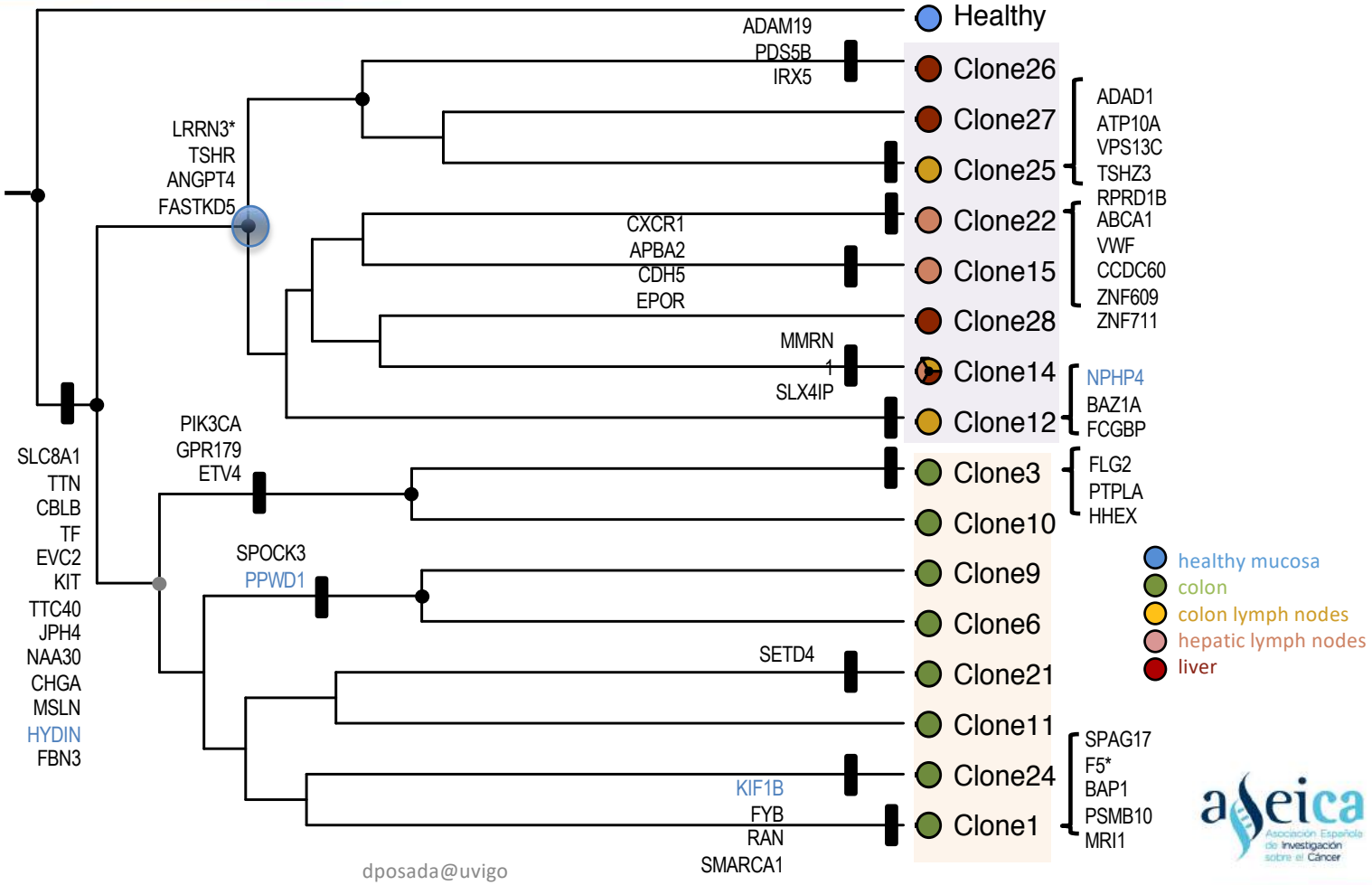
● Node posterior > 0.9
● Node posterior > 0.5

P1CRC Clonal Tree

***BEAST model:**

- Relaxed Exponential
- PopSizeChange
- Jones et al. Rate (Fixed)
- MCC (median heights)

- early and late non-synonymous mutations



Node posterior > 0.9

Node posterior > 0.5

Metastases

Primary Tumor

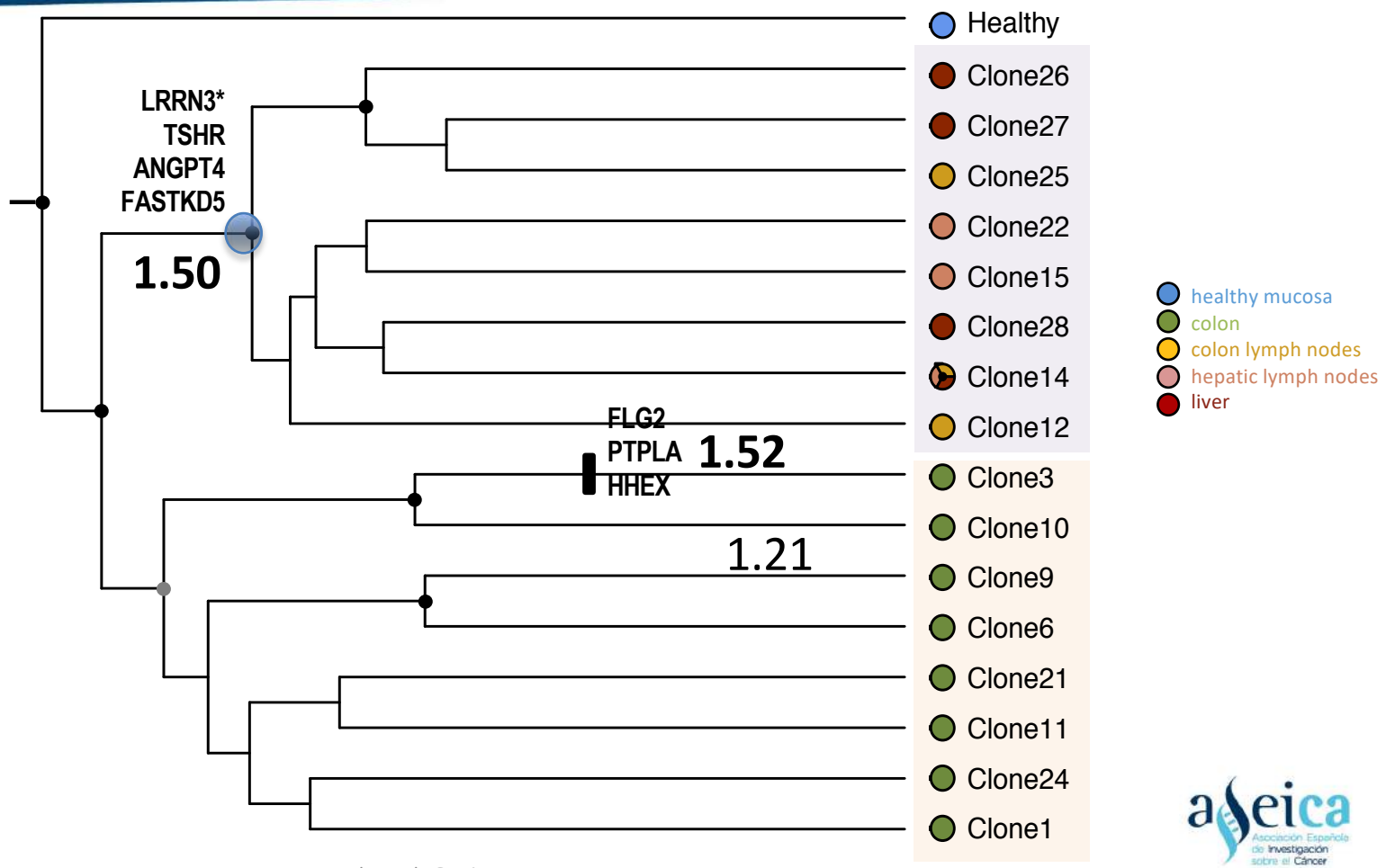
* Two distinct NS mutations affecting the same gene

Missense

Nonsense

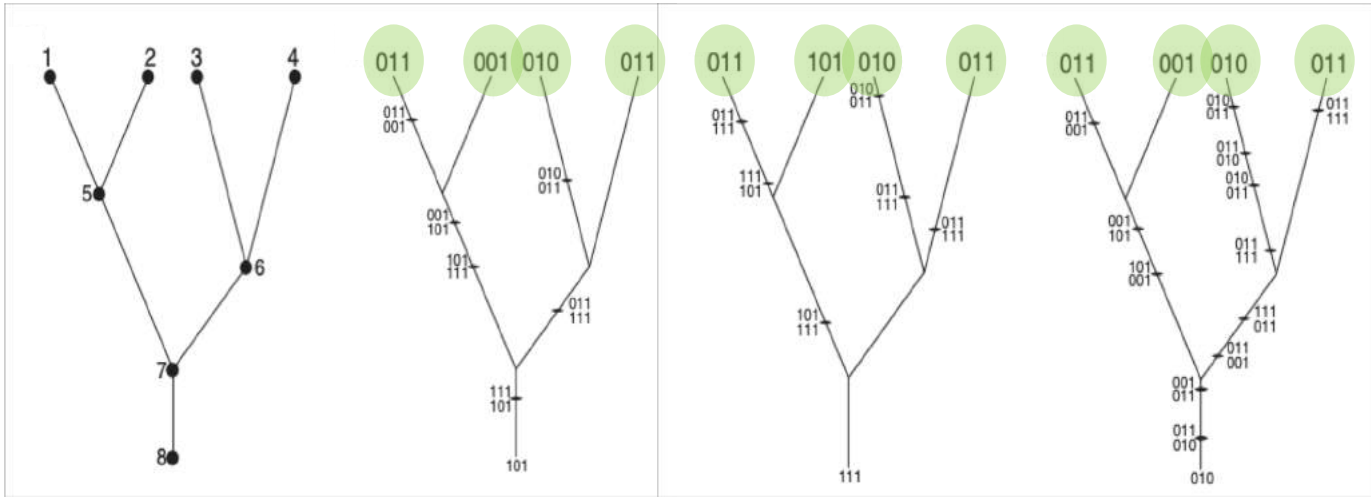
- dN/dS > 1 in some branches
  - positive selection
  - none of them known drivers

- LRRN3: leucine rich repeat neuronal 3
- TSHR: thyroid hormone receptor
- ANGPT4: endothelial growth factor
- FASTKD5: fast kinase domain, mitochondrial stress



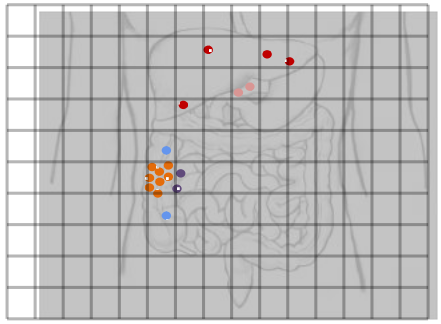
. Bayesian inference of historical biogeography

- BayArea: stochastic mapping + MCMC to sample geographical range evolution histories consistent with the observed ranges.
- => posterior distribution of a given history using a **distance-dependent** colonization approach.

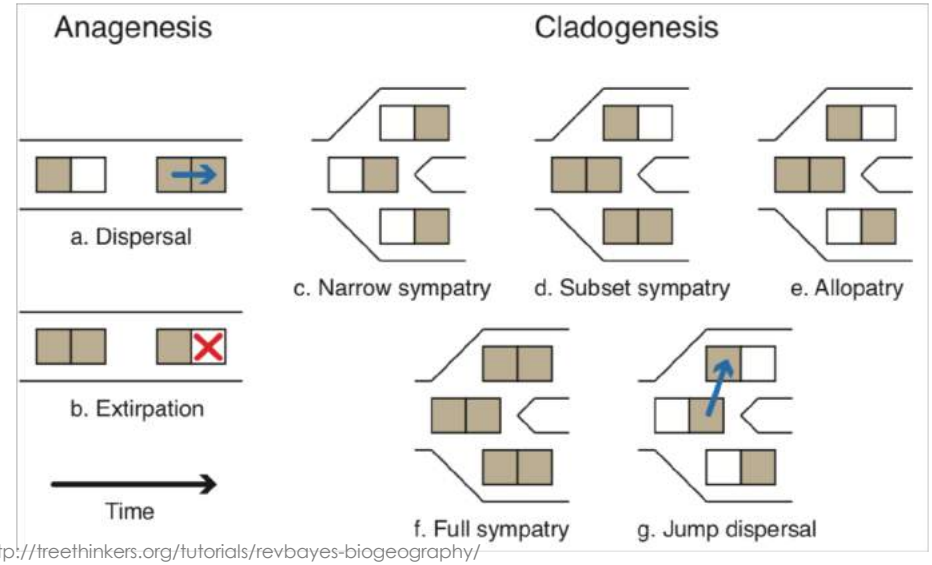


adapted from Landis *et al.* (2013)

"Geographic range" (Absence/Presence) + **"Geographical coordinates" (Latitude & Longitude)**



- colonization patterns: probabilistic **biogeographical** models
  - Dispersal-Extinction-Cladogenesis (DEC) model



http://treethinkers.org/tutorials/revbayes-biogeography/

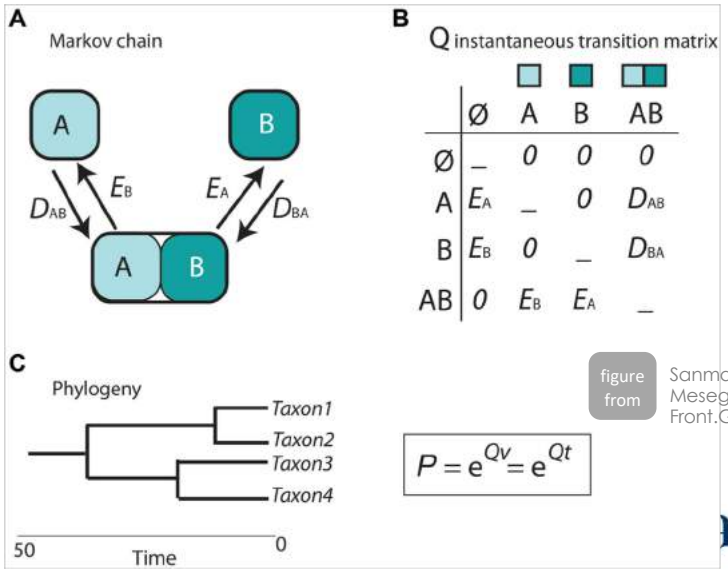


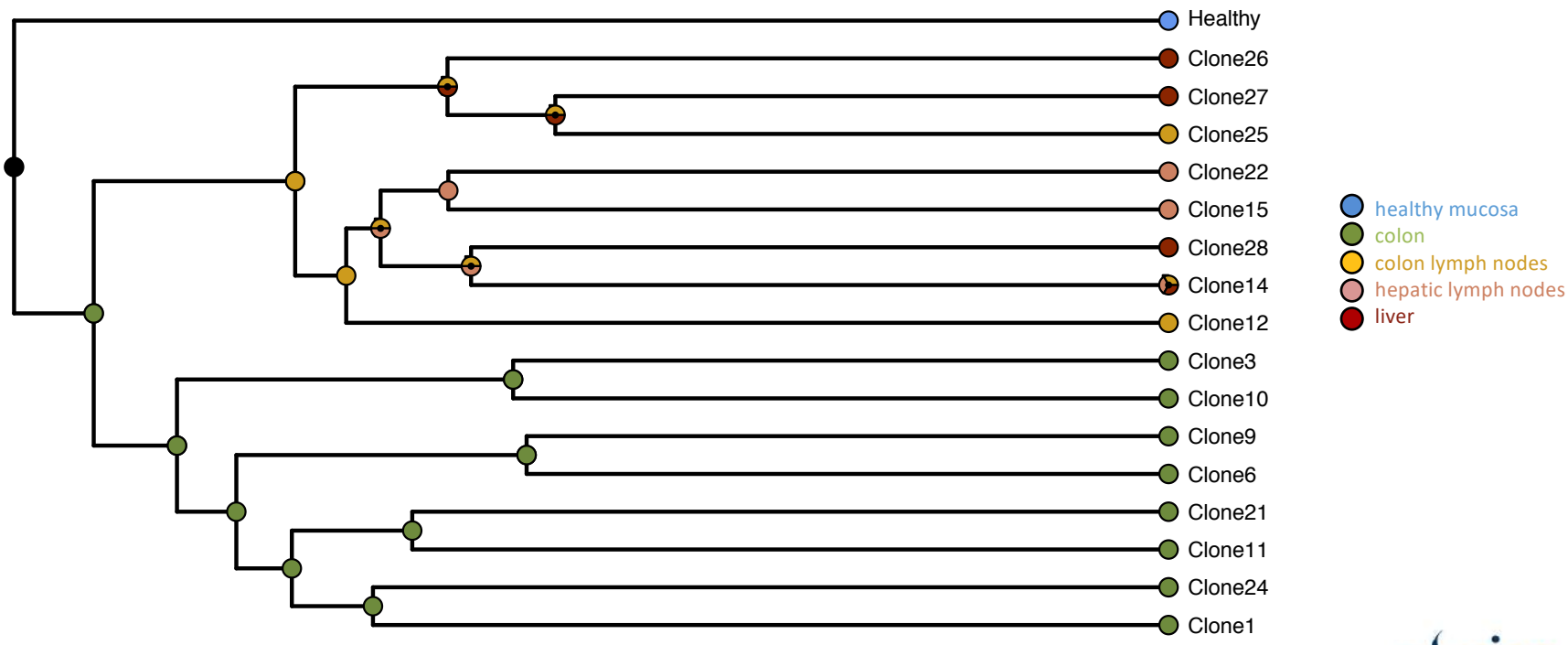
figure from Sanmartin & Meseguer. 2016. Front.Genet..

P1CRC colonization

BayArea:

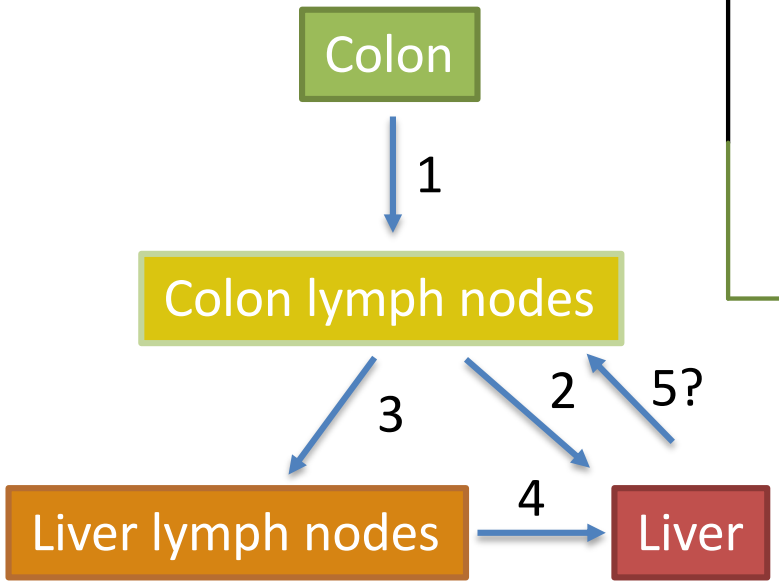
- MCMC: 1500000 (100000 burn-in)
- Model: distance dependent
- Only Posterior Probs > 0.5 shown

CRC01 – Bayarea Postprobs (distance–dependent)

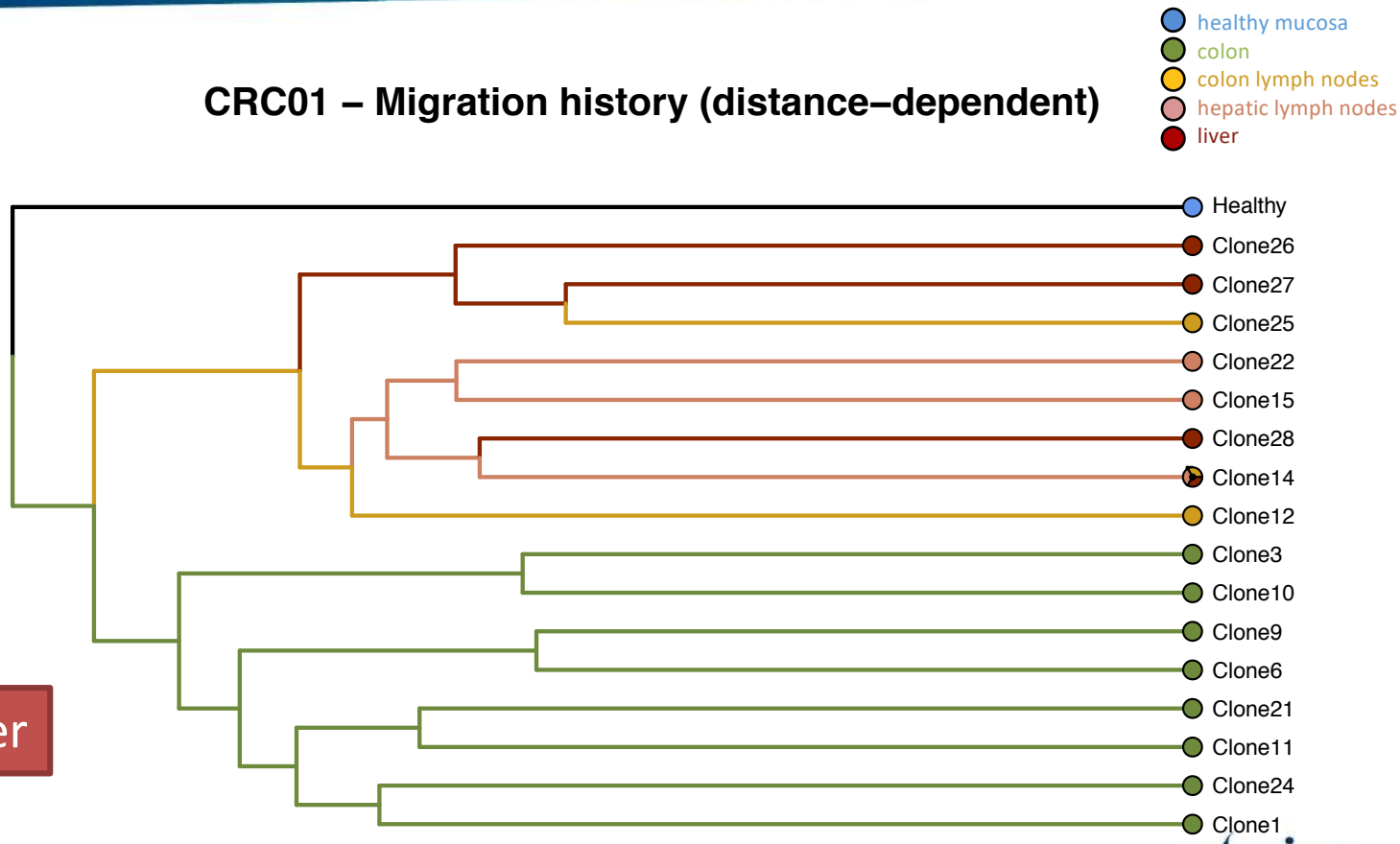


P1CRC Migration History

- Edges colored with location exhibiting higher PP



CRC01 – Migration history (distance–dependent)



.CRC01 dating and demography

BEAST (Bayesian evolutionary analysis by sampling trees)
It can be used as a method of reconstructing phylogenies but is also a framework for testing **evolutionary hypotheses**…

Model Selection
- **2 demographic models:** constant, exponential growth;
- **3 clock models:** strict clock, relaxed exponential & relaxed Log-normal.

Model	Description	MLE	logBF_A	logBF_B	logBF_C	logBF_D	logBF_E	logBF_F
A	Strict Constant	-80049239,15	0,00	-43,25	-37,78	-1,48	-54,63	-54,05
B	RelaxedExp Constant	-80049195,90	43,25	0,00	5,46	41,77	-11,38	-10,80
C	RelaxedLogNormal Constant	-80049201,37	37,78	-5,46	0,00	36,30	-16,84	-16,26
D	Strict PopSizeChange	-80049237,67	1,48	-41,77	-36,30	0,00	-53,15	-52,57
E	RelaxedExp PopSizeChange	-80049184,52	54,63	11,38	16,84	53,15	0,00	0,58
F	RelaxedLogNormal PopSizeChange	-80049185,10	54,05	10,80	16,26	52,57	-0,58	0,00

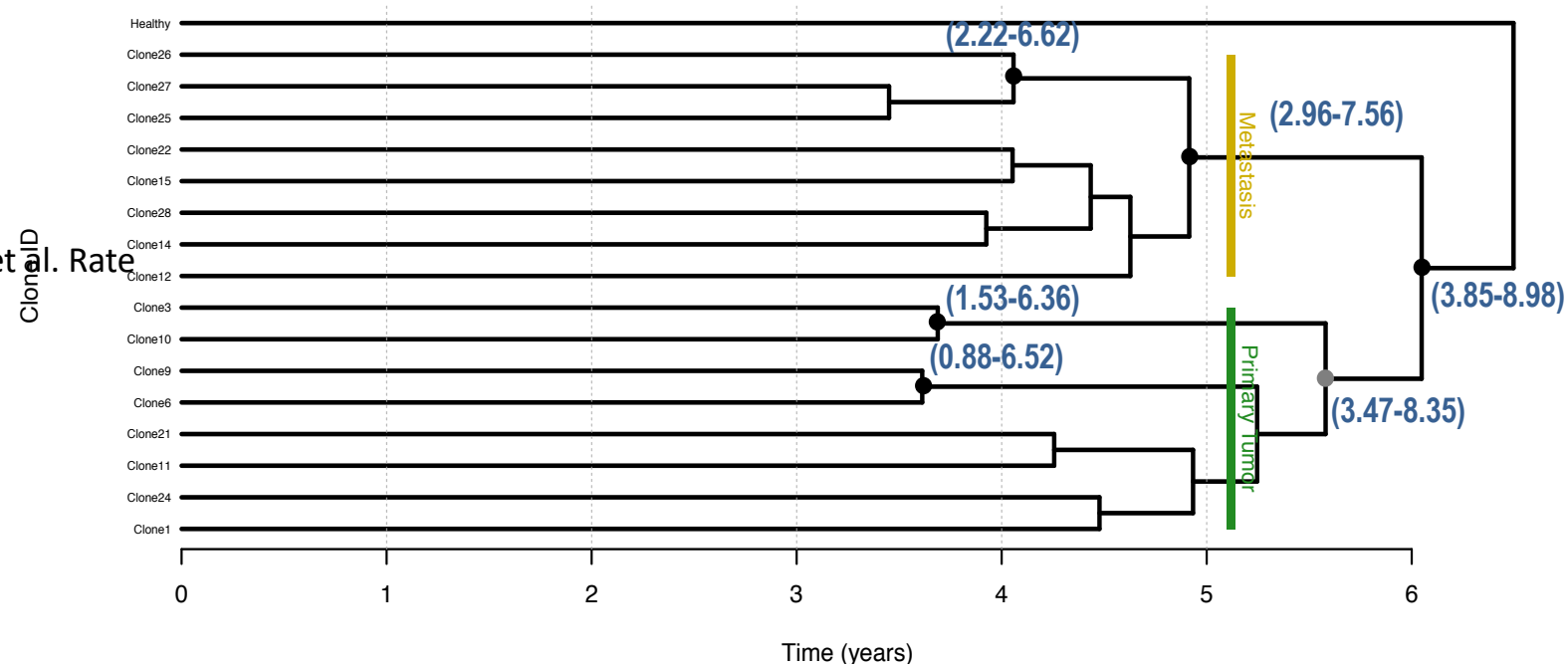
.time: relaxed clock

BEAST model:

- Exponential PopSizeChange
- Relaxed clock
- **4.6 x 10^{-10} Jones et al. 2008**
- MCC (median heights)

Clock conversion:

- 1 cell division = 4 days Jones et al. Rate



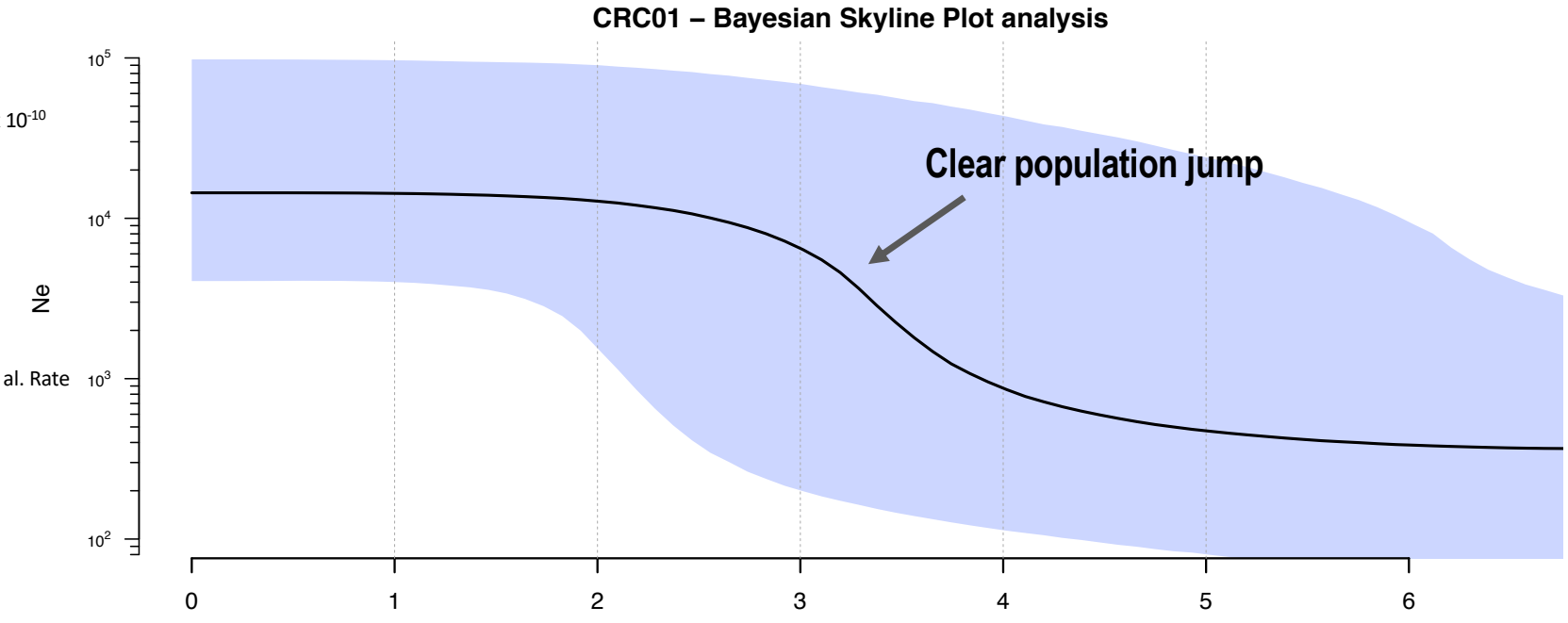
.demography: Bayesian skyline plot

P1CRC Clonal Tree

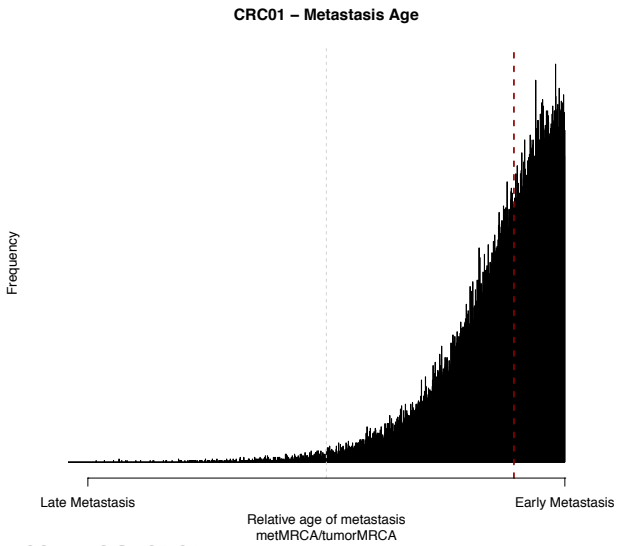
***BEAST model:**
- Relaxed Exponential
- PopSizeChange
- Jones et al. Rate (Fixed) 4.6 x 10^{-10}
- MCC (median heights)

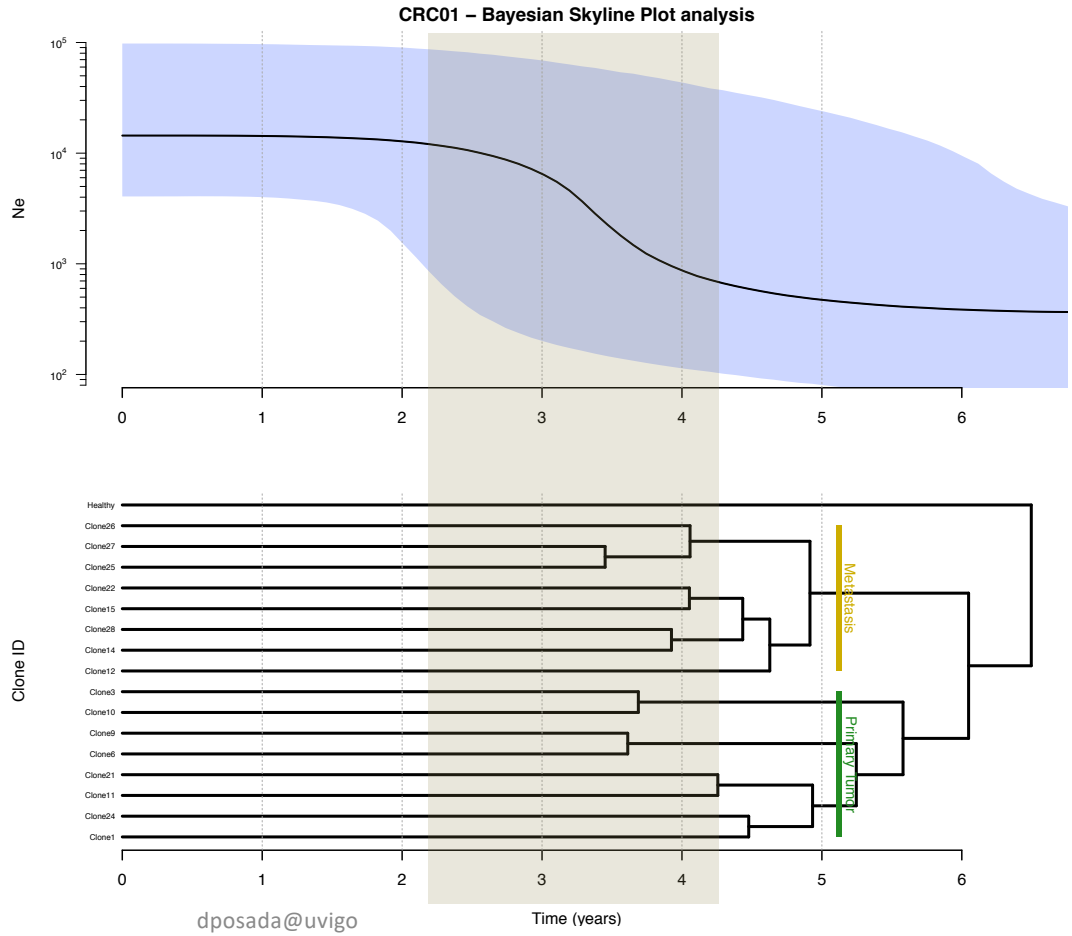
***BEAST BSP:**
- Relaxed Exponential
- PopSizeChange
- Jones et al. Rate (Fixed)
- Bins = 100

Clock conversion:
- 1 cell division = 4 days Jones et al. Rate

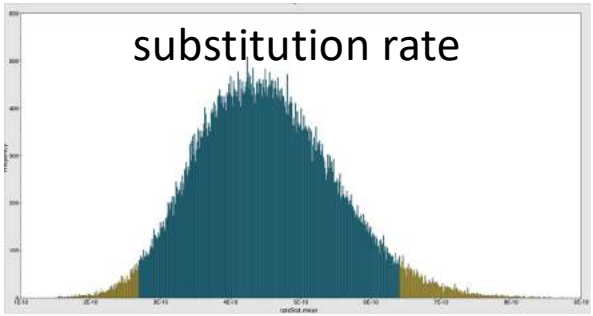


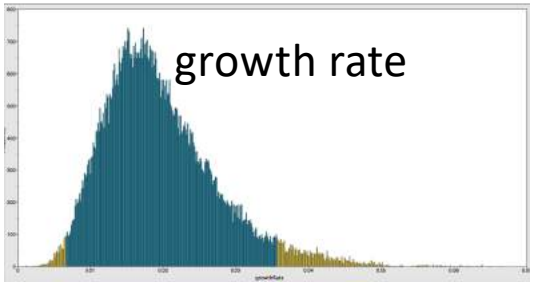
- early metastasis before main tumor growth/diversification

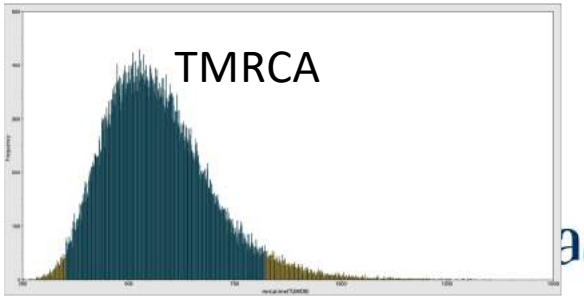


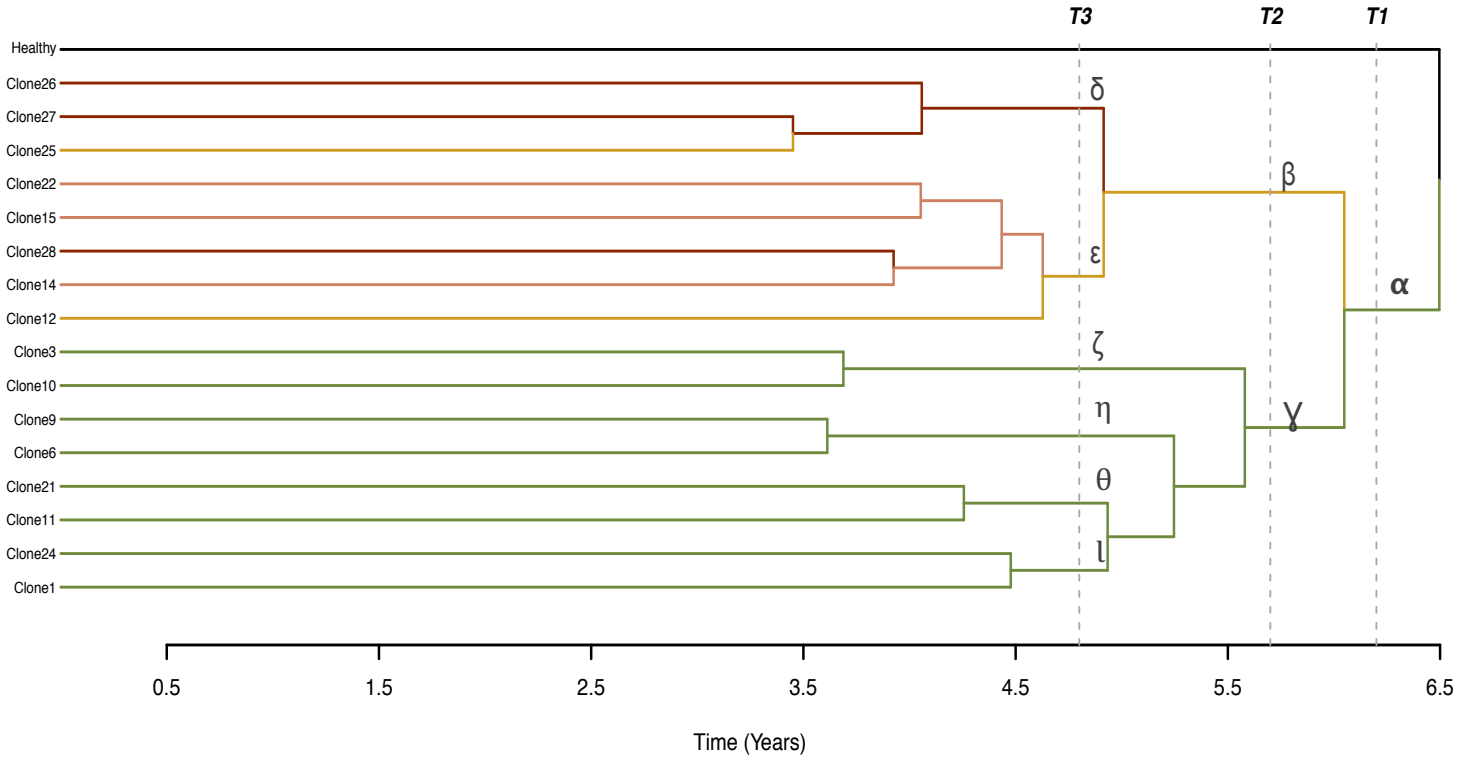


- **substitution rate**: 4.5e-10 [2.68e-10, 6.41e-10]
- **exponential growth rate**: 0.0196 [0.0066, 0.0357]
  - doubling-time ~ 138 days
- **TMRCA**: 6.23 years [3.86, 8.98]








T3
T2
T1
Healthy
Clone26
Clone27
Clone25
Clone22
Clone15
Clone28
Clone14
Clone12
Clone3
Clone10
Clone9
Clone6
Clone21
Clone11
Clone24
Clone1
δ
β
ε
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ζ
η
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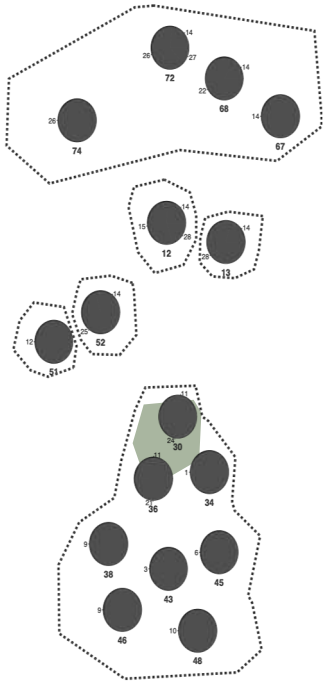
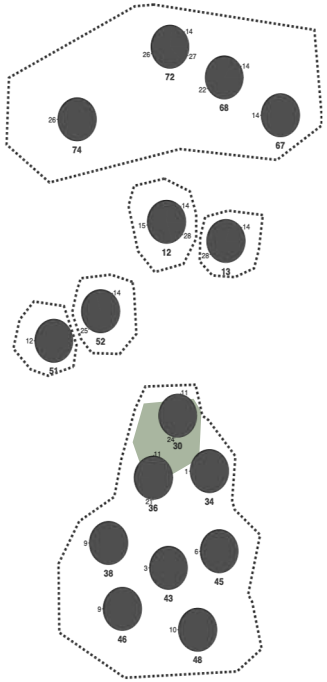
4.5
5.5
6.5
Time (Years)

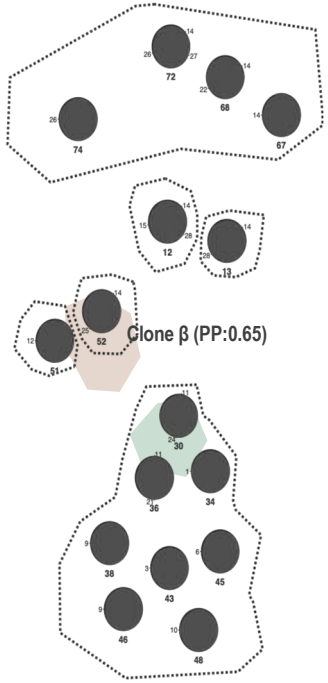
6.2 years



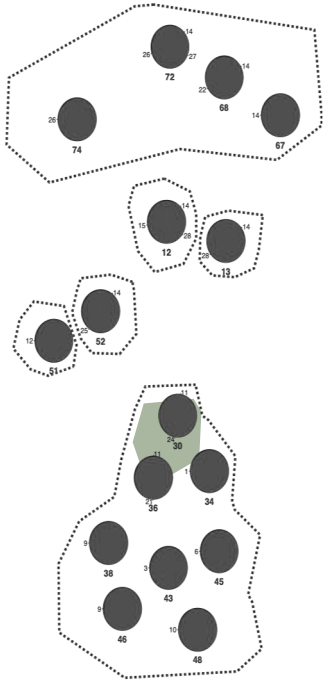
6.2 years



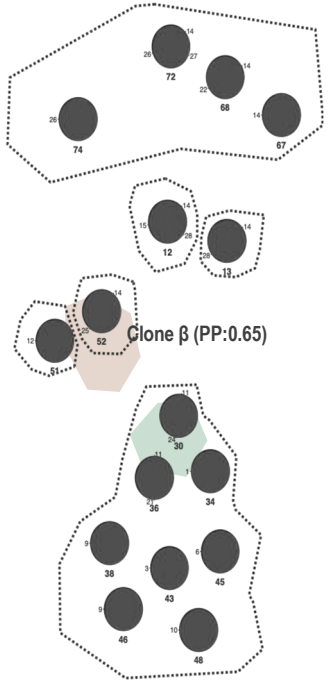
5.7 years



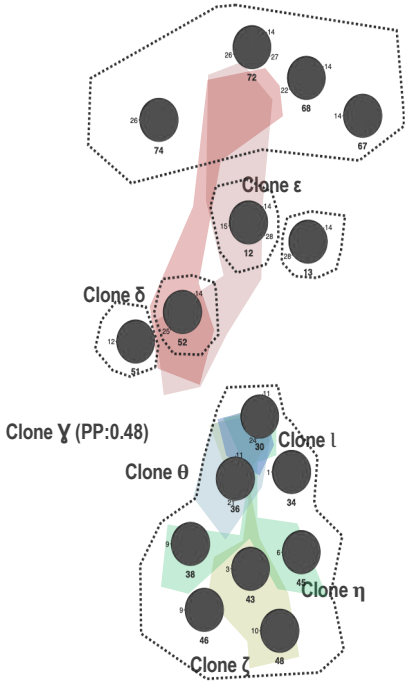
6.2 years

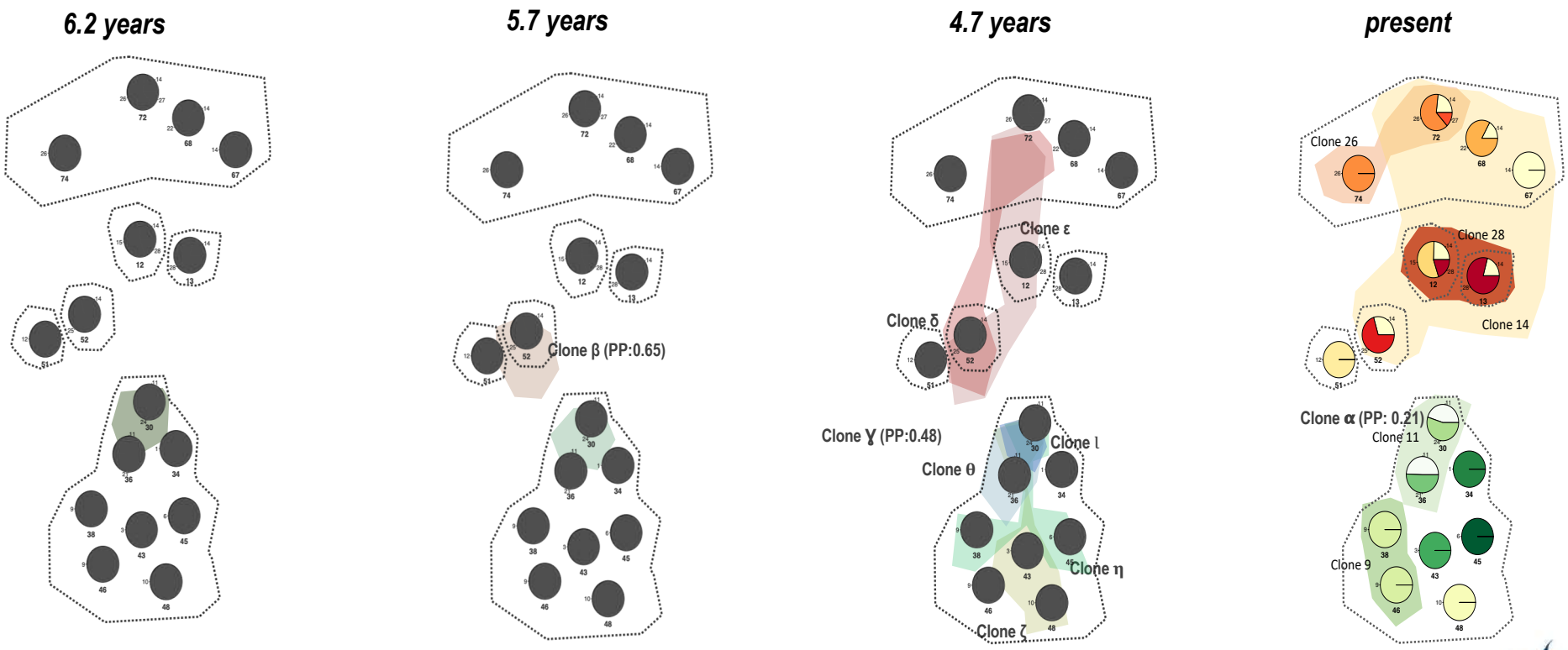


5.7 years



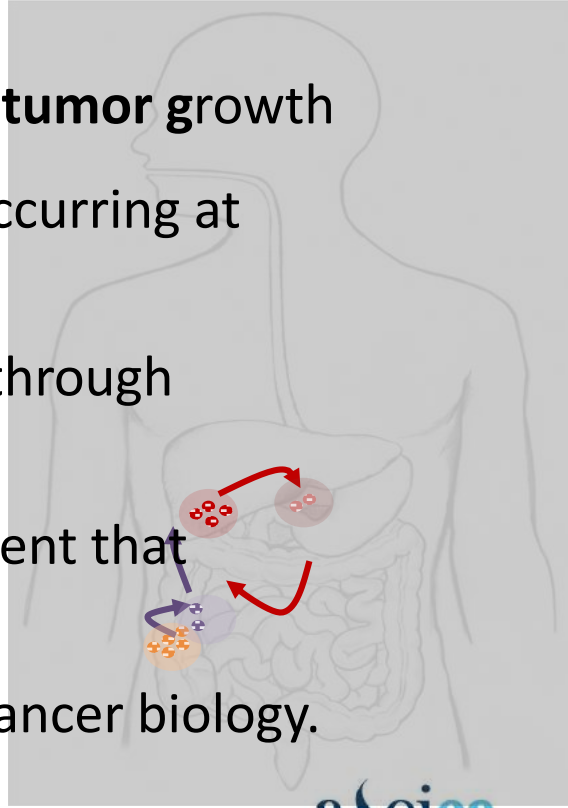
4.7 years




6.2 years
5.7 years
4.7 years
present
Clone β (PP:0.65)
Clone ε
Clone δ
Clone γ (PP:0.48)
Clone ι
Clone θ
Clone η
Clone ζ
Clone 26
Clone 28
Clone 14
Clone α (PP: 0.21)
Clone 11
Clone 9

.take home

1. in CRC, **metastasis can occur very early, linear, and before tumor g**rowth
2. we need to pay much attention also to **genomic changes** occurring at that time, before population expansion
3. spread to other organs could happen more than once and through **particular colonization routes**
4. we can measure **specific evolutionary parameters** in a patient that *might* be of clinical interest
5. **evolutionary biology** provides a complementary angle to cancer biology.



samples

- Jose M Cameselle (CRC) [CHUVI]
- Carmen Loureiro (CLL) [CHUVI]
- Tumor biobanks [CHUS, CHUVI]


NGS tumors

- Sonia Prado (single-cell)
- Nuria Estévez (single-cell)
- Pilar Alvariño (technician)



NGS analysis

- Joao Alves (NGS)
- Tamara Prieto (NGS)
- Laura Tomás (NGS)
- Jose Luis Soto (IHC, methyl.)
- Alberto Vicens (dN/dS)





simulations

- Andrés Pérez-Figueroa (popgen)
- Harald Detering (clonal sims)


collaborators

- Diana Valverde [UVIGO]
- Manuel Megías [UVIGO]


erc European Research Council

GOBIERNO DE ESPAÑA · MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

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phylocancer

XUNTA DE GALICIA

Universida de Vigo

Universida de Vigo cinbio

aseica Asociación Española de Investigación sobre el Cáncer

.thanks!!

“everything existing in the universe is the fruit of chance and necessity”

. Democritus (c. 460 b.C. – c. 370 b.C.)

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