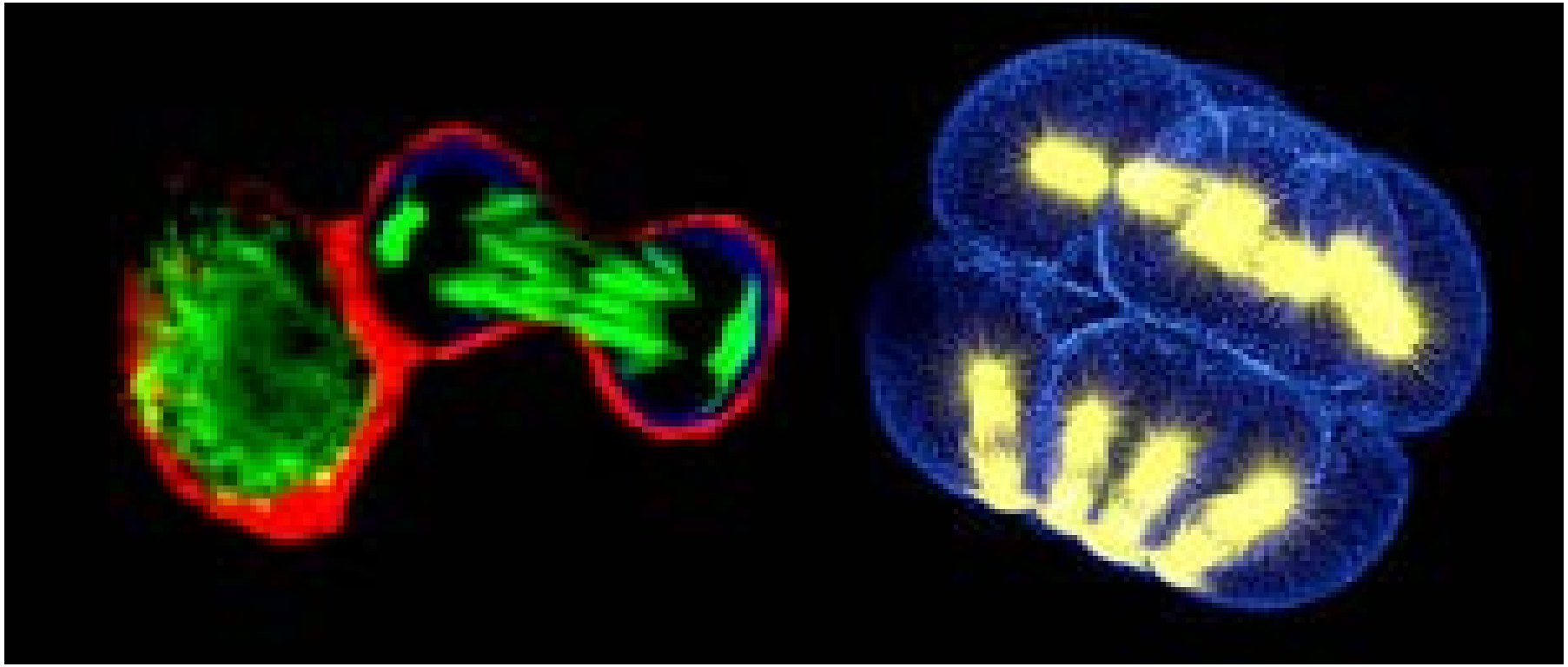
T cell fate determination



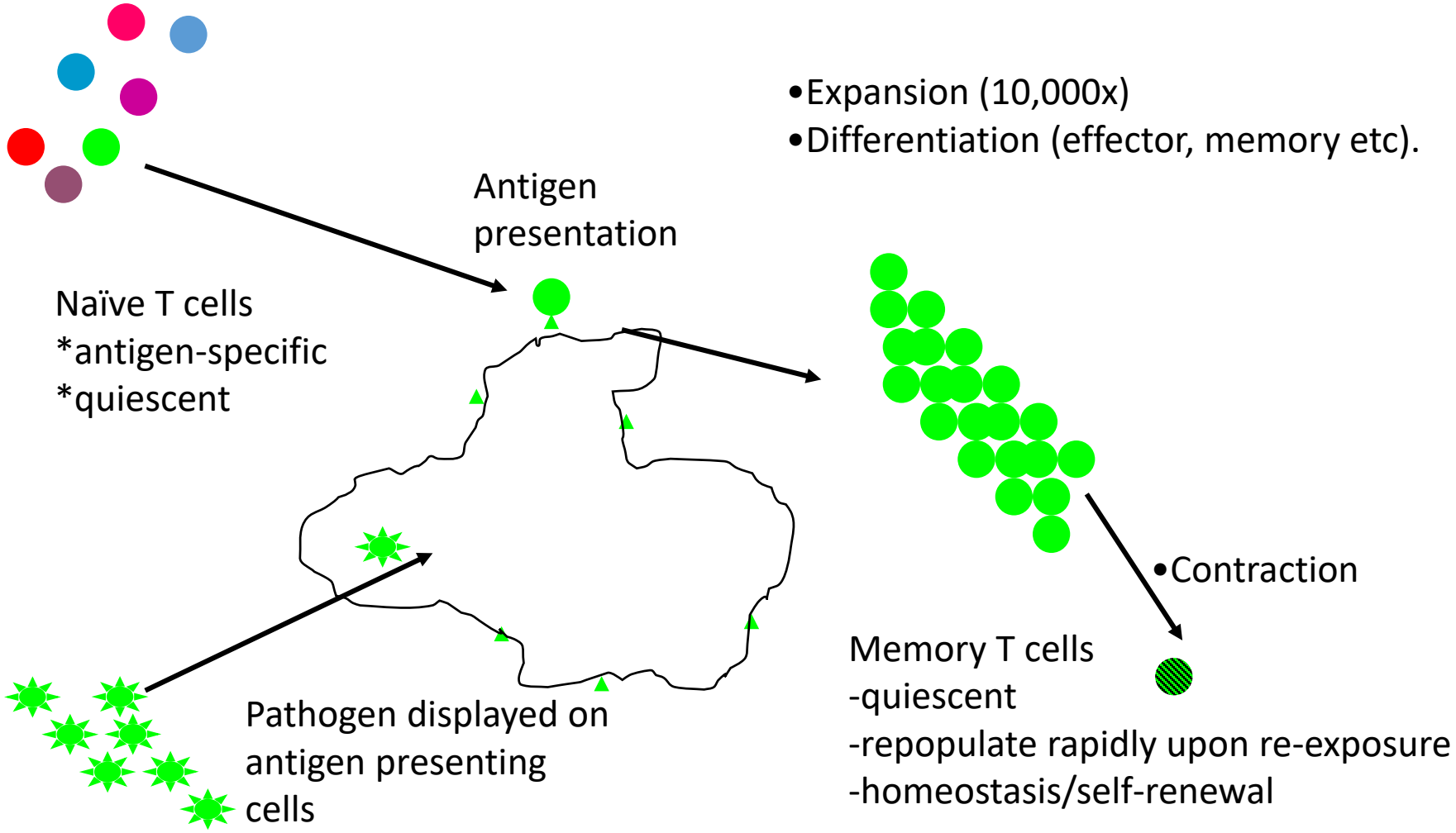
Sarah Russell

Peter MacCallum Cancer Centre

& Swinburne University of Technology

- 7up.mp4-00.01.33.000-00.02.04.355.mp4

Cell fate determination in immune responses.



Known knowns and known unknowns

- One clone can produce effector and memory cells
- Heterogeneity in proportion of effector and memory
- Heterogeneity in size of response

- N->E->M or N->M->E?????
- Is variability determined or random?
- If determined, at what stage?

The problem: Tracking cell fate changes

- Population-based analyses.
- Snapshots in time.
- Use of ever-changing ‘markers’ to badge cells.

Expansion and differentiation
of naive to effector and memory,
followed by contraction of effector cells.

Arguments in the T cell field – deterministic vs random?

OPINION

Lymphocyte fate specification as a deterministic but highly plastic process

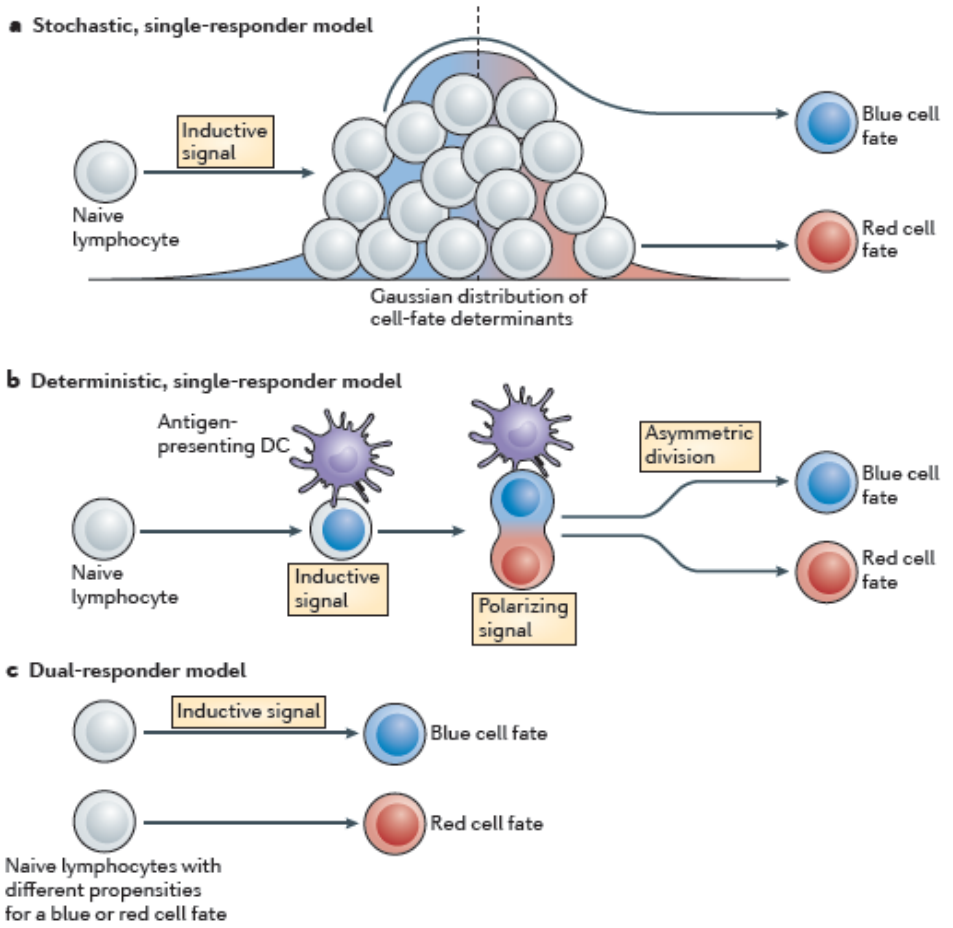
Steven L. Reiner and William C. Adams

Nature Reviews Immunology | AOP, published online 5 September 2014; doi:10.1038/nri3734

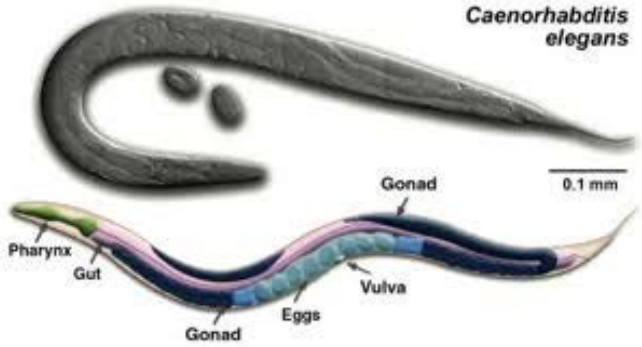
Why the immune system takes its chances with randomness

Philip D. Hodgkin, Mark R. Dowling and Ken R. Duffy

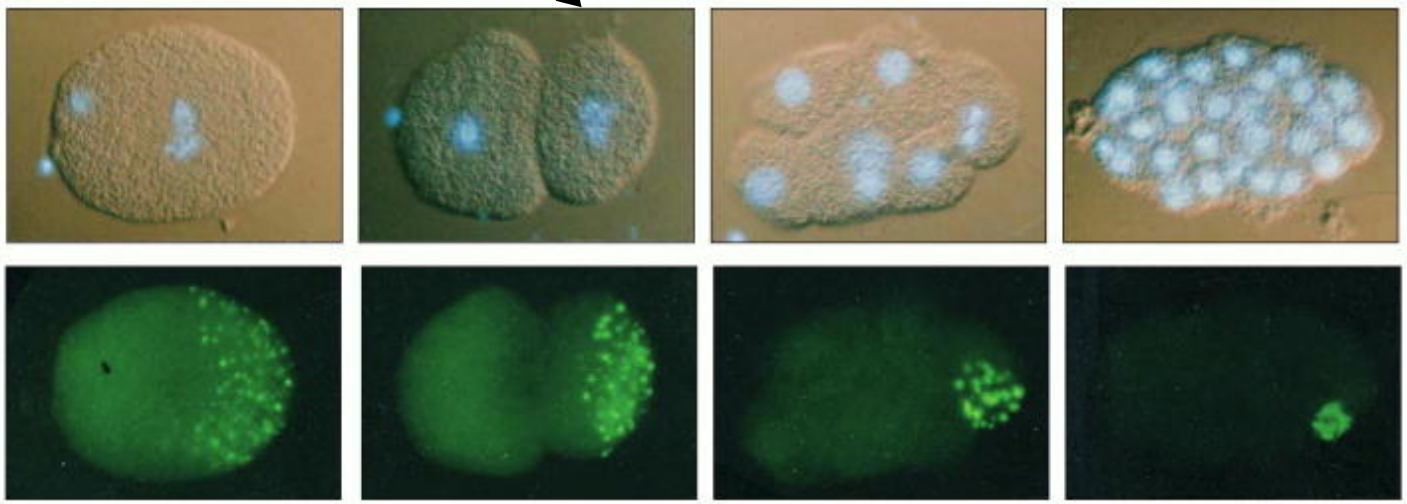
unlike them, however, we are gamblers, suspecting that the immune system does play a game of chance, albeit with the rules having evolved so that the odds are stacked in our favour.



Cell differentiation – a defining characteristic of multicellular organisms

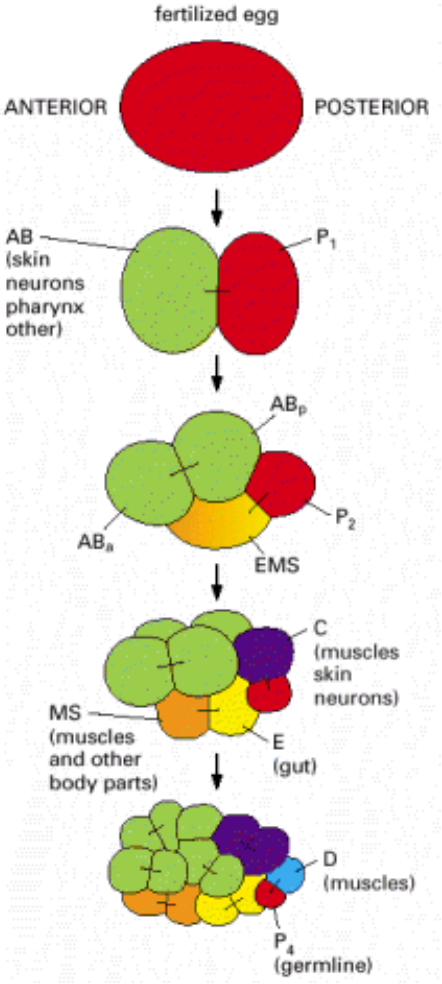


Asymmetric Cell Division (ACD)

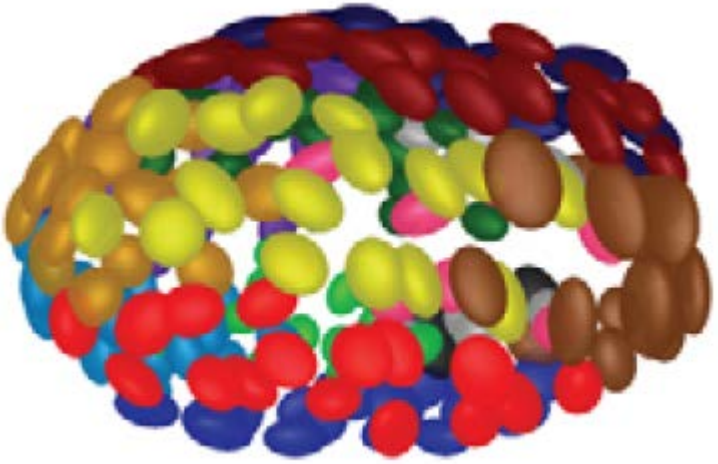


C.Elegans development
Strome, Wood. Cell 1983

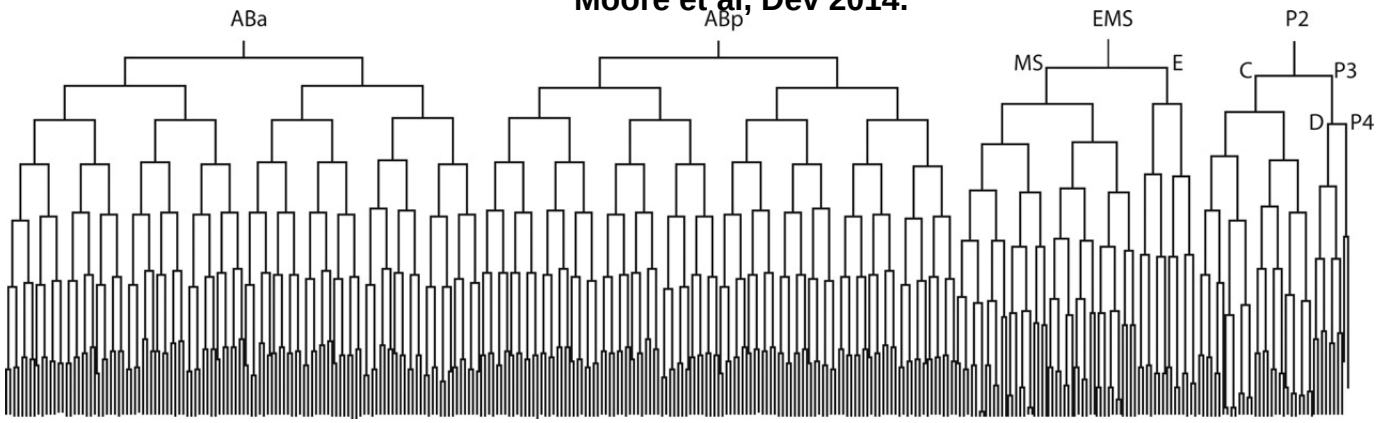
Single cell pedigree analysis.



Kemphues, Cell 2010

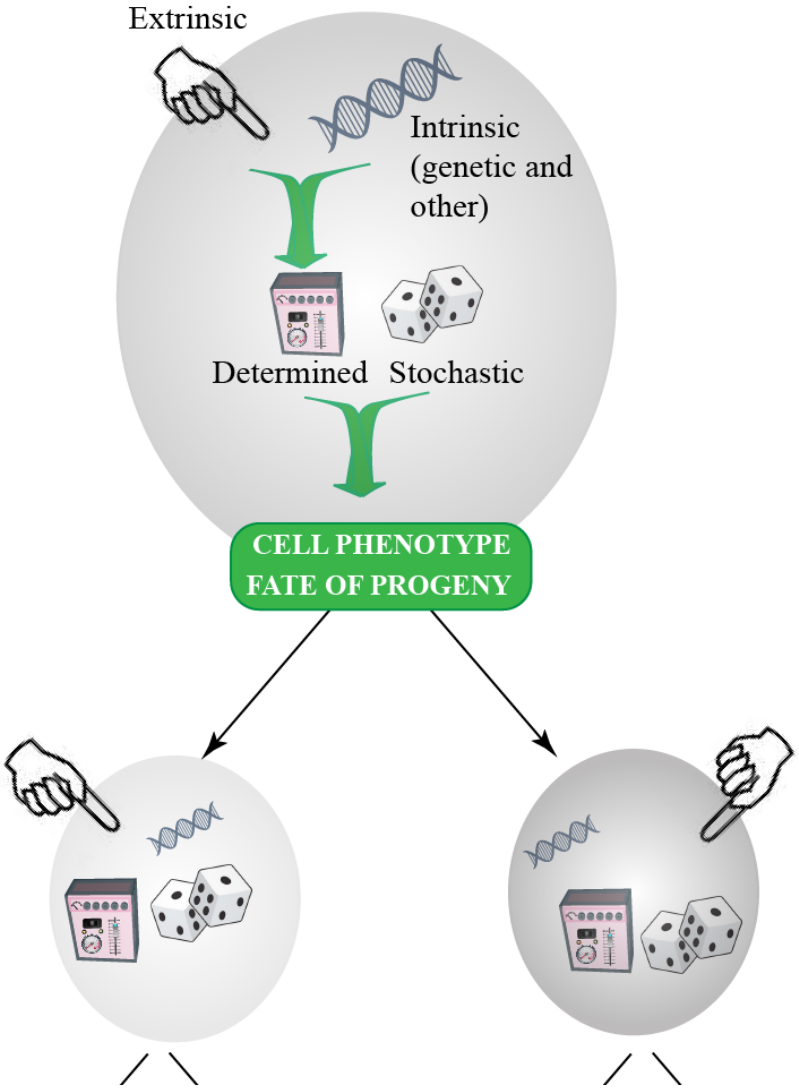


Moore et al, Dev 2014.

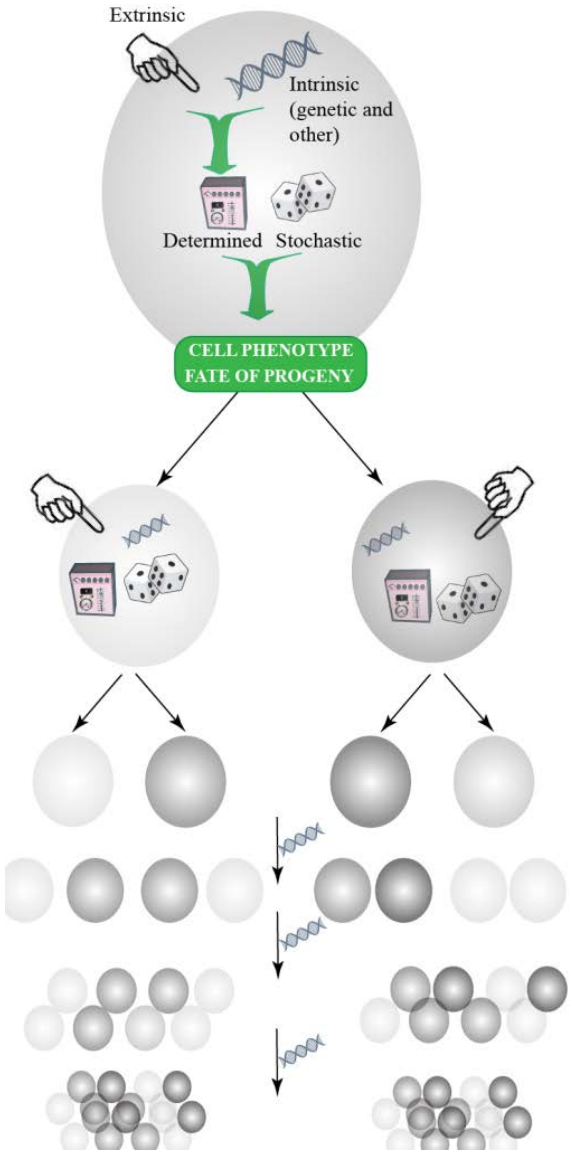


Bao Z et al. PNAS 2006

Extrinsic and intrinsic inputs, and stochasticity exert influences of varying degree and duration

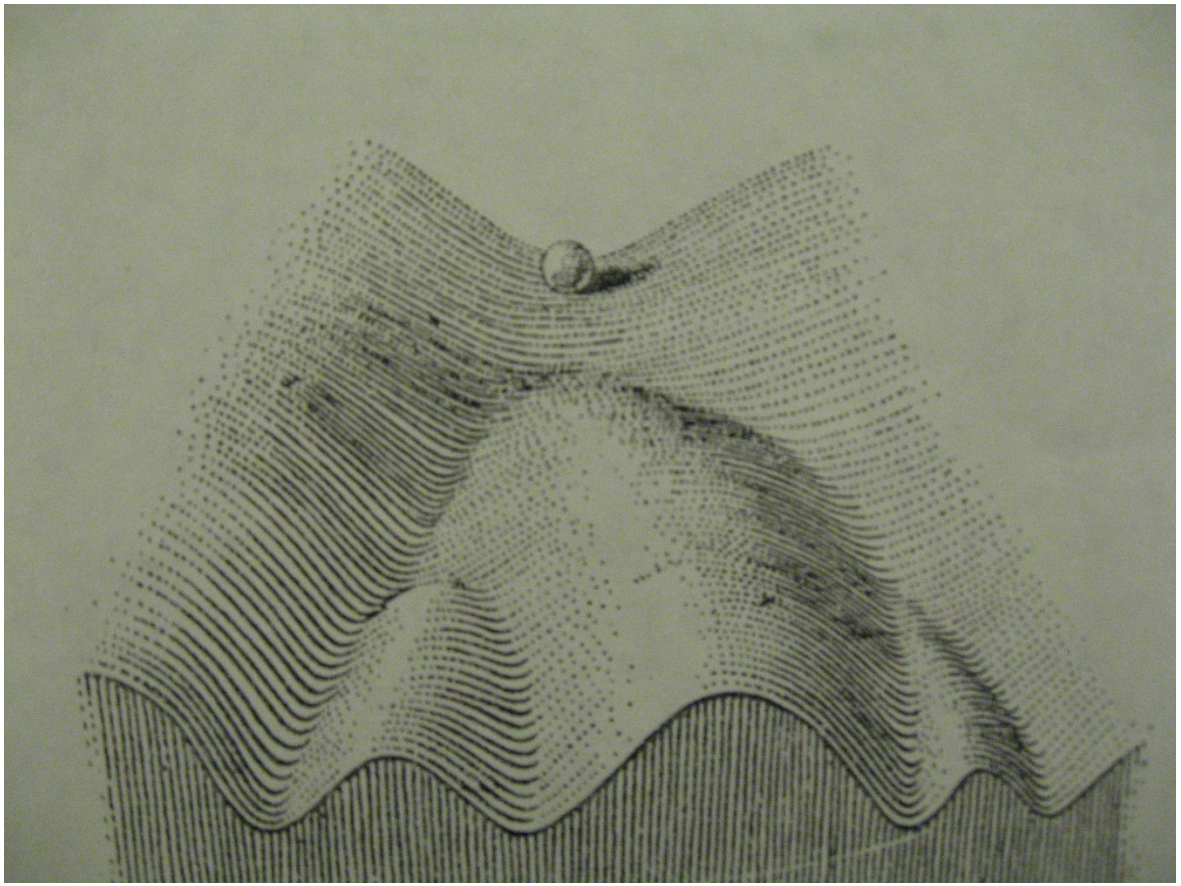


Cell fate over generations



- Duration of programming (seconds to generations)
  -cell state (phenotypic, metabolomics etc)
  -genetic
  -epigenetic
- Congruence of events (eg. an extrinsic input only has impact if it coincides with stochastically controlled expression of a signalling component.)
- Relative influence of inputs.
- Transmission to daughters.

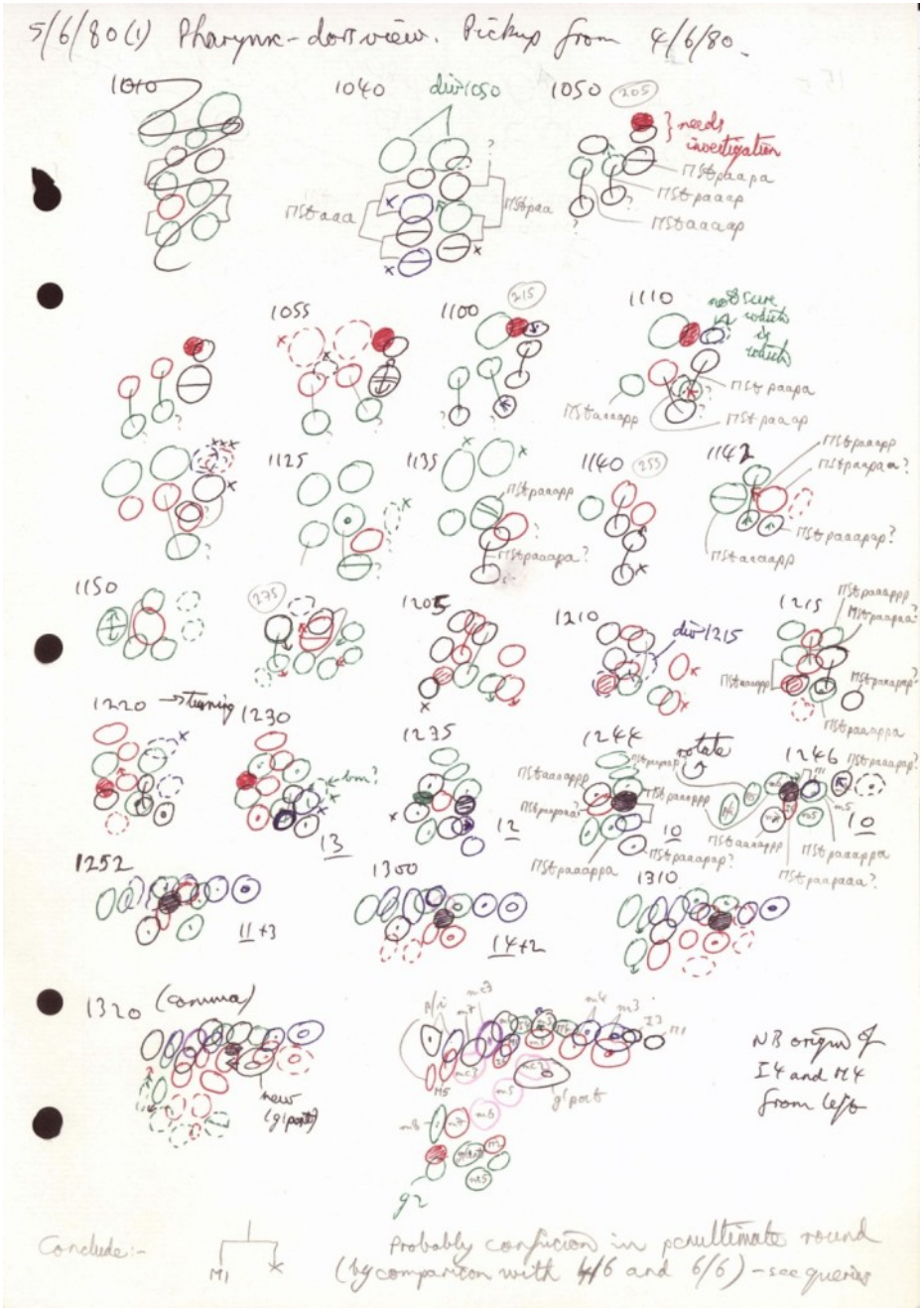
Waddington's Landscape



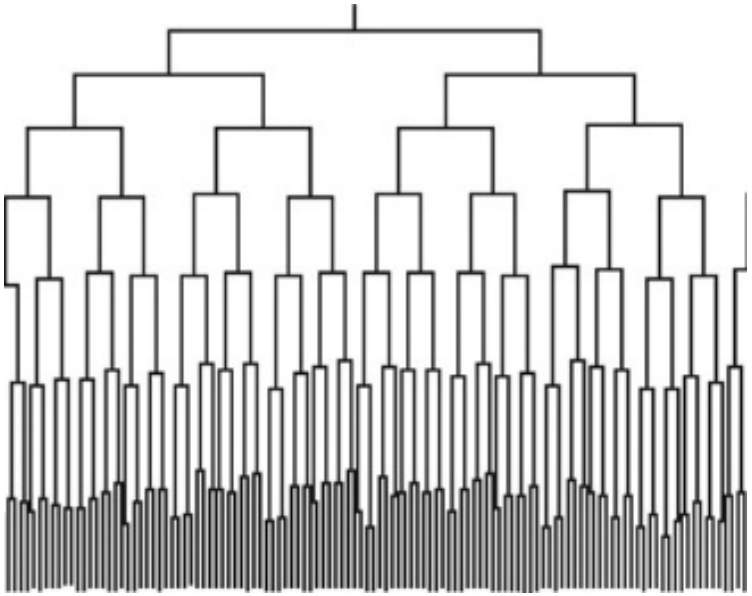
The Strategy of the Genes Conrad Waddington (Allen and Unwin, 1957)

Expansion and differentiation
of naive to effector and memory,
followed by contraction of effector cells.

WE NEED TO WATCH THE PROCESS AS IT UNFOLDS.



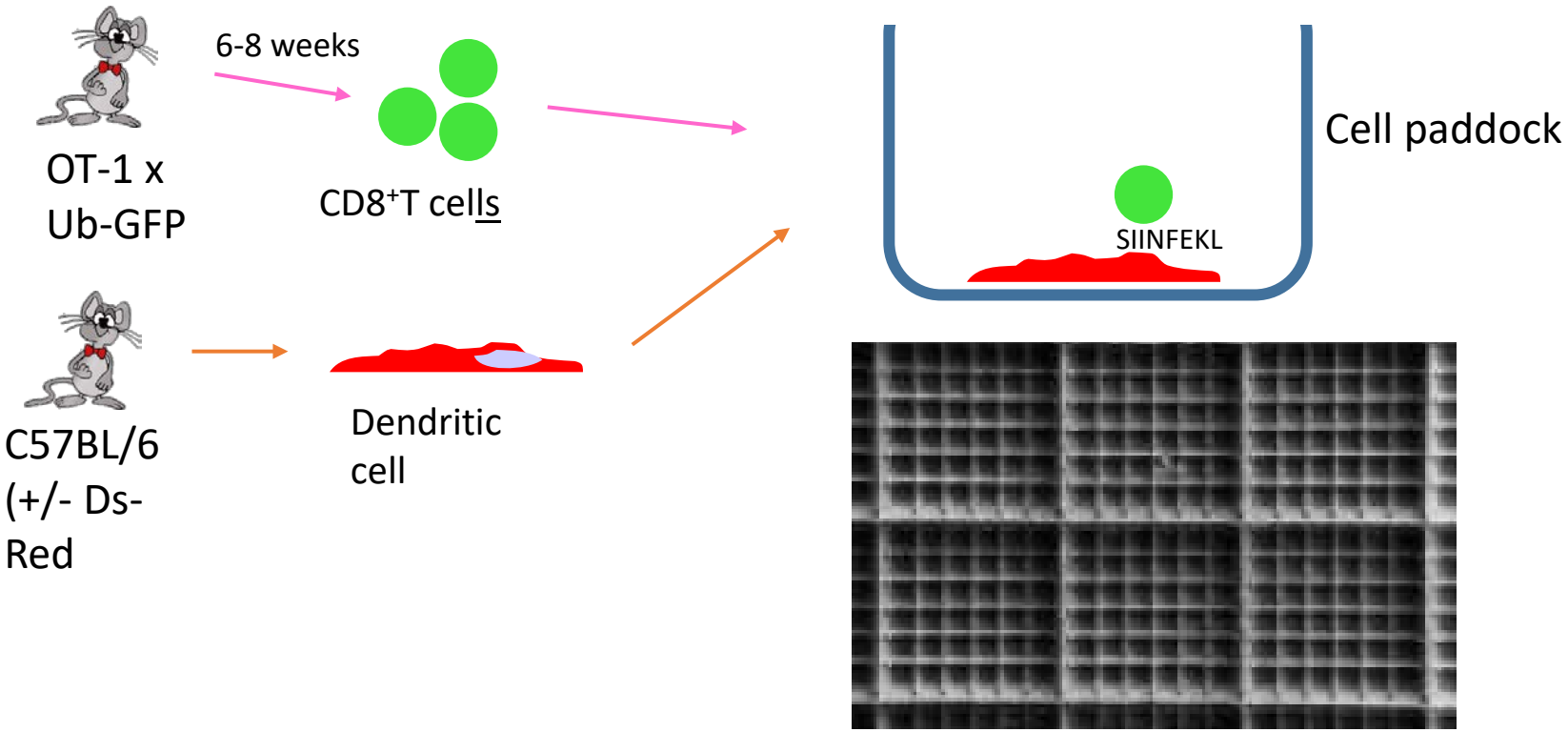
John Sulston-worm-notebook-page-1980 Wellcome Trust



Two major problems for T cells

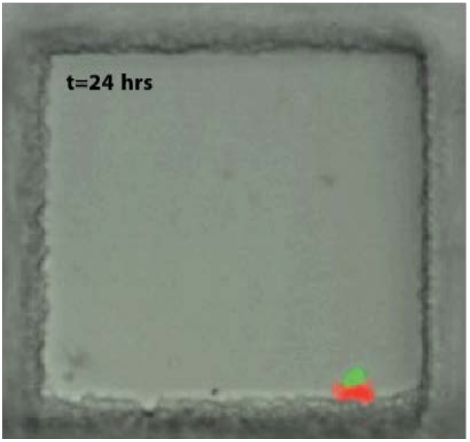
- Pedigrees are not invariant
- Cells migrate

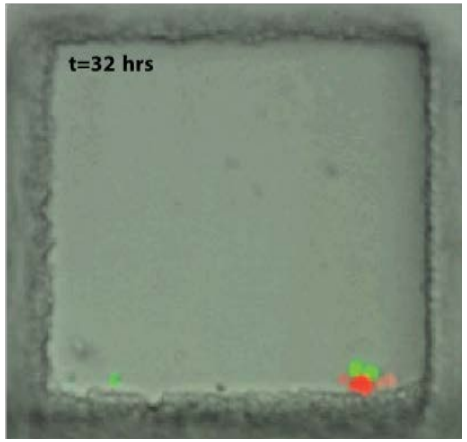
In vitro system to study CD8 T cell activation and differentiation.

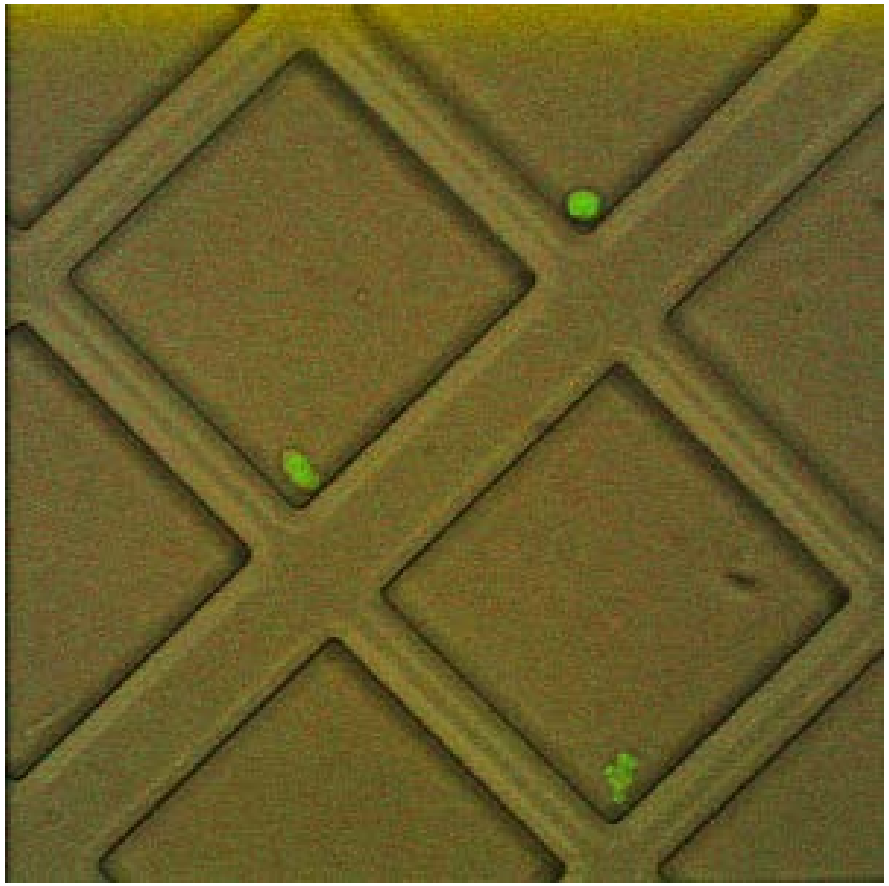


Day et al, ICB 2009

Tracking fate from the first daughter



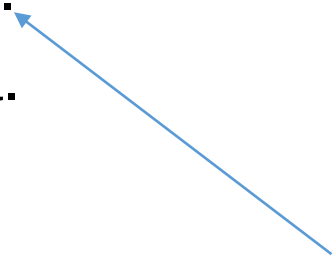




Mohammed Yassin

TACTICS – toolbox for image analysis

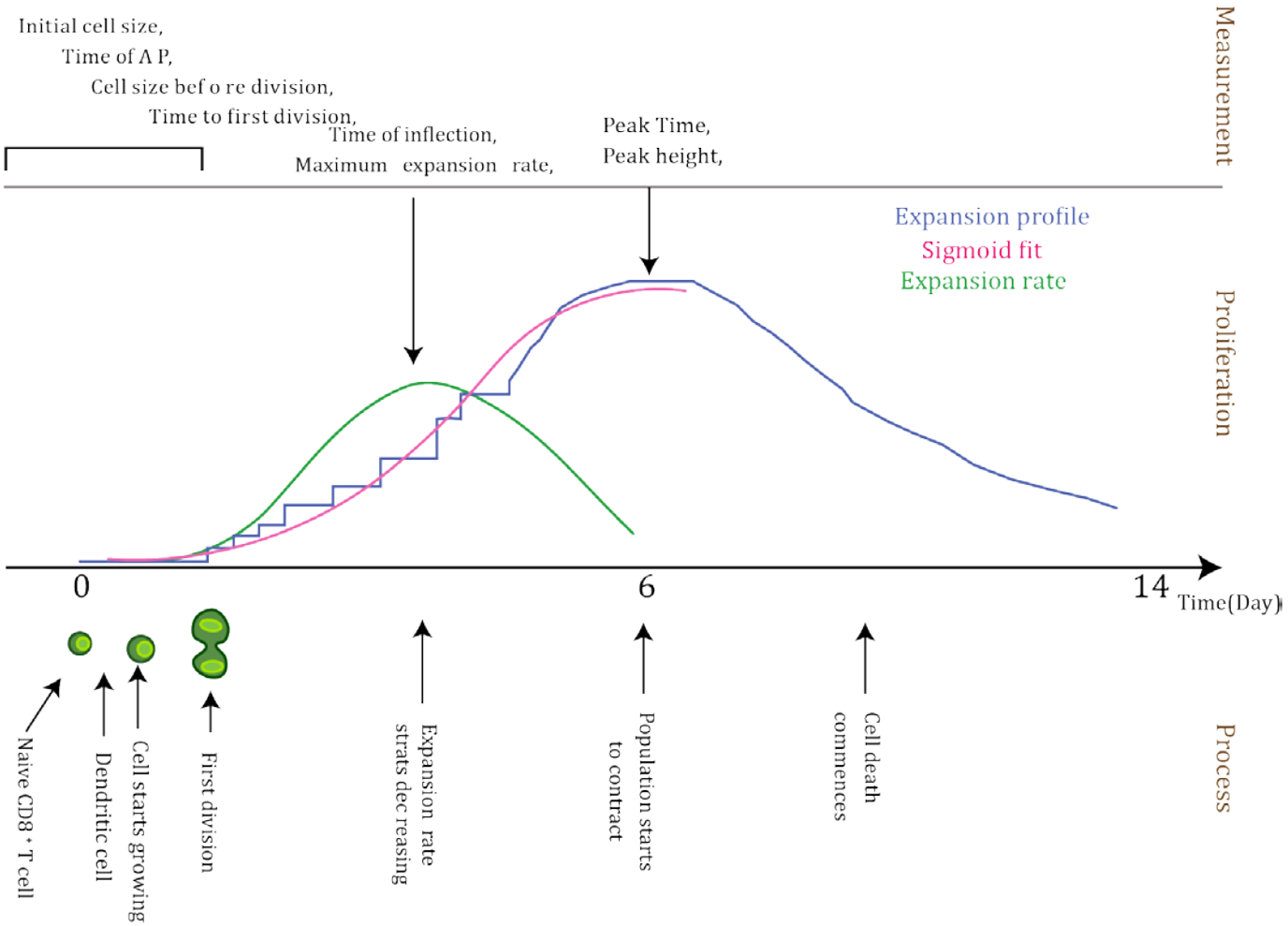
- Cell segmentation and tracking during time lapse imaging.
- Quantification of fluorescent intensities, localisation etc and cell size, morphology.
- High throughput but with quality control
  – Manual correction capacity.
- Ability to interrogate the data.
- Assembly of pedigrees.



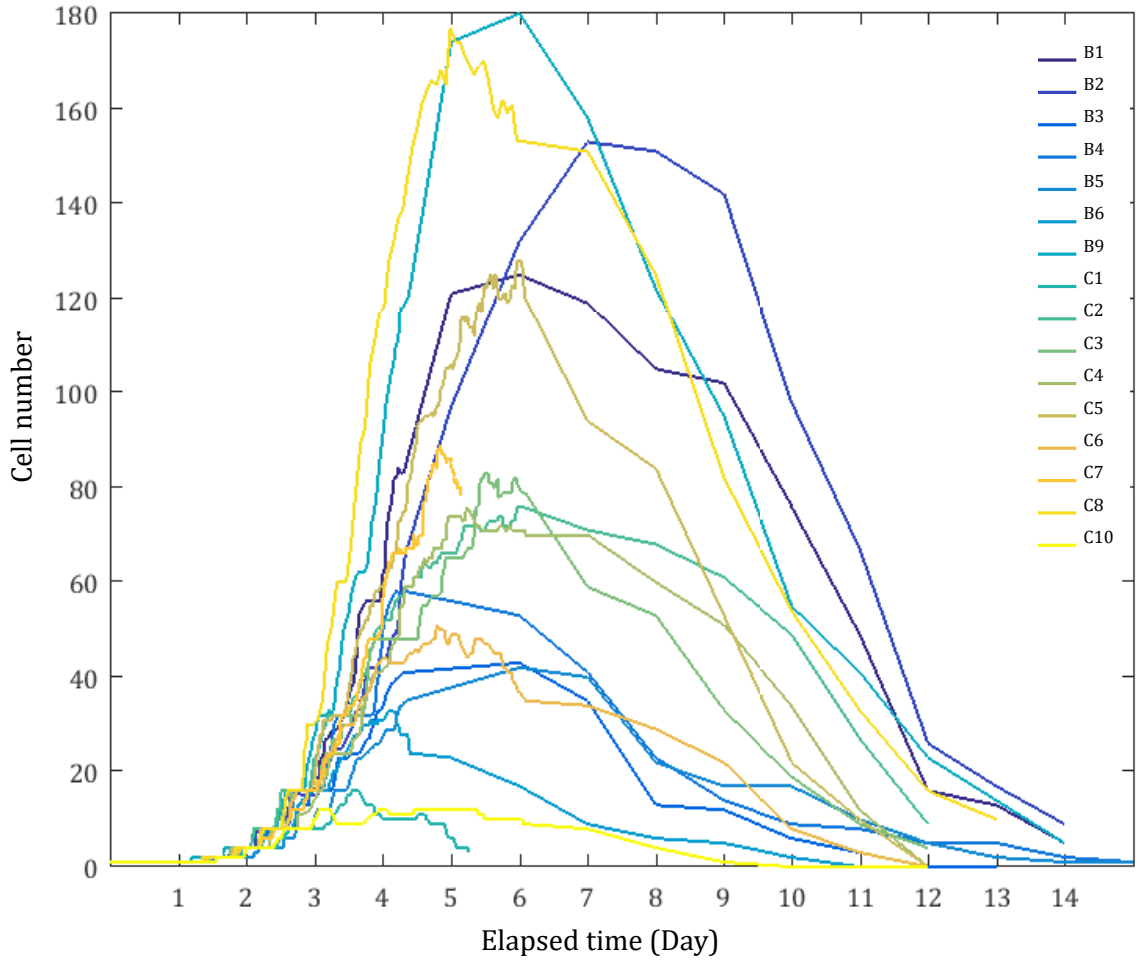
Thousands of hours of correction for 16 pedigrees

Pham, Shimoni et al, *Imm Cell Biol.* 2013
Shimoni, Pham, et al. *Bioinformatics*. 2013
Shimoni et al, *Plos One*, 2014

Measures of the clonal response



Quantifying clonal expansion and contraction



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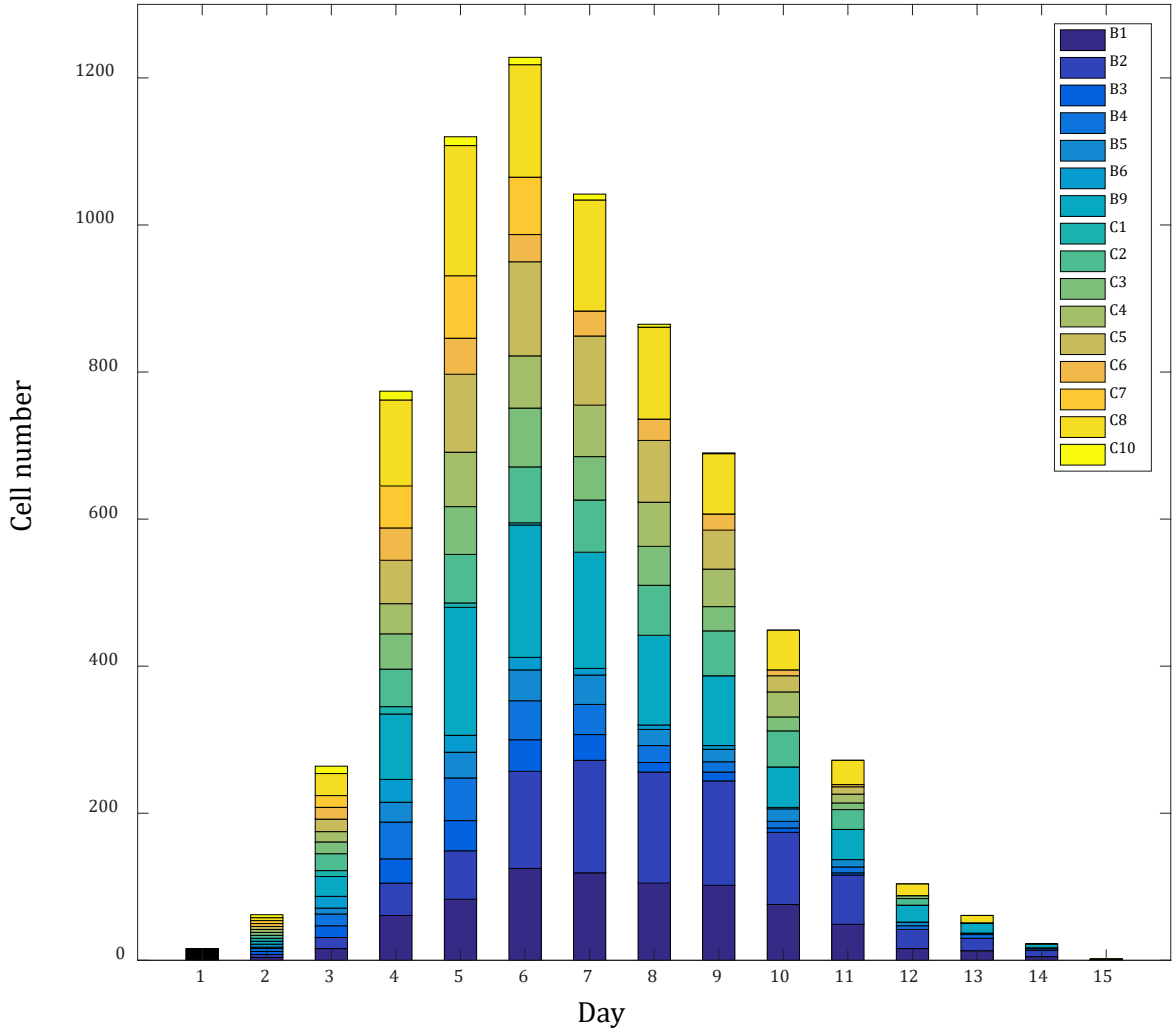
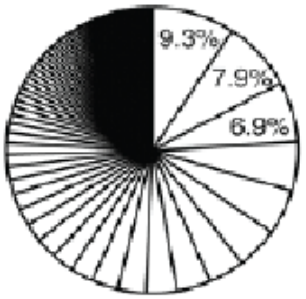
Founder heterogeneity matches that observed by DNA barcoding *in vivo*

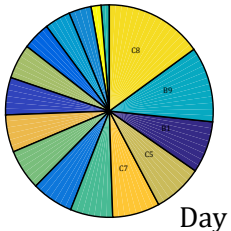
Clonal dominance changes over time

C

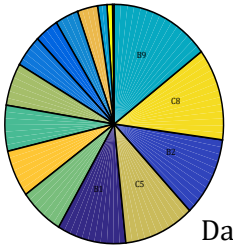




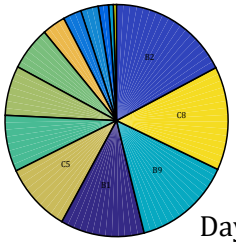
Gerlach et al, Science, 2013



Day 4



Day 6



Day 8

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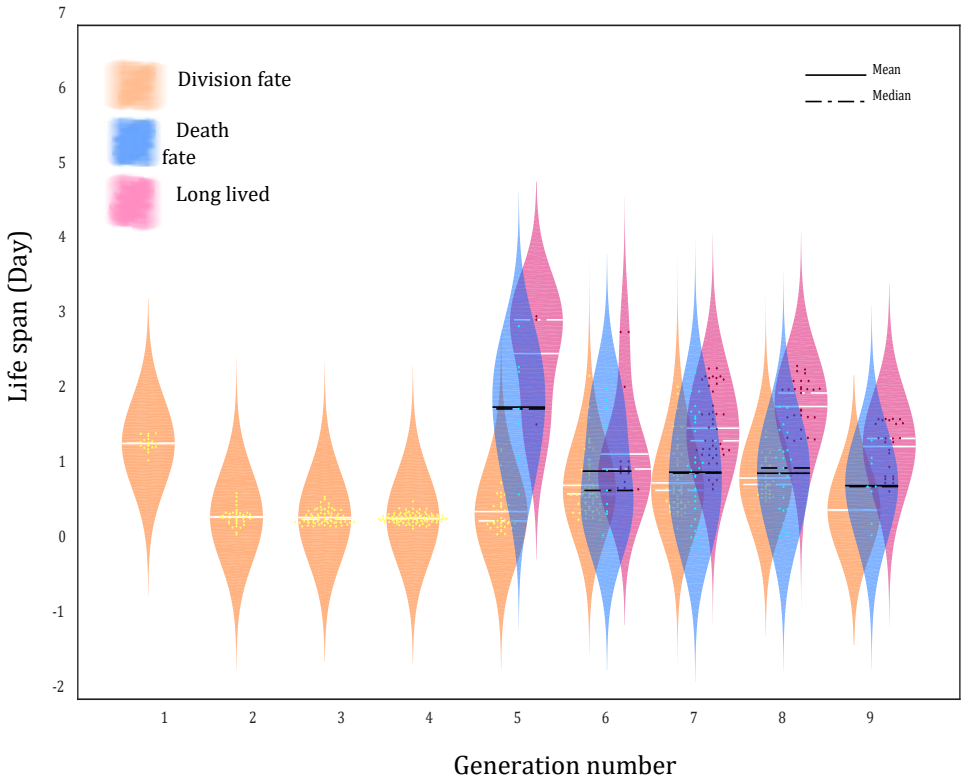
Cells exhibit uniform fate until Gen 5.



From 16 pedigrees (9 generations):

- 884 cells tracked until next division,
- 81 until death
- 381 tracked only part of their life, but of these 121 lived more than 18.5 hrs (greater than any cells in Gen 1-5)

- Remarkable homogeneity in the first generations.
- Cells divide slowly after Gen 5 – against the dogma (supported by in vivo data from Kinjyo et al, Nat Comms 2015).
- Can we take advantage of the transition from homogeneous to heterogeneous fate determination?

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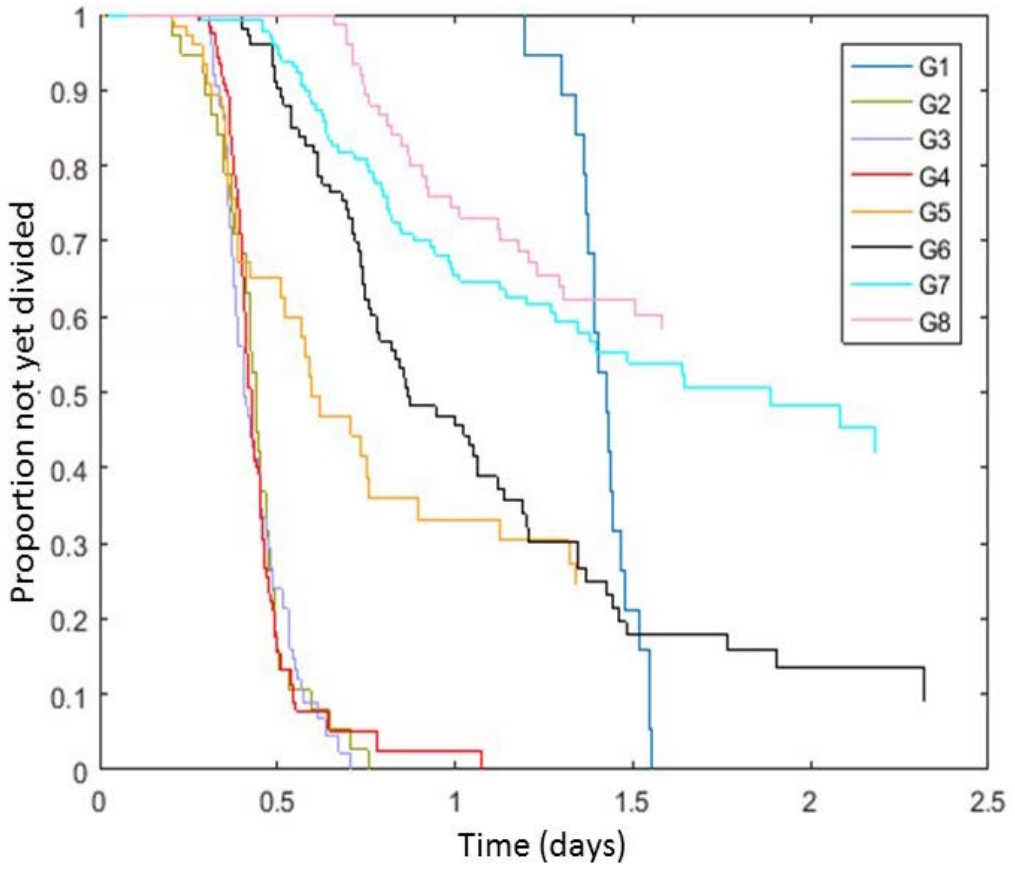
Cumulative distribution of life spans (in dividing cells) shows overlap of Gens 2-4



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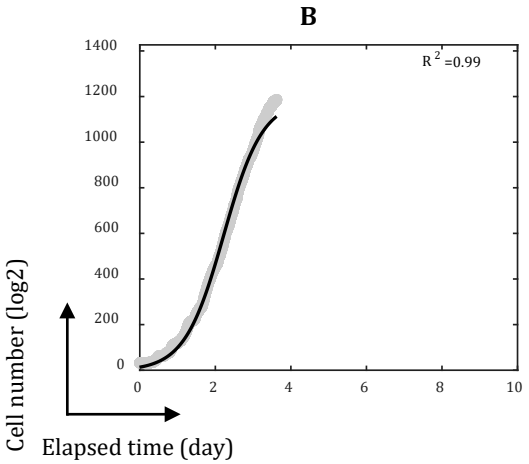
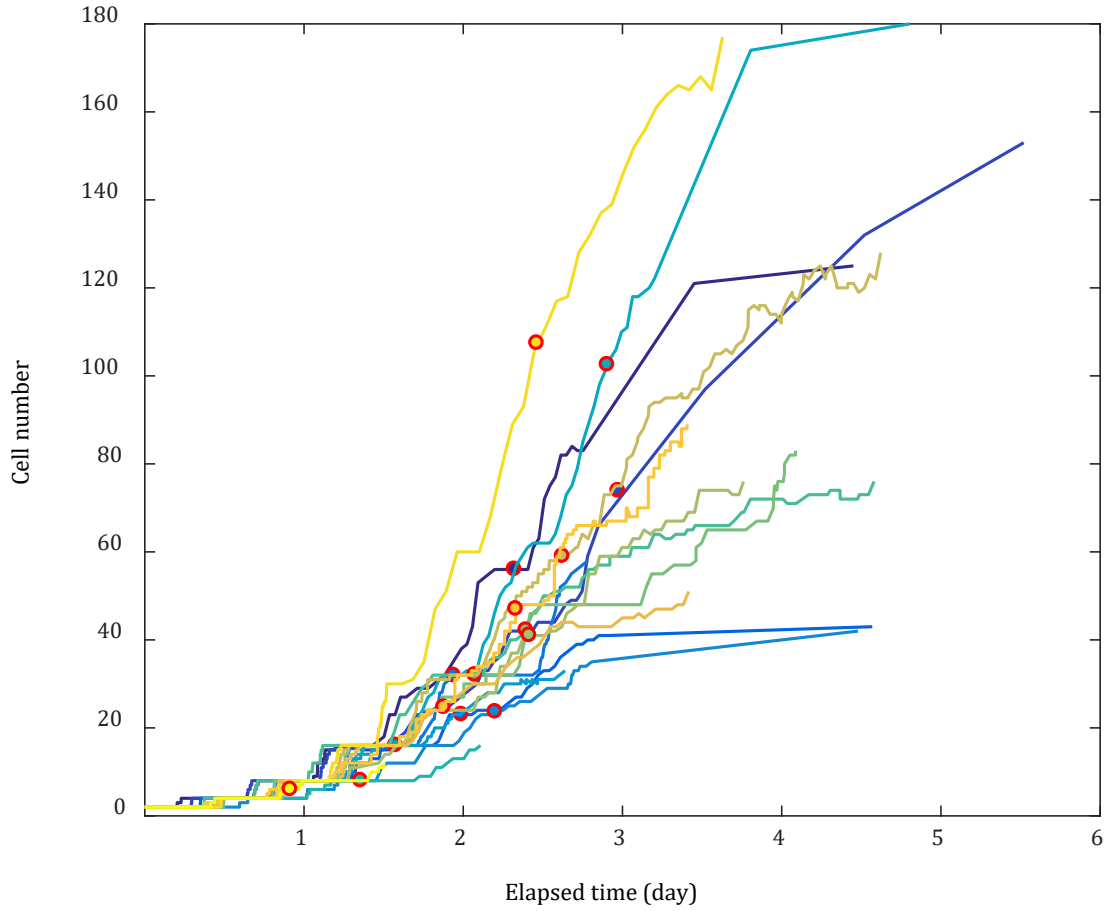
The growth phase fits a sigmoid curve

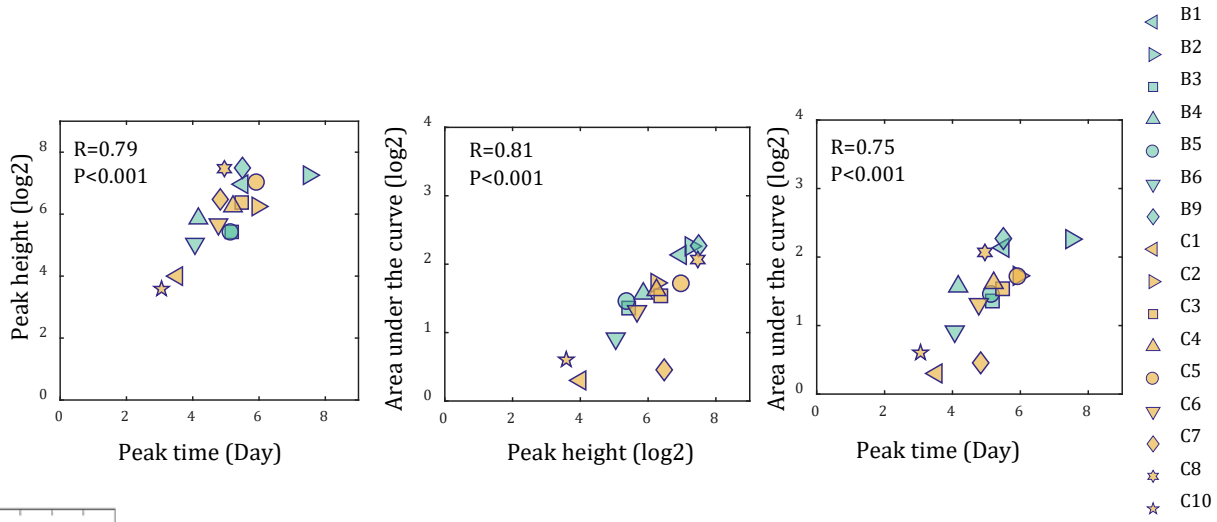


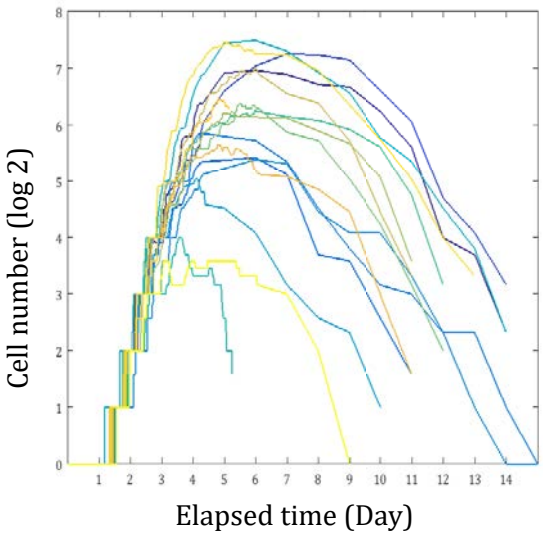


Time of inflection point (derived from sigmoid fit or smoothed data) reflects slowing of cell cycle (start of ‘Phase 2’)

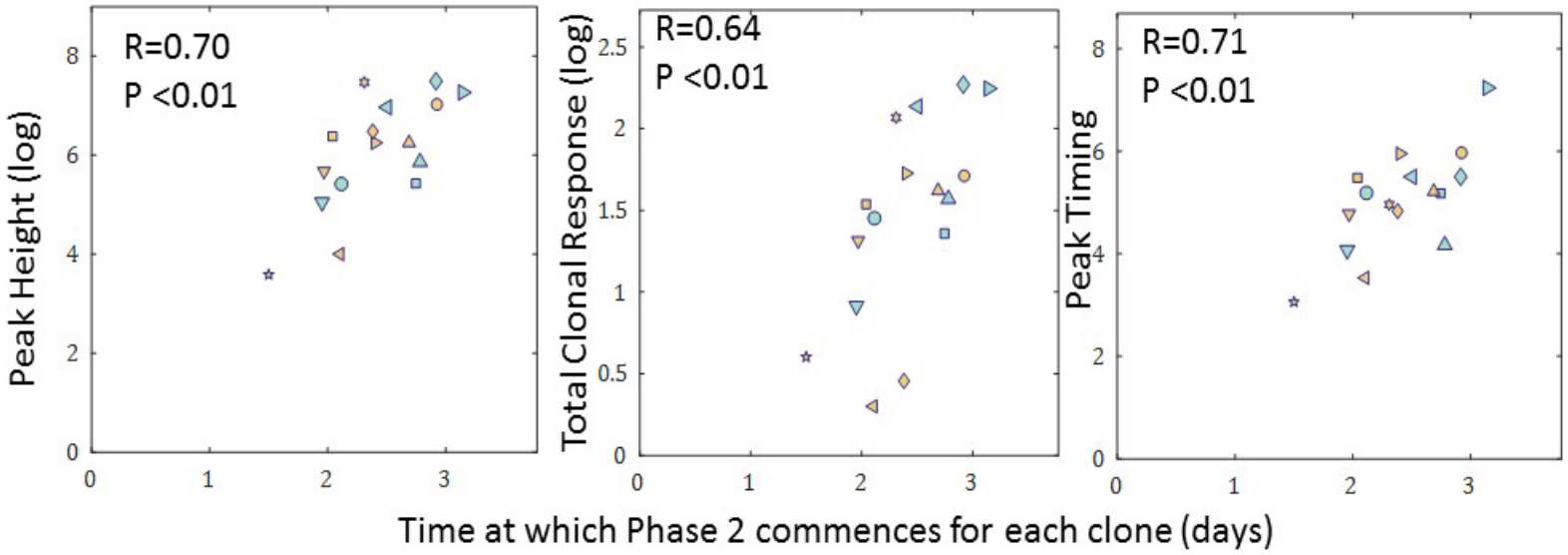
Mohammed Yassin, Kajal Zibaei, Federico Frascoli

Quantifying the rapidity, extent and duration of the immune response





The timing of cell cycle extension shows some correlation to the clonal response.



- This value might be a better means to predict clonal response from early timepoints.
- What controls the change in behaviour at Gen 5?
- 16 cells at Gen5 => presumably at least partly deterministic…
- Latent programming?

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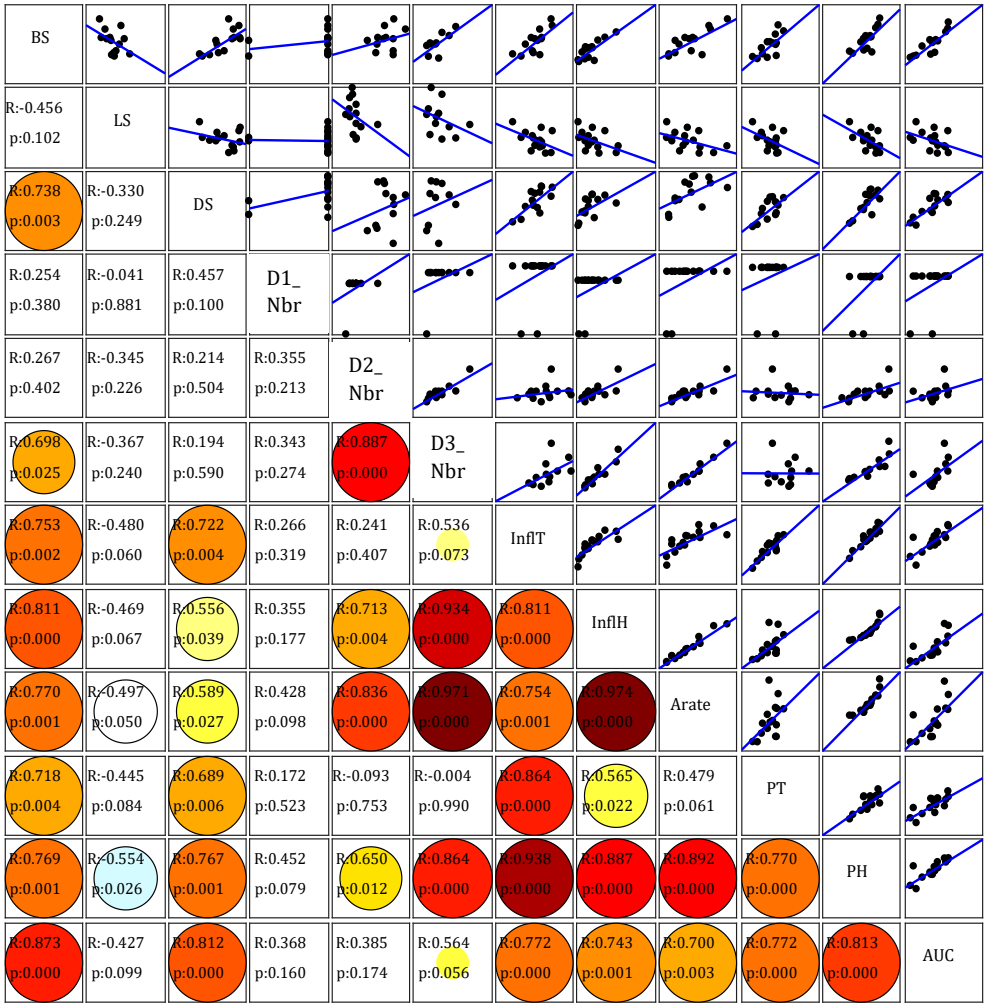
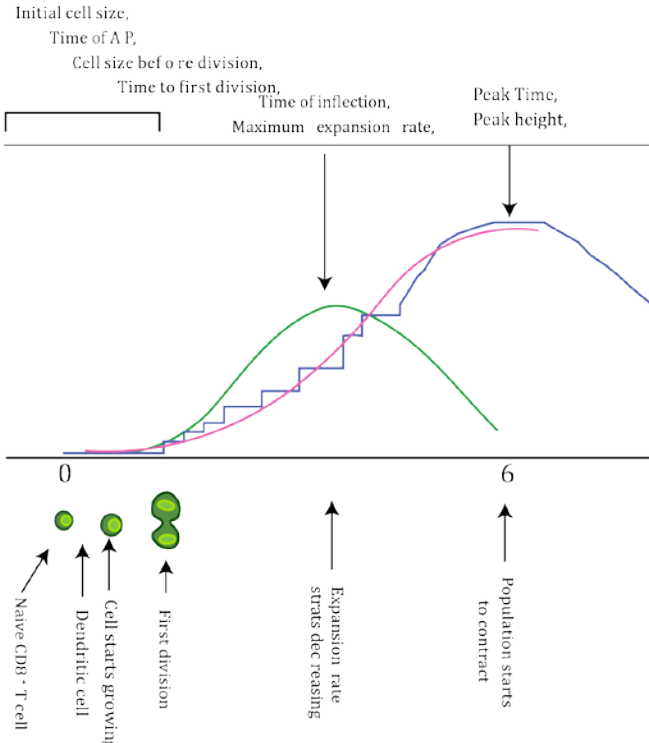
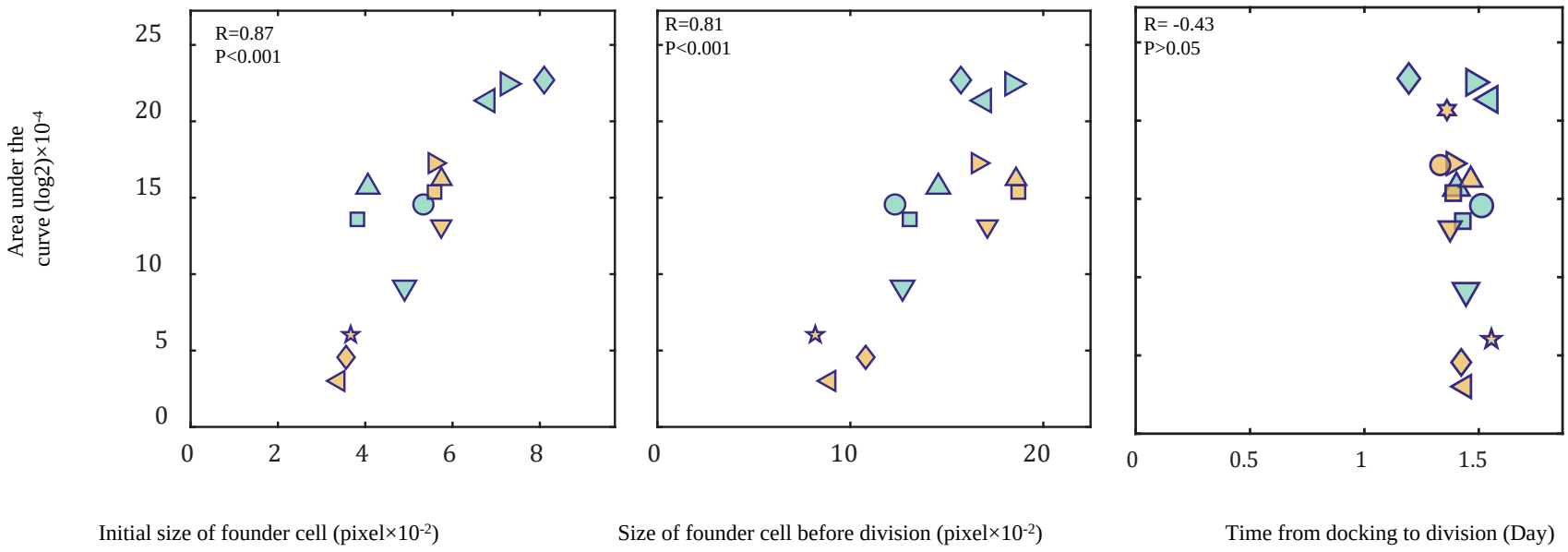
Correlations between different features of founder cells and different characteristics of the clonal response.



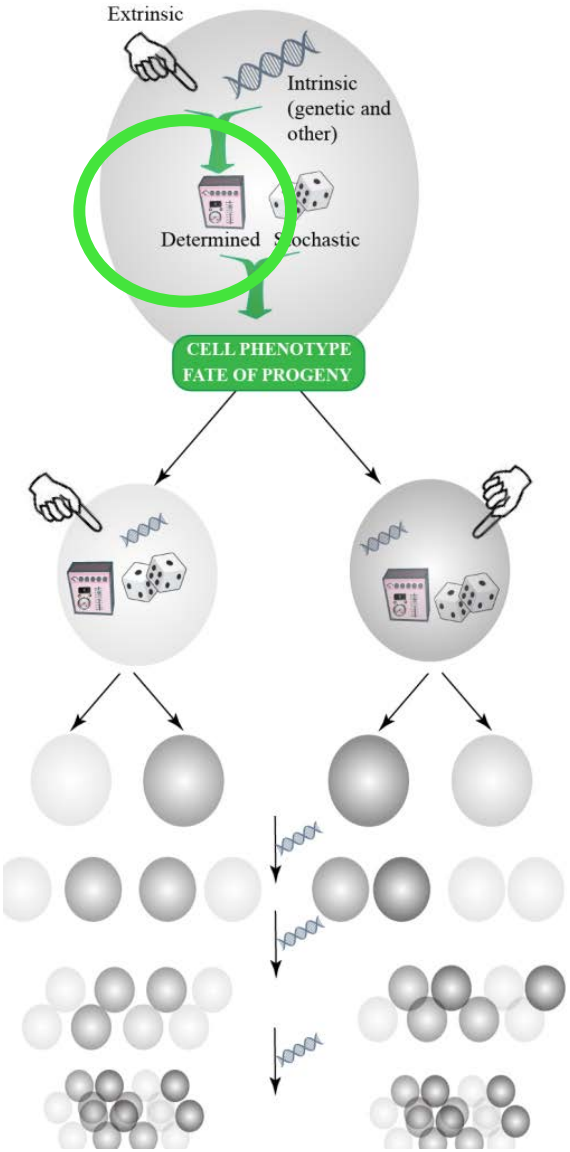


Total clonal response is partially predicted by naïve T cell size.



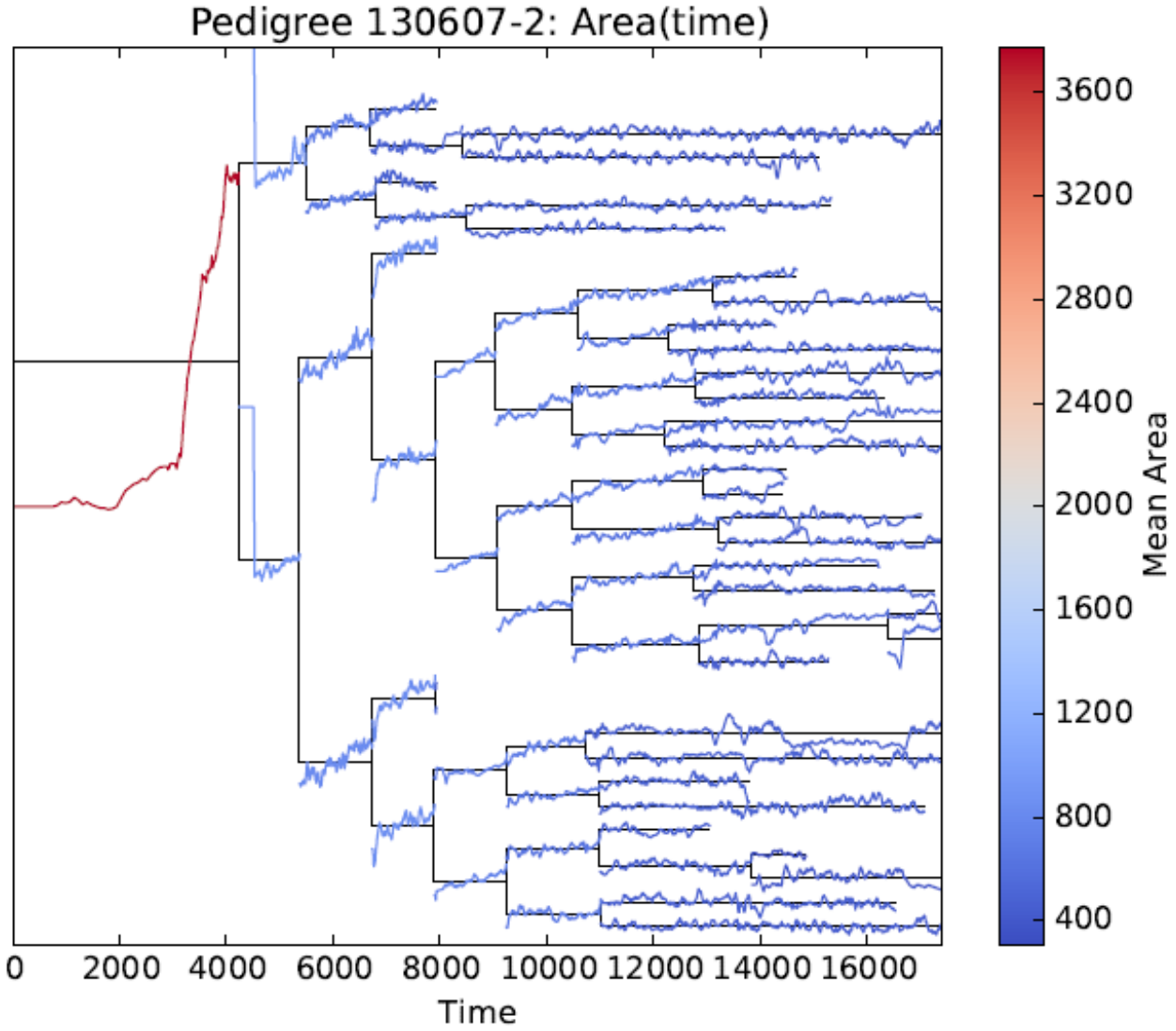
Can we sort for more effective T cells by selecting large cells?
-implications for eg. cancer immunotherapies.

Fate transmission: expansion and contraction



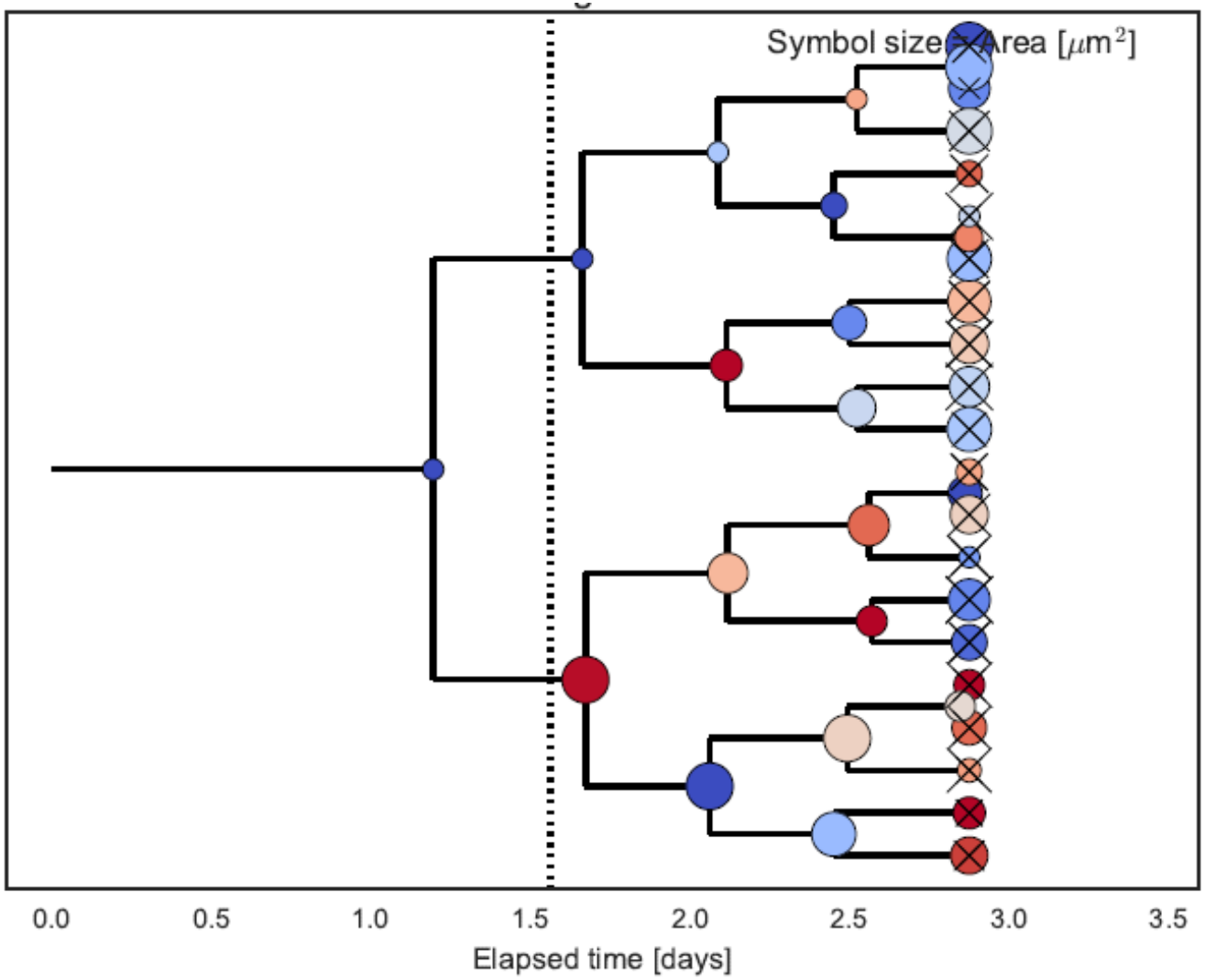
How do fates diverge within the clones?

Creating and quantifying pedigrees



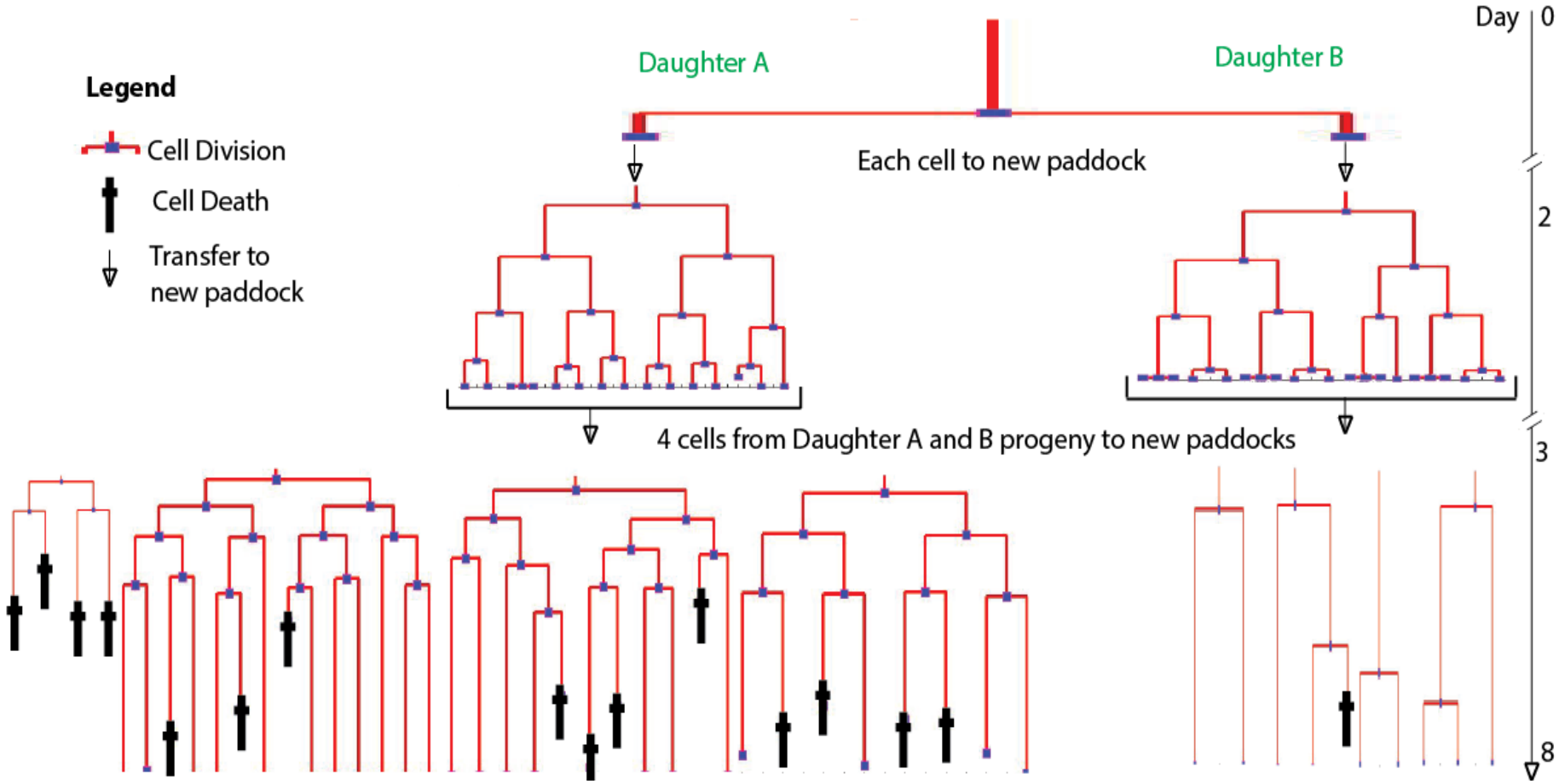
Mohammed Yassin, Raz Shimoni, Damien Hicks

Some pedigrees are extremely uniform



Mohammed Yassin, Raz Shimoni, Damien Hicks

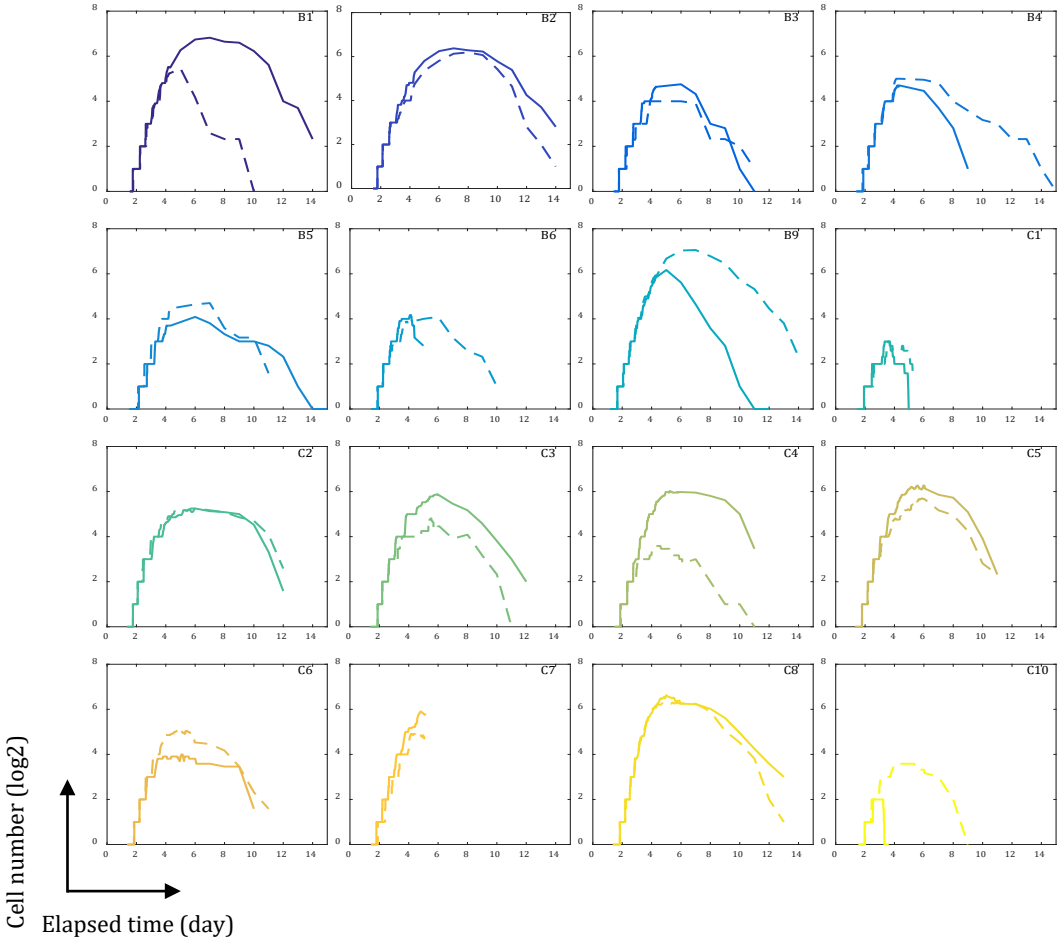
Other pedigrees are not uniform (asymmetry?)



???In almost all pedigrees, fate of Gen 7 cells is shared with other progeny from Gen 2

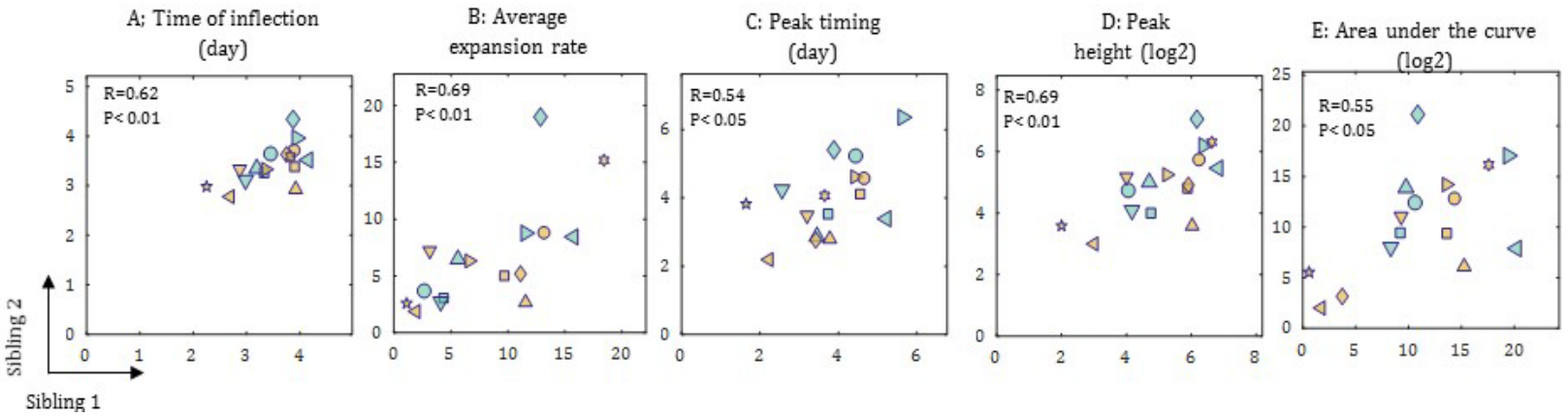
Mohammed Yassin, Raz Shimoni

Intraclonal fate decisions: expansion and contraction



Mohammed Yassin, Kajal Zibaei, Federico Frascoli

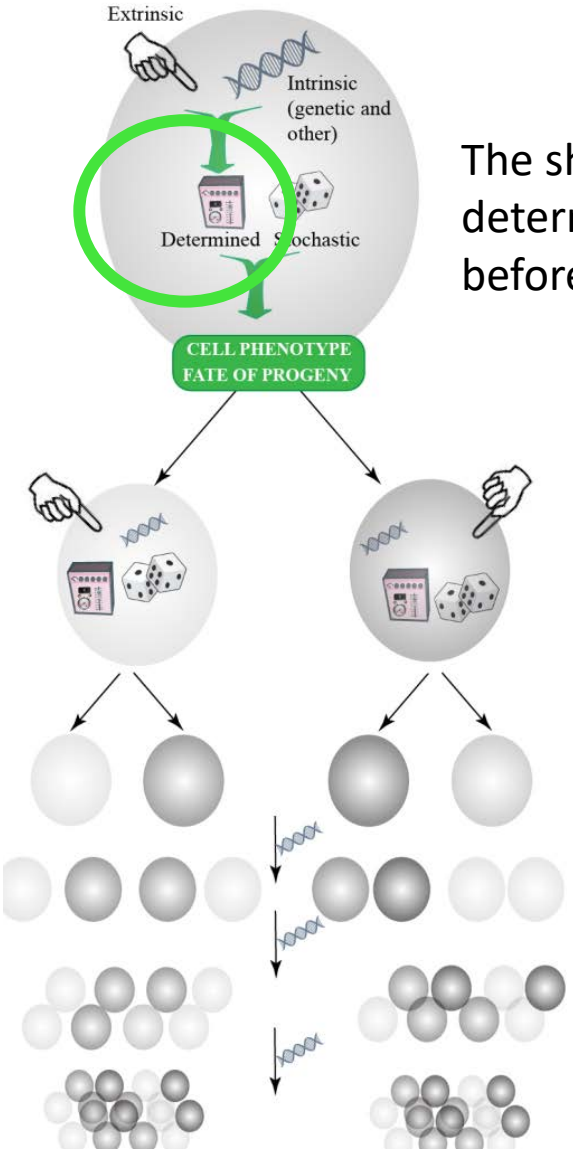
The two arms of the clone correlate reasonably for all 5 features of the clonal response.



- The correlations (R^2) suggest that almost half the variation in the proliferative response, and almost one quarter of the combined proliferative and death response, is explained by the choice of naïve cell.
- Now want to understand the generation by generation transmission of all attributes – need a systematic approach.
- Statistics on trees…

Mohammed Yassin, Kajal Zibaei, Federico Frascoli

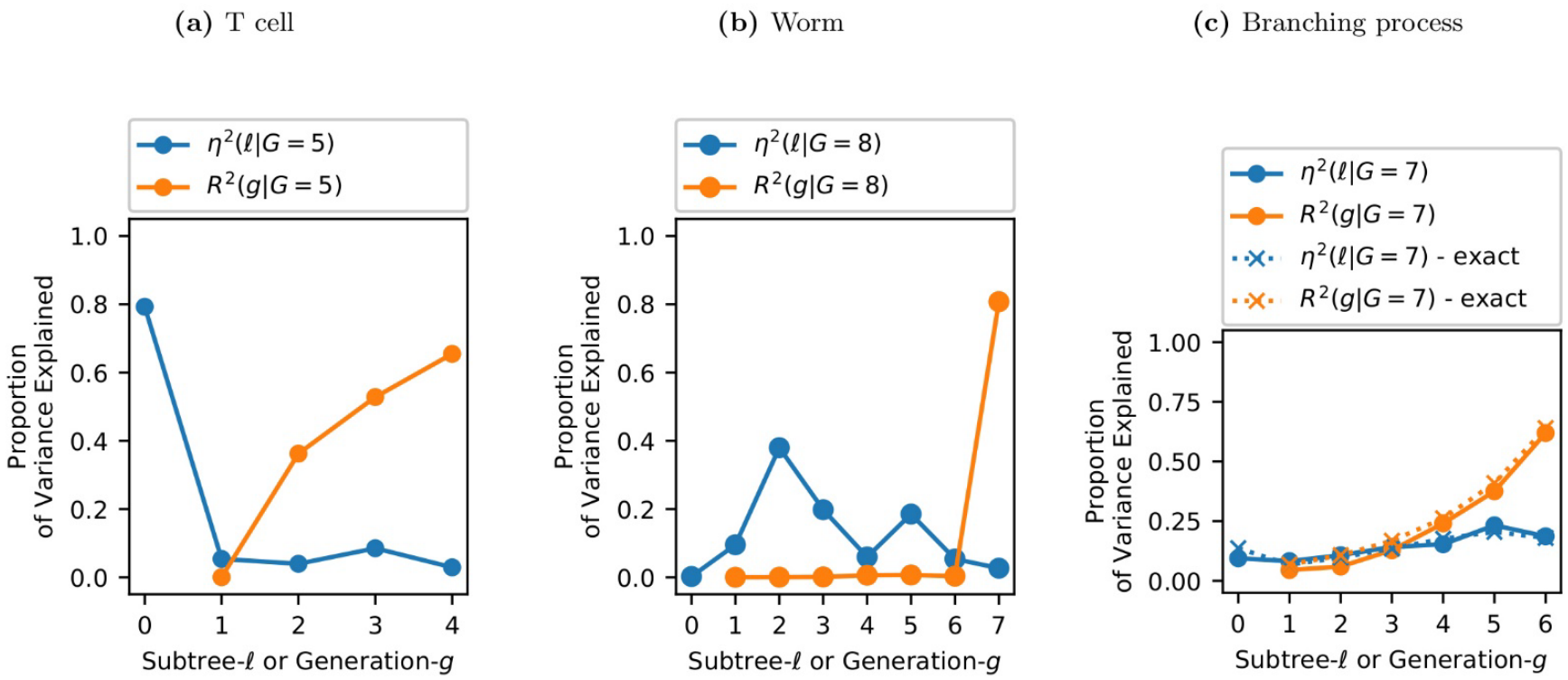
Fate transmission: expansion and contraction



The shape of the immune response is strongly determined by the size of the founder cell, even before antigen presentation.

How do fates diverge within the clones?
-mostly (but not all) symmetrical expansion profiles.
-how to quantify fate divergence in these pedigrees?

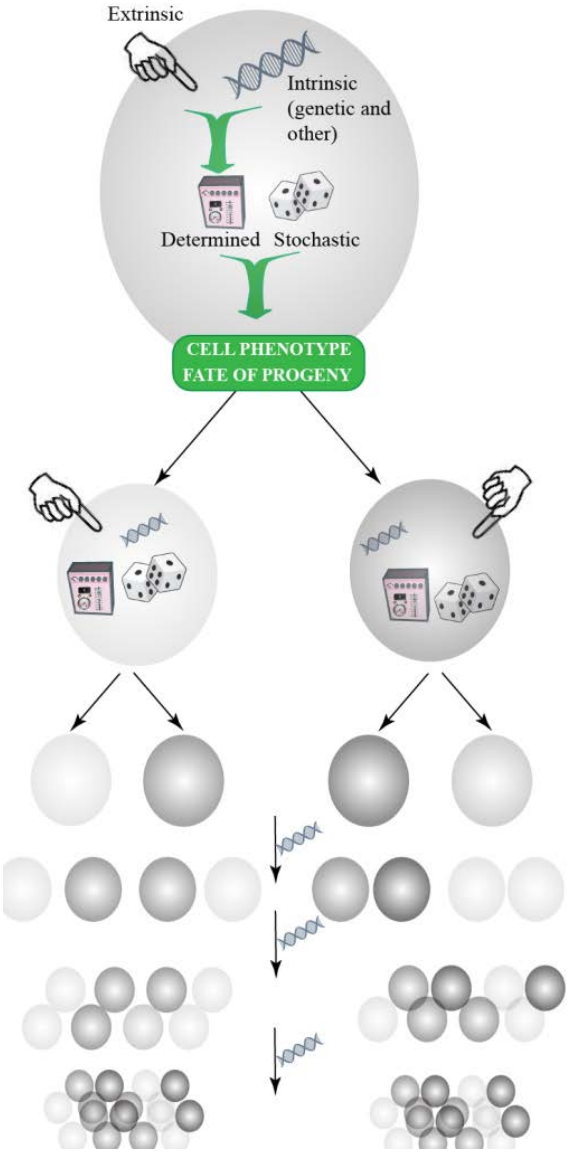
Maps of Variability in Lineage Trees
Hicks et al., PLOS Comp Bio (2019)



- T cells: 80 % of the variability in cell size at Gen 5 can be explained by the identity of the founder naïve T cell.
- Worm: variability in pharyngeal identity is predominantly explained by identity of Gen 2 and Gen 5, but not displayed until Gen7.

Damien Hicks, Terry Speed

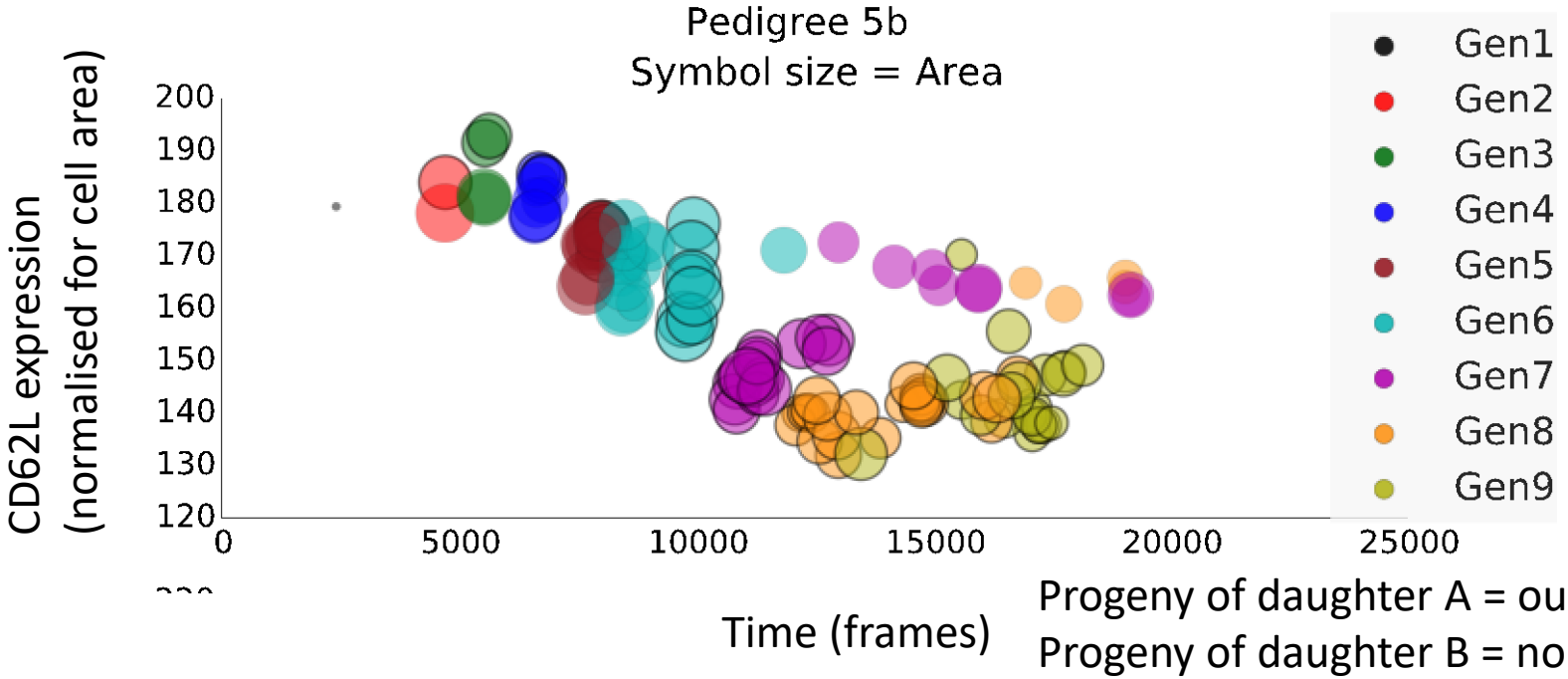
Fate transmission: differentiation



Can we identify memory cells in our system, and map their fate determination?

Memory cells:
-Become evident after effector population contracts.
-Marked by small cell size and higher CD62L (in some experimental systems).
-Quiescent.

Do the changes in cell cycle times reflect effector/memory decisions? Size and CD62L combined correlate with fate, and cluster according to ancestry at Gen 2



Progeny of daughter A = outlined
Progeny of daughter B = no outline

We might be seeing emergence of memory.
If so, memory cells arise from effector cells.
At least in some instances, this memory phenotype seems to be encoded in only one daughter (implications for the Asymmetric Cell Division hypothesis).

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Conclusions

- Cell proliferation is homogeneous in the first few generations.
- Clonal response is partially imposed by a change in fate (proliferation and death) at Gen 5.
- Fate is transmitted from the naïve founder T cell, but hidden until Gen 5.
- Clonal response can be predicted by size of the cell before antigen presentation.

Very tentative:

- Memory bifurcation might occur at Generation 2 in some clones (?ACD?)
- Memory cells seem to arise from effector cells.

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