A waiting time problem arising from the study of multi-stage carcinogenesis

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Outline of talk

1. History and background
2. The model
3. Main results and proof sketches
4. Generalizations
5. Open problems
History and Background

Muller (1951): “There are, however, reasons for inferring that many or most cancerous growths would require a series of mutations in order for cells to depart sufficiently from the normal.”

Armitage-Doll (1954): Proposed multi-stage model of cancer. If a cell has experienced \( j - 1 \) changes, \( j \)th change at rate \( u_j \). Cancer occurs after \( m \) changes.

- For small \( t \), probability that \( m - 1 \) changes happen before time \( t \) is approximately
  
  \[
  \frac{u_1 u_2 \ldots u_{m-1} t^{m-1}}{(m-1)!}.
  \]

- Probability that \( m \)th change happens in \( [t, t + dt] \) is \( u_m dt \).
- Incidence rate of cancer at time \( t \), for small \( t \), is
  
  \[
  r(t) dt \approx \frac{u_1 u_2 \ldots u_m t^{m-1}}{(m-1)!} dt.
  \]

Examined data on 17 types of cancer. Typically incidence rate increases like 5th or 6th power of age, suggesting 6 or 7 stages.
Knudson (1971) discovered that retinoblastoma is a result of two mutations.


Calabrese et. al. (2005): between 4 and 9 mutations required for colon cancer.

Sjoblom et. al. (2006): as many as 14 mutations involved in colon cancer, 20 in breast cancer.

Moolgavkar-Luebeck (1992): “the concept of multi-stage carcinogenesis is one of the central dogmas of cancer research.”

Regulatory sequence evolution (Durrett-Schmidt, 2007): DNA sequences (6-9 nucleotides) control how genes are expressed. Several mutations required to get a given regulatory sequence.
The Model

Population has fixed size $N$.

Moran model: each individual lives for an Exponential(1) time, then gets replaced by individual chosen at random.

Individuals experience mutations at rate $\mu$ (depends on $N$).

Let $\tau_m$ be the first time at which an individual has $m$ mutations.

Clearly $\tau_1 \sim \text{Exponential}(N\mu)$.

Problem: For $m \geq 2$, find limiting distribution of $\tau_m$ as $N \to \infty$.

Important simplifying assumptions:

- One mutation rate $\mu$, rather than $j$th mutation at rate $u_j$.
- Mutations offer no selective advantage.
Two ways to accumulate $m$ mutations

1. Fixation: one mutation spreads to the entire population. Then we have to wait for $m−1$ additional mutations.

2. Stochastic tunneling (Iwasa-Michor-Nowak, 2004): one individual gets $m$ mutations before any mutation fixates.

$4m−3$ different regimes for asymptotic distribution of $\tau_m$.

For $m=2$, results for 4 of 5 regimes in Iwasa-Michor-Komarova-Nowak (2005), Wodarz-Komarova (2005).

We have complete results for all $m$, with rigorous proofs.

Focus in this talk on $m=3$. 

Preliminaries: critical branching processes

When a mutation occurs, number of individuals with the mutation evolves as follows:

- $k \rightarrow k - 1$ at rate $k(N - k)/N$.
- $k \rightarrow k + 1$ at rate $k(N - k)/N$.

When $k \ll N$, approximate by a continuous-time branching process, each individual gives birth and dies at rate 1.

$\Pr(\text{number with the mutation reaches } L) = 1/L$.

$\Pr(\text{mutation lasts for time } t) \sim C/t$ (Kolmogorov, 1938).
Preliminaries: multitype branching processes

Consider the following multitype branching process:

- Initially there is a single type 1 individual.
- Every individual gives birth and dies at rate 1.
- Type \( k \) individual mutates to type \( k + 1 \) at rate \( \mu \).

Let \( p_m = P(\text{a type } m \text{ individual is born eventually}) \). Then

\[
p_m = \frac{1}{2 + \mu}(0) + \frac{1}{2 + \mu}(2p_m - p_m^2) + \frac{\mu}{2 + \mu}p_{m-1}.
\]

Can rewrite as

\[
p_m^2 + \mu p_m - \mu p_{m-1} = 0,
\]

so

\[
p_m = \frac{-\mu + \sqrt{\mu^2 + 4\mu p_{m-1}}}{2} \approx \sqrt{\mu p_{m-1}}.
\]

Since \( p_1 = 1 \), solving inductively gives

\[
p_m \approx \mu^{1-2^{-(m-1)}}.
\]
Exponential and Gamma limits for small $\mu$

By time $t$, there are approximately $N\mu t$ mutations, most successful lasts for a time $O(N\mu t)$ before disappearing or fixating.

If $N\mu \ll 1$, time between when a mutation occurs and when it disappears or fixates is much smaller than $t$, can be neglected.

Mutation fixates with probability $1/N$, has descendant with $m$ mutations with probability approximately $p_m$. Fixation happens first if $1/N \gg p_m$, or

$$ N\mu^{1-2^{-(m-1)}} \to 0. $$

Fixation before 2 mutations if $\mu \ll N^{-2}$, before 3 if $\mu \ll N^{-4/3}$.

**Theorem:** Let $Z_1$ and $Z_2$ be independent Exponential(1).

- If $\mu \ll N^{-2}$, then $\mu \tau_3 \to_d Z_1 + Z_2$.
- If $N^{-2} \ll \mu \ll N^{-4/3}$, then $\mu \tau_3 \to_d Z_1$.
- If $N^{-4/3} \ll \mu \ll N^{-1}$, then $N\mu^{7/4} \tau_3 \to_d Z_1$. 
Remarks about proof

Expected time for mutation to disappear or fixate is $O(\log N)$.

Expected time, up to time $t$, that there is a mutation in the population that has not fixated is $O(N\mu t \cdot \log N)$.

If $\mu \ll 1/(N \log N)$, ignore first mutations that occur while there is another mutation in the population, proofs are easy.

When $C/(N \log N) \leq \mu \ll 1/N$, many mutations in population at once. Need to show the events that they have a descendant with three mutations are approximately independent.
Poisson Approximation

Split $[0, t]$ into $M$ intervals, let $A_i$ be event that mutation in $i$th interval has descendant with 3 mutations.

Let $\beta_i$ be all intervals within a distance $C\mu^{-3/4}$ of the $i$th.

Result below gives exponential waiting time.

**Lemma** (Arratia-Goldstein-Gordon, 1989): Let $W$ be number of the $A_i$ that occur, $\lambda = E[W]$. Let $\mathcal{F}_i = \sigma((A_j)_{j \notin \beta_i})$. Define

$$b_1 = \sum_{i=1}^{M} \sum_{j \in \beta_i} P(A_i)P(A_j),$$

$$b_2 = \sum_{i=1}^{M} \sum_{i \neq j \in \beta_i} P(A_i \cap A_j),$$

$$b_3 = \sum_{i=1}^{M} E[|P(A_i|\mathcal{F}_i) - P(A_i)|].$$

Then $|P(W = 0) - e^{-\lambda}| \leq b_1 + b_2 + b_3$. 


The borderline cases

Suppose $\mu \sim CN^{-2}$. After the first fixation, each mutation fixates with probability $1/N$ and has a descendant with 3 mutations but does not fixate with probability $O(1/N)$.

Let $X(t)$ be the number of individuals with the mutation at time $t$. Births and deaths at rate $X(t)(N-X(t))/N$. Additional mutations happen at rate $\mu X(t)$.

Consider instead a simple random walk $(Y(t), t \geq 0)$ which jumps at rate 1. Mutation rate

$$\mu Y(t) \cdot \frac{N}{2Y(t)(N-Y(t))} = \frac{\mu}{2(1-Y(t)/N)}.$$

Probability of no fixation or additional mutation is

$$E\left[ \exp \left( -\frac{\mu}{2} \int_0^T \frac{1}{1-Y(t)/N} \, dt \right) \mathbf{1}_{\{Y(T)=0\}} \right],$$

where $T$ is the first time the walk hits 0 or $N$. 
If $Y(0) = \lfloor Nx \rfloor$, limit as $N \to \infty$ is

$$u(x) = E \left[ \exp \left( -\frac{C}{2} \int_0^U \frac{1}{1 - B(t)} \, dt \right) 1\{B(U) = 0\} \right],$$

where $(B(t), t \geq 0)$ is Brownian motion with $B(0) = x$ and $U$ is the first time Brownian motion hits 0 or 1.

Use Feynman-Kac to get differential equation for $u(x)$, obtain series solution, calculate

$$\alpha = \lim_{x \to 0} \frac{1 - u(x)}{x} = \sum_{k=1}^{\infty} \frac{C^k}{(k-1)! (k-1)!} / \sum_{k=1}^{\infty} \frac{C^k}{k! (k-1)!}. $$

**Theorem:** Let $Z \sim \text{Exponential}(1)$, $Y \sim \text{Exponential}(\alpha)$.

- If $\mu \sim CN^{-2}$, then $\mu \tau_3 \to_d Z + Y$.
- If $\mu \sim CN^{-4/3}$, then $\mu \tau_3 \to_d Y$ (use $C^{3/2}$ in definition of $\alpha$).
The case $N\mu \not\to 0$

Limit not exponential because we can’t ignore the time between the first mutation and the third mutation.

Let $X_k(t)$ be number of individuals with $k$ mutations at time $t$.

$$E[X_1(t)] \approx N\mu t, \quad E[X_2(t)] \approx \mu \int_0^t E[X_1(s)] \, ds \approx \frac{N\mu^2 t^2}{2}.$$ 

Fluctuations primarily from births and deaths, so

$$\text{Var}(X_1(t)) = O(N\mu t^2), \quad \text{Var}(X_2(t)) = O(N\mu^2 t^3).$$

$X_1(t) \approx E[X_1(t)]$ when $\sqrt{N\mu t^2} \ll N\mu t$, or $N\mu \gg 1$.

$X_2(t) \approx E[X_2(t)]$ when $\sqrt{N\mu^2 t^3} \ll N\mu^2 t^2$, or $N\mu^2 t \gg 1$. 
If $N\mu^2 t \gg 1$, we have $X_2(t) \approx E[X_2(t)] \approx N\mu^2 t^2/2$, so

$$P(\tau_3 > t) \approx \exp \left( -\mu \int_0^t E[X_2(s)] \, ds \right) \approx \exp \left( -\frac{N\mu^3 t^3}{6} \right).$$

This is relevant when $N\mu^2 (N^{-1/3} \mu^{-1}) \gg 1$, or $\mu \gg N^{-2/3}$.

When $N^{-1} \ll \mu \ll N^{-2/3}$, we have $X_1(t) \approx E[X_1(t)] \approx N\mu t$. Second mutations are in the population for only a short time, so

$$P(\tau_3 > t) \approx \exp \left( -\mu p_2 \int_0^t E[X_1(s)] \, ds \right) \approx \exp \left( -\frac{N\mu^{5/2} t^2}{2} \right).$$

**Theorem:**

- If $\mu \gg N^{-2/3}$, then
  $$\lim_{N \to \infty} P(N^{1/3} \mu \tau_3 > t) = \exp(-t^3/6).$$

- If $N^{-1} \ll \mu \ll N^{-2/3}$, then
  $$\lim_{N \to \infty} P(N^{1/2} \mu^{5/4} \tau_3 > t) = \exp(-t^2/2).$$
Two more borderline cases

When $\mu \sim CN^{-1}$, get stochastic effects both from the number of individuals with one mutation, and from the time between the first and third mutations.

When $\mu \sim CN^{-2/3}$, get stochastic effects both from the number of individuals with two mutations, and from the time between the second and third mutations.

Consider the following two-type branching process:

- Initially there is a single type 1 individual.
- Every individual gives birth and dies at rate 1.
- A type 1 individual mutates to type 2 at rate $r$.

Let $f(r, t) = P(\text{a type 2 individual appears by time } t)$.

Solving Kolmogorov’s backward equations gives (for small $r$),

$$f(r, t) \approx \sqrt{r} \cdot \frac{1 - e^{-2\sqrt{rt}}}{1 + e^{-2\sqrt{rt}}}.$$
When $\mu \sim CN^{-1}$, mutations happen at rate $N\mu$. Mutation at time $s$ has probability approximately $f(\mu p_2, t - s)$ of having a descendant with 3 mutations by time $t$, so

$$P(\tau_3 > t) \approx \exp \left( - \int_{0}^{t} N\mu f(\mu p_2, t - s) \, ds \right).$$

When $\mu \sim CN^{-2/3}$, there are $N\mu s$ individuals with one mutation at time $s$. Second mutations happen at rate $N\mu^2 s$, so

$$P(\tau_3 > t) \approx \exp \left( - \int_{0}^{t} N\mu^2 s f(\mu, t - s) \, ds \right).$$

**Theorem:**

- If $\mu \sim CN^{-1}$, then

$$\lim_{N \to \infty} P(\mu^{3/4} \tau_3 > t) = \exp \left( - C \int_{0}^{t} \frac{1 - e^{-2(t-s)}}{1 + e^{-2(t-s)}} \, ds \right).$$

- If $\mu \sim CN^{-2/3}$, then

$$\lim_{N \to \infty} P(\mu^{1/2} \tau_3 > t) = \exp \left( - C^{3/2} \int_{0}^{t} \frac{s(1 - e^{-2(t-s)})}{1 + e^{-2(t-s)}} \, ds \right).$$
Results for general $m$

**Theorem:** Let $S_j \sim \Gamma(j, 1)$ and $Y_j \sim \text{Exponential}(\alpha_j)$, 

$$\alpha_j = \frac{\sum_{k=1}^{\infty} C2^k(1-2^{-(j-1)})}{\sum_{k=1}^{\infty} \frac{C2^k(1-2^{-(j-1)})}{k!(k-1)!}}.$$

- If $\mu \ll N^{-2}$, then $\mu \tau_m \rightarrow_d S_{m-1}$.
- If $N^{-2j^{-1}}/(2^{j-1}-1) \ll \mu \ll N^{-2j}/(2^j-1)$ for $j = 2, \ldots, m-1$, then $\mu \tau_m \rightarrow_d S_{m-j}$.
- If $N^{-2m^{-1}}/(2^{m-1}-1) \ll \mu \ll N^{-1}$, then $N\mu^{2-2^{-(m-1)}} \tau_m \rightarrow_d S_1$.
- If $\mu \sim CN^{-2j^{-1}}/(2^{j-1}-1)$ for some $j = 2, \ldots, m$ and $C > 0$, then $\mu \tau_m \rightarrow_d S_{m-j} + Y_j$. 

• If $\mu \gg N^{-2/m}$, then

$$\lim_{N \to \infty} P(\tau_m > N^{-1/m}\mu^{-1}t) = \exp \left( -\frac{t^m}{m!} \right).$$

• If $N^{-1}/(1+(m-j-2)2^{-(j+1)}) \ll \mu \ll N^{-1}/(1+(m-j-1)2^{-j})$ for some $j = 1, \ldots, m-2$, then

$$\lim_{N \to \infty} P(\tau_m > N^{-1/(m-j)}\mu^{-1}(1-2^{-j})/(m-j)t) = \exp \left( -\frac{t^{m-j}}{(m-j)!} \right).$$

• If $\mu \sim CN^{-1}/(1+(m-j-1)2^{-j})$ for some $j = 1, \ldots, m-1$, then

$$\lim_{N \to \infty} P(\tau_m > \mu^{-1}(1-2^{-j})t)$$

$$= \exp \left( -\frac{C^{1+(m-j-1)2^{-j}}}{(m-j-1)!} \int_0^t s^{m-j-1}(1-e^{-2(t-s)})/1+e^{-2(t-s)} \, ds \right).$$

The case $\mu \gg N^{-2/m}$ agrees with Armitage-Doll (1954), but $P(\tau_m \leq t)$ can grow like $C t^k$ for any $k = 1, 2, \ldots, m$. 
Partial results for general mutation rates

Suppose an individual with $j - 1$ mutations acquires a $j$th mutation at rate $u_j$.

**Theorem:** Suppose $Z_1$ has the Exponential(1) distribution and

- $Nu_1 \to 0$.
- $Nu_2^{1/2}u_3^{1/4} \cdots u_m^{1/2^{m-1}} \to \infty$.
- For $j = 1, \ldots, m - 1$, there is a $b_j$ such that $u_j/u_{j-1} > b_j$ for all $N$.
- There is an $a > 0$ such that $N^a u_m \to 0$.

Then $Nu_1^{1/2}u_2^{1/4} \cdots u_m^{1/2^{m-1}} \tau_m \to_d Z_1$.

We have a result when $Nu_1 \to 0$ and $Nu_2^{1/2}u_3^{1/4} \cdots u_m^{1/2^{m-1}} \to C$. In this case fixation may or may not occur.

We do not have results for general $u_j$ when $Nu_1 \to \infty$. 
Open Problems

Complete results for general mutation rates $u_j$.

Allow individuals with mutations to have a selective advantage.

- When $\mu$ is large, model as supercritical, multitype branching process (Moolgavkar-Dewanji-Venzon (1988), Moolgavkar-Luebeck (1990, 1992)).

- When $\mu$ is small, fixations are possible. Assume individual with $j$ mutations selected with probability proportional to $1+js$. Beerenwinkel et. al. (2007) conjecture traveling wave behavior. If $Y_j(t)$ denotes the fraction of the population with $j$ mutations at time $t$, then

$$Y_j(t) \approx C \exp \left( - \frac{(j - vt)^2}{2\sigma^2} \right)$$

and

$$v \approx \frac{2s \log N}{\log(s/\mu)^2}.$$