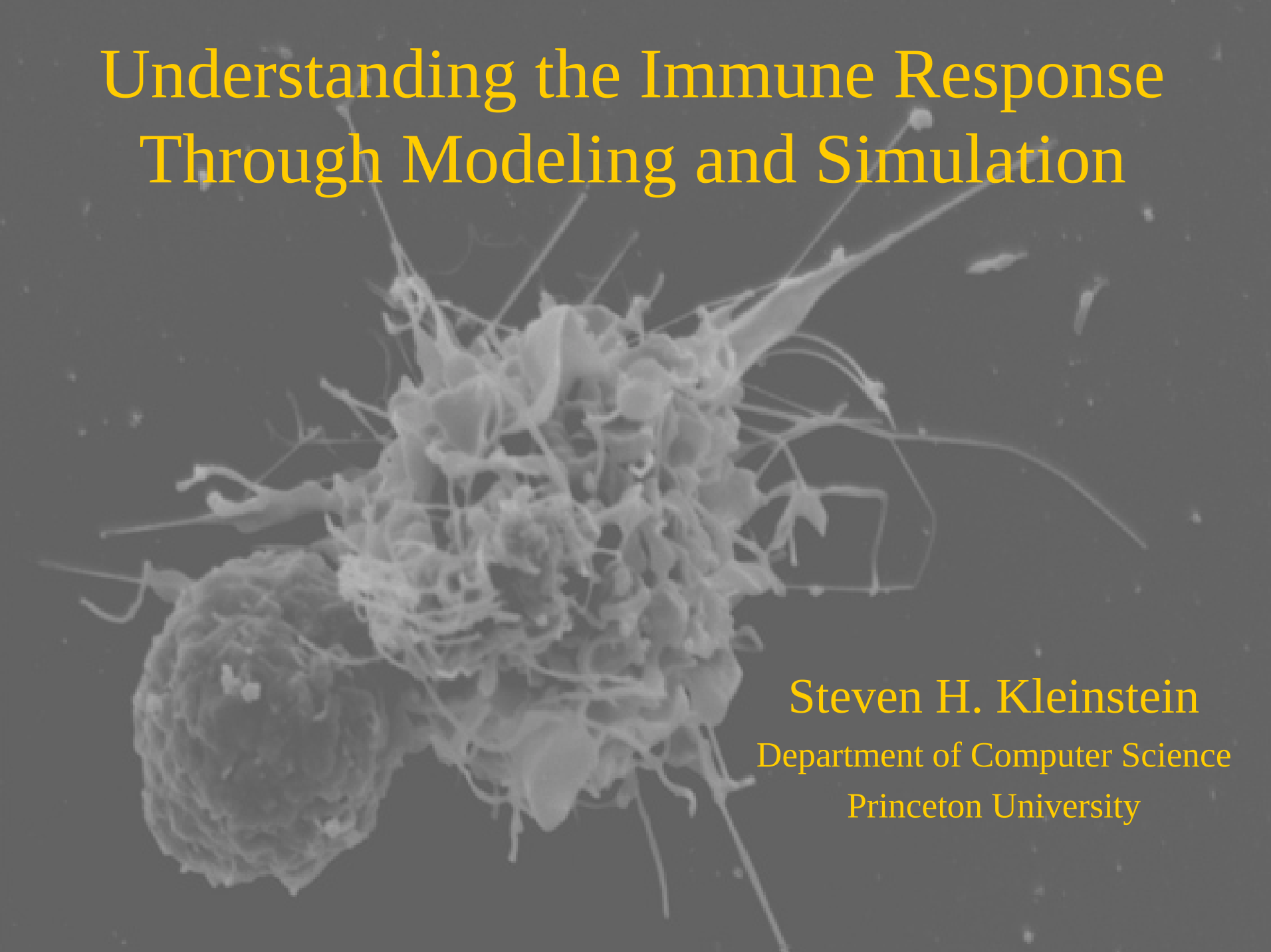
Understanding the Immune Response Through Modeling and Simulation



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The Immune System

Relatively new science, began with Jenner in 1796

- Protects the body from damaging pathogens
  - viruses, bacteria, parasites
- Provides basis for vaccines (e.g., flu)
- Implicated in disease:
  - Autoimmune (Lupus, MS, Rheumatoid Arthritis)
  - Sepsis, Cancer

Understanding will lead to better diagnostics and therapies

Why Model the Immune System?

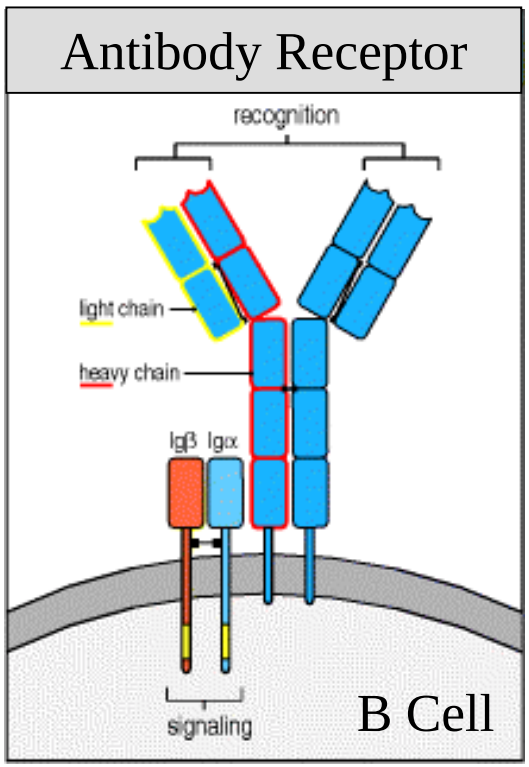
Experiments provide only a static window onto the real dynamics of immunity

- Immune response involves the collective and coordinated response of $\approx 10^{12}$ cells and molecules
- Distributed throughout body
  - blood, lymph nodes, spleen, thymus, bone marrow, etc.
- Interactions involve feedback loops and non-linear dynamics
- Experiments often require artificial constructs
- High variability observed in experimental results

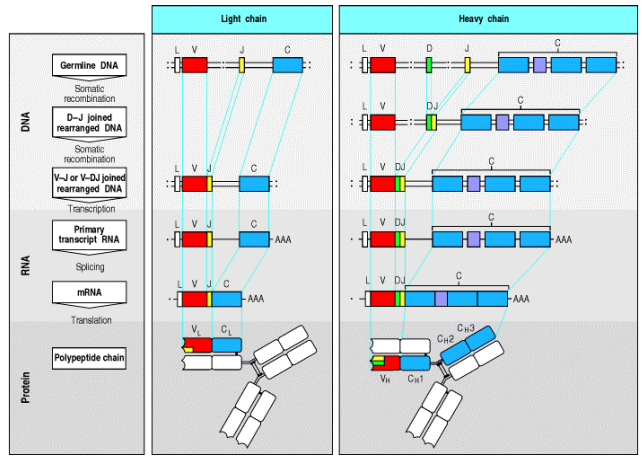
Somatic Hypermutation: important component of response

B cells Antibody Receptors “Recognize” Antigens

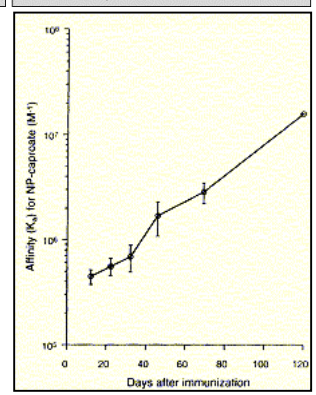
1. B cell’s must recognize universe of pathogens (antigens)
2. Response to any specific antigen must be efficient



Rearrangement creates initial diversity…



Hypermutation and selection lead to affinity increase over time…

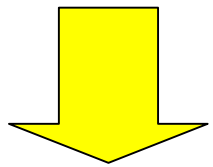


Hypermutation & Selection ≈ Darwinian Evolution, but in 3 weeks!

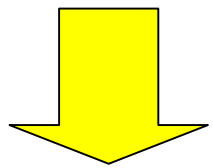
What might go wrong?

Autoimmunity is a response against body's own proteins, DNA, etc.

Somatic Hypermutation



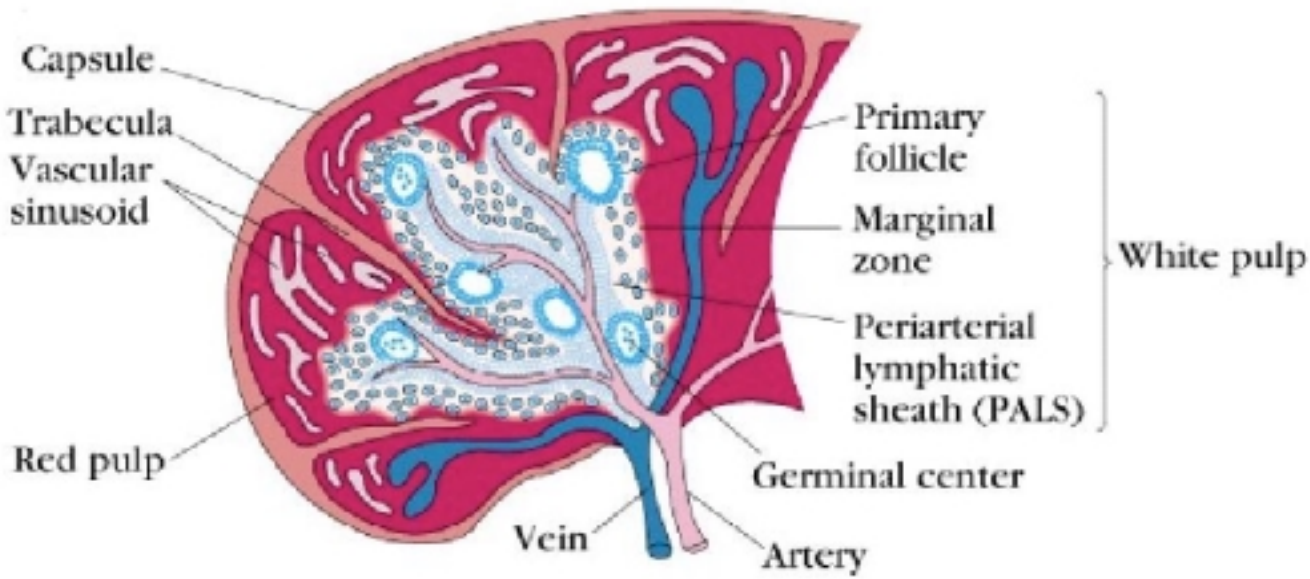
Antibody Receptors Against Self-antigens

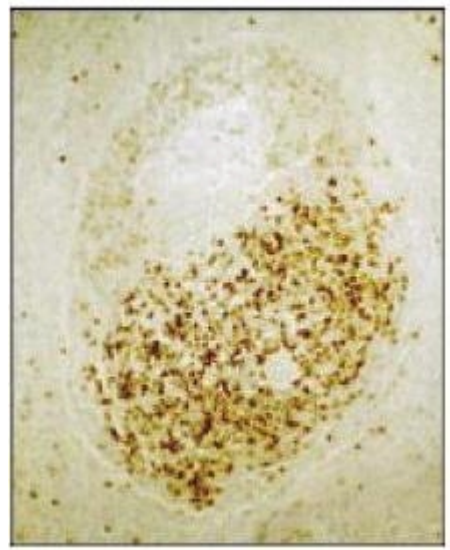


Autoimmune Disease

Commonly Accepted:
Somatic Hypermutation Restricted to Germinal Centers

Germinal Centers Form in the Spleen





http://mcb.berkeley.edu/courses/mcb150/Lect10/Lect10.pdf

Commonly Accepted:

Germinal Centers are the Site of Somatic Hypermutation and Selection of Higher-Affinity B Cells

Motivating Experiment

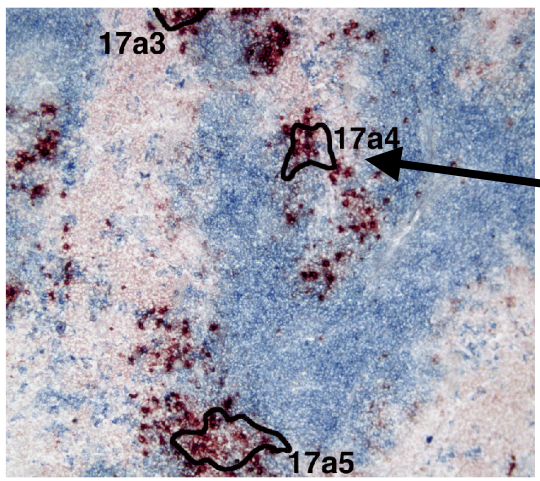
In auto-immune mouse model, observed mutating B cells in extra-follicular areas of spleen (not germinal centers)

Auto-immune Mouse

MRL/lpr AM14 heavy chain transgenic

(William, Euler, Christensen, and Shlomchik. Science. 2002)

Extra-Follicular Areas



Microdissection (10 cells)

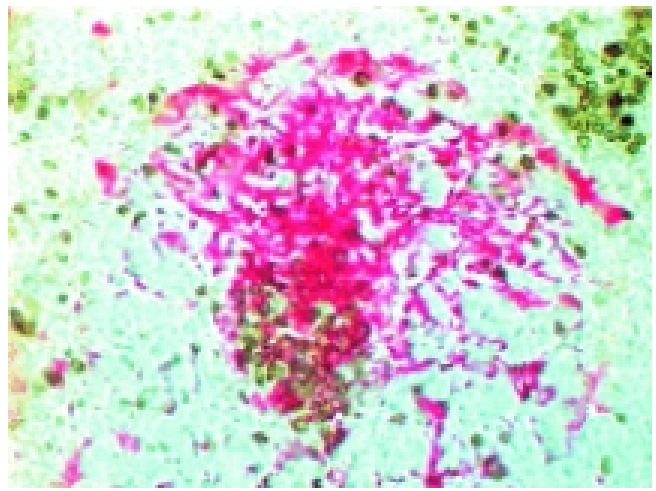
B cells T cells

Control

Primary anti-hapten response to NP

(Jacob et al., 1991; Jacob and Kelsoe, 1992; Jacob et al., 1993; Radmacher et al., 1998)

Germinal Centers

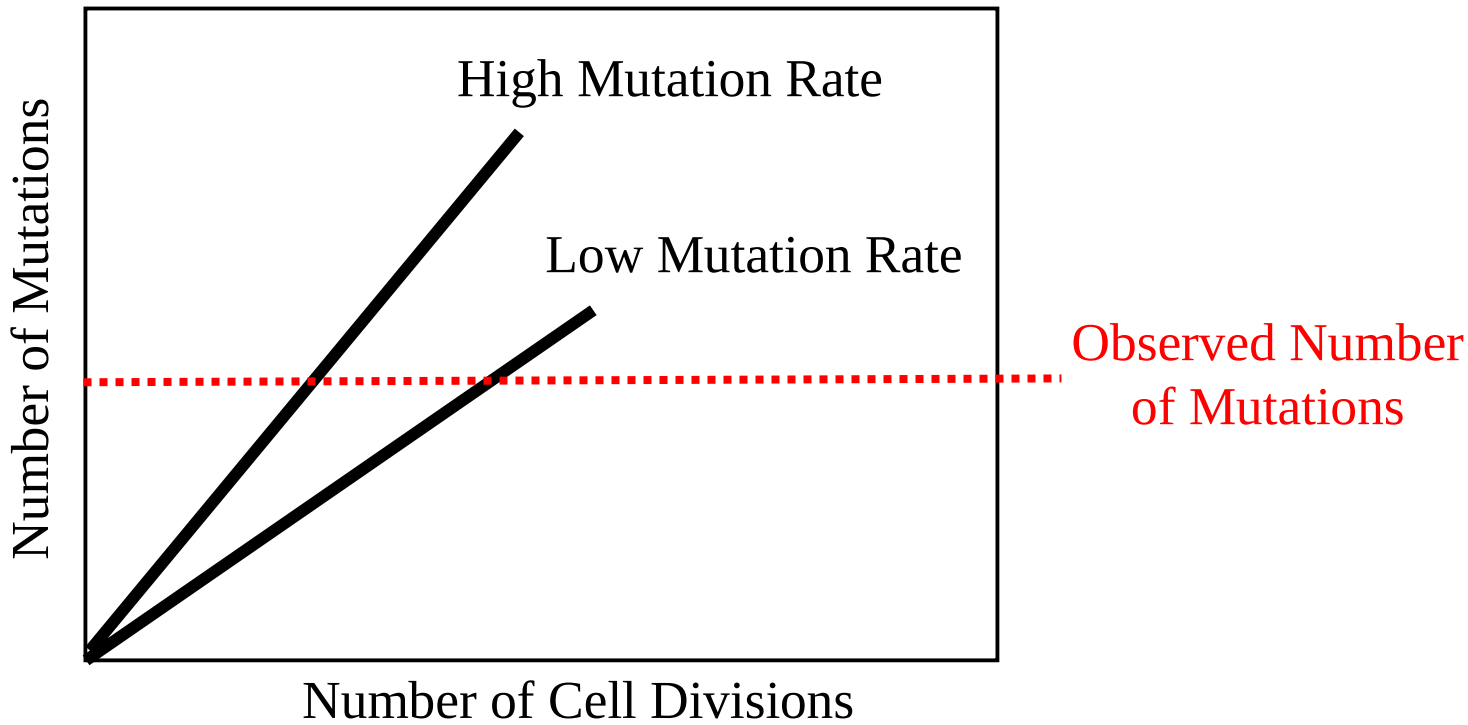


Dividing B cells FDC

Estimate mutation rate to show (hyper?)mutation

What's hard about estimating the mutation rate?

The number of divisions in vivo is unknown



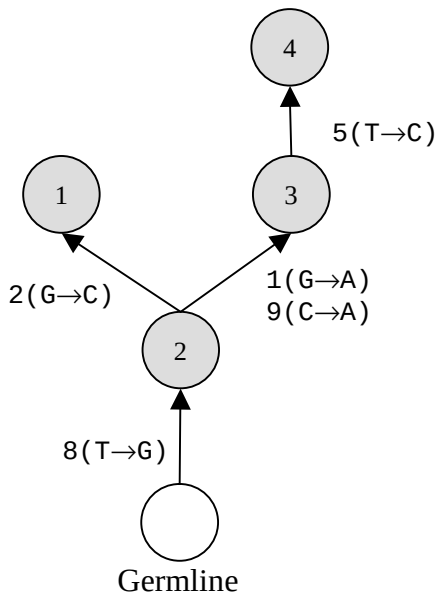
Most recognized *in vivo* estimates took educated guesses

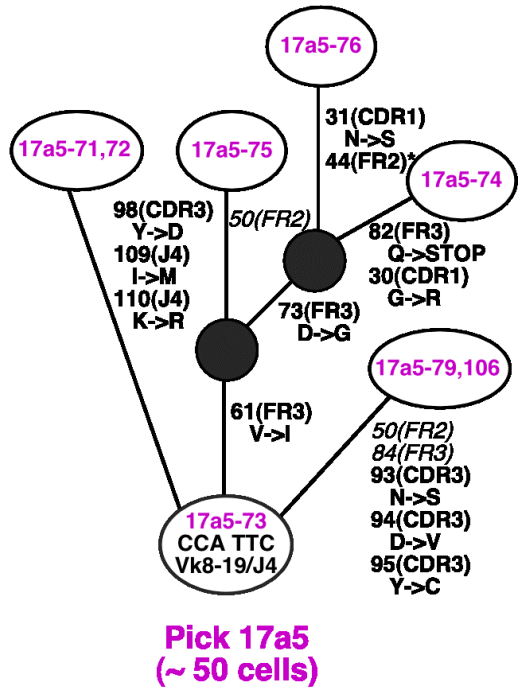
(McKean et al, 1984 and Sablitzky et al, 1985)

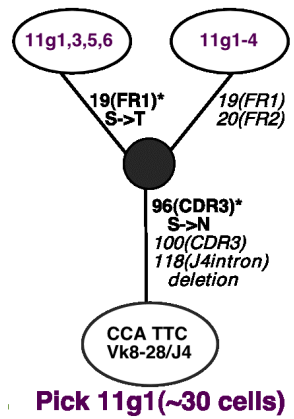
Clonal Trees Provide Needed Information

Analyze pattern of shared and unique mutations among sequences from each microdissection

Germline	GGGATTCTC
1	-C-----G-
2	-------G-
3	A------GA
4	A---C--GA





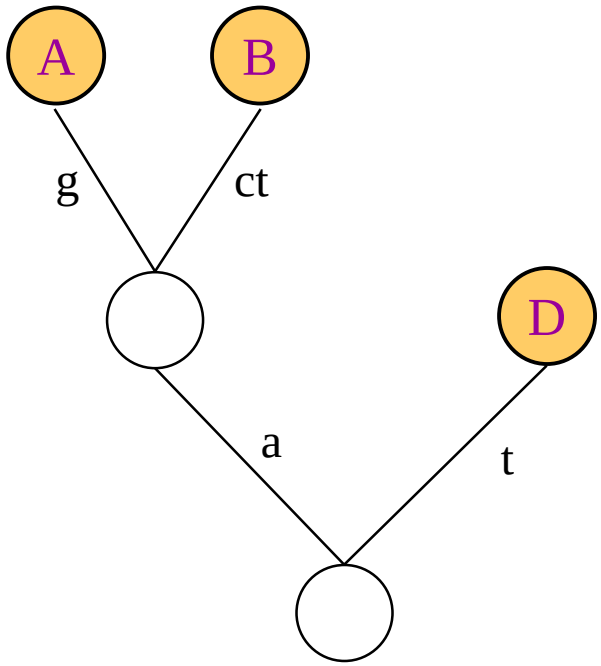


Clonal tree ‘shapes’ reflect underlying dynamics

Relating Tree Shapes to Underlying Dynamics

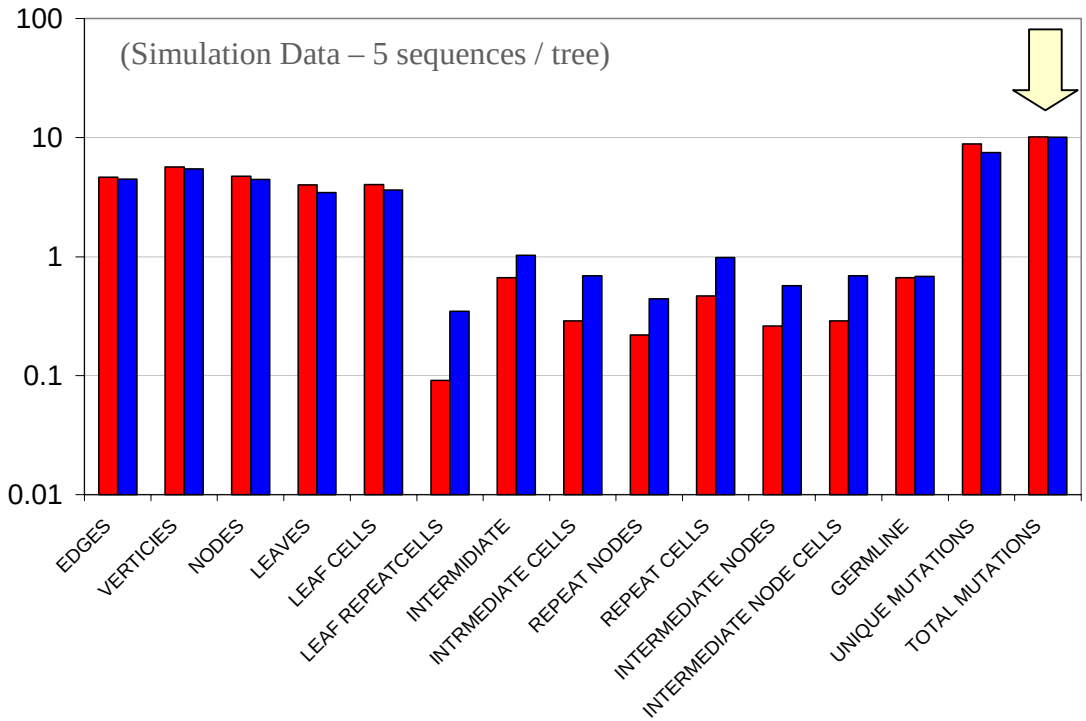
Investigate with computer simulation of B cell clonal expansion

Parameters: mutation rate (μ), lethal frequency (λ), # divisions (d), pick size (p)



Initial Sequence

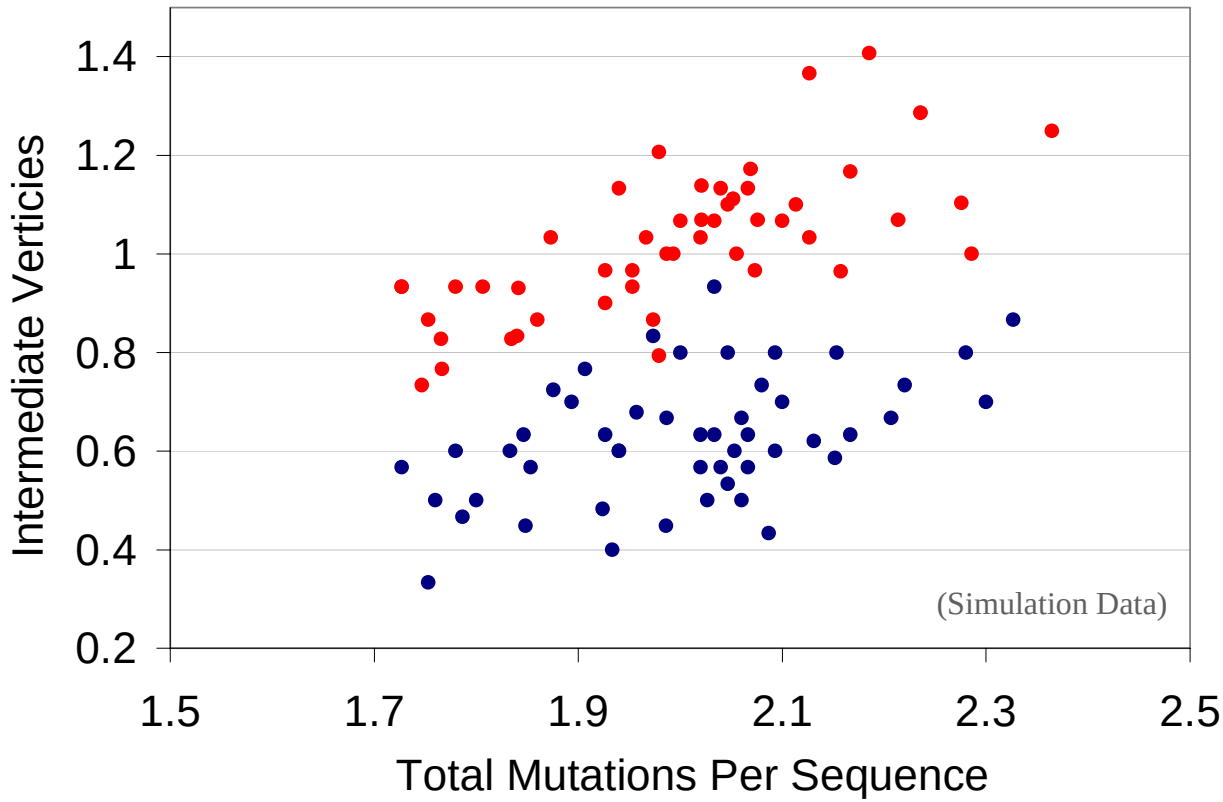
Compare: Rate of 0.2 division^{-1} for 14 divisions
Rate of 0.4 division^{-1} for 7 divisions



Relevant shape measures can differentiate similar clones

Intermediate Vertices is Useful Measure

Compare: Rate of 0.2 division^{-1} for 14 divisions
Rate of 0.4 division^{-1} for 7 divisions



Shape measures can supplement information from mutation counting

Method for Estimating Mutation Rate (μ)

Find mutation rate that produces distribution of tree ‘shapes’ most equivalent to observed set of trees

Mutation Rate

Experimental Observations

Simulation of B cell expansion

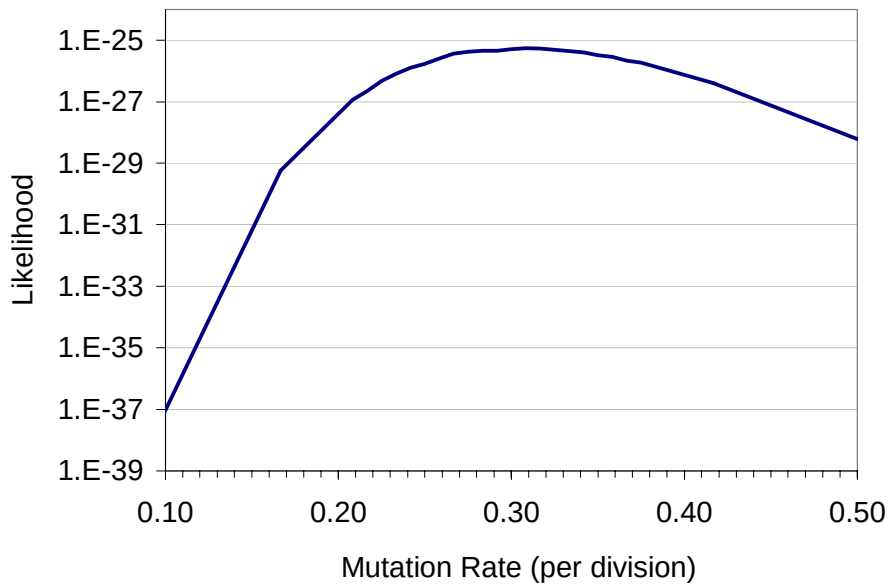
Set of Observed Tree Shapes

=

Distribution of Tree Shapes

Number of mutations
Intermediate vertices
Sequences at root

Assumes equivalent mutation rate in all trees, although number divisions may differ



Also developed analytical method based on same underlying idea

(***The Journal of Immunology*** **(2003) Vol. 171 No. 9, 4639-4649.)**

Details of the Simulation Method

For each value of the mutation rate (μ), calculate likelihood by…

Equivalent Matrix, E(t,d)

 # simulated trees 'equivalent' to observed tree after d divisions

divisions (d)

t	1	2	…	D
Tree 1	0	0	0	0
Tree 2	0	0	0	0
…	0	0	0	0
Tree T	0	0	0	0

1. Run simulation many times to fill in equivalent matrix

2. Likelihood of experimentally observed tree t:

$$L(t \mid \mu) = \frac{\sum_d E(t,d)}{\sum_d O(t,d)}$$

← **Sample space is subset of all simulation runs**

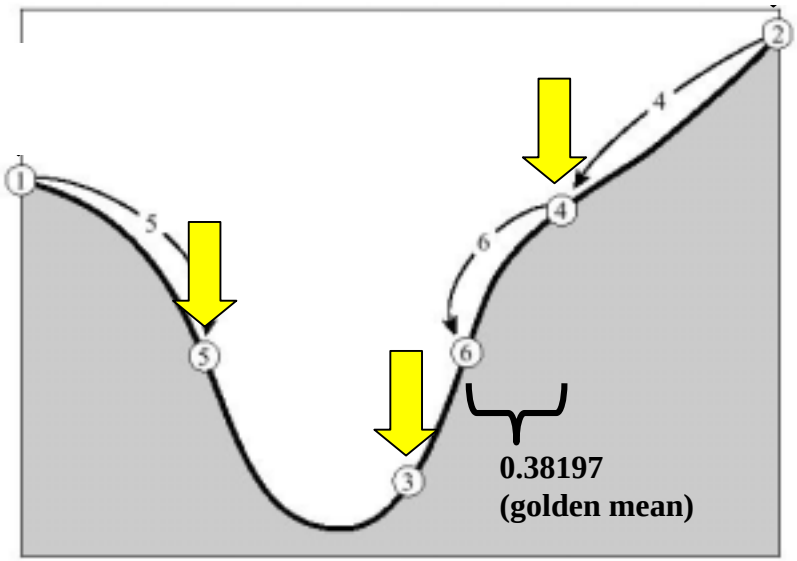
3. Likelihood of experimental dataset:

$$L(\mu) = \prod_t L(t \mid \mu)$$

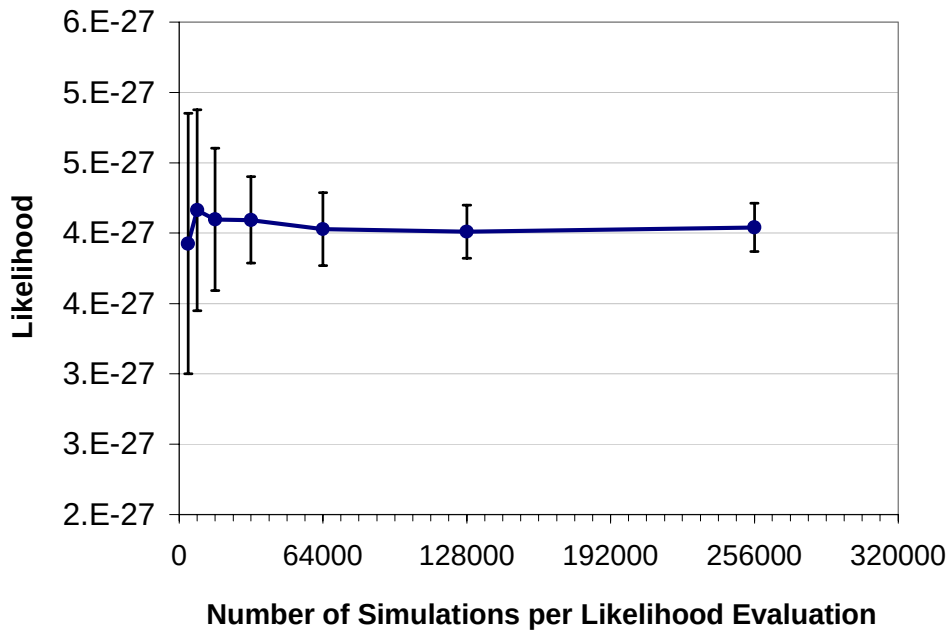
Use Golden Section Search to optimize mutation rate (μ)

Finding the Optimal Mutation Rate

Golden Section Search works by successive bracketing of minimum/maximum



http://lib-www.lanl.gov/numerical/bookcpdf/c10-1.pdf



Direct Search Method (No Derivative)
Simple Implementation, Linear Convergence

Not tolerant of noise,
Make sure evaluation is precise

Method is effective with 128,000 simulations per Likelihood

Details of the Analytical Method

Formulas to approximate tree shapes…

The average number of mutations per sequence (M) $M = (1-\lambda_1)\mu d$

The average number of sequences present at the root of the tree (R) $R = S_t \times \left(\frac{e^{-\mu}}{(1-\lambda_1 \mu)} \right)^d$

Total number of sequences in nodes with repeated sequences (P) $P = S_t \left[1-(1-p_1)^{S_t - 1} \right]$

Minimize error X(μ) over all experimentally observed trees (t)

Observed shape Calculated shape

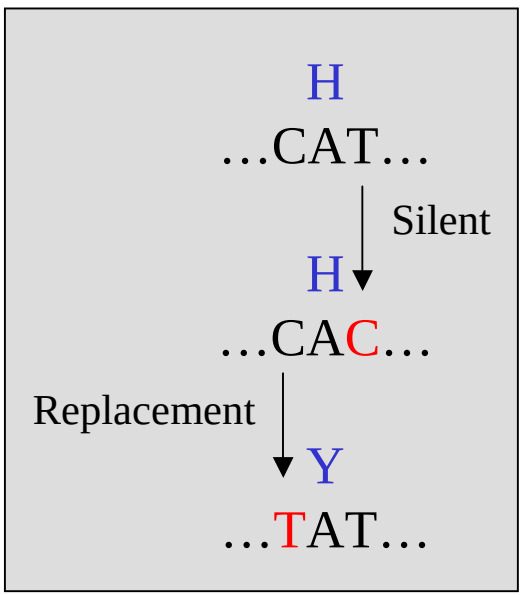
$$X(\mu) = \sum_t \underset{d}{MIN} \left(\frac{(M_t - M)^2}{VAR(M_t)} + \frac{(R_t - R)^2}{VAR(R_t)} + \frac{(P_t - P)^2}{VAR(P_t)} \right)$$

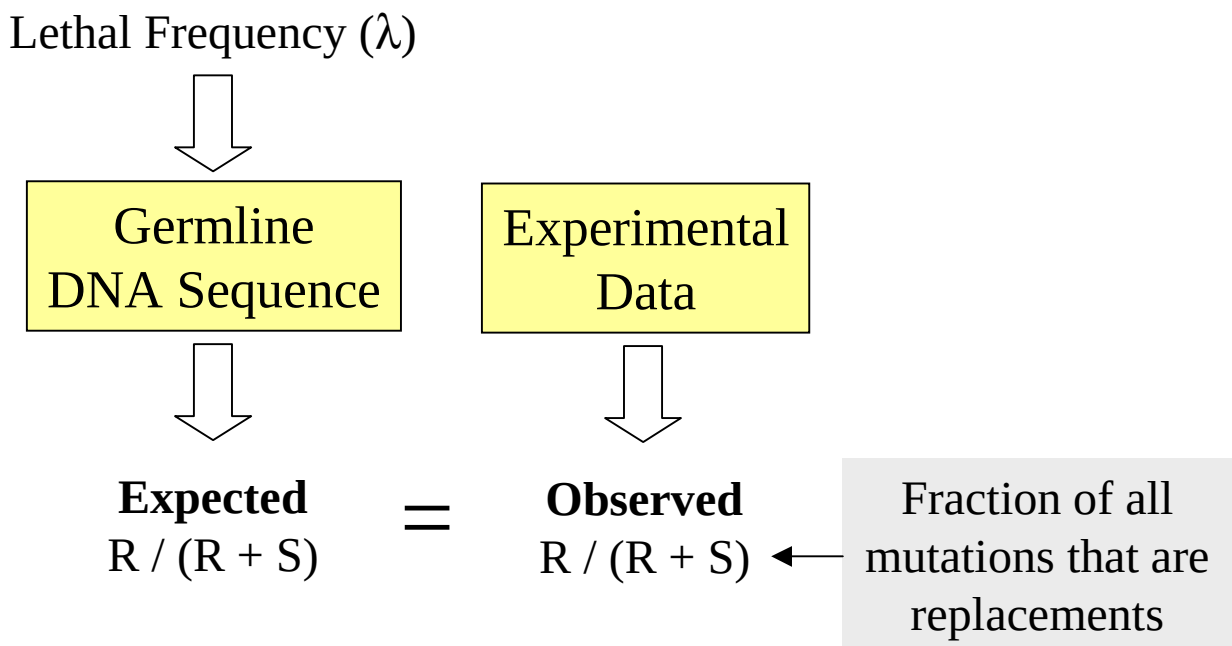
For each observed tree, choose number of divisions to minimize error

Estimating the Lethal Frequency (λ)

Simulation Model Parameters:
mutation rate (μ), # divisions (d), # sequences (s), lethal frequency (λ)

Only replacement mutations can be lethal, so…

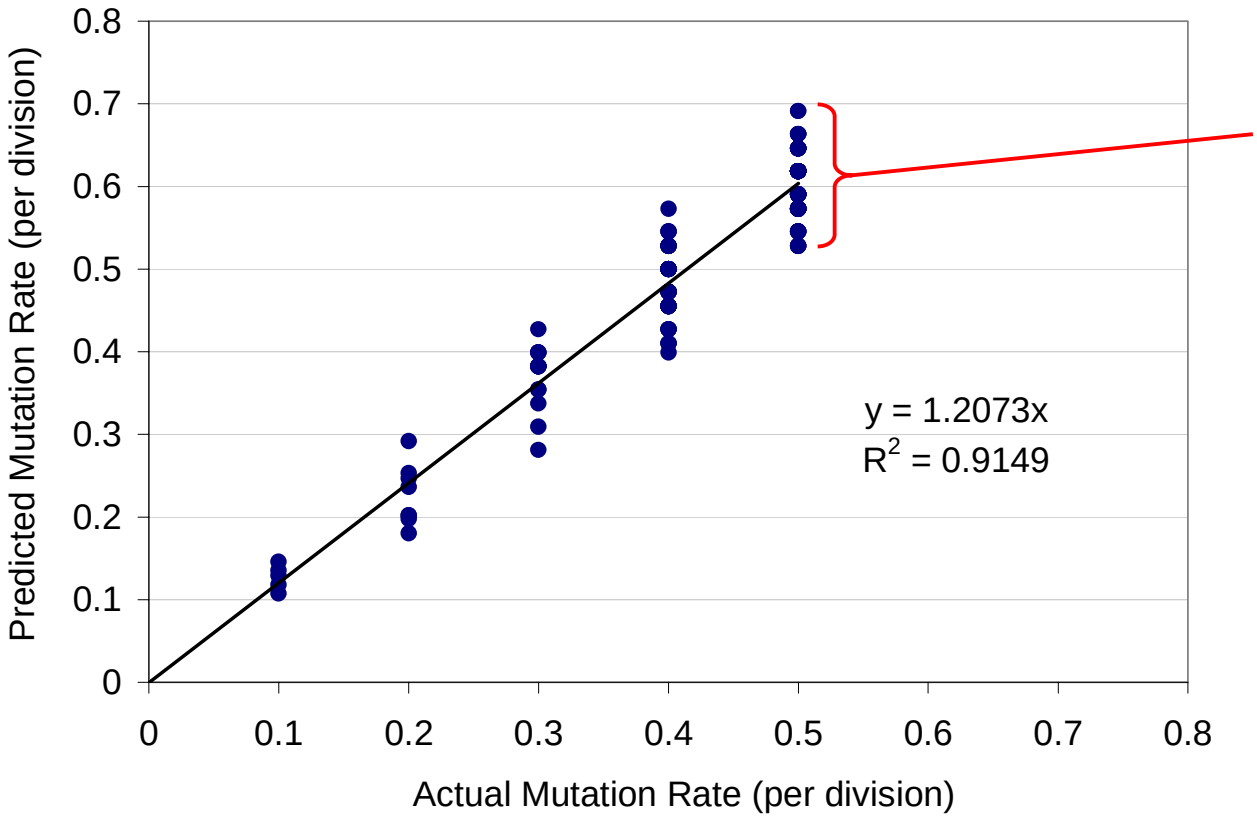




Choose λ so expected R/(R+S) equals observed value over all mutations

Validating the Simulation Method

Use simulation to construct synthetic data sets with limited number of trees/sequences reflecting currently available experimental data



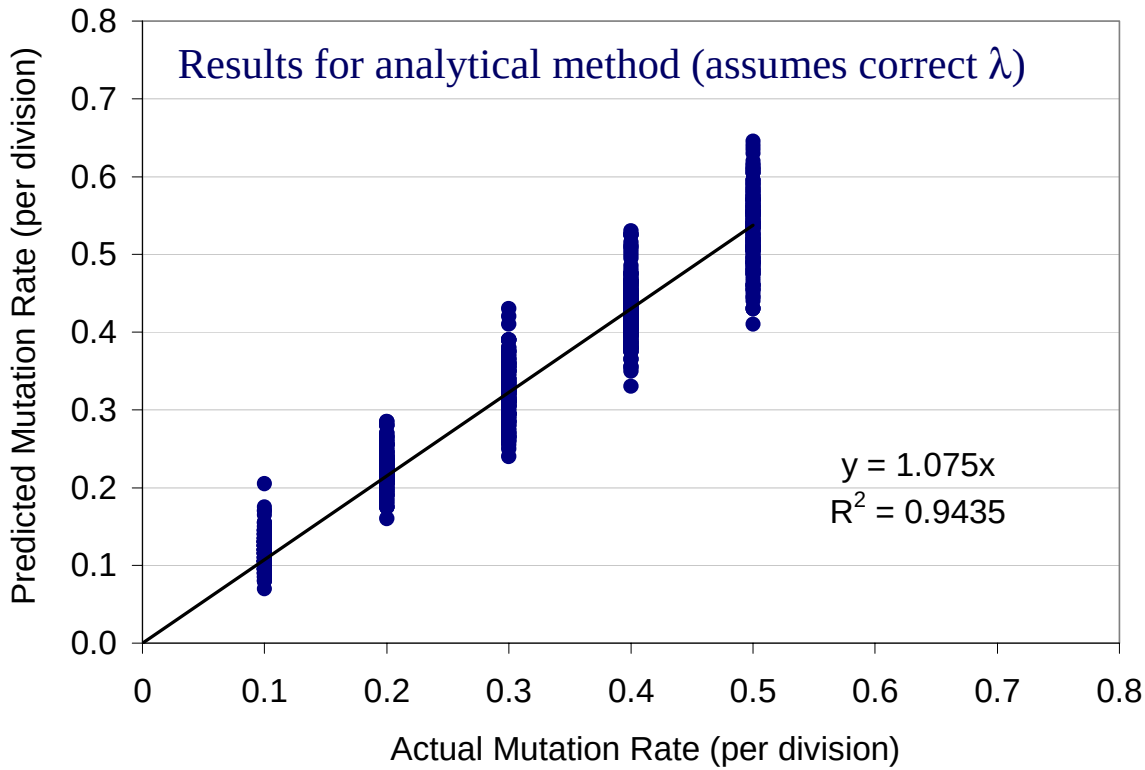
Method Precision

(SD = 0.035 division^{-1})

Method works even with limited number of clonal trees and sequences

Validating the Analytical Method

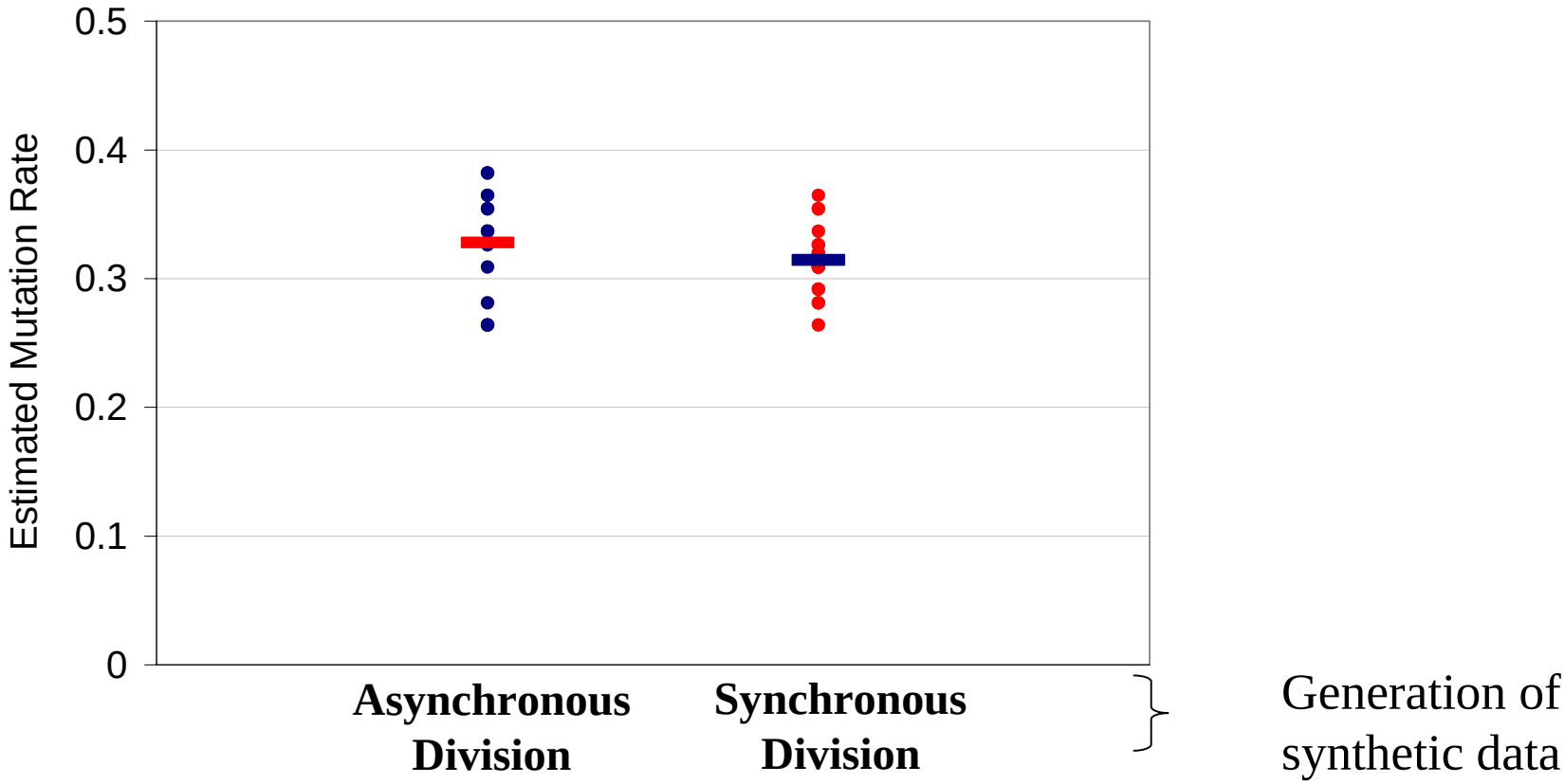
Use simulation to construct artificial data sets with limited number of trees/sequences reflecting currently available experimental data



Method works even with limited number of clonal trees and sequences

Testing Method Assumption…

All cells in single microdissection divided same number of times (i.e., division is synchronous)

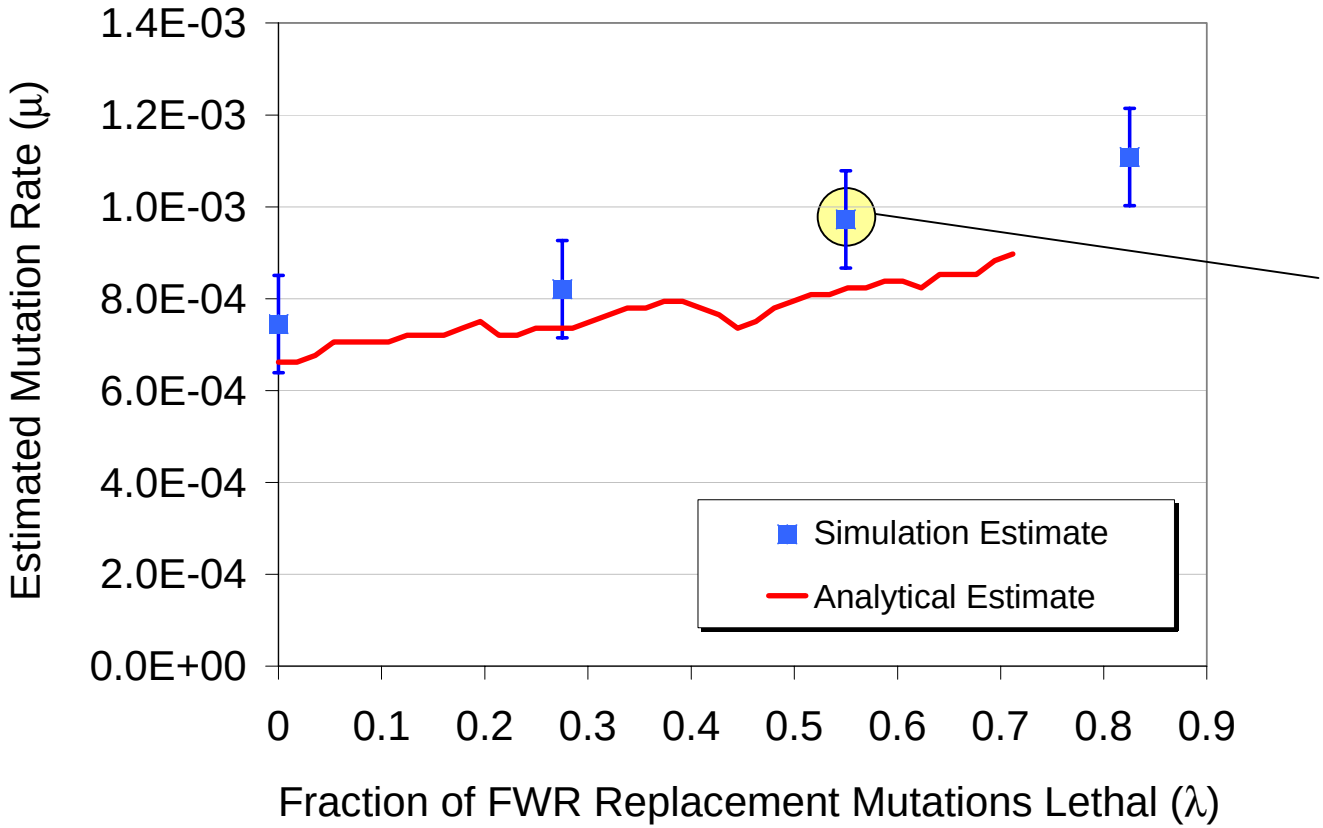


Assumption does not significantly impact rate estimate

Mutation Rate in Autoimmune Response

Experimental data set: 31 trees from 7 mice, ≈6 sequences / tree from extra-follicular areas

(Williams et al, Science, 2002)



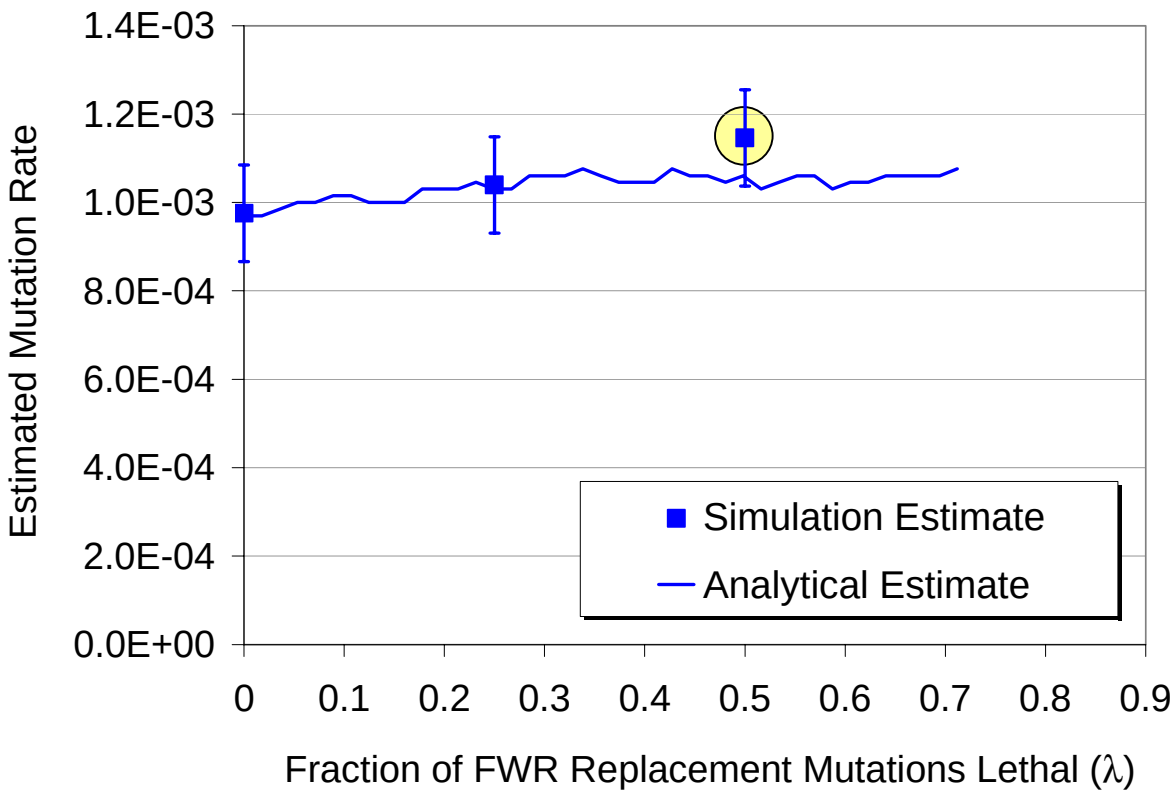
Estimated λ ≈ 0.55
based on R/(R+S)

Estimated mutation rate is $1.0 \pm 0.1 \times 10^{-3}$ base-pair^{-1} division^{-1}

Mutation Rate in Primary NP Response

Experimental data set: 23 trees, ≈7 sequences / tree from germinal centers

(Jacob et al., 1991; Jacob and Kelsoe, 1992; Jacob et al., 1993; Radmacher et al., 1998)



Testing impact on estimate:

- Data based on larger picks
- Positive selection may be factor

Estimated mutation rate is $1.1 \pm 0.1 \times 10^{-3}$ base-pair^{-1} division^{-1}

Summary

- Developed simulation and analytical methods to estimate *in vivo* mutation rates (and lethal frequencies)
  - First rigorous method for *in vivo* estimates
- Synthetic datasets used to show that…
  - Methods are precise ($\pm$ 0.1 x 10^{-3} base-pair^{-1} division^{-1})
  - Assumption of synchronous division does not impact results
- Extra-follicular B cells in autoimmune mouse hypermutate
  - Mutation rate (0.9 $\pm$ 0.1 x 10^{-3}) similar to NP response (1.1 $\pm$ 0.1 x 10^{-3})
- Future improvements in precision with additional data

Rigorous method to compare mutation rates under varying experimental conditions

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For more information:

Kleinstein, Louzoun and Shlomchik
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