Waiting for k mutations

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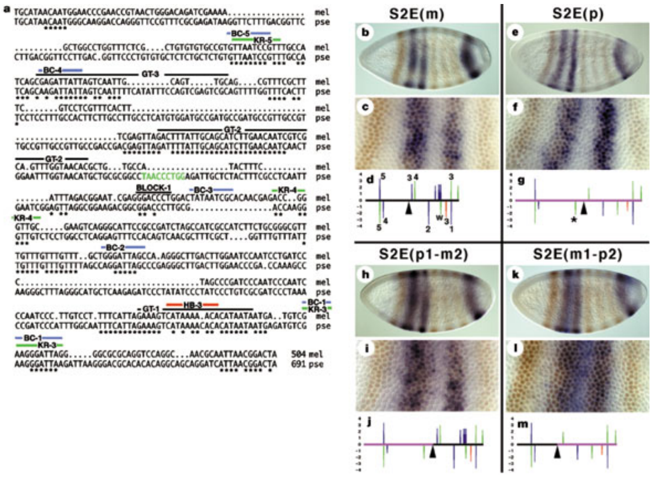
The Problem

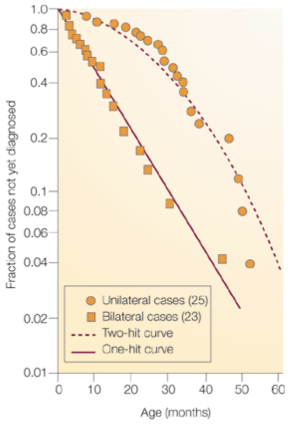
Given a population of size N, how long does it take until τ_k the first time we have an individual with a prespecified sequence of k mutations?

- Initially all individuals are type 0.
- Each individual is subject to replacement at rate 1.
- A copy is made of an individual chosen at random from the population.
- Type $j-1$ mutates to type j at rate u_j.

Even-skipped stripe 2 enhancer in Drosophila



Incidence of Retinoblastoma



Nature Reviews | Cancer

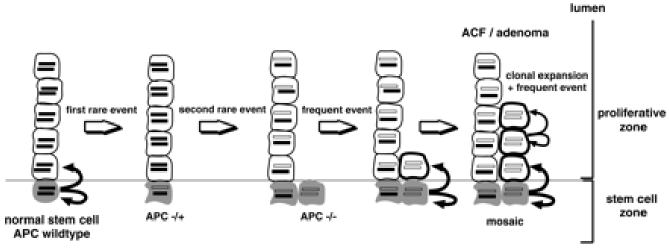
The Limits of Darwinism

The malaria parasite *Plasmodium falciparum* has evolved resistance to chloroquine. This is due to two amino acid altering substituions in PfCRT. Michael Behe in his book *The Edge of Evolution* calls such an event a *chloroquine complexity cluster*, or CCC. He concludes:

"There are 5000 species of modern mammals. If each species had an average of a million members and if a new generation appeared every year, and if this went on for two hundred million years, the likelihood of a single CCC appearing in the whole bunch over that entire time would only be 1 in a hundred."

Progression to Colon Cancer

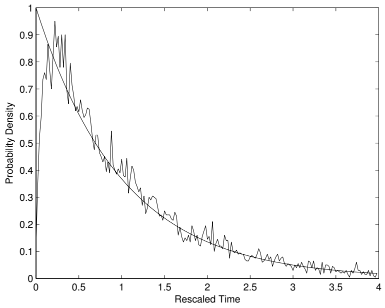
Luebeck and Moolgavakar (2002) PNAS fit a four stage model to incidence of colon cancer by age.



Theorem 1. If $Nu_1 \to 0$ and $N\sqrt{u_2} \to \infty$

$$P(\tau_2 > t/Nu_1\sqrt{u_2}) \to e^{-t}$$

10,000 simulations of $n = 10^3$, $u_1 = 10^{-4}$, $\sqrt{u_2} = 10^{-2}$



Behe is wrong

If $N = 10^6$, $u_1 = u_2 = 10^{-9}$, waiting time is exponential $10^{7.5} = 31.6$ million years for one prespecified pair of mutations in one species.

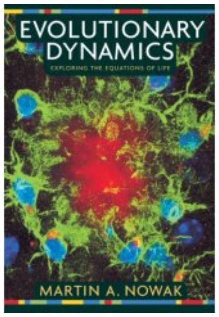
"There are 5000 species of modern mammals. If each species had an average of a million members and if a new generation appeared every year, and if this went on for two hundred million years, the likelihood of a single CCC appearing in the whole bunch over that entire time would only be 1 in a hundred."

References for k=2 result

Komarova, Sengupta, Nowak (2003) J. Theor. Biol. 223, 433–450

Iwasa, Michor, Nowak (2004) Genetics. 166, 1571–1579

Iwasa, Michor, Komorova, and Nowak (2005) J. Theor. Biol. 233, 15–23



Idea of Proof

Since 1's mutate to 2's at rate u_2, τ_2 will occur when there have been $O(1/u_2)$ births of individuals of type 1.

The number of 1's is roughly a symmetric random walk, so τ_2 will occur when the number of 1's reaches $O(1/\sqrt{u_2})$.

$N \gg 1/\sqrt{u_2}$ guarantees that up to τ_2 the number of 1's is $o(N)$, so 1 mutations occur at rate Nu_1.

The waiting time from the 1 mutation until the 2 mutant appears is of order $1/\sqrt{u_2}$. For this to be much smaller than the overall waiting time $1/Nu_1\sqrt{u_2}$ we need $Nu_1 \ll 1$.

A few details

Consider the multitype branching process in which individuals die at rate 1, give birth to a new individual of the same type at rate 1, and individuals mutate from type $j-1$ to type j at rate u_j.

The probability q that an individual of type 1 eventually has a descendant of type 2 satisfies

$$\begin{aligned} q &= \frac{1}{2+u_2}(2q-q^2) + \frac{u_2}{2+u_2} \\ 0 &= q^2 + u_2 q - u_2 \\ q &= \frac{-u_2 + \sqrt{u_2^2 + 4u_2}}{2} \sim \sqrt{u_2} \end{aligned}$$

The probability that an individual of type 1 eventually has a descendant of type 2, $\sim \sqrt{u_2}$.

If there were always N individuals of type 0, 1 mutants occur at times of a Poisson process with rate Nu_1. The time σ_2 of the birth of the 1 individual that has a 2 descendant will be exponential with rate $\sim Nu_1\sqrt{u_2}$.

If $Nu_1 \ll 1$, $\tau_2 - \sigma_2 = O(1/\sqrt{u_2}) = o(1/Nu_1\sqrt{u_2})$.

If we wait for fixation, replace u_2 by $u_2\beta$, where $\beta =$ fixation probability. Small surprise is time is only increased by $1/\sqrt{\beta}$.

If 1's are mildly deleterious, which means fitness $1 - \rho\sqrt{u_2}$, instead of the usual $1 - O(1/N)$, time is increased by $1/R$ where $R = (-\rho + \sqrt{4+\rho^2})/2$

Drosophila

Suppose a transcription factor binding site consists of 10 nucleotides. Taking 10^{-8} as mutation rate, $u_1 = 10^{-7}$ and $u_2 = (1/3) \times 10^{-8}$.

$N = 5 \times 10^6$ chromosomes, so waiting time has mean $1/Nu_1\sqrt{u_2} = 34,600$ generations or 3,460 years assuming 10 generations per year.

Nu_1 is not small, but Theorem 2 and simulations suggest this adds 25% to total $= 4,325$ years.

In neutral case $\beta = 1/2N$ increasing time by a factor of $1/\sqrt{\beta} = 2200$ to 9 million years.

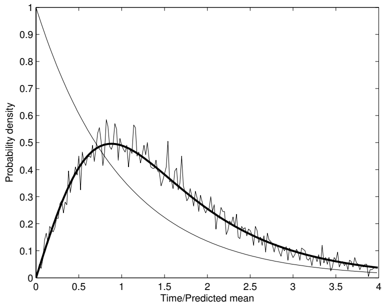
If two fitnesses are $1 - 10^{-4}$ and $1 + 10^{-4}$ answer is roughly 1 million years.

When $Nu_1 \not\to 0$

Theorem 2. Suppose that $Nu_1 \to \lambda \in (0, \infty)$, $u_2 \to 0$, and $N\sqrt{u_2} \to \infty$ as $N \to \infty$. Then $\tau_2 \cdot Nu_1\sqrt{u_2}$ converges to a limit that has density function

$$f_2(t) = h(t) \exp\left(-\int_0^t h(s)\, ds\right) \quad \text{where} \quad h(s) = \frac{1 - e^{-2s/\lambda}}{1 + e^{-2s/\lambda}}$$

10,000 simulations of $N = 10^3$, $u_1 = 10^{-3}$, $\sqrt{u_2} = 10^{-2}$. The exponential with mean $1/Nu_1\sqrt{u_2}$ is given by the thin line. The approximation from Theorem 2 by the thick line.



Sketch of proof

Let Q_1 be the law of the process starting from a single 1 and modified to have no further 1 mutations. Let $g_2(t) = Q_1(\tau_2 \le t)$.

$$g_2'(t) = -u_2 g_2(t) - g_2(t)^2 + u_2$$

Solve the ODE and then compute

$$P(\tau_2 \le t) = 1 - \exp\left(-Nu_1 \int_0^t g(s)\, ds\right)$$

Wodarz and Komorova (2005), *Computational Biology of Cancer*. World Scientific, solve hyperbolic PDE for generating function.

Waiting for k mutations

Total progeny of a critical binary branching process has $P(\xi > k) \sim Ck^{-1/2}$, so the sum of M such random variables is $O(M^2)$.

To get 1 individual of type 4, we need of order

$1/u_4$ births of type 3.

$1/\sqrt{u_4}$ mutations to type 3.

$1/u_3\sqrt{u_4}$ births of type 2.

$1/u_3^{1/2}u_4^{1/4}$ mutations to type 2.

$1/u_2u_3^{1/2}u_4^{1/4}$ births of type 1.

$1/u_2^{1/2}u_3^{1/4}u_4^{1/8}$ mutations to type 1.

Probability type j has a type k descendant.

$$\sim r_{j,k} = u_{j+1}^{1/2} u_{j+2}^{1/4} \cdots u_k^{1/2^{k-j}} \quad \text{for } 1 \le j < k$$

Theorem 3. Let $k \ge 2$. Suppose that:

(i) $Nu_1 \to 0$.

(ii) For $j = 1, \ldots, k-1$, $u_{j+1}/u_j > b_j$ for all N.

(iii) There is an $a > 0$ so that $N^a u_k \to 0$.

(iv) $Nr_{1,k} \to \infty$.

Then for all $t > 0$, $\lim_{N\to\infty} P(\tau_k > t/Nu_1 r_{1,k}) = \exp(-t)$.

Explanation of the Conditions

$$r_{j,k} = u_{j+1}^{1/2} u_{j+2}^{1/4} \cdots u_k^{1/2^{k-j}} \quad \text{for } 1 \le j < k$$

(i) $Nu_1 \to 0$ implies we can ignore $\tau_k - \sigma_k$, where σ_k is the birth time of the type 1 with a type k descenation

(iv) $Nr_{1,k} \to \infty$ guarantees the number of mutants stays $o(N)$.

(ii) $u_{j+1}/u_j > b_j$ for all N. In cancer applications later mutation rates are larger, but in regulatory sequence example $u_2 = u_1/30$.

(iii) $N^a u_k \to 0$ for some $a > 0$. Mutation rates can't be too big.

Ideas in Proof

$$r_{j,k} = u_{j+1}^{1/2} u_{j+2}^{1/4} \cdots u_k^{1/2^{k-j}} \quad \text{for } 1 \le j < k$$

In the branching process, the probability a type j has a type k descendant.

$$p_{j,k} = \frac{1}{2 + u_{j+1}}(2p_{j,k} - p_{j,k}^2) + \frac{u_{j+1}}{2 + u_{j+1}} p_{j+1,k}.$$

Solving gives

$$p_{j,k} = \frac{-u_{j+1} + \sqrt{u_{j+1}^2 + 4u_{j+1}p_{j+1,k}}}{2}.$$

Using (ii) and (iii) we conclude $p_{j,k} \sim r_{j,k}$

If $k = 4$ and $u_j = \mu$ then there are $\mu^{-1/2}$ 3's; $\mu^{-3/4}$ 2's; $\mu^{-7/8}$ 1's.

Processes live on different time scales.

Use induction to reduce to two type case.

Back to reality

Our results are appropriate for the regulatory sequence application since one is interested in the typical amount of time that the process takes.

However, most cancers occur in less than 1% of the population so we are looking at the lower tail of the distribution. Let $g_k(t) = Q_1(\tau_k \le t)$ where Q_1 is the probability for the branching process started with one type 1. In the case $u_j \equiv \mu$

$$g_j'(t) = \mu g_{j-1}(t) - (1-\mu) g_j(t)^2 - 2\mu g_j(t)$$

One can inductively solve the differential equations and finds

If $t \ll \mu^{-1/2}$ then $g_k(t) \approx \mu^{k-1} t^{k-1}/(k-1)!$

When $Nr_{1,k} \not\to \infty$

Fixation of 1 before τ_k and stochastic tunneling each have positive probability.

Theorem 4. Let $k \ge 2$. Assume

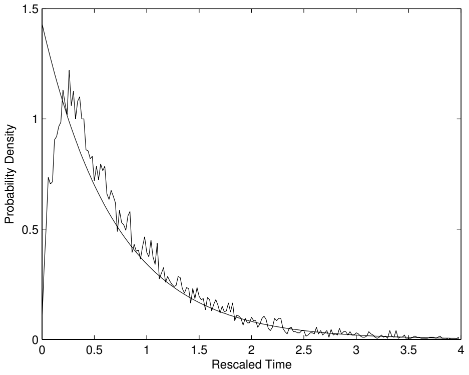
(i) $Nu_1 \to 0$.
(ii) For $j = 1, \dots, k-1$, $u_{j+1}/u_j > b_j$ for all N.
(iii) There is an $a > 0$ so that $N^a u_k \to 0$.
(iv) $(Nr_{1,k})^2 \to \gamma > 0$, and we let

$$\alpha = \sum_{k=1}^{\infty} \frac{\gamma^k}{(k-1)!(k-1)!} \Big/ \sum_{k=1}^{\infty} \frac{\gamma^k}{k!(k-1)!} > 1$$

then for all $t > 0$, $\lim_{N\to\infty} P(u_1 \tau_k > t) = \exp(-\alpha t)$.

10,000 simulations with $N = 10^3$, $u_1 = 10^{-4}$, $\sqrt{u_2} = 10^{-3}$

$\gamma = 1$, $\alpha = 1.433$



Let $X_j(t)$ be the number of type j at time t.

If $X_1(0) = N\epsilon$ then $N^{-1}X_1(Nt) \to Z_t$ where Z_t is the Wright-Fisher diffusion process with infinitesimal generator $x(1-x)d^2/dx^2$.

When $X_1(Nt) = Nx$, mutations to type 2 that eventually lead to a type k individual occur at rate approximately

$$N \cdot Nx \cdot u_2 r_{2,k} \sim N^2 r_{1,k}^2 x \to \gamma x,$$

so if we let $u(x)$ be the probability that the process Z_t hits 0 before reaching 1 or generating a type m mutation, then $u(x)$ satisfies

$$x(1-x)u''(x) - \gamma x u(x) = 0, \qquad u(0) = 1, \quad u(1) = 0$$

The constant $\alpha = \lim_{\epsilon \to 0}(1 - u(\epsilon))/\epsilon$.