Math 186:
4.2 Poisson Distribution: Counting Crossovers in Meiosis

4.2 Exponential and 4.6 Gamma Distributions:
Distance Between Crossovers

Math 283: Ewens & Grant 1.3.7, 4.1-4.2

Prof. Tesler

Math 186 and 283
Winter 2019
**Chromosomal coordinate system**

- **Mouse chr. 11: 55.50–55.70 cM.**

- The unit *Morgan* is defined so that crossovers occur at an average rate 1 per Morgan (M) or .01 per centi-Morgan (cM).

- 1911–1913: Alfred H. Sturtevant developed the first genetic map of a chromosome, *D. melanogaster* (fruit fly), as an undergrad in Thomas Hunt Morgan’s lab.

- 1919: J.B.S. Haldane improved on it and renamed the units to Morgans and centi-Morgans.
Morgans and centi-Morgans

- Morgans (M) and centi-Morgans (1 cM = .01 M) are a coordinate system for chromosomes based on recombination rates during meiosis.
- They are expressed as a real number \( \geq 0 \).
- Two genes on the same chromosome at positions \( d_1 \) and \( d_2 \) in Morgans, have an average of \( |d_1 - d_2| \) crossovers between them.
- It’s more common to measure it in centi-Morgans, so two genes located 123 cM apart would have an average of 1.23 crossovers between them.
- **Units of (centi-) Morgans were developed prior to the discovery that DNA is comprised of a large but finite number of discrete nucleotides.**
Counting crossovers

**Assumption 1:** Crossovers between two genes on the same chromosome occur at a rate

\[ \lambda = 0.01 \text{ per cM} = 0.01 \text{ cM}^{-1} \]

\[ = 1 \text{ per M} = 1 \text{ M}^{-1} \]

If genes \( A \) and \( B \) are \( d = 123 \) cM apart, the average number of crossovers between them per meiosis over the whole population is

\[ \lambda d = (0.01 \text{ cM}^{-1})(123 \text{ cM}) = 1.23 \]

\( \lambda d = 1.23 > 0 \) is a pure number without units.

**Assumption 2:** If genes are in order \( A, B, C \), then crossovers between \( A \) and \( B \) are independent of crossovers between \( B \) and \( C \).

What is the probability that \( k \) crossovers occur between \( A \) and \( B \)?
Counting crossovers

Let $X = 0, 1, 2, \ldots$ be the number of crossovers occurring between $A$ and $B$ in a particular meiosis.

$X$ is a discrete random variable. We will develop a discrete pdf for it called the *Poisson distribution*.

We’ll use the average number of crossovers between them (e.g., $\lambda d = 1.23$) to determine the distribution of $X$. 

![Diagram of A and B with X crossovers]
Counting crossovers with the binomial distribution

Split the interval from $A$ to $B$ into $n$ “equal” pieces and assume that:
- The probability of 2 or more crossovers in a piece is essentially 0. *(This requires $n$ to be large.)*
- Crossovers in different pieces occur independently.
- Crossover probabilities are the same in each piece. *(This is what makes the pieces “equal.”)*

In this model, the number of crossovers, $X$, follows a binomial distribution:
- There are $n$ pieces, each with (unknown) equal probability $p$ of having a crossover, so the average number of crossovers is $np$.
- The average is also $\lambda d$, so $np = \lambda d$ and $p = \lambda d / n$.
- For $k = 0, 1, 2, \ldots$

\[
P(X = k) = \binom{n}{k} p^k (1 - p)^{n-k} = \binom{n}{k} \left( \frac{\lambda d}{n} \right)^k \left( 1 - \frac{\lambda d}{n} \right)^{n-k}
\]
Suppose $d = 123$ cM (so $\lambda d = 1.23$) and $k = 3$.

We don’t know $n$; however, as $n \to \infty$, the digits of $P(X = 3)$ stabilize:

<table>
<thead>
<tr>
<th>$n$</th>
<th>$p = \lambda d/n$</th>
<th>$P(X = 3) = \binom{n}{3} p^3 (1 - p)^{n-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.23</td>
<td>0</td>
</tr>
<tr>
<td>$10^1$</td>
<td>$1.23 \cdot 10^{-1}$</td>
<td>0.08910328876</td>
</tr>
<tr>
<td>$10^2$</td>
<td>$1.23 \cdot 10^{-2}$</td>
<td>0.09058485007</td>
</tr>
<tr>
<td>$10^3$</td>
<td>$1.23 \cdot 10^{-3}$</td>
<td>0.09064683438</td>
</tr>
<tr>
<td>$10^4$</td>
<td>$1.23 \cdot 10^{-4}$</td>
<td>0.09065233222</td>
</tr>
<tr>
<td>$10^5$</td>
<td>$1.23 \cdot 10^{-5}$</td>
<td>0.09065287510</td>
</tr>
<tr>
<td>$10^6$</td>
<td>$1.23 \cdot 10^{-6}$</td>
<td>0.09065292933</td>
</tr>
<tr>
<td>$10^7$</td>
<td>$1.23 \cdot 10^{-7}$</td>
<td>0.09065293476</td>
</tr>
<tr>
<td>$10^8$</td>
<td>$1.23 \cdot 10^{-8}$</td>
<td>0.09065293534</td>
</tr>
</tbody>
</table>
Theorem (Poisson Limit)

\[
\lim_{n \to \infty} \left( \frac{n}{k} \right) \left( \frac{\mu}{n} \right)^k \left( 1 - \frac{\mu}{n} \right)^{n-k} = \frac{e^{-\mu} \mu^k}{k!}
\]

- Set \( \mu = \lambda d \).
- For \( \mu = \lambda d = 1.23 \) and \( k = 3 \), this gives

\[
e^{-1.23} \frac{1.23^3}{3!} \approx 0.09065293537
\]

- The values in the table converge to this as \( n \to \infty \).
- Even for \( n = 10 \), the value in the table was within 2% of this limit.
Poisson limit – Proof for $k = 3$

\[
\binom{n}{3} \left( \frac{\mu}{n} \right)^3 \left( 1 - \frac{\mu}{n} \right)^{n-3} = \frac{n(n-1)(n-2)}{3!} \frac{\mu^3}{n^3} \left( 1 - \frac{\mu}{n} \right)^n \\
= \frac{\mu^3}{3!} \frac{n(n-1)(n-2)}{n^3} \left( 1 - \frac{\mu}{n} \right)^n \\
= \frac{\mu^3}{3!} \frac{n(n-1)(n-2)}{n^3} \left( 1 - \frac{\mu}{n} \right)^n
\]

Limits of each piece:

- $n(n-1)(n-2)/n^3 \to 1$
- $(1 - \frac{\mu}{n})^3 \to (1 - 0)^3 = 1$
- $(1 - \frac{\mu}{n})^n \to 1^{\infty}$; need L’Hospital’s rule!
- L’Hospital’s rule gives $\lim_{n \to \infty} \left( 1 + \frac{x}{n} \right)^n = e^x$, so $\lim_{n \to \infty} \left( 1 - \frac{\mu}{n} \right)^n = e^{-\mu}$

\[
\binom{n}{3} \left( \frac{\mu}{n} \right)^3 \left( 1 - \frac{\mu}{n} \right)^{n-3} \to \frac{\mu^3}{3!} \cdot 1 \cdot \frac{e^{-\mu}}{1} = \frac{\mu^3}{3!} e^{-\mu}
\]
Poisson limit – Proof for $k = 3$

L’Hospital’s rule

- $L = \lim_{n \to \infty} \left(1 + \frac{x}{n}\right)^n$ has the form $(1 + 0)^\infty = 1^\infty$
- The logarithm $\ln L = \lim_{n \to \infty} n \ln \left(1 + \frac{x}{n}\right)$ has form $\infty \cdot \ln(1) = \infty \cdot 0$
- Convert that to $\ln L = \lim_{n \to \infty} \frac{\ln \left(1 + \frac{x}{n}\right)}{1/n}$, which has form $\frac{0}{0}$.
- Now we may apply L’Hospital’s Rule!
  Differentiate the top and bottom separately with respect to $n$:

$$
\ln L = \lim_{n \to \infty} \frac{\ln \left(1 + \frac{x}{n}\right)}{1/n} = \lim_{n \to \infty} \frac{\ln \left(1 + \frac{x}{n}\right)}{1/n} \cdot \left(-\frac{x}{n^2}\right) = \lim_{n \to \infty} \frac{x}{1 + \frac{x}{n}} = \lim_{n \to \infty} \frac{x}{1 + 0} = x
$$

- So $L = e^x$. Thus $\lim_{n \to \infty} \left(1 + \frac{x}{n}\right)^n = e^x$,
- We used this with $x = -\mu$. 

Prof. Tesler
Application

Historically, people approximated the binomial distribution by

\[ \binom{n}{k} p^k (1 - p)^{n-k} \approx \frac{e^{-np} (np)^k}{k!} \]

for \( n \geq 50 \) and \( np \leq 5 \).

Due to modern calculators and computers, this is not used as much.
The second method to count crossovers is to define a new distribution based on the limiting process we just studied. The Poisson distribution with parameter $\mu$ (a positive real #) is

$$P(X = k) = \begin{cases} \frac{e^{-\mu} \mu^k}{k!} & \text{for } k = 0, 1, 2, \ldots; \\ 0 & \text{otherwise.} \end{cases}$$

It’s a valid pdf since it’s always $\geq 0$ and the total probability is 1:

$$\sum_{k=0}^{\infty} P(X = k) = \sum_{k=0}^{\infty} \frac{e^{-\mu} \mu^k}{k!} = e^{-\mu} \cdot \sum_{k=0}^{\infty} \frac{\mu^k}{k!} = e^{-\mu} e^\mu = 1$$

Taylor series of $e^\mu$
Poisson distribution – rates

Poisson processes often involve rates, and the parameter may be described in terms of a rate:

Crossovers

- \( \lambda = .01 \text{ cM}^{-1} = 1 \text{ M}^{-1} \) is called the rate of a Poisson process.
- \( \mu = \lambda d \) is the Poisson parameter. It is a unitless number.

Other rates

- \( \lambda \) could be the average number of events per unit time, length, area, volume, etc., giving \( \mu = \lambda t, \mu = \lambda l, \mu = \lambda A, \mu = \lambda V \), etc.
- E.g., collect rain on a rectangular area for 1 second, and let \( \lambda \) be the average number of raindrops per unit area per second:

  ![Raindrops](image)

  Then for area \( A \) and time \( t \), the expected number of raindrops is \( \mu = \lambda A t \).
Probabilities of different numbers of crossovers

- This table shows the probability of $k$ crossovers occurring during meiosis, for two genes located 123 cM apart ($\mu = 1.23$).
- If we look at 100 gametes formed independently (say in different individuals), the expected # exhibiting $k$ crossovers is $100P(X = k)$.

<table>
<thead>
<tr>
<th># crossovers $k$</th>
<th>Theoretical proportion (pdf) $P(X = k) = e^{-1.23}(1.23)^k/k!$</th>
<th>Frequency $100P(X = k)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>.2922925777</td>
<td>29.2292577</td>
</tr>
<tr>
<td>1</td>
<td>.3595198706</td>
<td>35.95198706</td>
</tr>
<tr>
<td>2</td>
<td>.2211047204</td>
<td>22.11047204</td>
</tr>
<tr>
<td>3</td>
<td>.09065293537</td>
<td>9.065293537</td>
</tr>
<tr>
<td>4</td>
<td>.02787577763</td>
<td>2.787577763</td>
</tr>
<tr>
<td>5</td>
<td>.006857441295</td>
<td>0.6857441295</td>
</tr>
<tr>
<td>6</td>
<td>.001405775465</td>
<td>0.1405775465</td>
</tr>
<tr>
<td>7</td>
<td>.0002470148317</td>
<td>0.02470148317</td>
</tr>
</tbody>
</table>

$P(X = 1.5) = 0$, $P(X = -2) = 0$ (not non-negative integers)

$P(X \geq 3) = 1 - P(X = 0) - P(X = 1) - P(X = 2) = 0.1270828313$

Theoretical frequency of $X \geq 3$ is $100P(X \geq 3) = 12.70828313$. 
We will show that:

- The mean of the Poisson distribution equals the Poisson parameter (which is why we can call the parameter \( \mu \)).
- The variance is \( \sigma^2 = \mu \) and the standard deviation is \( \sigma = \sqrt{\mu} \).

**Example:** \( d = 123 \) cM

- the average number of crossovers between the two sites is \( \mu = \lambda d = 1.23 \);
- the variance of that is \( \sigma^2 = 1.23 \);
- the standard deviation is \( \sigma = \sqrt{1.23} \approx 1.11 \)
Mean and standard deviation of Poisson Distribution

Deriving the formula for the mean

- \( E(X) = \sum_{k=0}^{\infty} k \cdot \frac{e^{-\mu} \mu^k}{k!} \)
- Simplify \( \frac{k}{k!} \):
  - \( \frac{5}{5!} = \frac{5}{1 \cdot 2 \cdot 3 \cdot 4 \cdot 5} = \frac{1}{1 \cdot 2 \cdot 3 \cdot 4} = \frac{1}{4!} \). Similarly, \( \frac{k}{k!} = \frac{1}{(k-1)!} \) for \( k > 0 \).
  - For \( k = 0 \), it’s \( \frac{0}{0!} = 0 \), so the \( k = 0 \) term vanishes.

- \( E(X) = \sum_{k=1}^{\infty} \frac{e^{-\mu} \mu^k}{(k-1)!} = \mu e^{-\mu} \sum_{k=1}^{\infty} \frac{\mu^{k-1}}{(k-1)!} \)
  - \( = \mu e^{-\mu} \left( \frac{\mu^0}{0!} + \frac{\mu^1}{1!} + \frac{\mu^2}{2!} + \cdots \right) = \mu e^{-\mu} e^\mu = \mu \)
- Note that the Taylor series of \( e^x \) around \( x = 0 \) is \( e^x = \sum_{n=0}^{\infty} \frac{x^n}{n!} \).

Deriving the formula for the variance

- \( E(X^2) = \mu(\mu + 1) \) can be shown in a similar fashion, so the variance is \( \text{Var}(X) = E(X^2) - (E(X))^2 = \mu(\mu + 1) - \mu^2 = \mu \).
Theorem (Sum of Poisson Random Variables)

Let $X$, $Y$ be independent Poisson random variables with parameters $\mu$ and $\nu$. Then $W = X + Y$ is Poisson with parameter $\mu + \nu$.

Example

The # crossovers between $A$ & $B$ is Poisson with parameter 1.23

$B$ & $C$ 2.45

$A$ & $C$ 3.68
Theorem (Sum of Poisson Random Variables)

Let $X, Y$ be independent Poisson random variables with parameters $\mu$ and $\nu$. Then $W = X + Y$ is Poisson with parameter $\mu + \nu$.

Proof.

\[
P(W = k) = \sum_{m=0}^{k} P(X = m)P(Y = k - m)
= \sum_{m=0}^{k} \frac{e^{-\mu} \mu^m}{m!} \cdot \frac{e^{-\nu} \nu^{k-m}}{(k-m)!}
= e^{-(\mu+\nu)} \sum_{m=0}^{k} \frac{\mu^m \nu^{k-m}}{m! (k-m)!}
= \frac{e^{-(\mu+\nu)}}{k!} \sum_{m=0}^{k} \frac{k!}{m! (k-m)!} \mu^m \nu^{k-m}
= \frac{e^{-(\mu+\nu)}}{k!} \sum_{m=0}^{k} \binom{k}{m} \mu^m \nu^{k-m}
= e^{-(\mu+\nu)} (\mu + \nu)^k
\]

$\square$
Determining the Poisson parameter from data

Suppose that we had a way to count many crossovers occurred between two genes in individual meioses, and we count it in 100 independent gametes as shown in the table below. How far apart are the genes in cM?

<table>
<thead>
<tr>
<th>$k$</th>
<th>Obs. Freq.</th>
<th>Obs. Prop.</th>
<th># Crossovers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>64</td>
<td>0.64</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>29</td>
<td>0.29</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0.06</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.01</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>1.00</td>
<td>44</td>
</tr>
</tbody>
</table>

- **Observed Frequency**: # gametes with exactly $k$ crossovers between $A$ and $B$
- **Observed Proportion**: frequency / total # gametes
- **# Crossovers**: Total number of crossovers accounted for $= k$ times observed frequency
The total number of crossovers between $A$ and $B$ among all 100 gametes is

$$0(64) + 1(29) + 2(6) + 3(1) = 44.$$  

The average number of crossovers per gamete is $44/100 = 0.44$.

The Poisson parameter $\mu = \lambda d$ is $\mu = 0.44$, so

$$d = \frac{44}{\lambda} = \frac{44}{1 \text{ cM}^{-1}} = 44 \text{ cM} \quad \text{or} \quad = \frac{44}{1 \text{ M}^{-1}} = 0.44 \text{ M}$$

Note: This demonstrates the general procedure to fit the Poisson distribution, but it's not realistic to count the number of crossovers. So linkage maps are constructed using markers $\ll 1 \text{ cM}$ apart so that only $k = 0$ and $k = 1$ arise.
Determining the Poisson parameter from data

Compare the original data with the values predicted by the Poisson distribution for $d = 44$ cM. Observed and theoretical values are close:

<table>
<thead>
<tr>
<th>$k$</th>
<th>Obs. Freq.</th>
<th>Obs. Prop.</th>
<th># Crossovers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>64</td>
<td>0.64</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>29</td>
<td>0.29</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0.06</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.01</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>1.00</td>
<td>44</td>
</tr>
</tbody>
</table>

Theoretical proportion (pdf)  

<table>
<thead>
<tr>
<th>$k$</th>
<th>$P(X = k) = e^{-0.44}(0.44)^k/k!$</th>
<th>Theoretical frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6440364211</td>
<td>64.40364211</td>
</tr>
<tr>
<td>1</td>
<td>0.2833760253</td>
<td>28.33760253</td>
</tr>
<tr>
<td>2</td>
<td>0.06234272555</td>
<td>6.234272555</td>
</tr>
<tr>
<td>3</td>
<td>0.009143599749</td>
<td>0.9143599749</td>
</tr>
<tr>
<td>4</td>
<td>0.001005795973</td>
<td>0.1005795973</td>
</tr>
<tr>
<td>5</td>
<td>0.00008851004558</td>
<td>0.008851004558</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
When $\mu \geq 5$, the Poisson distribution is also well-approximated by the normal distribution with the same $\mu$ and with $\sigma = \sqrt{\mu}$:
4.2 Exponential distribution

- How far is it from the start of a chromosome to the first crossover?
- How far is it from one crossover to the next?
- Let $D$ be the random variable giving either of those. It is a real number $> 0$, with the *exponential distribution*

$$f_D(d) = \begin{cases} \lambda e^{-\lambda d} & \text{if } d \geq 0; \\ 0 & \text{if } d < 0. \end{cases}$$

where crossovers happen at a rate $\lambda = 1 \text{ M}^{-1} = 0.01 \text{ cM}^{-1}$.

- **General case**
  - **Mean** $E(D) = 1/\lambda = 100 \text{ cM} = 1 \text{ M}$
  - **Variance** $\text{Var}(D) = 1/\lambda^2 = 10000 \text{ cM}^2 = 1 \text{ M}^2$
  - **Standard Dev.** $\text{SD}(D) = 1/\lambda = 100 \text{ cM} = 1 \text{ M}$

- **Crossovers**
  - $E(D) = 1/\lambda = 100 \text{ cM} = 1 \text{ M}$
  - $\text{Var}(D) = 1/\lambda^2 = 10000 \text{ cM}^2 = 1 \text{ M}^2$
  - $\text{SD}(D) = 1/\lambda = 100 \text{ cM} = 1 \text{ M}$
4.2 Exponential distribution

Exponential distribution

- $\mu$
- $\mu \pm \sigma$
- Exponential: $\lambda = 0.01$

pdf

0 100 200 300 400
0.002
0.004
0.006
0.008
0.01
0.012
In general, if events occur on the real number line \( x \geq 0 \) in such a way that the expected number of events in all intervals \([x, x + d]\) is \( \lambda d \) (for \( x > 0 \)), then the exponential distribution with parameter \( \lambda \) models the time/distance/etc. until the first event.

It also models the time/distance/etc. between consecutive events.

Chromosomes are finite; to make this model work, treat “there is no next crossover” as though there is one but it happens somewhere past the end of the chromosome.
Let \( d > 0 \) be any real number.

Let \( N(d) \) be the number of crossovers that occur in the interval \([0, d]\).

If \( N(d) = 0 \) then there are no crossovers in \([0, d]\), so \( D > d \).

If \( D > d \) then the first crossover is after \( d \) so \( N(d) = 0 \).

Thus, \( D > d \) is equivalent to \( N(d) = 0 \).

\[
P(D > d) = P(N(d) = 0) = e^{-\lambda d} (\lambda d)^0 / 0! = e^{-\lambda d}
\]
since \( N(d) \) has a Poisson distribution with parameter \( \lambda d \).

The cdf of \( D \) is

\[
F_D(d) = P(D \leq d) = 1 - P(D > d) = \begin{cases} 1 - e^{-\lambda d} & \text{if } d \geq 0; \\ 0 & \text{if } d < 0. \end{cases}
\]

Differentiating the cdf gives pdf \( f_D(d) = F_D'(d) = \lambda e^{-\lambda d} \) (if \( d \geq 0 \)).
### Discrete and Continuous Analogs

<table>
<thead>
<tr>
<th></th>
<th>Discrete</th>
<th>Continuous</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Success”</td>
<td>Coin flip at a position is heads</td>
<td>Point where crossover occurs</td>
</tr>
<tr>
<td>Rate</td>
<td>Probability $p$ per flip</td>
<td>$\lambda$ (crossovers per Morgan)</td>
</tr>
<tr>
<td># successes</td>
<td>Binomial distribution: # heads out of $n$ flips</td>
<td>Poisson distribution: # crossovers in distance $d$</td>
</tr>
<tr>
<td>Wait until 1st success</td>
<td>Geometric distribution</td>
<td>Exponential distribution</td>
</tr>
<tr>
<td>Wait until $r^{th}$ success</td>
<td>Negative binomial distribution</td>
<td>Gamma distribution</td>
</tr>
</tbody>
</table>
4.6 Gamma distribution

- How far is it from the start of a chromosome until the \( r \)th crossover, for some choice of \( r = 1, 2, 3, \ldots \)?

- Let \( D_r \) be a random variable giving this distance.

- It has the gamma distribution with pdf

\[
f_{D_r}(d) = \begin{cases} \frac{\lambda^r}{(r-1)!} d^{r-1} e^{-\lambda d} & \text{if } d \geq 0; \\ 0 & \text{if } d < 0. \end{cases}
\]

- **Mean** \( E(D_r) = r/\lambda \)
- **Variance** \( \text{Var}(D_r) = r/\lambda^2 \)
- **Standard deviation** \( \text{SD}(D_r) = \sqrt{r}/\lambda \)

- The gamma distribution for \( r = 1 \) is the same as the exponential distribution.

- The sum of \( r \) i.i.d. exponential variables, \( D_r = X_1 + X_2 + \cdots + X_r \), each with rate \( \lambda \), gives the gamma distribution.
4.6 Gamma distribution

\begin{align*}
\text{Gamma distribution} & \\
\text{pdf} & \quad \mu \\
\text{d} & \quad \mu \pm \sigma \\
\text{Gamma: } r=3, \lambda=0.01
\end{align*}
Let \( d > 0 \) be any real number.

\( D_3 > d \) is the event that the third crossover does not happen until sometime after position \( d \).

When \( D_3 > d \), the number \( N(d) \) of crossovers in the chromosome interval \([0, d]\) is less than 3, so it’s 0, 1, or 2.

\( D_3 > d \) is equivalent to \( N(d) < 3 \).

\( D_3 \leq d \) is equivalent to \( N(d) \geq 3 \).
Let $d > 0$ be any real number.

$D_3 > d$ is the event that the third crossover does not happen until sometime after position $d$.

When $D_3 > d$, the number $N(d)$ of crossovers in the chromosome interval $[0,d]$ is less than 3, so it’s 0, 1, or 2:

$$P(D_3 > d) = P(N(d) = 0) + P(N(d) = 1) + P(N(d) = 2)$$

$$= e^{-\lambda d} \left( \frac{(\lambda d)^0}{0!} + \frac{(\lambda d)^1}{1!} + \frac{(\lambda d)^2}{2!} \right)$$

The cdf of $D_3$ is $P(D_3 \leq d) = 1 - P(D_3 > d)$.

Differentiating the cdf and simplifying gives the pdf

$$f_{D_3}(d) = \begin{cases} 
\lambda^3 d^2 e^{-\lambda d} / 2! & \text{if } d \geq 0; \\
0 & \text{if } d < 0.
\end{cases}$$