On Possible Indicators of Negative Selection in Germinal Centers

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What is a Germinal Center?

GC: Keystone of Adaptive Immunity

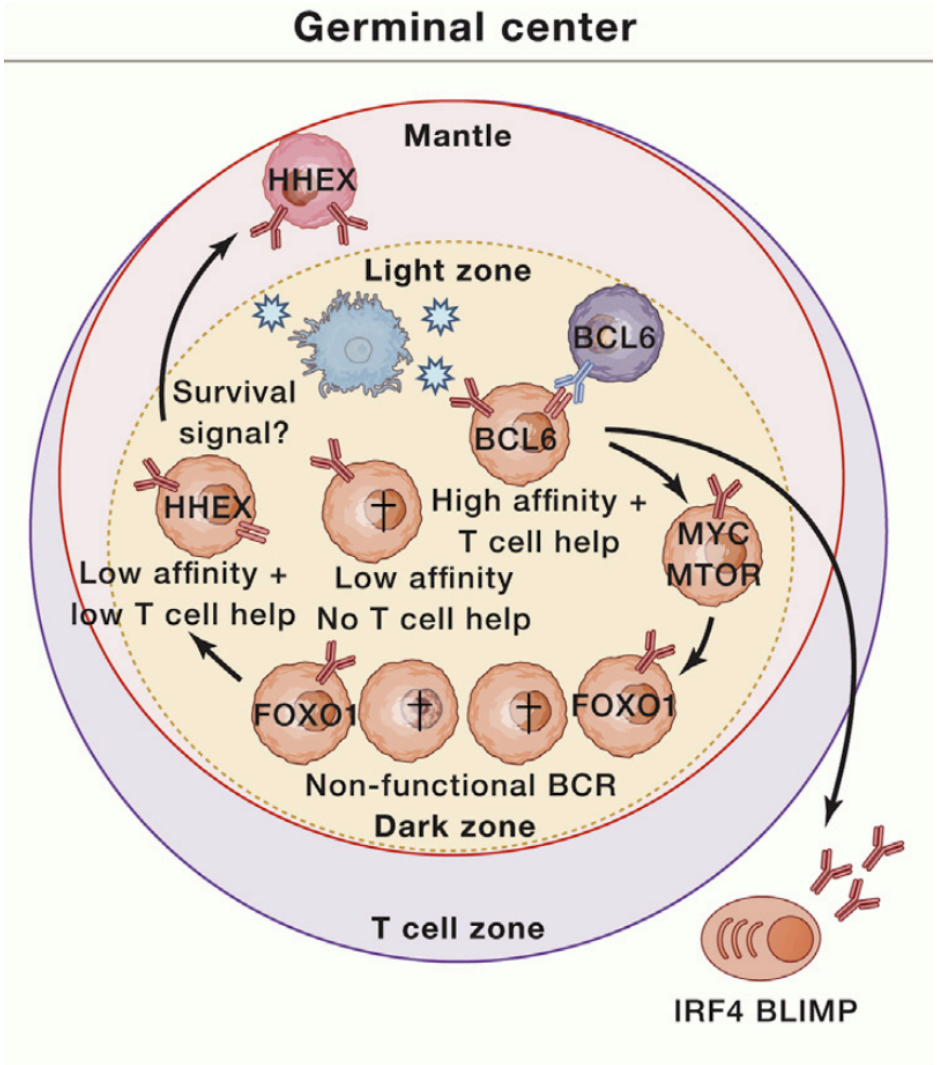

Figure: Young (2021)

GC: Small Scale Evolutionary Optimization Alg.

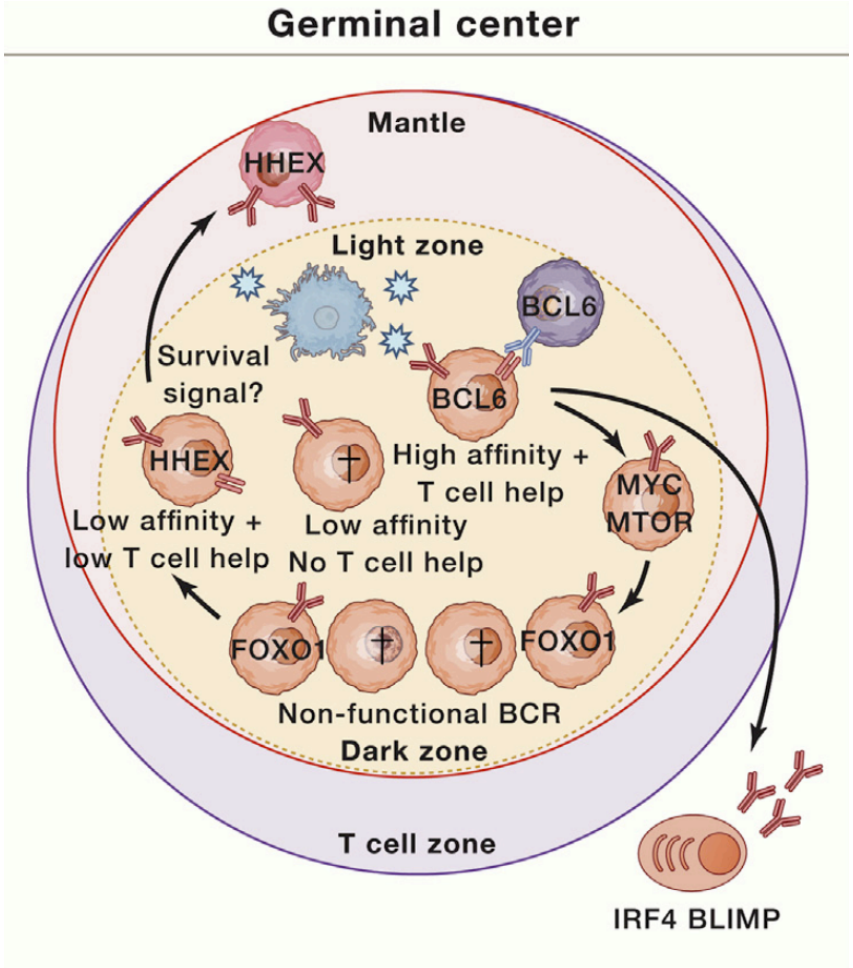

Figure: Young (2021)

- Body gets hit by pathogen.
- Anitgen gets presented to B-Cells in Germinal Center.
- Speedy evolution for **Affinity** (binding ability).
- Get high quality antibodies (yay!)

GCs are Neat (For Theory)

- Fast & small scale evolution.
- Contrained system.
- Known objective function.

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The fitness of a B cell should be related to its affinity.

Question: How *Does* Selection Take Place?

Standard Models: Single Parameter

Traditional population growth model:

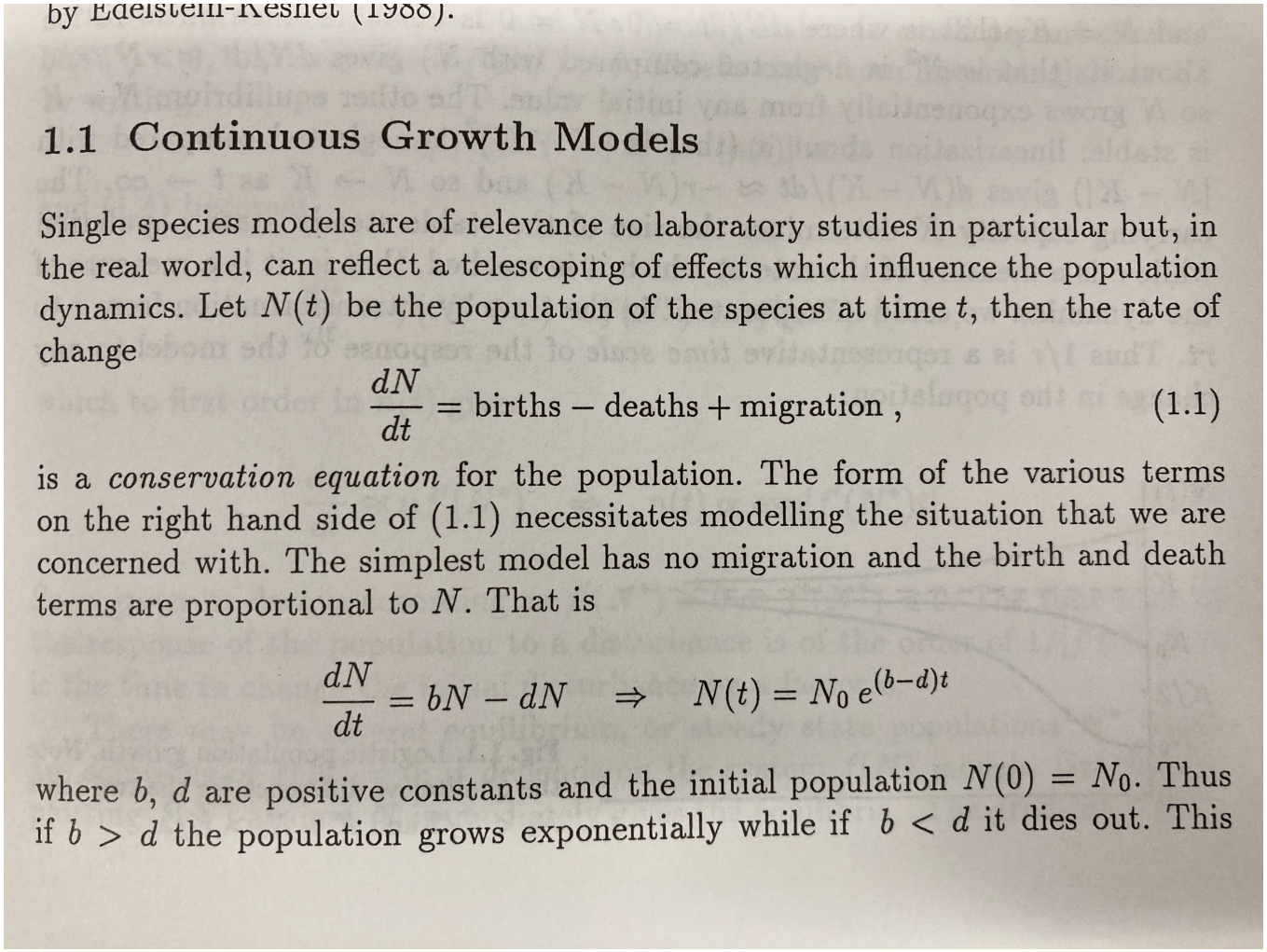
$$\text{New cell rate} = (\text{Cell ``Fitness''}) * (\text{Number of Cells})$$

Standard Models: Single Parameter

Traditional population growth model:

$$\text{New cell rate} = (\text{birth rate} - \text{death rate}) * (\text{Number of Cells})$$

Normal models: Single Parameter



by Edelstein-Keshet (1988).

1.1 Continuous Growth Models

Single species models are of relevance to laboratory studies in particular but, in the real world, can reflect a telescoping of effects which influence the population dynamics. Let $N(t)$ be the population of the species at time t, then the rate of change

$$\frac{dN}{dt} = \text{births} - \text{deaths} + \text{migration}\,, \tag{1.1}$$

is a *conservation equation* for the population. The form of the various terms on the right hand side of (1.1) necessitates modelling the situation that we are concerned with. The simplest model has no migration and the birth and death terms are proportional to N. That is

$$\frac{dN}{dt} = bN - dN \quad \Rightarrow \quad N(t) = N_0\, e^{(b-d)t}$$

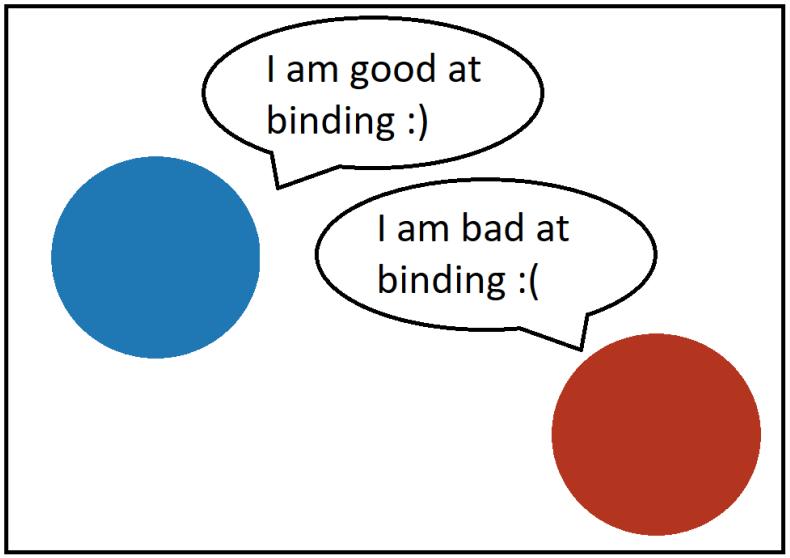
where b, d are positive constants and the initial population $N(0) = N_0$. Thus if $b > d$ the population grows exponentially while if $b < d$ it dies out. This

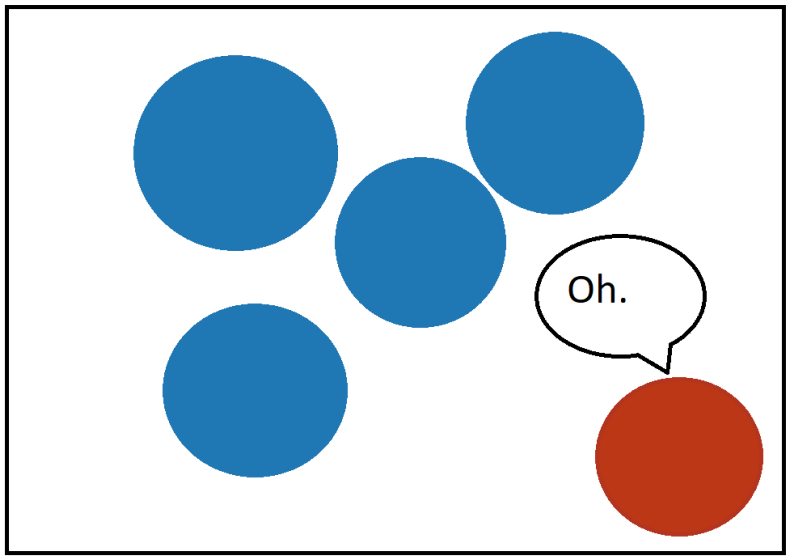
Figure: Murray (1993)

Birth and Death Are Not The Same

Birth and Death Are Not The Same

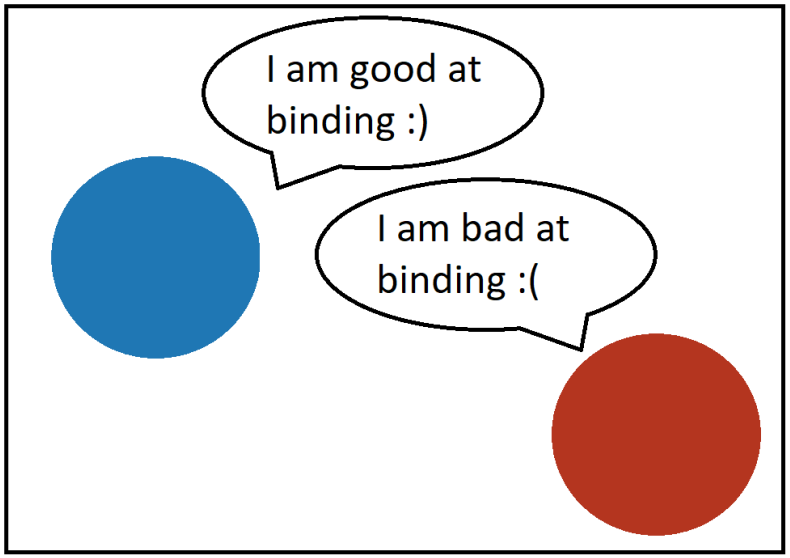
Birth Selection/Positive Selection: High fitness = divide faster

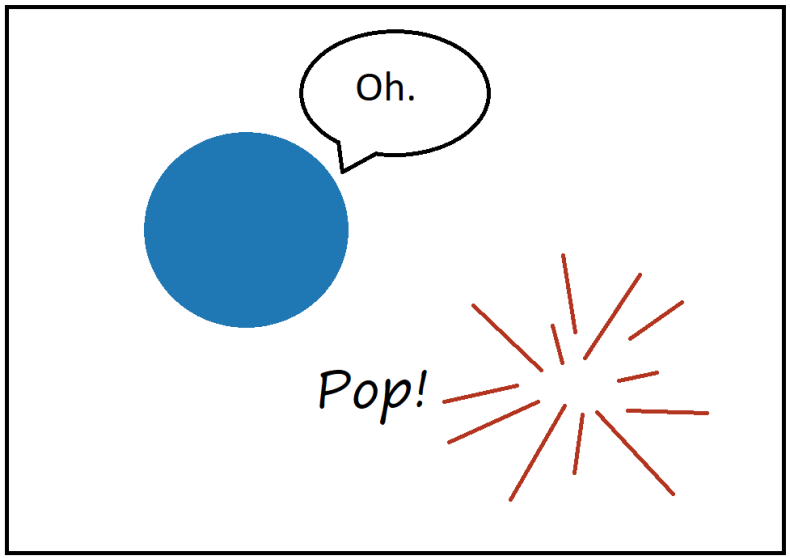




Birth and Death Are Not The Same

Death Selection/Negative Selection: High fitness = die slower





Detour: The Moran Model

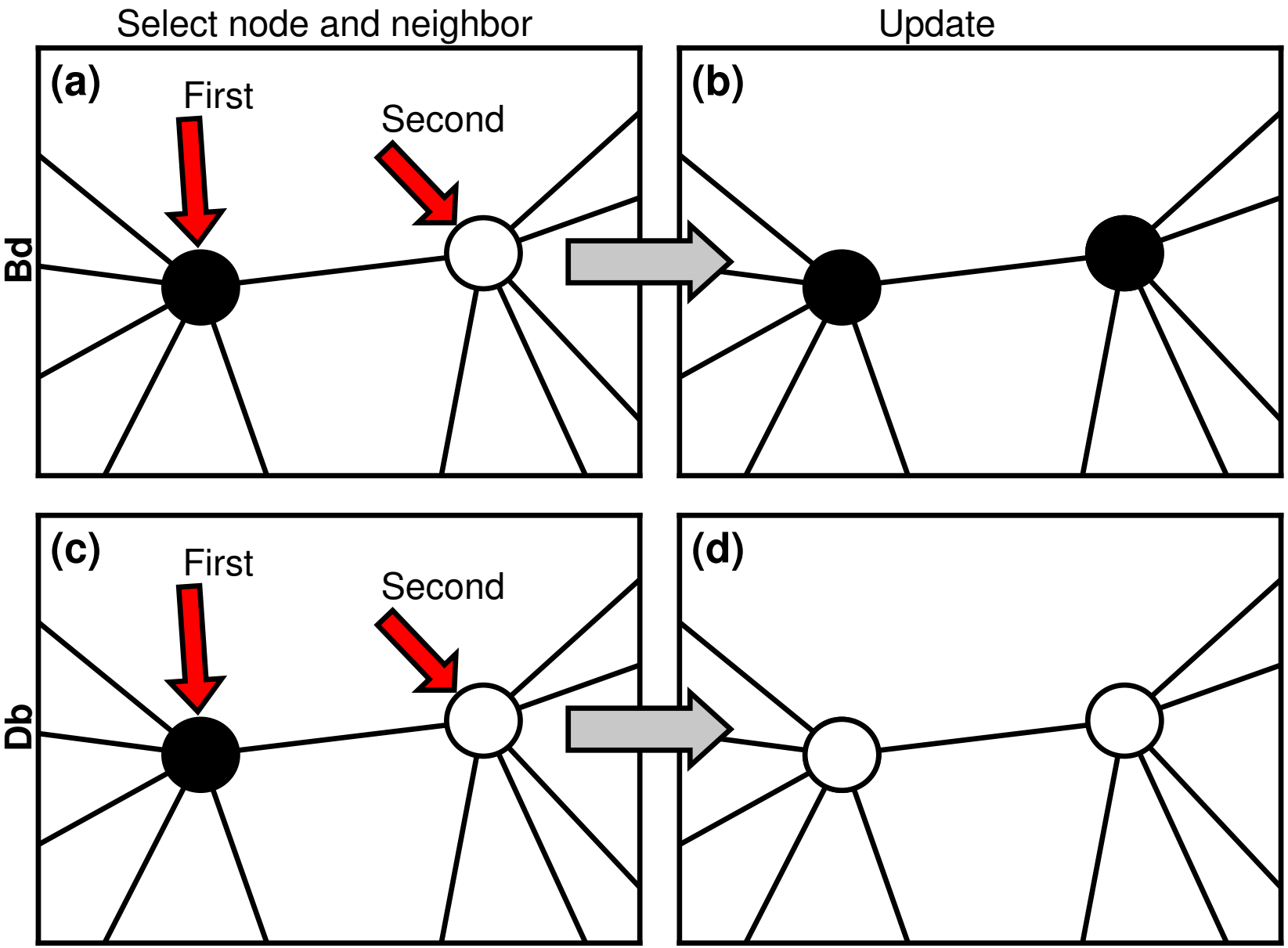
Detour: The Moran Model

Definition

The **Moran Model** is a simple model of cancer development.

- Looks at how mutants strains spread through tissues.
- Perserves tissue structure.
- Slooooooow mutation rate.
- Evolves with simple birth-death rules.

Detour: Moran Model(s)

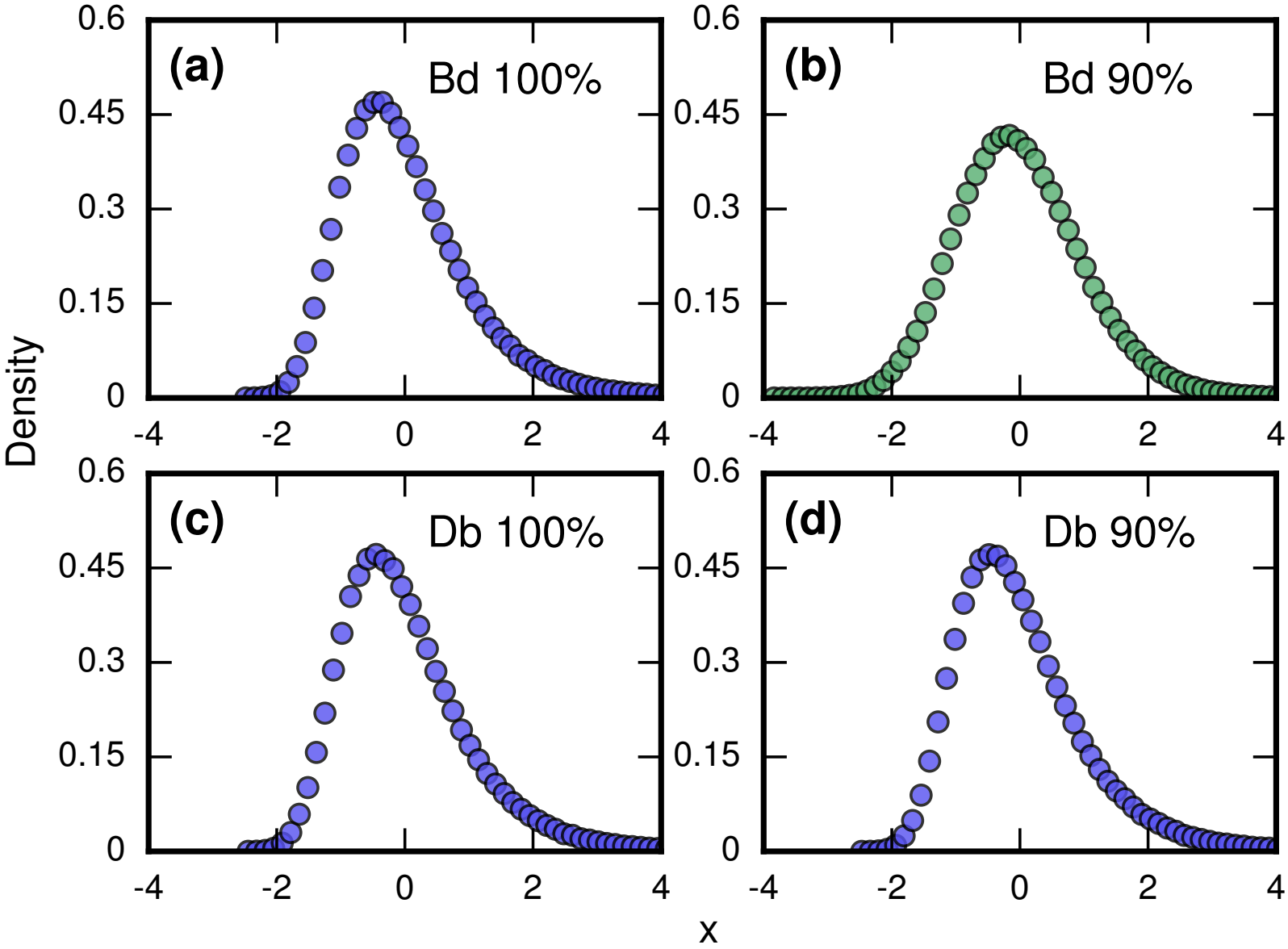


Detour: Key Metric

Definition

The **Partial Takeover Time** measures how long it takes for a novel mutant to take over X% of the tissue.

Detour: Fixation Times Distinguish Birth and Death



End of Detour: Birth and Death Are Not The Same

Intuition:

- Birth Selection starts fast, slows down near total fixation.
- Death Selection starts slow, but fixates fast.
- Truncation skip slow regime for Birth Selection, changing the qualitative shape.
- (Connection to the *Coupon Collector's Problem* in probability theory).

The Goal

Can we find a convenient signature of Death Selection in Germinal Centers?

Method

We want a model that...

1. Recreates the basic dynamics of GCs, and
2. Includes are few parts as possible.

We want a model that will produce the strongest possible signal of selection, with a minimum of confounding variables.

A Model for Affinity Selection

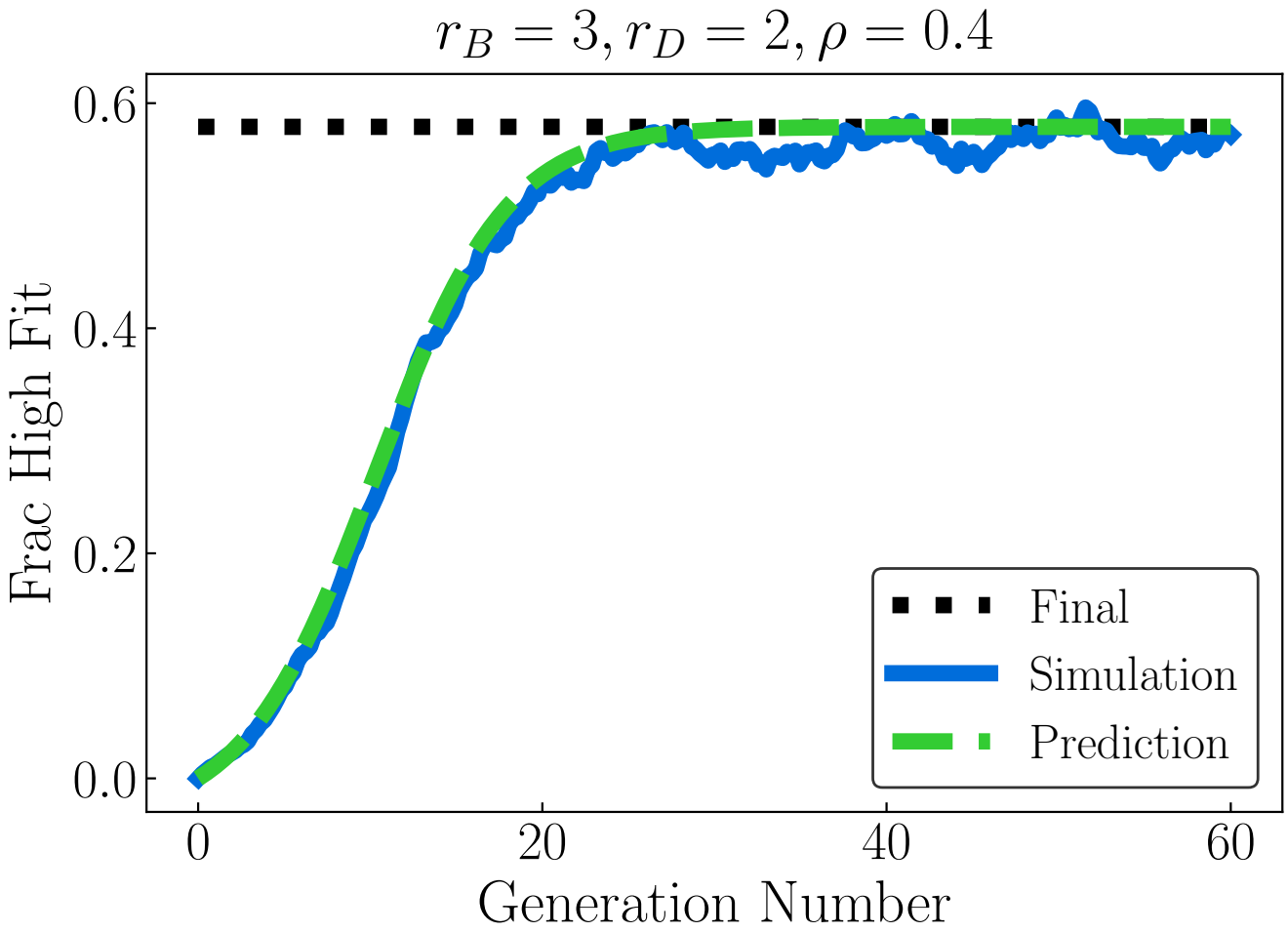
Only two affinities, High (H) and Low (L), to produce the maximum possible signal.

GC has three potential selection mechanisms:

- Birth Selection (r_B),
- Death Selection (r_D),
- Mutational Selection (??).

Typical Selection Curve

h = fraction of population which is high affinity.



Mutational Selection?

- Mutations in GC occur 10^6 times more often than default. (1 mutation per 1000 base pairs, on $\approx 350 - 700$ base pairs)
- GCs occasionally have **Clonal Bursts**, where one cell line repeatedly divides.
- When bursting, the line has a **notably lower** mutation rate than normal for the GC.
- This opens the possibility of a **tunable mutation rate**.

Asymetric Mutation Rates

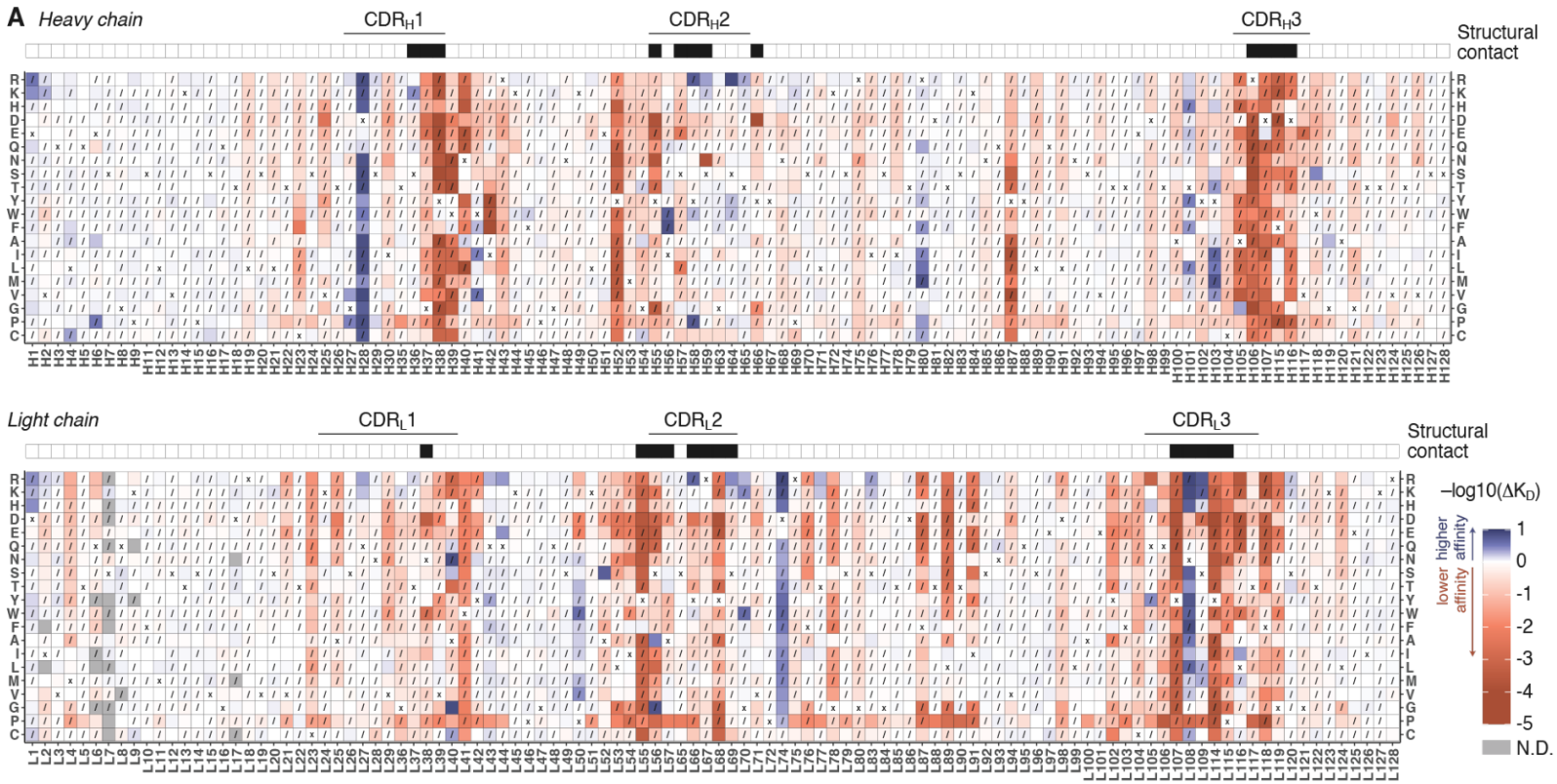


Figure: Tatsuya (2022)

Asymetric Mutation Rates

When an H cell divides, each daughter has one of three options:

1. It mutates into an L (w.p. β_H).
2. It aquires a neutral mutation (w.p. α_H).
3. It is a regular daughter (w.p. $1 - \alpha_H - \beta_H$).

Average Selection

$\beta_H = H->L$ mutation rate, $\beta_L = L->H$ mutation rate
Let $\ell = 1 - h$, then the average selection dynamics are given by:

$$\boxed{\frac{dh}{dt} = \frac{\beta_L}{1 + n/N}\frac{\ell}{r_B h + \ell} + \frac{1 - \beta_H}{1 + n/N}\frac{r_B h}{r_B h + \ell} - \frac{n/N}{1 + n/N}\frac{h/r_D}{h/r_D + \ell}}$$

Skew Hypothesis

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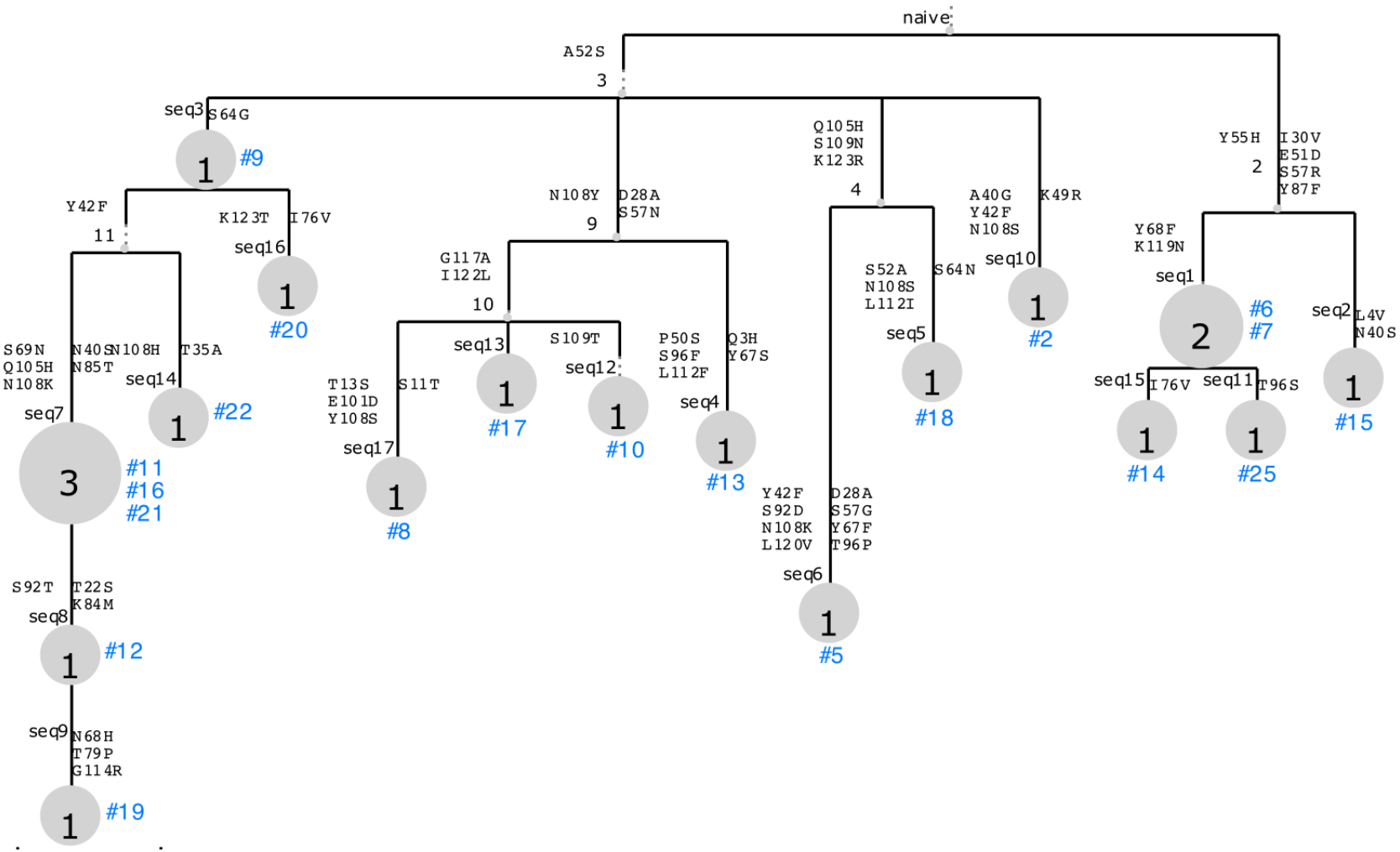
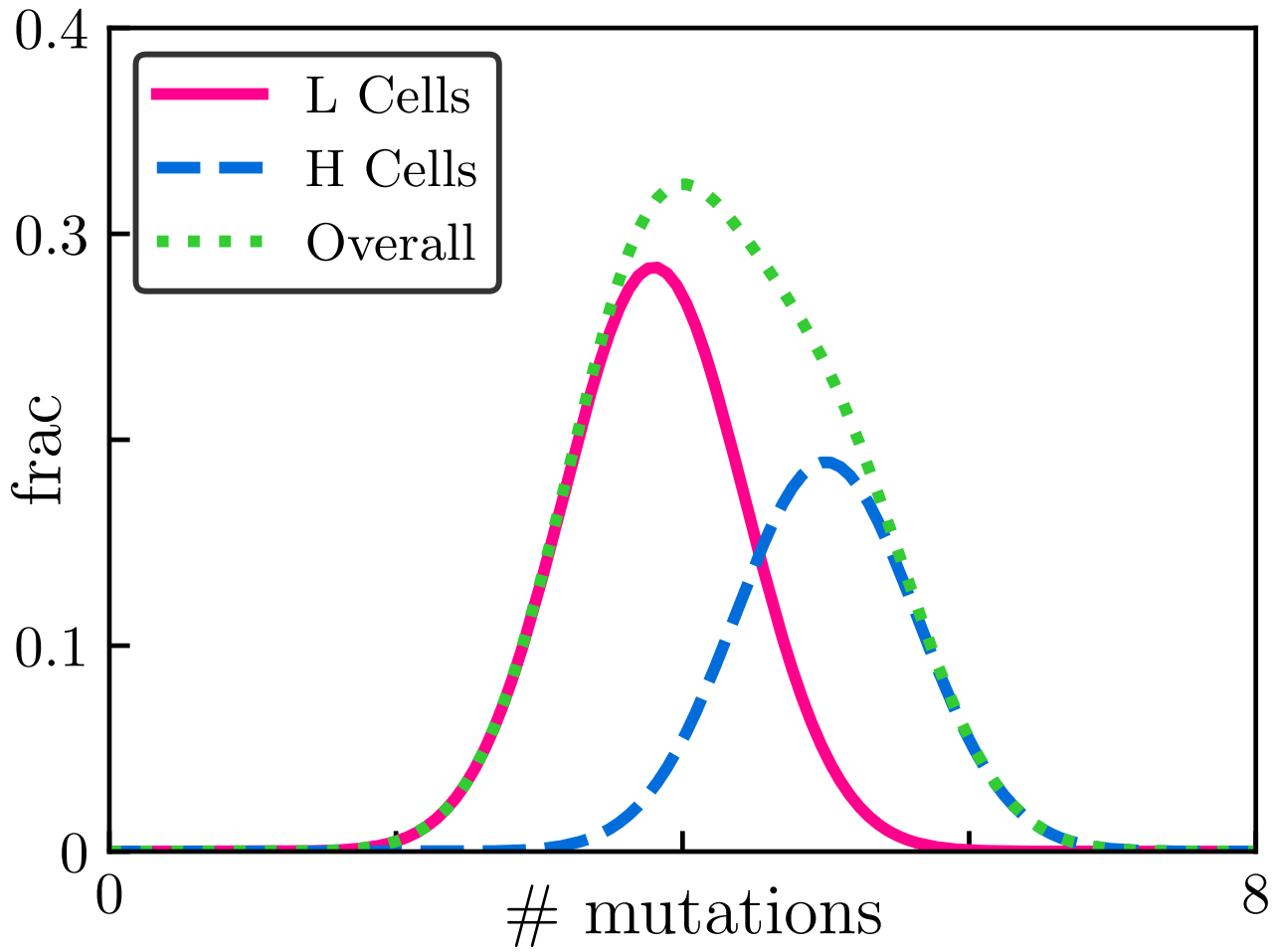


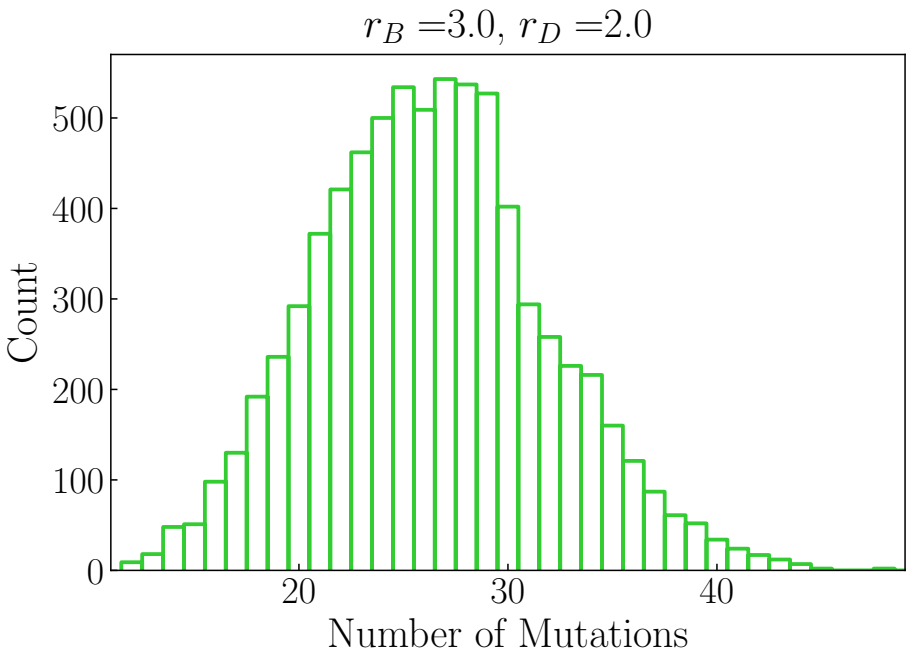
Figure: Tatsuya (2022)

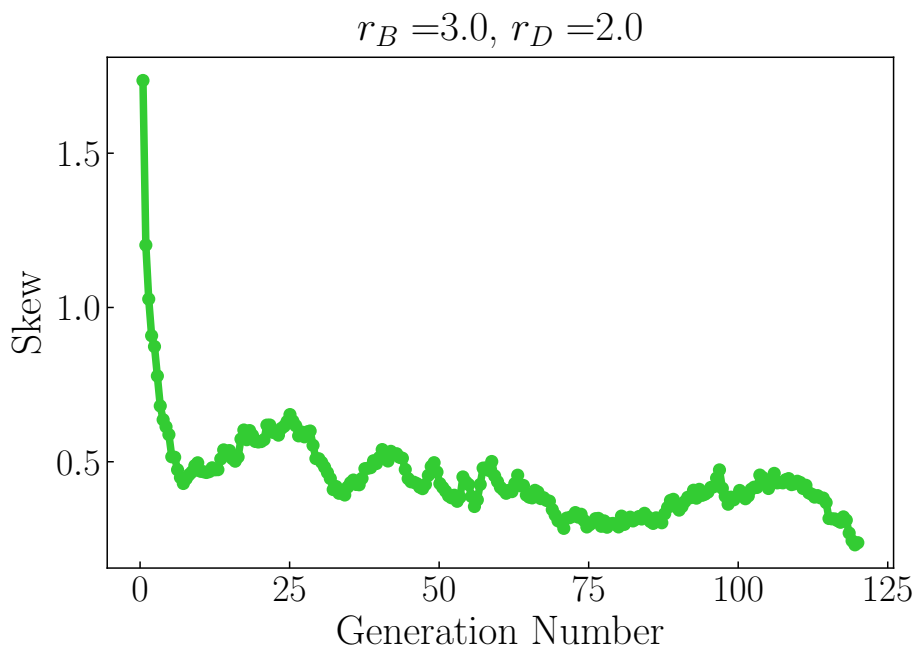
Skew Hypothesis

Since L and H have different levels of mutational activity, would they seperate?



Skew Hypothesis: Pilot Data





How is Skew Measured?

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Definition

The kth **Moment** of N numbers is:

$$\langle x^k \rangle = \frac{{x_1}^k + {x_2}^k + \ldots + {x_N}^k}{N}$$

How is Skew Measured?

Definition

The shape of a distribution with mean μ is determined by its **Central Moments**:

$$C(x^k) = \frac{(x_1 - \mu)^k + (x_2 - \mu)^k + \ldots + (x_N - \mu)^k}{N}$$

- The second central moment measures the width of the distribution (the **Variance**).

How is Skew Measured?

Definition

The **Skew** of a distribution is given by:

$$\text{Skew}(x) = \frac{C(x^3)}{C(x^2)^{3/2}}$$

Calculation Outline: Mutation Dynamics

$$h_m = \frac{\text{number of H cells with m mutations}}{\text{number of cells}}$$

$$\ell_m = \frac{\text{number of L cells with m mutations}}{\text{number of cells}}$$

$$\begin{aligned} \langle m^k \rangle &= \text{k'th moment of mutation distribution} \\ &= \sum_m (h_m + \ell_m)\, m^k \end{aligned}$$

Calculation Outline: Linear Structure

$$\partial_t \langle m_H^k \rangle = A_H \langle m_H^k \rangle + B_H \sum_{w=0}^{k} \binom{k}{w} \langle m_H^k \rangle + C_H \sum_{w=0}^{k} \binom{k}{w} \langle m_L^k \rangle$$

$$\partial_t \langle m_L^k \rangle = A_L \langle m_L^k \rangle + B_L \sum_{w=0}^{k} \binom{k}{w} \langle m_H^k \rangle + C_L \sum_{w=0}^{k} \binom{k}{w} \langle m_L^k \rangle$$

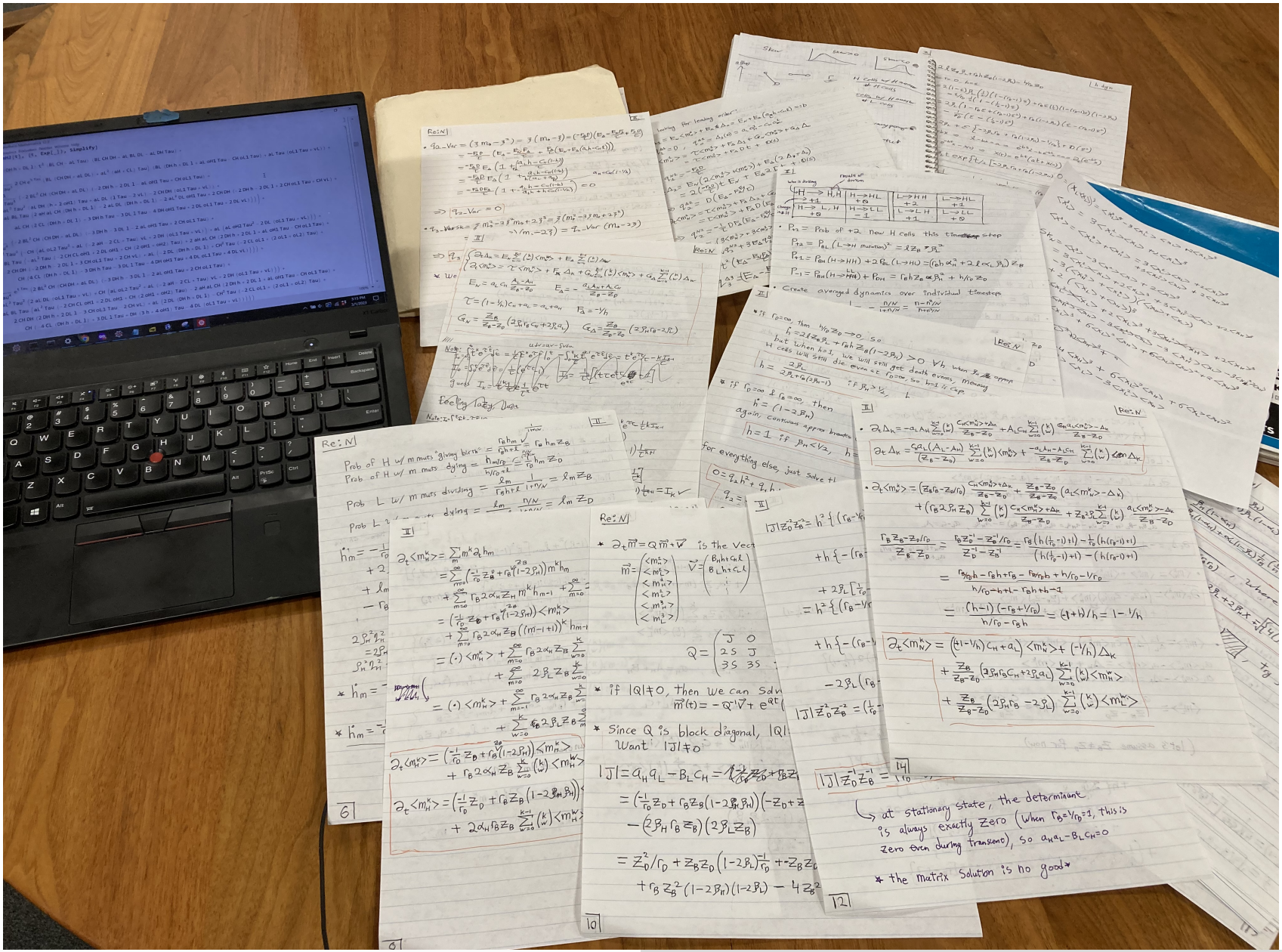
Calculation Outline: Back of Envelope

$$\text{Skew} = \frac{C(m^3)}{C(m^2)^{3/2}}$$

$$C(m^3) \approx \langle m^3 \rangle = \mathbb{O}(t^3)$$
$$C(m^2) \approx \langle m^2 \rangle = \mathbb{O}(t^2)$$

Therefore Skew $\rightarrow$ Constant

N O P E


Re: N
Prob of H w/ m mates giving birth = $\frac{r_B h_m}{r_B h + 1} = r_B h_m z_B$
Prob of H w/ m mates dying = $\frac{h_m r_D}{h r_D + 1} \approx r_D h_m z_D$
Prob L w/ m mates dividing = $\frac{\lambda_m}{r_B h + 1} \frac{1}{1 + r_D N} = \lambda_m z_B$
Prob L w/ m mates dying = $\frac{l_m}{1 + r_D N} = l_m z_D$
$\dot{h}_m = -\frac{1}{r_D} h_m + \lambda_{m-1} z_B h_{m-1} - \lambda_m z_B h_m + r_B 2\alpha_{m+1} z_B h_{m+1} - r_B 2\alpha_m z_B h_m$
$\dot{h}_m = -\frac{1}{r_D} h_m + \lambda_{m-1} z_B h_{m-1} - \lambda_m z_B h_m + r_B 2\alpha_{m+1} z_B h_{m+1} - r_B 2\alpha_m z_B h_m$
$\partial_t \langle m_H^k \rangle = \sum_m m^k \partial_t h_m$
$= \sum_{m=0}^{\infty} \left(-\frac{1}{r_D} z_B^0 + r_B^0 (1-2\beta_H)\right) m^k h_m + \sum_{m=0}^{\infty} r_B 2\alpha_H z_H m^k h_{m-1} + \sum_{m=0}^{\infty} \ldots$
$= \left(\frac{1}{r_D} z_B + r_B^0 (1-2\beta_H)\right) \langle m_H^k \rangle + \sum_{m=0}^{\infty} r_B 2\alpha_H z_B \left((m-1+1)\right)^k h_{m-1}$
$= (\cdot) \langle m_H^k \rangle + \sum_{m=0}^{\infty} r_B 2\alpha_H z_B \sum_{w=0}^{k} \ldots$
$= (\cdot) \langle m_H^k \rangle + \sum_{m=-1}^{\infty} r_B 2\alpha_H z_B \sum_{w=0}^{k} \ldots + \sum_{w=0}^{k} r_B 2\beta_L z_B \ldots$
$\partial_t \langle m_H^k \rangle = \left(\frac{-1}{r_D} z_D + r_B^0 (1-2\beta_H)\right) \langle m_H^k \rangle + r_B 2\alpha_H z_B \sum_{w=0}^{k} \binom{k}{w} \langle m_H^w \rangle$
$\partial_t \langle m_H^k \rangle = \left(\frac{-1}{r_D} z_D + r_B z_B \left(1 - 2\beta_H r_B z_B\right)\right) + 2\alpha_H r_B z_B \sum_{w=0}^{k-1} \binom{k}{w} \langle m_H^w \rangle$
Re: N
$\partial_t \vec{m} = Q \vec{m} + \vec{V}$ is the Vec
$\vec{m} = \begin{pmatrix} \langle m_H^1 \rangle \\ \langle m_L^1 \rangle \\ \langle m_H^2 \rangle \\ \langle m_H^1 m_L^1 \rangle \\ \langle m_L^2 \rangle \\ \langle m_H^3 \rangle \\ \langle m_L^3 \rangle \end{pmatrix}$ $\vec{V} = \begin{pmatrix} B_H h_t c_H L \\ B_L h_t c_L L \\ \vdots \end{pmatrix}$
$Q = \begin{pmatrix} J & 0 & \\ 2S & J & \\ 3S & 3S & \ddots \end{pmatrix}$
* if $|Q| \neq 0$, then we can Sdv
$\vec{m}(t) = -Q^{-1}\vec{V} + e^{Qt}(\ldots)$
* Since Q is block diagonal, $|Q|$
Want $|J| \neq 0$
$|J| = a_H q_L - B_L C_H = \ldots$
$= \left(\frac{-1}{r_D} z_D + r_B z_B \left(1 - 2\beta_H r_B z_B\right)\right)\left(-z_D + z \ldots\right)$
$- \left(2\beta_H r_B z_B\right)\left(2\beta_L z_B\right)$
$= z_D^2 / r_D + z_B z_D \left(1 - 2\beta_L\right) \frac{1}{r_D} + \ldots z_B z_D$
$+ r_B z_B^2 (1 - 2\beta_H)(1 - 2\beta_L) - 4 z_B^2$
$\partial_t \Delta_k = -a_L \Delta_k \sum_{w=0}^{k-1} \binom{k}{w} \frac{C_H \langle m_H^w \rangle + \Delta_k}{z_B - z_D} + A_L C_H \sum_{w=0}^{k-1} \binom{k}{w} \frac{a_H \langle m_H^w \rangle - \Delta_k}{z_B - z_D}$
$\partial_t \Delta_k = \frac{a_H \beta_L (A_L - A_H)}{(z_B - z_D)} \sum_{w=0}^{k-1} \binom{k}{w} \langle m_H^w \rangle - \frac{a_L A_H + A_L C_H}{z_B - z_D} \sum_{w=0}^{k-1} \binom{k}{w} \Delta_k$
$\partial_t \langle m_H^k \rangle = \left(z_B r_B - z_D / r_D\right) \frac{C_H \langle m_H^k \rangle + \Delta_k}{z_B - z_D} + \frac{z_B - z_D}{z_B - z_D} \left(a_L \langle m_H^k \rangle - \Delta_k\right)$
$+ \left(r_B \beta_H r_B z_B\right) \sum_{w=0}^{k-1} \binom{k}{w} \frac{C_H \langle m_H^w \rangle + \Delta_k}{z_B - z_D} + z_B \beta_L \sum_{w=0}^{k-1} \binom{k}{w} \frac{a_L \langle m_H^w \rangle - \Delta_k}{z_B - z_D}$
$\frac{r_B z_B - z_D / r_D}{z_B - z_D} = \frac{r_B z_D^{-1} - z_B^{-1} / r_D}{z_D^{-1} - z_B^{-1}} = \frac{r_B \left(h\left(\frac{1}{r_D} - 1\right) + 1\right) - \frac{1}{r_D}\left(h\left(r_B - 1\right) + 1\right)}{\left(h\left(\frac{1}{r_D} - 1\right) + 1\right) - \left(h\left(r_B - 1\right) + 1\right)}$
$= \frac{r_B / r_D h - r_B h + r_B - r_B / r_D h + h / r_D - 1 / r_D}{h / r_D - h + 1 - r_B h + h - 1}$
$= \frac{(h-1)\left(-r_B + 1/r_D\right)}{h / r_D - r_B h} = \frac{-(1+h)}{h} = 1 - 1/h$
$\partial_t \langle m_H^k \rangle = \left(\left(1 - 1/h\right) C_H + a_L\right) \langle m_H^k \rangle + \left(-1/h\right) \Delta_k$
$+ \frac{z_B}{z_B - z_D} \left(a_H \beta_H r_B C_H + 2\beta_H a_L\right) \sum_{w=0}^{k-1} \binom{k}{w} \langle m_H^w \rangle$
$+ \frac{z_B}{z_B - z_D} \left(2\beta_H r_B - 2\beta_L\right) \sum_{w=0}^{k-1} \binom{k}{w} \langle m_H^w \rangle$
$|J| z_D^{-1} z_B^{-1} = \frac{1}{r_D}$
↳ at stationary state, the determinant is always exactly zero (when $r_B = 1/r_D = 1$, this is zero even during transens), so $a_H q_L - B_L C_H = 0$
* the matrix solution is no good
6
10
12
14

BJOL

Calculation Outline: Problem

$$\partial_t \vec{m} = Q\vec{m} + \vec{v}$$

Truncating to the third moment gives:

$$\boxed{|Q| = |J|^3 = \left[Z_D Z_B \left(1/r_D - r_B\right) \partial_t h\right]^3 \to 0}$$

So basic methods are poor in steady-state.

Calculation Outline: Better Method

Instead of computing $\langle m_H^k \rangle$ and $\langle m_L^k \rangle$, instead compute:

$$\mathcal{M}_k := \langle m_H^k \rangle + \langle m_L^k \rangle,$$
$$\mathcal{S}_k := (r_B Z_B \beta_H / \ell)\langle m_H^k \rangle + (Z_B \beta_L / h)\langle m_L^k \rangle.$$

Calculation Outline: Better Method

$$\partial_t \mathcal{M}_k = -\tau \mathcal{M}_k + \mathcal{S}_k + M_M \sum_{w=0}^{k-1} \binom{k}{w} \mathcal{M}_w + M_S \sum_{w=0}^{k-1} \binom{k}{w} \mathcal{S}_w,$$

$$\partial_t \mathcal{S}_k = S_M \sum_{w=0}^{k-1} \binom{k}{w} \mathcal{M}_w + S_S \sum_{w=0}^{k-1} \binom{k}{w} \mathcal{S}_w.$$

Calculation Outline: Better Method

$$\partial_t \mathcal{M}_k = -\tau \mathcal{M}_k + \mathcal{S}_k + M_M \sum_{w=0}^{k-1} \binom{k}{w} \mathcal{M}_w + M_S \sum_{w=0}^{k-1} \binom{k}{w} \mathcal{S}_w,$$

$$\partial_t \mathcal{S}_k = S_M \sum_{w=0}^{k-1} \binom{k}{w} \mathcal{M}_w + S_S \sum_{w=0}^{k-1} \binom{k}{w} \mathcal{S}_w.$$

- $\partial_t \mathcal{M}_k$ depends on terms $\{k, \ldots, 0\}$, and $\partial_t \mathcal{S}_k$ depends on terms $\{k-1, \ldots, 0\}$.
- So this is iteratively solvable.

Calculation Outline: Better Method

$C(m^N)[t^W]$ = the coefficient of the t^W term of the N'th central moment.

Calculation Outline: Better Method

$C(m^N)[t^N]$ = the coefficient of the t^W term of the N'th central moment.

$$C(m^N)[t^N] = 0,$$
$$C(m^N)[t^{N-1}] = \delta_{N,2}\left[\mu + \frac{2S_M}{\tau}\left(\frac{M_M}{\tau} + M_S - \frac{\mu}{\tau}\right)\right].$$

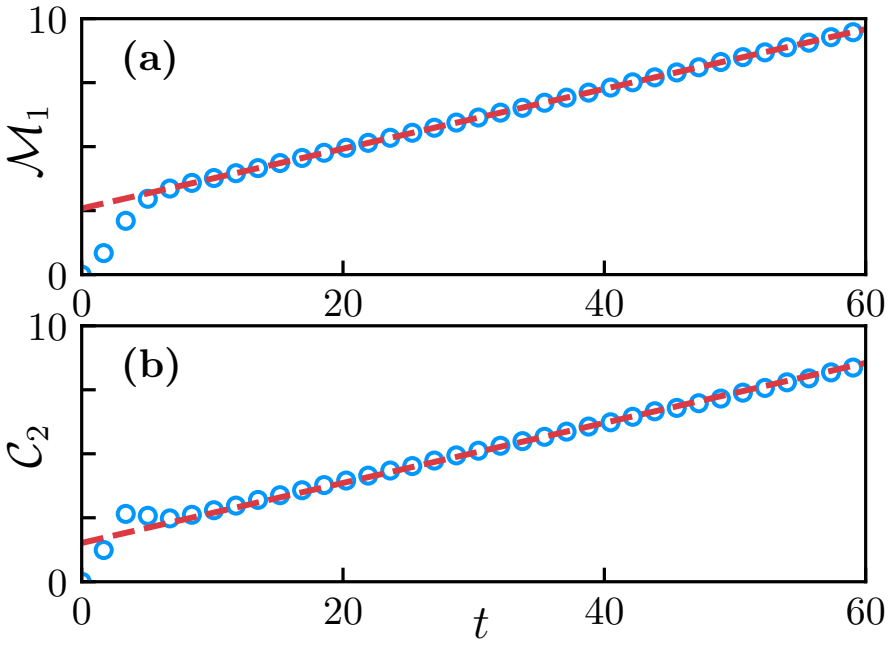
Calculation Outline: Using Careful Methods

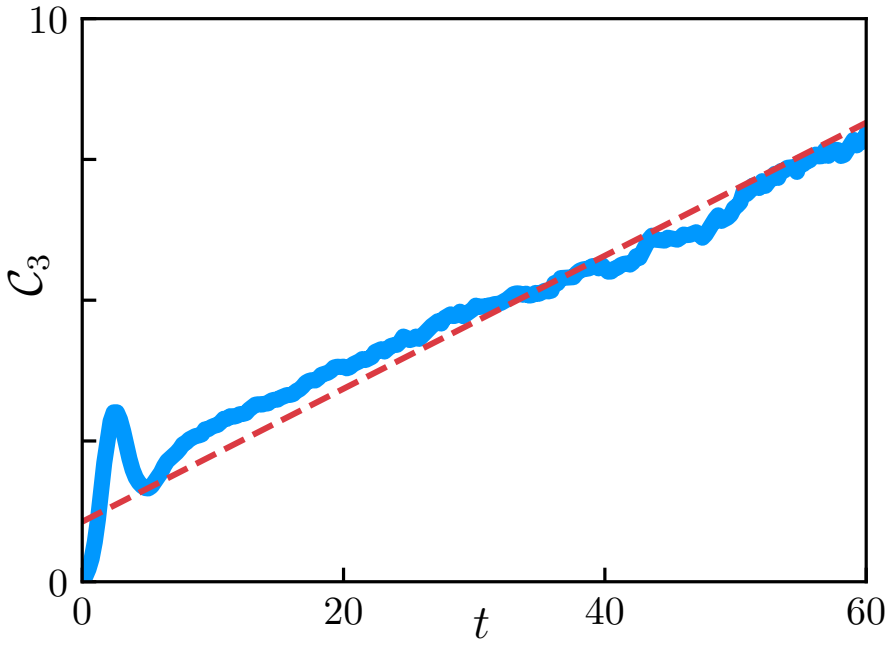
$$\text{Skew} = \frac{C(m^3)}{C(m^2)^{3/2}}$$

where

$$C(m^3) = \langle m^3 \rangle - 3\langle m^2 \rangle \langle m \rangle + 2\langle m \rangle^3 = \mathbb{O}(t)$$
$$C(m^2) = \langle m^2 \rangle - 2\langle m \rangle^2 = \mathbb{O}(t)$$

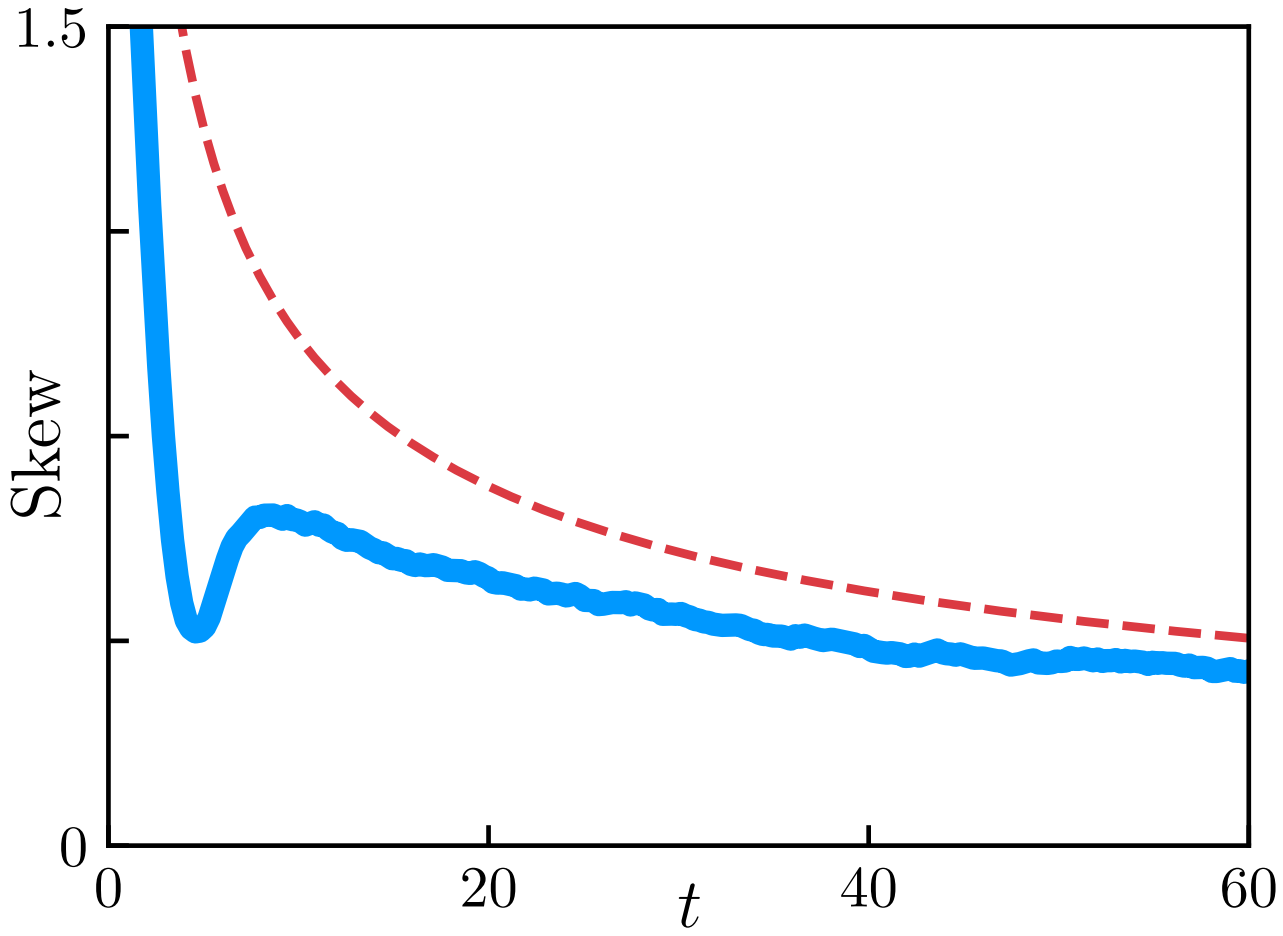
Mutations: The Truth



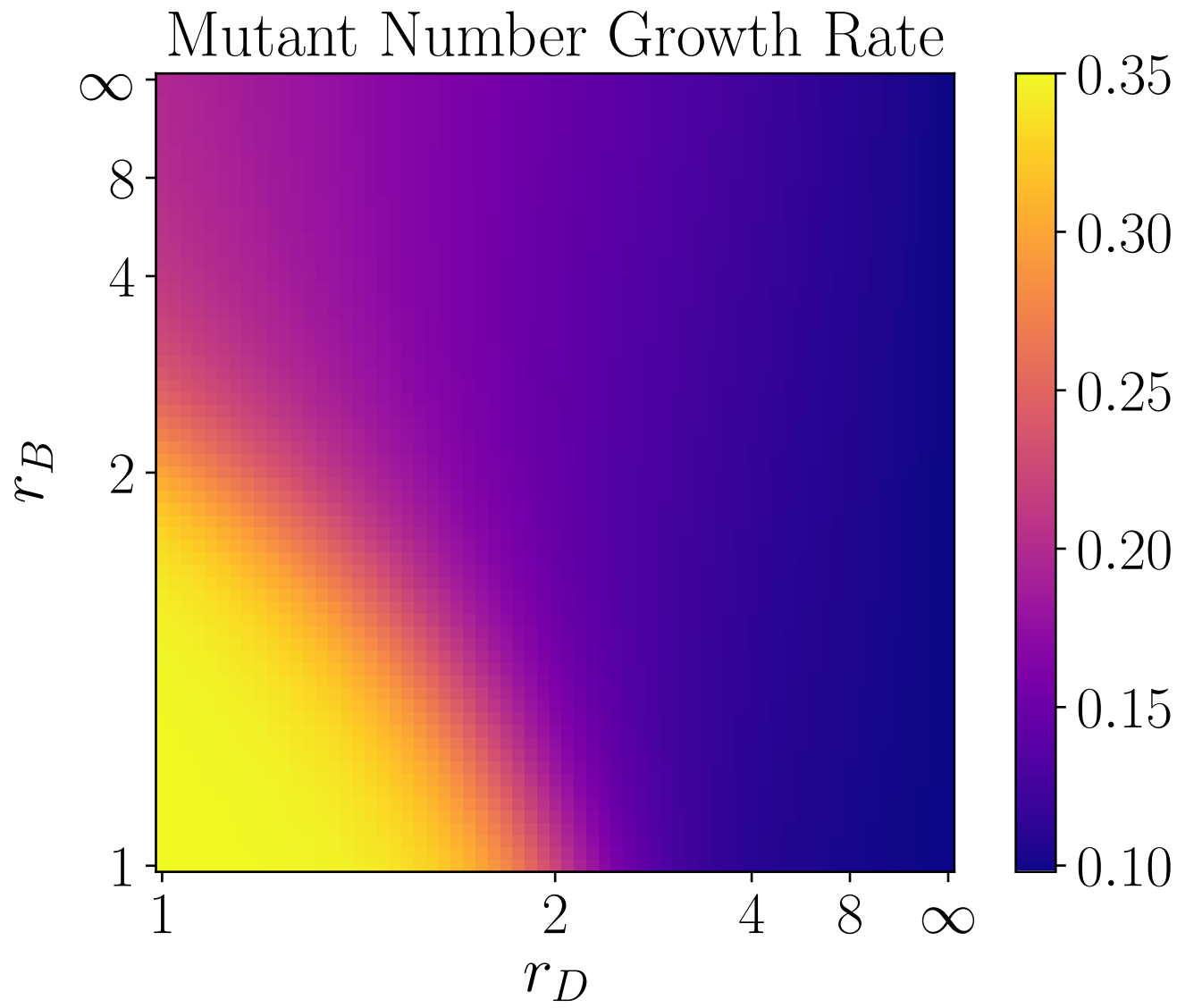


Mutations: The Truth

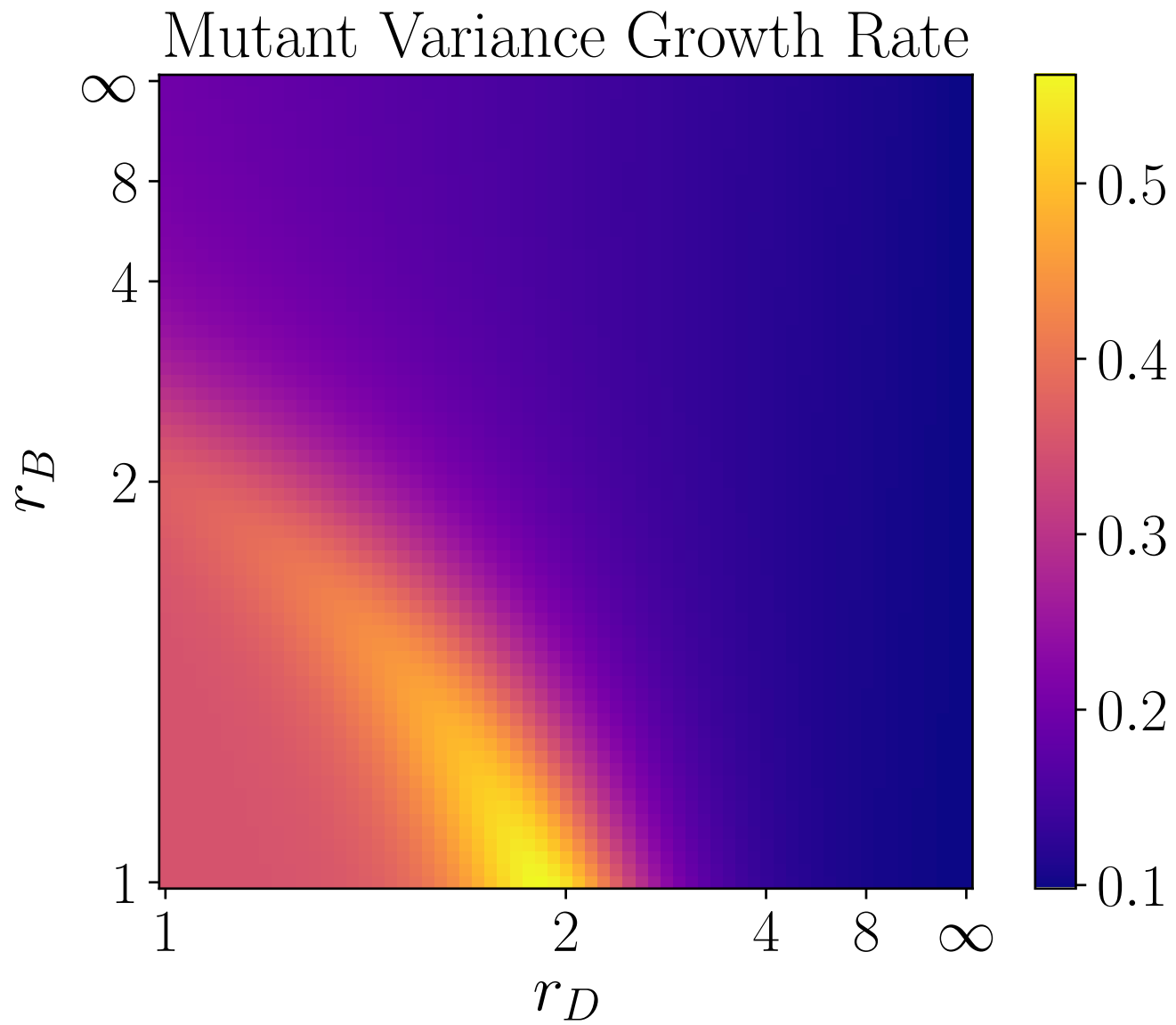
$$\text{Skew} = \mathbb{O}(t^{-1/2}) \to 0$$



Dynamical Footprint of Selection



Dynamical Footprint of Selection



Ancestry Hypothesis

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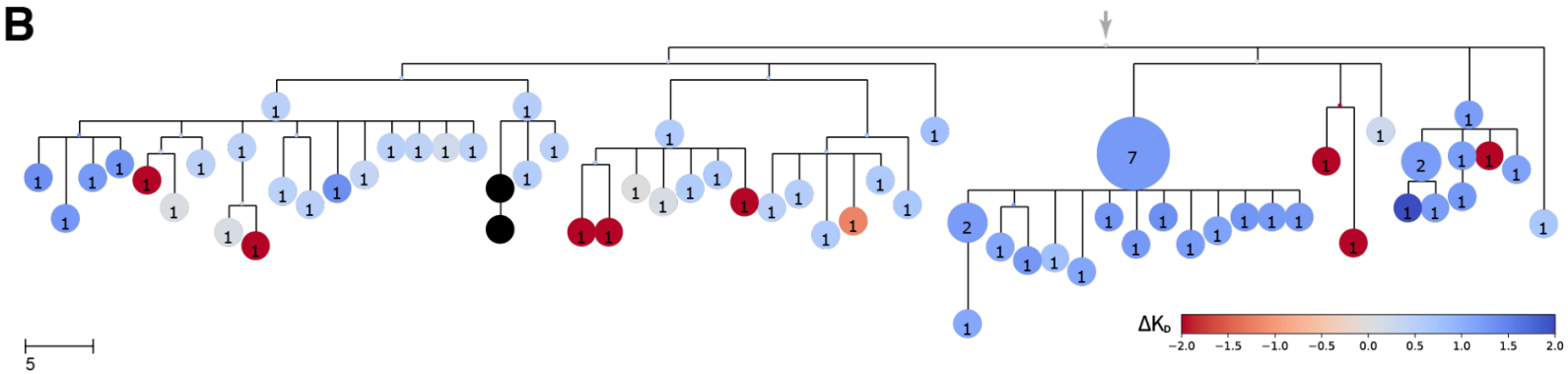


Figure: Tatsuya (2022)

Ancestry Hypothesis

$$F_H = \frac{\text{number of H cells with H ancestors}}{\text{number of H cells}}$$

$$F_L = \frac{\text{number of L cells with H ancestors}}{\text{number of L cells}}$$

Ancestry Hypothesis

The ancestry fractions are:

$$F_H = \frac{\alpha_H}{\alpha_H + \beta_L g(r_B r_D)},$$

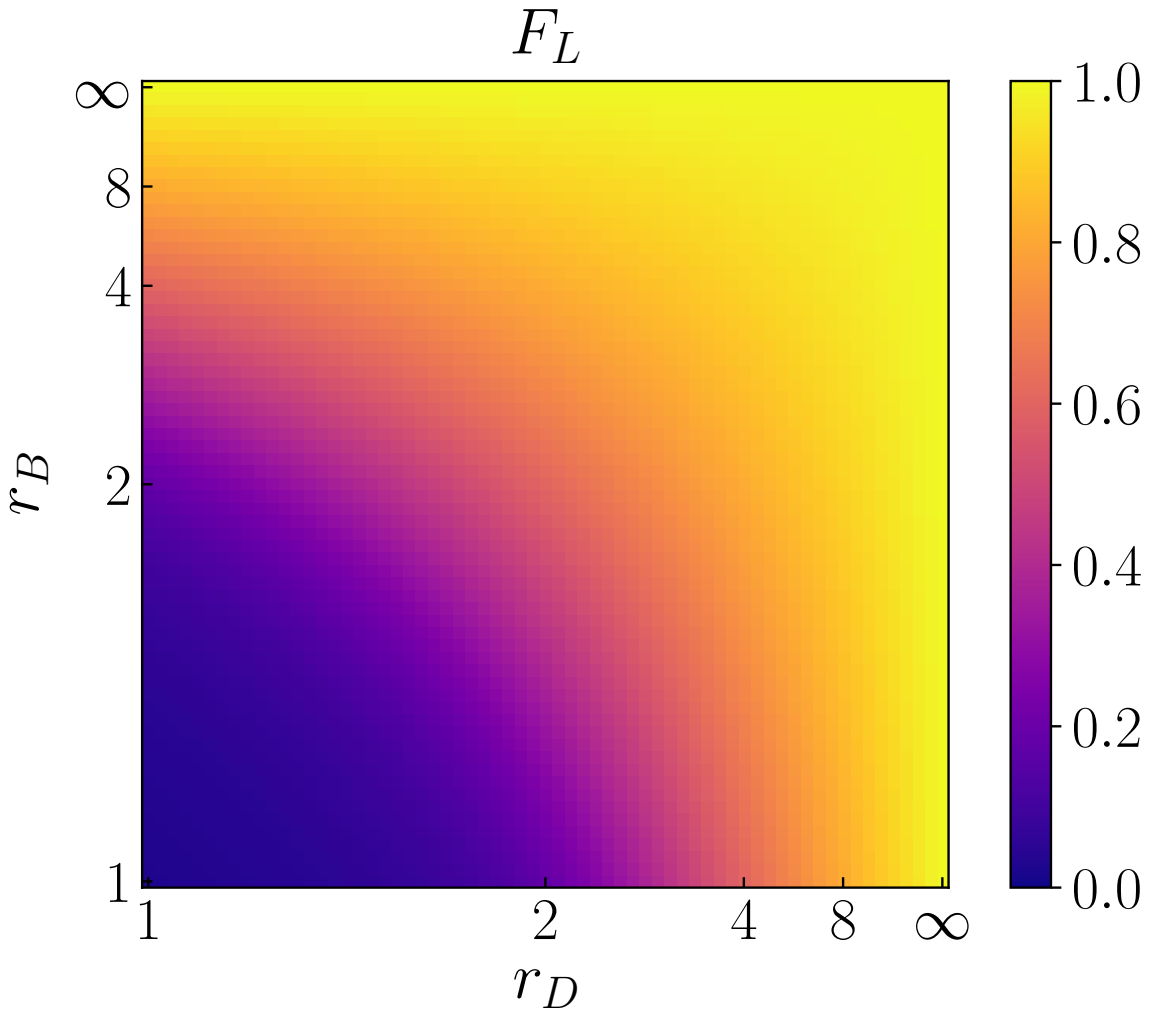
and

$$F_L = \frac{\beta_H}{\beta_H + \alpha_L g(r_B r_D)},$$

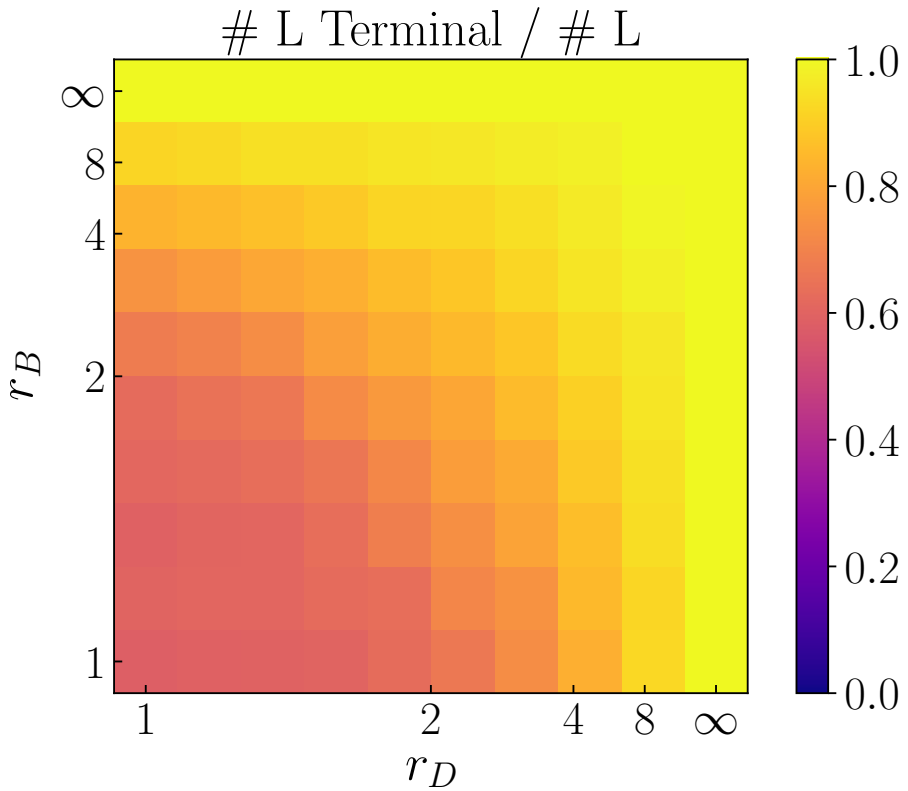
Where α_H is the rate of $H \to H$ mutations, ditto for α_L.

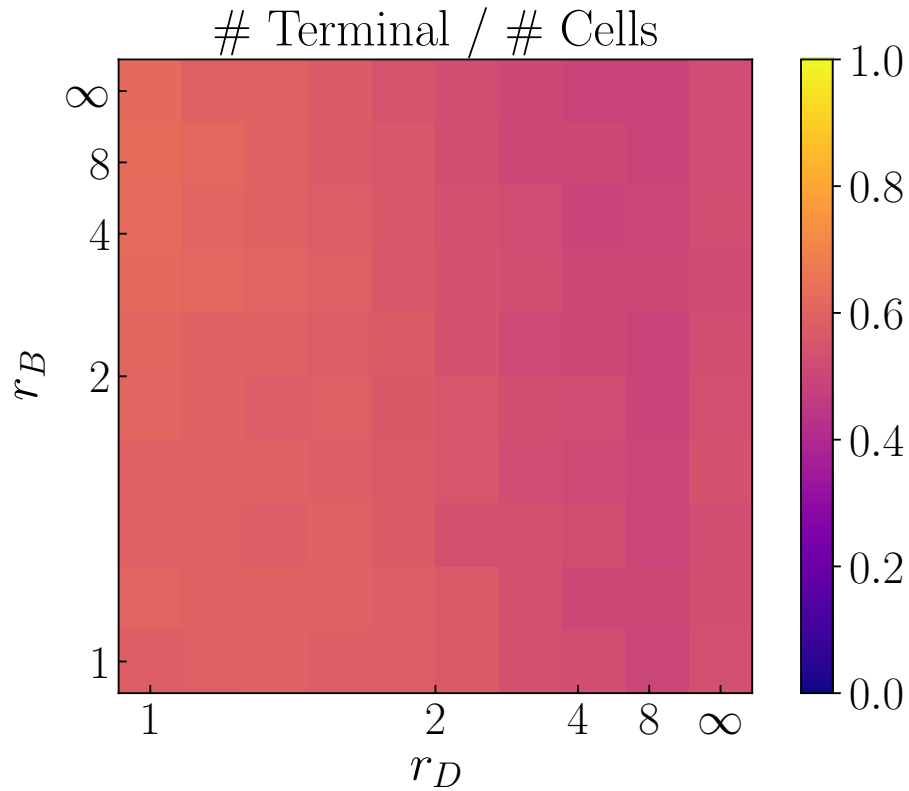
Ancestry Hypothesis: False

Notably, F_H and F_L **only** depend on the combined $r_B r_D$.



Ancestry Hypothesis: Alternates



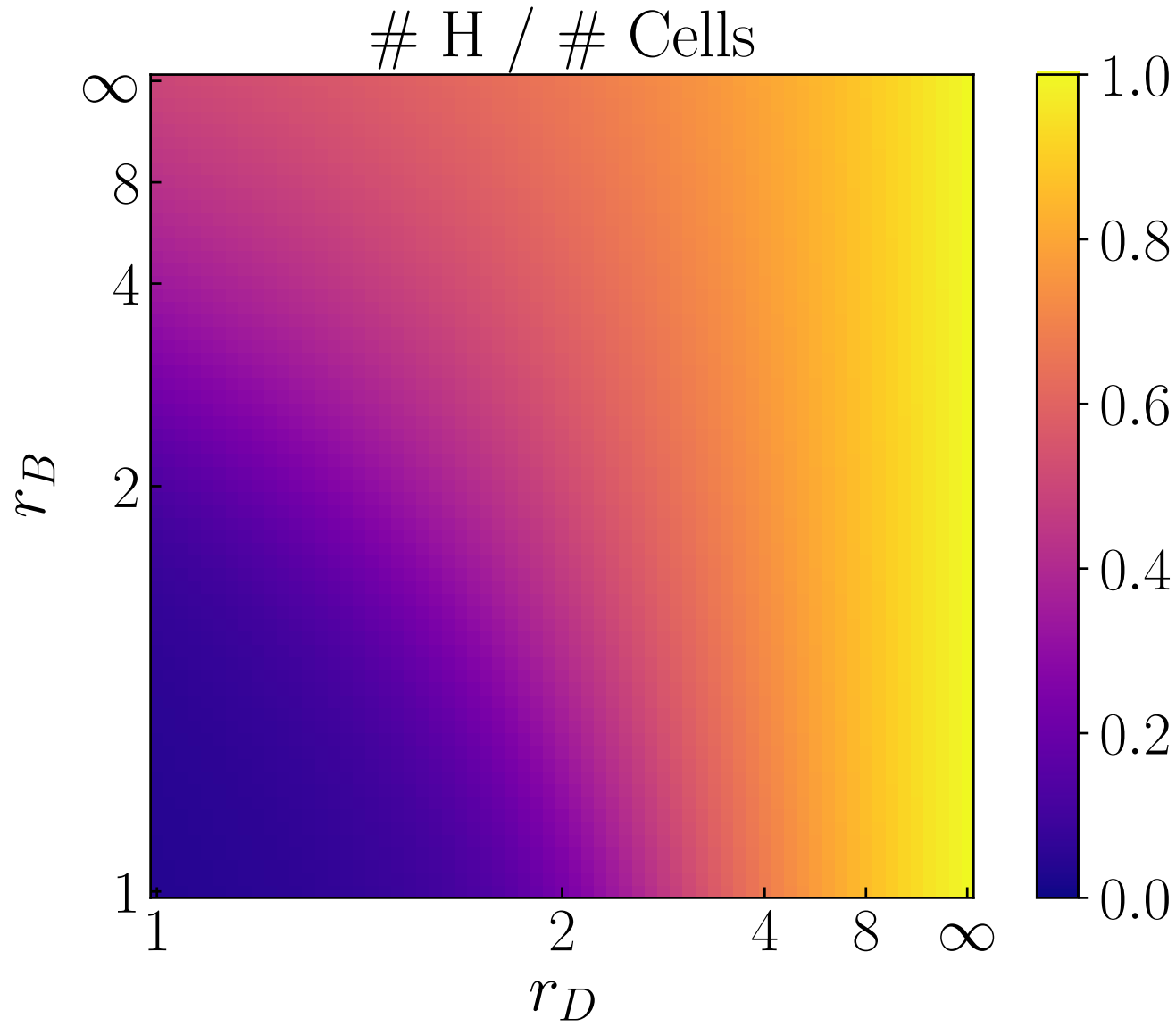


Is there anything static that distiguishes r_D and r_B?

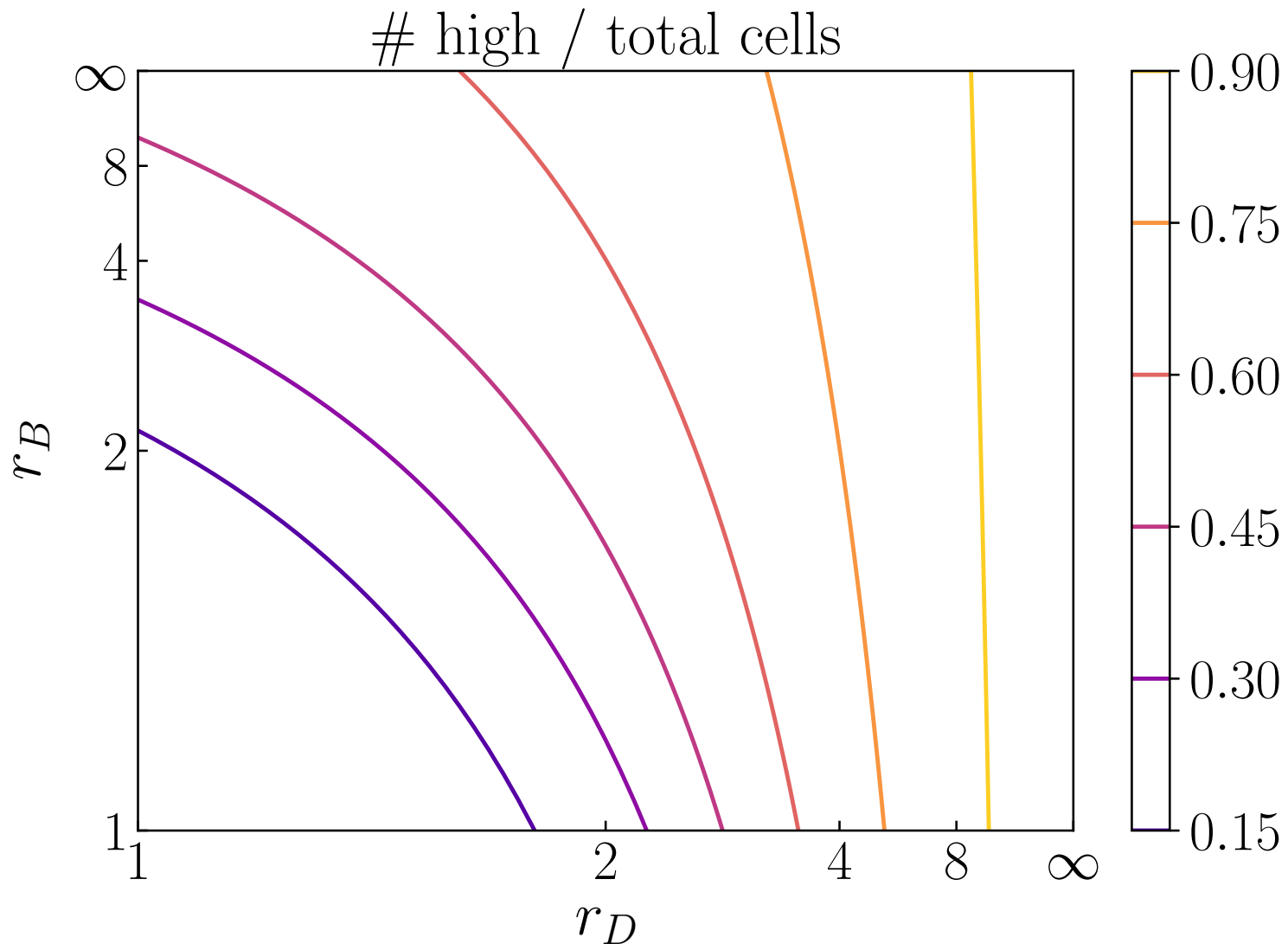
Overall selectivity

$$\text{Fraction of cells which are High affinity} = h = \frac{1}{1 + r_B g(r_B r_D)}$$

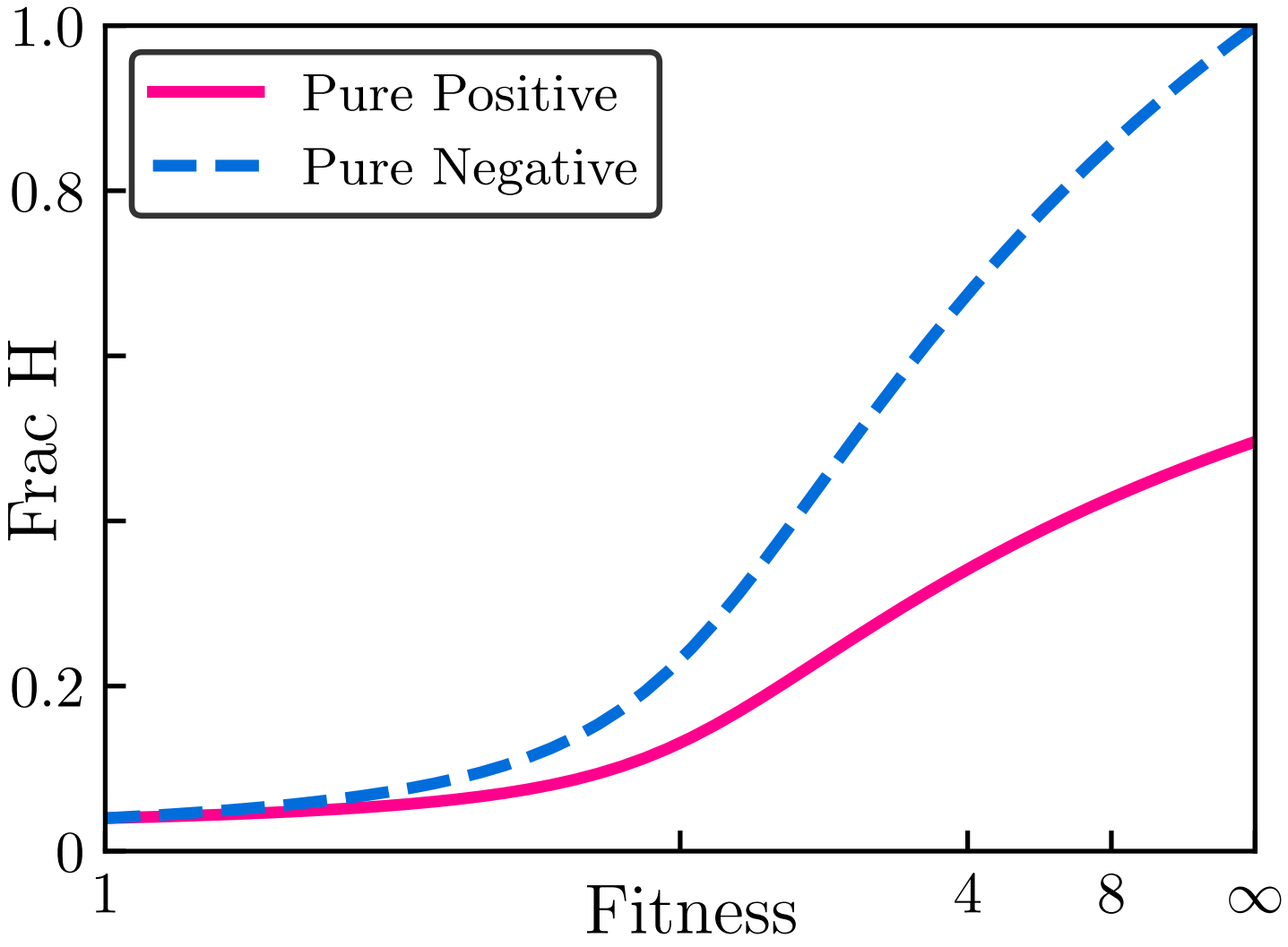
Overall selectivity



Overall selectivity



Overall selectivity



Ceiling of Selection

$$\lim_{r_B \to \infty} h(r_B, r_D) = \left(1 + \frac{\beta_H}{r_D(1-\beta_H)}\right)^{-1} < 1.$$

So if

$$h_{\text{observed}} > h(\infty, 1) = 1 - \beta_H,$$

then said system **must have negative selection** $r_D > 1$, since the force of positive selection alone is insufficient to attain that level of pressure.

Ceiling of Selection

In the case of weak mutation ($\beta_H + \beta_L \leq 1$), we can bound the strength of negative selection via

$$r_D \leq \frac{h}{1-h} \frac{\beta_H h + (1-\beta_L)(1-h)}{(1-\beta_H)h + \beta_L(1-h)}.$$

Meanwhile, if mutation is high ($\beta_H + \beta_L > 1$), then

$$r_D \leq \frac{h}{1-h} \frac{\beta_H}{1-\beta_H}.$$

Example?

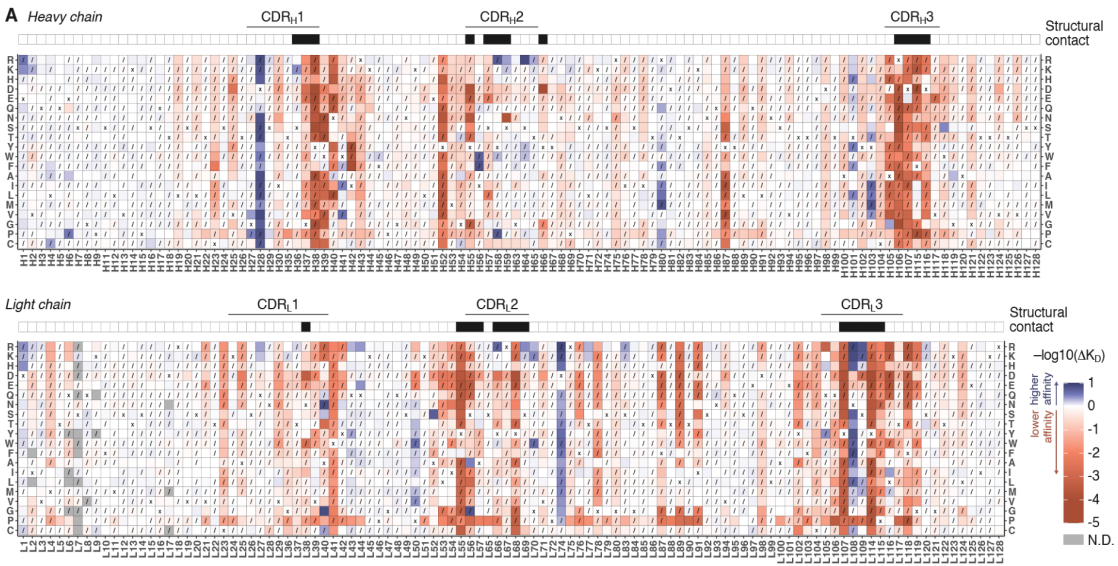


Figure: Tatsuya (2022)

$$\beta_H \approx \frac{700}{1000} \times 0.25 \approx 0.18$$

$$\beta_L \approx \frac{700}{1000} \times 0.024 \approx 0.02$$

Example?

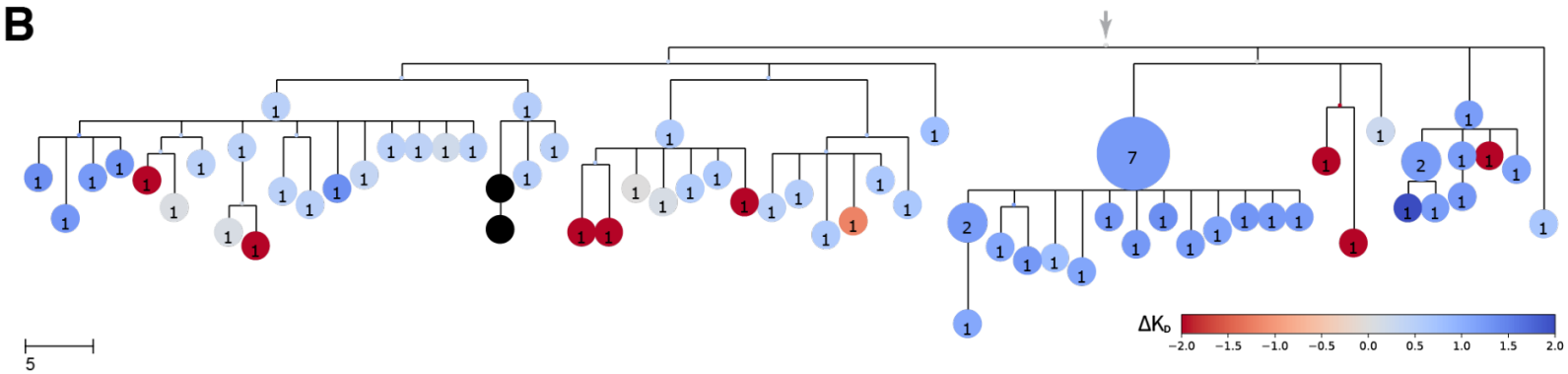


Figure: Tatsuya (2022)

$$h_{\text{observed}} \approx \frac{68}{79} \approx 0.86$$

Back of Envelope Calc.

Taking these numbers at face value:

$$0.86 \approx h_{\text{observed}} \gtrsim 1 - \beta_H \approx 0.825$$

and

$$\frac{h}{1-h}\frac{\beta_H h + (1-\beta_L)(1-h)}{(1-\beta_H)h + \beta_L(1-h)} \approx 2.49.$$

Back of Envelope Calc.

Therefore, we have that

$$1 < r_D \lesssim 2.49$$

This suggests that negative selection is present, and gives an upper bound on it's strength.

Back of Envelope Calc.

Therefore, we have that

$$1 < r_D \lesssim 2.49$$

This suggests that negative selection is present, and gives an upper bound on it's strength. **HOWEVER...**

LZ and DZ

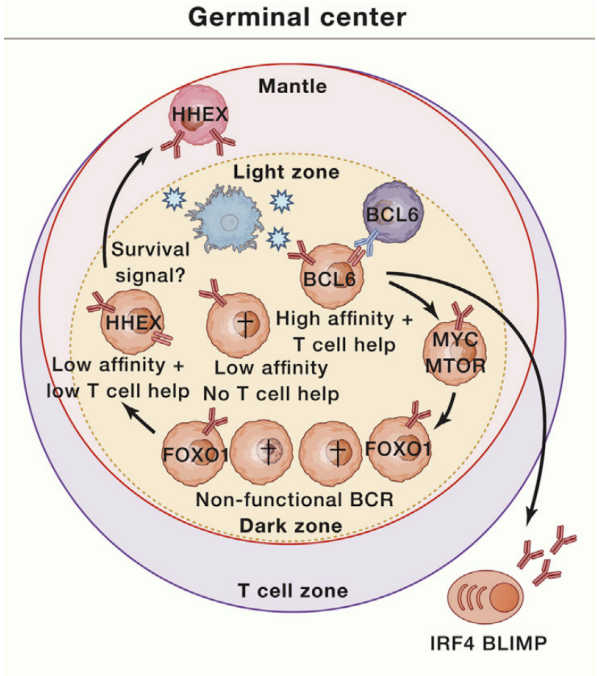


Figure: Young (2021)

LZ and DZ

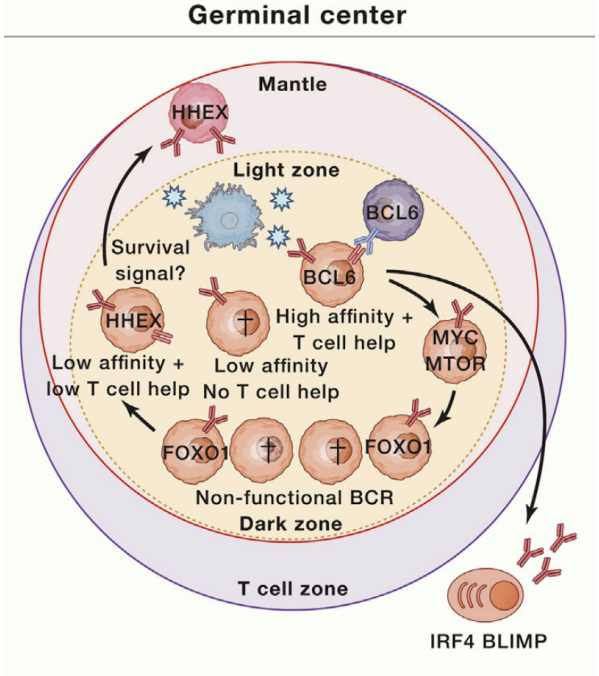


Figure: Young (2021)

B cells can be induced to divide rapidly in the DZ, without reentering the LZ.

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- B cells can be induced to divide rapidly in the DZ, without reentering the LZ.
- But preliminary studies indicates that they do not acquire more mutations than ordinary B cells...
- This implies that mutation count and division count are not correlated...

Limits of Theory...?

Limits of Theory...?

- We need to interrogate the commonly quoted mutation rate.
- Simple is better when it comes to measuring selection.

Static Signals of Selection Scheme?

Criteria	Status
Mutation Distribution Shape	Nope
Mutation Dynamics	Sure
Preferential Ancestry	Not Really
Overall Selection Strength	Yep

Thanks to Tatsuya Araki, Kevin O'Keffee, Daniel Abrams, Juhee Pae, & Arup Chakraborty for the comments.

Selected References & Image Sources


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Talk (will be) available at: ottinoloffler.com