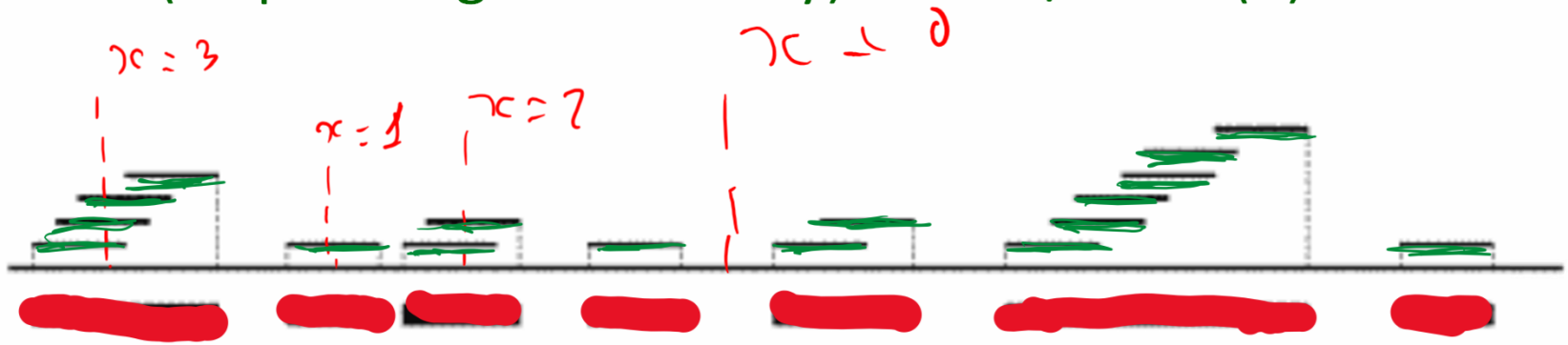
Where is the Poisson?

- *G - genome length (in bp)*
- *L - short read average length*
- *N – number of short read sequenced*
- *λ – sequencing coverage redundancy = LN/G*
- *x- number of short reads covering a given site on the genome*

$$P(x) = \frac{\lambda^x e^{-\lambda}}{x!}$$

Poisson as a limit of Binomial: For a given site on the genome for each short read Prob(site covered): p=L/G is very small. Number of attempts (short reads): N is very large. Their product (sequencing redundancy): λ = NL/G is O(1).



Ewens, Grant, Chapter 5.1

How long should be the length L_{ov} of the overlap to connect two short reads into a contig?

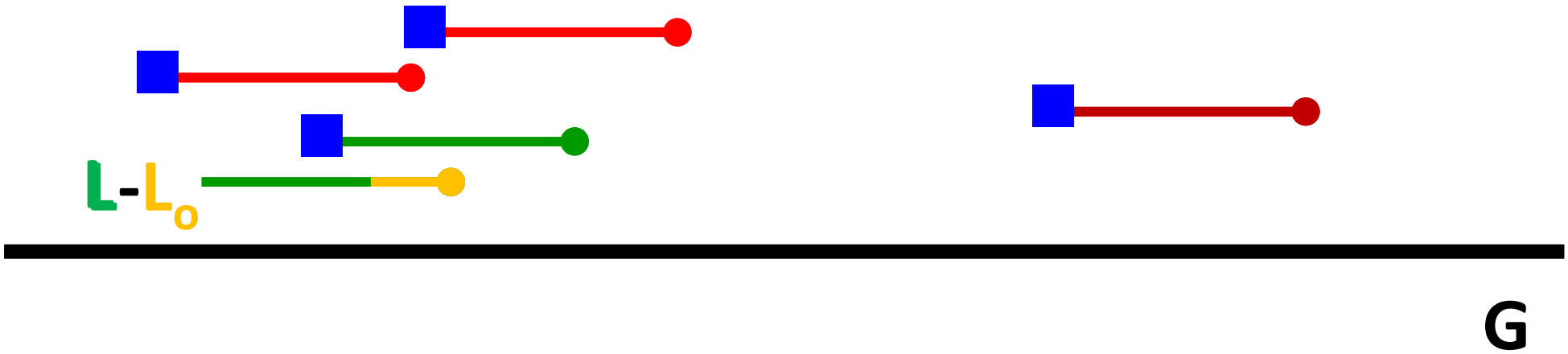
If DNA was a random chain with $p_A = p_C = p_G = p_T = 1/4$

L_{ov}~16-20 would be enough

$$2 \cdot G \cdot 4^{-Lov} = 2 \cdot 3x10^9 \cdot 4^{-16} = 1.4$$

$$2 \cdot 3x10^9 \cdot 4^{-20} = 0.0055 << 1$$

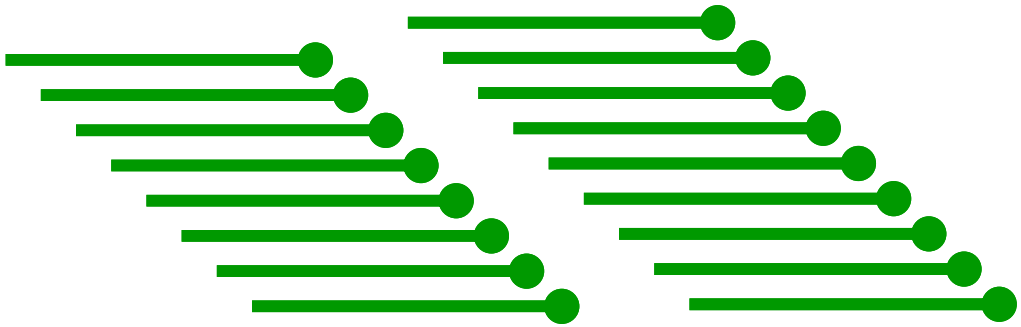
How many contigs?



$$\text{P(short read can be extended by another short read)} = \frac{L - L_o}{G} = \text{p}$$

$$\text{P(short read cannot be extended by any short reads)} = e^{-pN} \approx Ne^{-\lambda}$$

$$\text{number of contigs} = Ne^{-pN} \approx Ne^{-\lambda}$$



How many contigs?

- A given short read is the right end of a contig if and only if no left ends of other short reads fall within it.
- The left end of another short read has the probability $p=(L-1)/G$ to fall within a given read. There are $N-1$ other reads. Hence the expected number of left ends inside a given shot read is $p\cdot (N-1)=(N-1)\cdot(L-1)/G \approx \lambda$
- If significant overlap required to merge two short reads is L_{ov}, modified λ is given by $(N-1)\cdot(L-L_{ov})/G$
- Probability that no left ends fall inside a short read is $exp(-\lambda)$. Thus the Number of contigs is $N_{contigs}=Ne^{-\lambda}$:

λ	0.5	0.75	1	1.5	2	3	4	5	6	7
Mean number of contigs	60.7	70.8	73.6	66.9	54.1	29.9	14.7	6.7	3.0	1.3

Table 5.2. The mean number of contigs for different levels of coverage, with $G = 100{,}000$ and $L = 500$.

Average length of a contig?

- Length of a genome covered:
  $G_{covered}=G\cdot P(X>0)=G\cdot(1-\exp(-\lambda))$
- *Number of contigs* $N_{contigs}=N\cdot e^{-\lambda}$
- Average length of a contig =
  $\langle L\rangle=\sum_i L_i/N_{contigs}=G_{covered}/N_{contigs}=$

$G\cdot(1-\exp(-\lambda))/N\cdot e^{-\lambda}=L\cdot(1-\exp(-\lambda))/\lambda\cdot e^{-\lambda}$

λ	2	4	6	8	10
Mean contig size	1,600	6,700	33,500	186,000	1,100,000

Table 5.3. The mean contig size for different values of a for the case $L = 500$.

Estimate

- Human genome is $3x10^9$ bp long
- Chromosome 1 is about $G=0.25x10^9$ bp
- Illumina generates short reads $L=100$ bp long
- What number of reads N are needed to completely assemble the 1st chromosome?
- The formula to use is: $1=N_{contigs}=Ne^{-\lambda}=Ne^{-NL/G}$
- Answer: $N=4.4x10^7$ short (100bp) reads
  Test: 4.4e7*exp(-4.4e7*100/0.25e9)=0.9997
- What coverage redundancy λ will it be?
  Answer: $\lambda=NL/G=17.6$ coverage redundancy

How much would it cost to assemble human genome now?

- Human Genome Project: $2.7 billion in 1991 dollars.
- Now a de novo full assembly of the whole human genome would now cost $3 \times 10^9 \times 17.6 / 10^9 \times 10\$/GBase = \$ 530$
- 2nd genome (and after) would be even cheaper as we would already have a reference genome to which we can map short reads. (Puzzle: picture on the box)
- But this is a naïve estimate. In reality, there are complications. See the next slides:

What spoils these estimates?

```
>gi|224514922|ref|NT_024477.14| Homo sapiens chromosome 12 genomic
contig, GRCh37.p13 Primary Assembly (displaying 3′ end)
CGGGAAATCAAAAGCCCCTCTGAATCCTGCGCACCGAGATTCTCCCCAGCCAAGGTGAGGCGGCAGCAGT
GGGAGATCCACACCGTAGCATTGGAACACAAATGCAGCATTACAAATGCAGACATGACACCGAAAATATA
ACACACCCCATTGCTCATGTAACAAGCACCTGTAATGCTAATGCACTGCCTCAAAACAAAATATTAATAT
AAGATCGGCAATCCGCACACTGCCGTGCAGTGCTAAGACAGCAATGAAAATAGTCAACATAATAACCCTA
ATAGTGTTAGGGTTAGGGTCAGGGTCCCGGTCCGGGTCGGGGTCCGGGTCCGGGGTCCGGGTCAGGGTGA
GGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGT
TAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGG
GTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTA
GGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGT
TAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGG
GTTAGGGTTAGGGTTAGGGTTAG
```

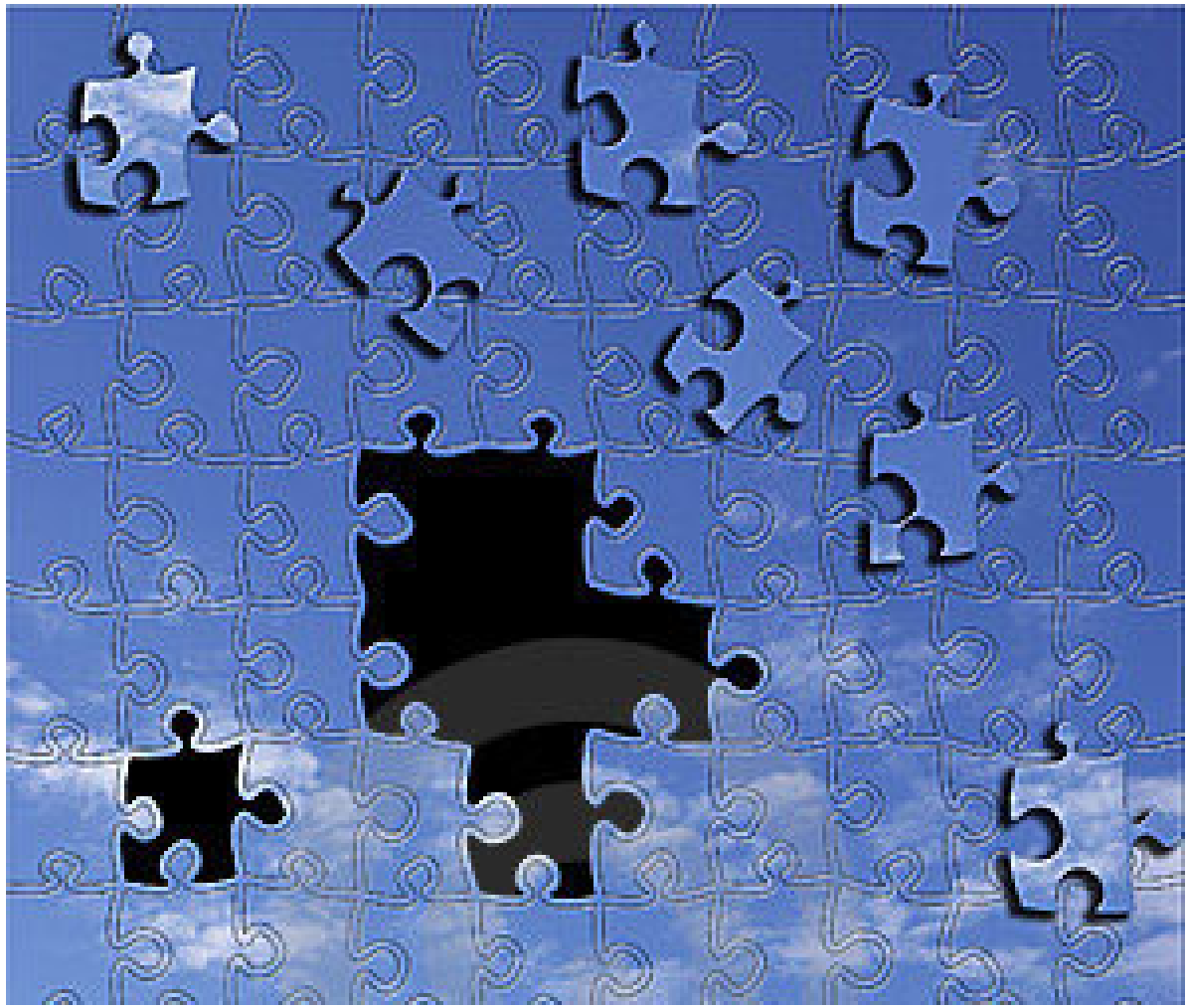
FIGURE 8.11 A BLASTN search of the human genome (all assemblies) database was performed at the NCBI website using TTAGGGTTAGGGTTAGGG as query (i.e., three TTAGGG repeats). There were matches to hundreds of genomic scaffolds. This figure shows an example (NT_024477.14) assigned to the telomere of chromosome 12q having many dozens of TTAGGG repeats. These occurred at the 3′ end of the genomic contig sequence.

There were 100s of matches while one expects << 1 match:

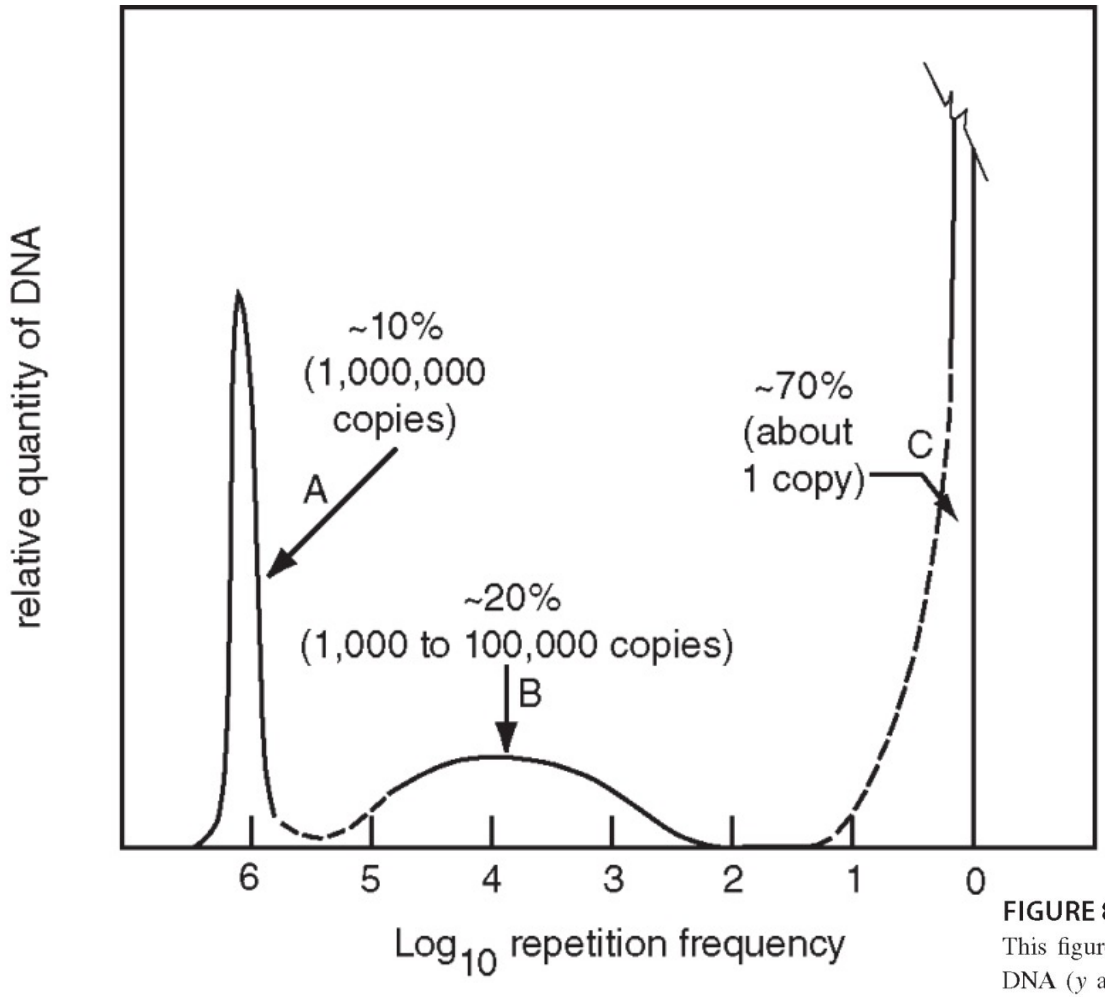
$$2 \cdot 3\text{x}10^{9} \cdot 4^{-18}=0.08<<1$$

DNA repeats make assembly difficult

Repeats are like sky puzzle pieces

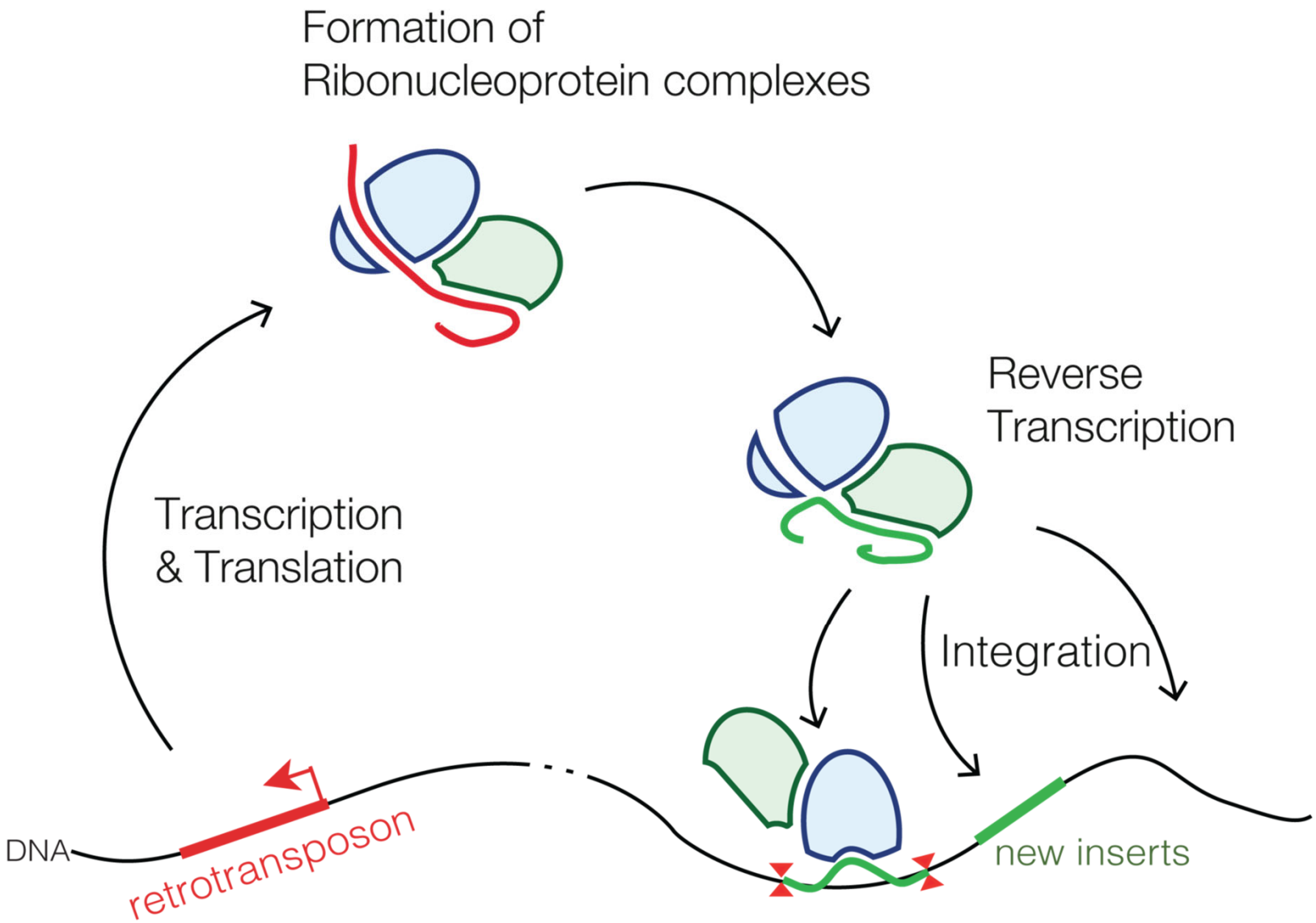


How many repeats are in eukaryotic genomes?

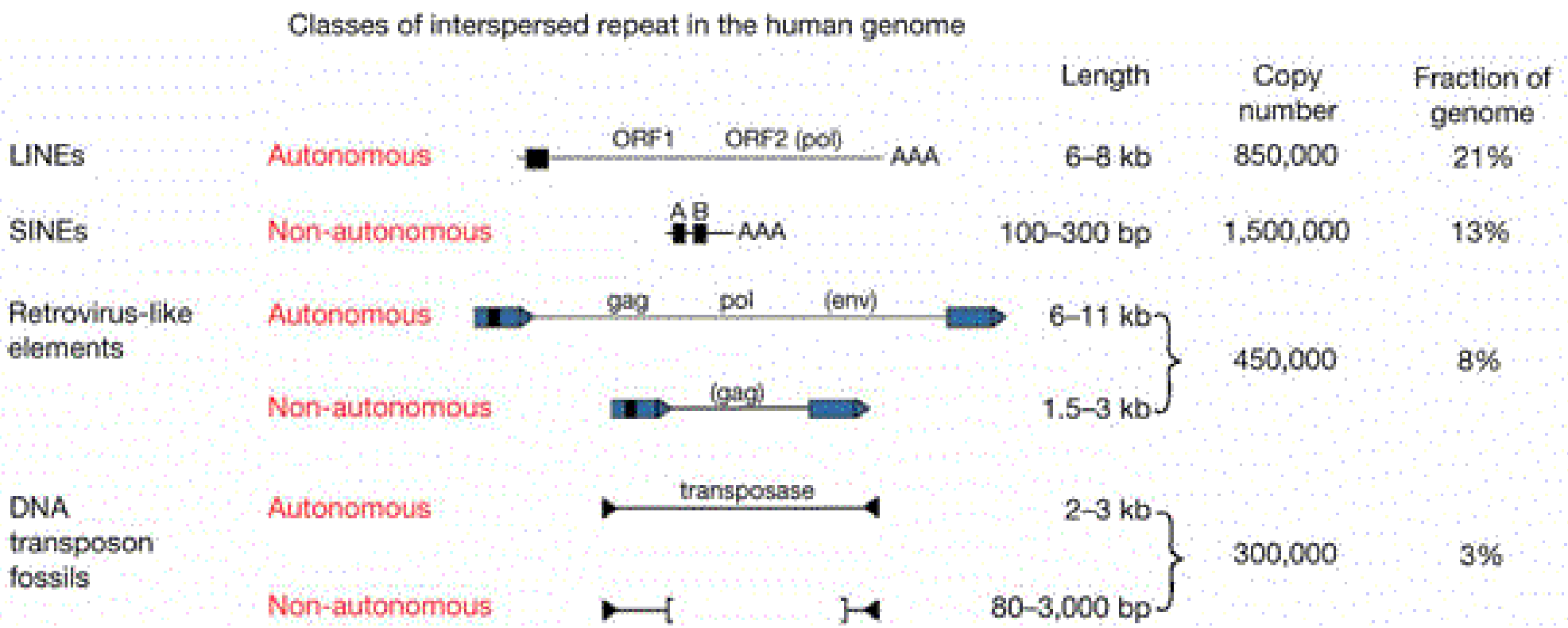


Data for mouse genome obtained in 1961 (sic!) using DNA denaturation and renaturation curves

FIGURE 8.6 The complexity of genomic DNA can be estimated by denaturing then renaturing DNA. This figure (redrawn from Britten and Kohne, 1968) depicts the relative quantity of mouse genomic DNA (*y* axis) versus the logarithm of the frequency with which the DNA is repeated. The data are derived from a $C_0\,t_{1/2}$ curve, which describes the percent of genomic DNA that reassociates at particular times and DNA concentrations. A large $C_0\,t_{1/2}$ value implies a slower reassociation reaction. Three classes are apparent. The fast component accounts for 10% of mouse genomic DNA (arrow **A**), and represents highly repetitive satellite DNA. An intermediate component accounts for about 20% of mouse genomic DNA and contains repeats having from 1000 to 100,000 copies. The slowly reassociating component, comprising 70% of the mouse genome, corresponds to unique, single-copy DNA. Britten and Kohne (1968) obtained similar profiles from other eukaryotes, although distinct differences were evident between species. Used with permission.


Formation of
Ribonucleoprotein complexes
Reverse
Transcription
Transcription
& Translation
Integration
DNA
retrotransposon
new inserts

Almost all transposable elements in mammals fall into one of four classes



Slide by Ross Hardison, Penn State U.

How to assemble a real genome with repeats?

Here we assume a "de novo" assembly without help from the previously assembled genomes

Nicolaas Govert de Bruijn (1918 – 2012) was a Dutch mathematician, noted for his many contributions in the fields of graph theory, analysis, number theory, combinatorics and logic

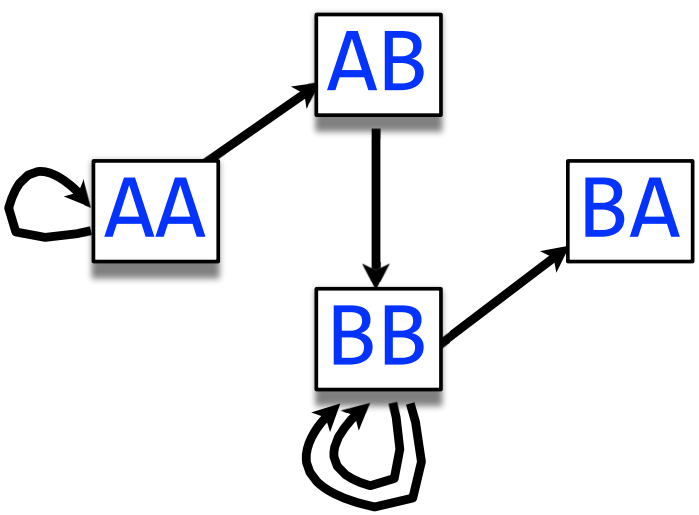
http://www.langmead-lab.org/teaching-materials/

De Bruijn graph

genome: AAABBBBA

3-mers: AAA, AAB, ABB, BBB, BBB, BBA

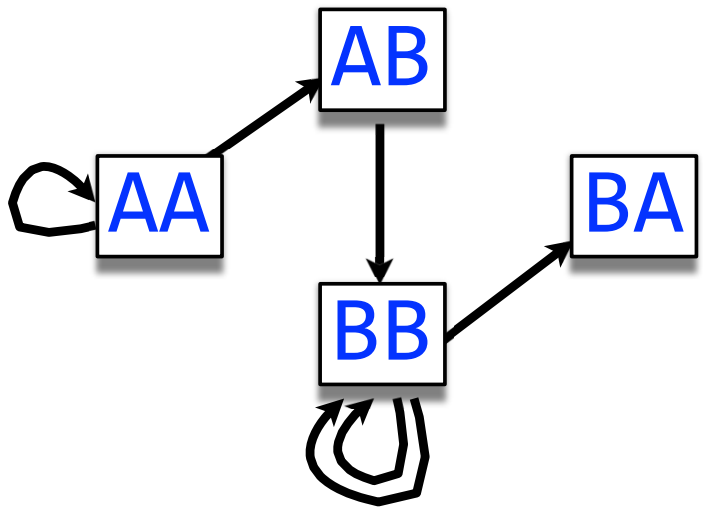
L/R 2-mers: AA, AA AA, AB AB, BB BB, BB BB, BB BB, BA



One edge per every *k*-mer

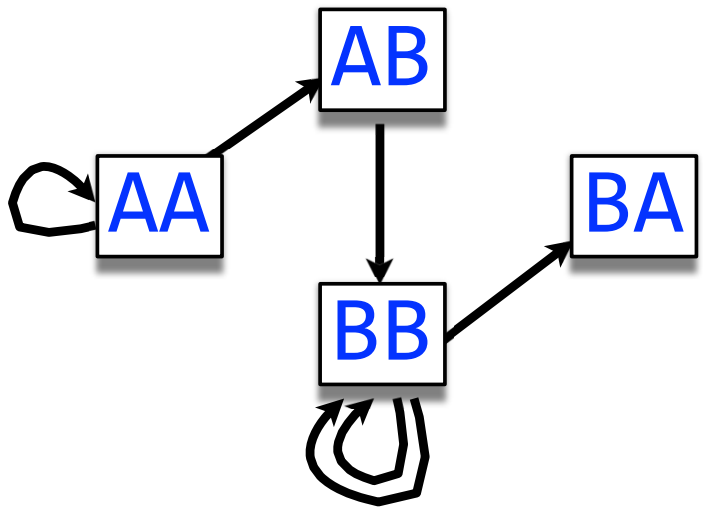
One node per distinct *k*-1-mer

De Bruijn graph



Walk crossing each edge exactly once gives a reconstruction of the genome

Assembly = Eulerian walk on De Bruijn graph



AAABBBBA

Walk crossing each edge exactly once gives a reconstruction of the genome. This is an *Eulerian walk*.

Edge-disjoint loops are a problem: multiple solutions

graph can have multiple Eulerian walks, only one of which corresponds to original superstring

Right: graph for ZABCDABEFABY, k=2

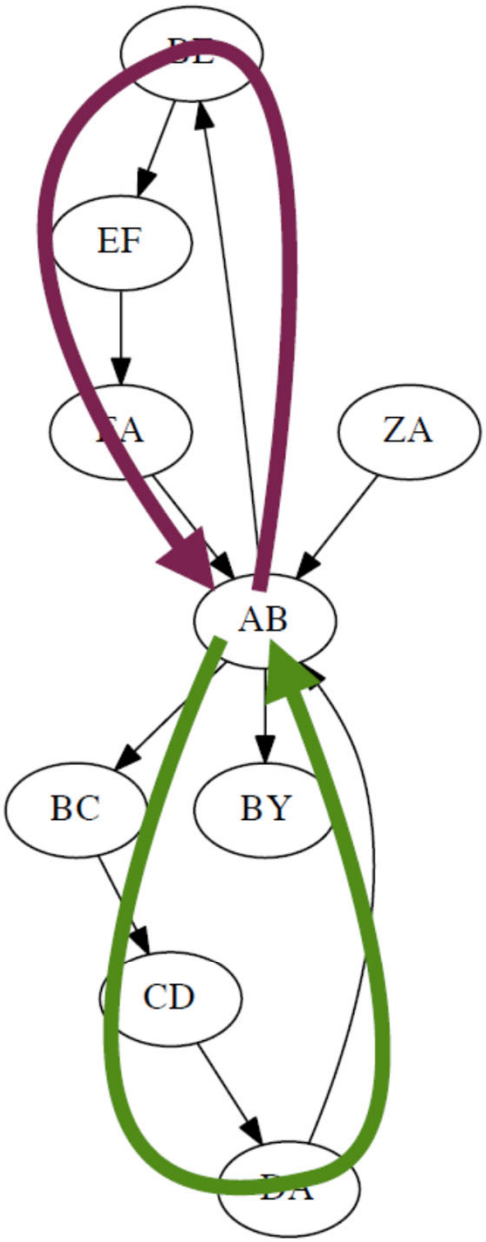
Alternative Eulerian walks:

ZA → AB → BE → EF → FA → AB → BC → CD → DA → AB → BY

ZA → AB → BC → CD → DA → AB → BE → EF → FA → AB → BY

These correspond to two edge-disjoint directed cycles joined by node AB

AB is a repeat: ZABCDABEFABY

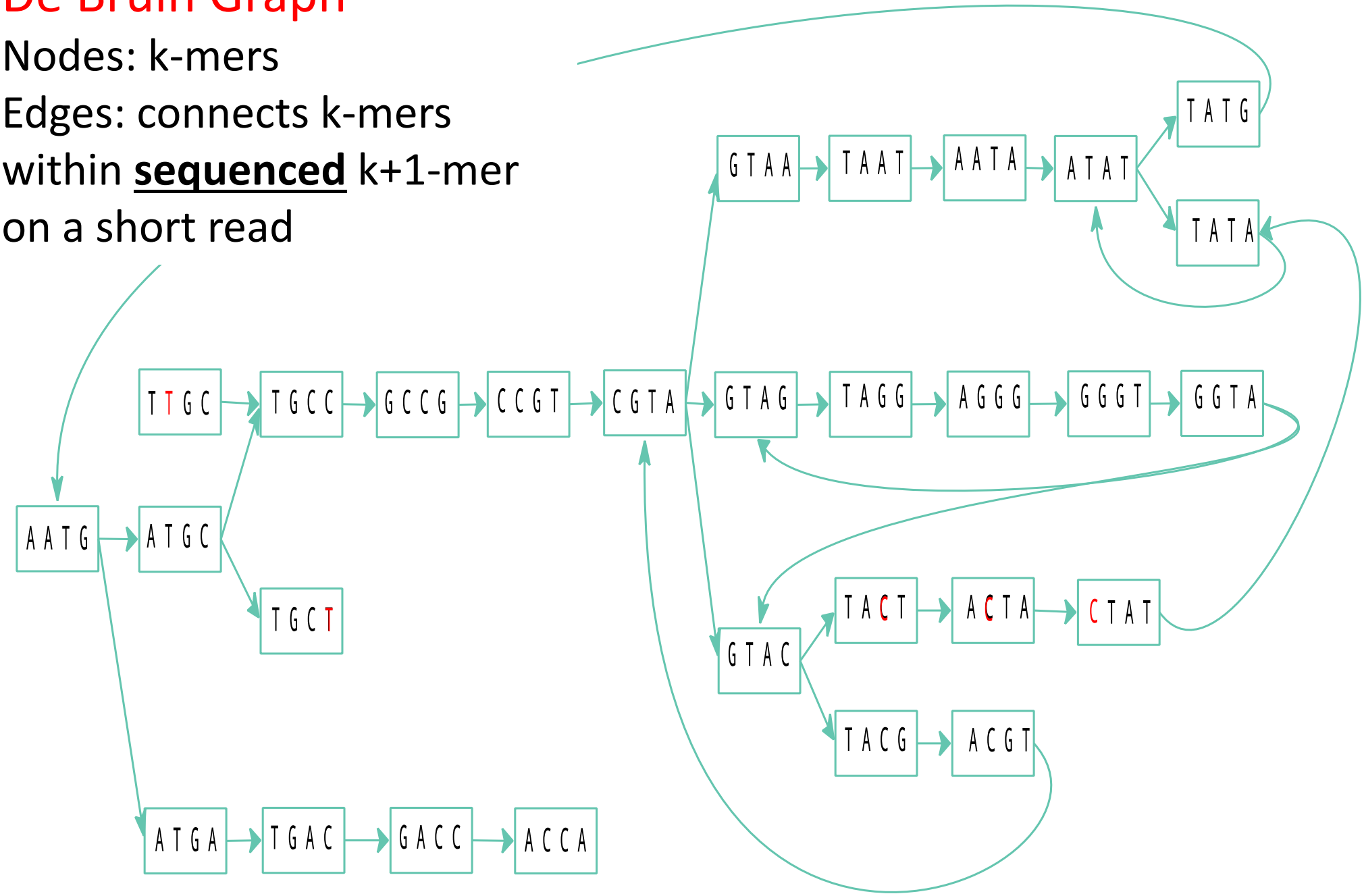


Adapted from a slide by Ben Langmead, Johns Hopkins U.

De Bruin Graph

Nodes: k-mers

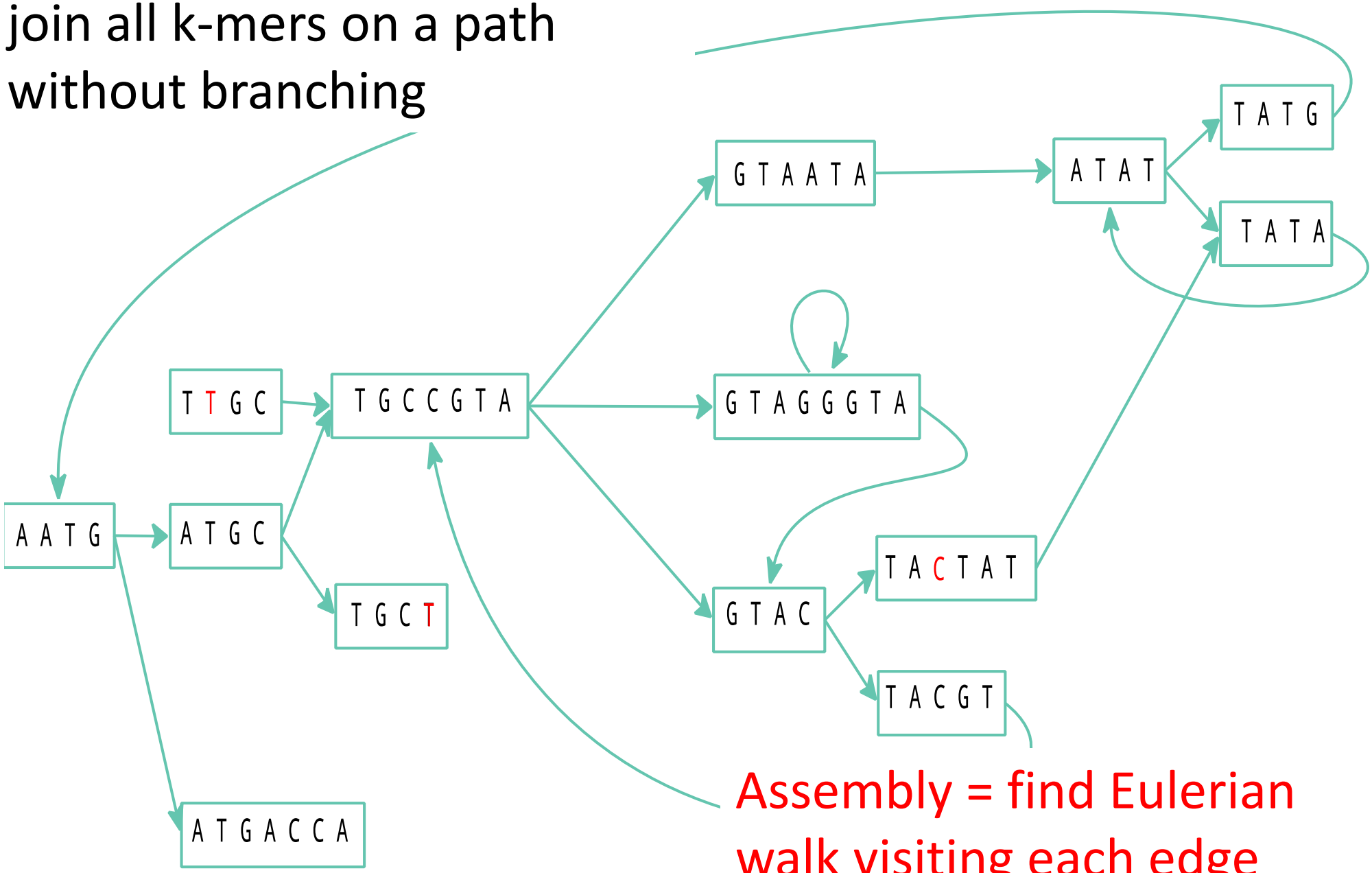
Edges: connects k-mers within **sequenced** k+1-mer on a short read



Slide by Sorin Istrail, Brown U.

Simplified De Bruin Graph

join all k-mers on a path without branching



Assembly = find Eulerian walk visiting each edge once

Slide by Sorin Istrail, Brown U.

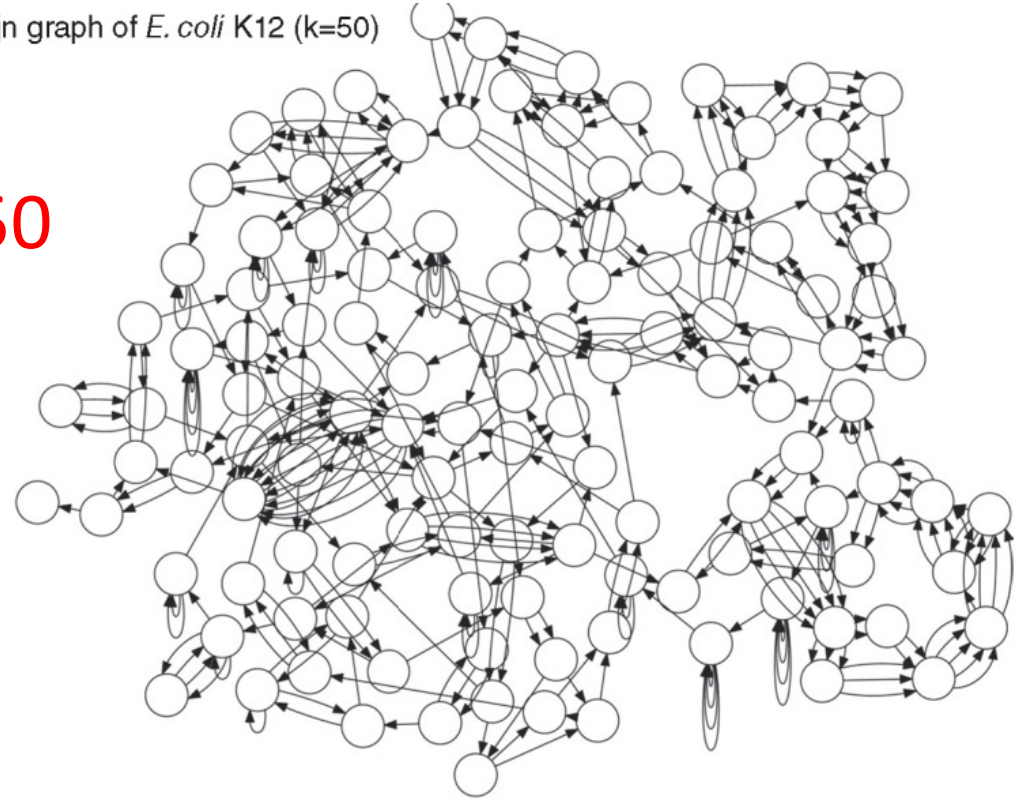
How to assemble a genome with repeats?

- Answer:
  longer reads
- But:
  cheap sequencing
  =
  short reads

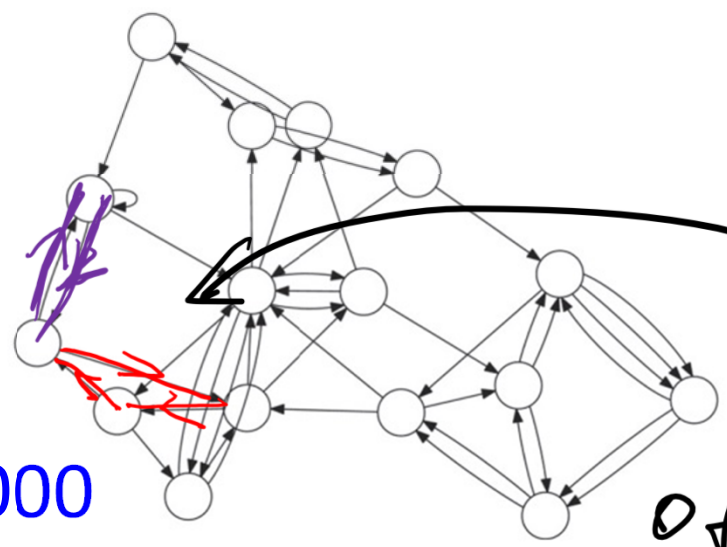
Technology	Read length (bp)
Roche 454	700
Illumina	50–250
SOLiD	50
Ion Torrent	200
Pacific Biosciences	2900
Sanger	400–900

(a) de Bruijn graph of *E. coli* K12 (k=50)

k=50



(b) de Bruijn graph (k=1,000)



k=1000

(c) de Bruijn graph (k=5,000)

k=5000

example of disjoint loops

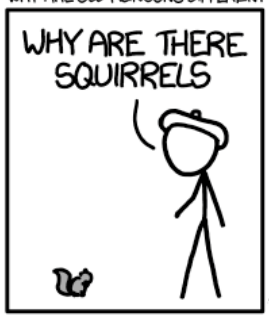
Credit: XKCD comics

QUESTIONS

FOUND IN GOOGLE AUTOCOMPLETE

WHY DO WHALES JUMP
WHY ARE WITCHES GREEN
WHY ARE THERE MIRRORS ABOVE BEDS
WHY DO I SAY UH
WHY IS SEA SALT BETTER
WHY ARE THERE TREES IN THE MIDDLE OF FIELDS
WHY IS THERE NOT A POKEMON MMO
WHY IS THERE LAUGHING IN TV SHOWS
WHY ARE THERE DOORS ON THE FREEWAY
WHY ARE THERE SO MANY SVCHOST.EXE RUNNING
WHY AREN'T THERE ANY COUNTRIES IN ANTARCTICA
WHY ARE THERE SCARY SOUNDS IN MINECRAFT
WHY IS THERE KICKING IN MY STOMACH
WHY ARE THERE TWO SLASHES AFTER HTTP
WHY ARE THERE CELEBRITIES
WHY DO SNAKES EXIST
WHY DO OYSTERS HAVE PEARLS
WHY ARE DUCKS CALLED DUCKS
WHY DO THEY CALL IT THE CLAP
WHY ARE KYLE AND CARTMAN FRIENDS
WHY IS THERE AN ARROW ON AANG'S HEAD
WHY ARE TEXT MESSAGES BLUE
WHY ARE THERE MUSTACHES ON CLOTHES
WHY ARE THERE MUSTACHES ON CARS
WHY ARE THERE MUSTACHES EVERYWHERE
WHY ARE THERE SO MANY BIRDS IN OHIO
WHY IS THERE SO MUCH RAIN IN OHIO
WHY IS OHIO WEATHER SO WEIRD
WHY ARE THERE MALE AND FEMALE BIKES
WHY ARE THERE BRIDESMAIDS
WHY DO DYING PEOPLE REACH UP
WHY AREN'T THERE VARICOSE ARTERIES
WHY ARE OLD KLINGONS DIFFERENT

WHY ARE THERE SQUIRRELS



WHY IS PROGRAMMING SO HARD
WHY IS THERE A 0 OHM RESISTOR
WHY DO AMERICANS HATE SOCCER
WHY DO RHYMES SOUND GOOD
WHY DO TREES DIE
WHY IS THERE NO SOUND ON CNN
WHY AREN'T POKEMON REAL
WHY AREN'T BULLETS SHARP
WHY DO DREAMS SEEM SO REAL

WHY AREN'T THERE DINOSAUR GHOSTS
WHY DO IGUANAS DIE
WHY IS THERE HELL IF GOD FORGIVES

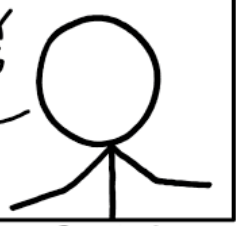
WHY ARE THERE TINY SPIDERS IN MY HOUSE
WHY DO SPIDERS COME INSIDE
WHY ARE THERE HUGE SPIDERS IN MY HOUSE
WHY ARE THERE LOTS OF SPIDERS IN MY HOUSE
WHY ARE THERE SPIDERS IN MY ROOM
WHY ARE THERE SO MANY SPIDERS IN MY ROOM
WHY DO SPIDER BITES ITCH
WHY IS DYING SO SCARY
WHY IS THERE NO GPS IN LAPTOPS
WHY DO KNEES CLICK
WHY AREN'T THERE E GRADES
WHY IS ISOLATION BAD
WHY DO BOYS LIKE ME
WHY DON'T BOYS LIKE ME
WHY IS THERE ALWAYS A JAVA UPDATE
WHY ARE THERE RED DOTS ON MY THIGHS
WHY IS LYING GOOD

WHY IS GPS FREE

WHY IS SEX SO IMPORTANT

WHY ARE THERE SLAVES IN THE BIBLE
WHY DO TWINS HAVE DIFFERENT FINGERPRINTS
WHY ARE AMERICANS AFRAID OF DRAGONS
WHY IS HTTPS CROSSED OUT IN RED
WHY IS THERE A LINE THROUGH HTTPS
WHY IS THERE A RED LINE THROUGH HTTPS ON FACEBOOK
WHY IS HTTPS IMPORTANT
WHY IS YKK ON ALL ZIPPERS

WHY AREN'T MY ARMS GROWING



WHY ARE THERE WEEKS
WHY DO I FEEL DIZZY
WHY ARE THERE SWARMS OF GNATS
WHY IS THERE PHLEGM
WHY AREN'T ECONOMISTS RICH
WHY DO AMERICANS CALL IT SOCCER
WHY ARE MY EARS RINGING
WHY ARE THERE SO MANY AVENGERS
WHY ARE THE AVENGERS FIGHTING THE X MEN
WHY IS WOLVERINE NOT IN THE AVENGERS
WHY IS THERE LAVA
WHY ARE THERE SO MANY CROWS IN ROCHESTER, MN
WHY IS PSYCHIC WEAK TO BUG
WHY DO CHILDREN GET CANCER
WHY IS POSEIDON ANGRY WITH ODYSSEUS
WHY IS THERE ICE IN SPACE
WHY ARE THERE ANTS IN MY LAPTOP
WHY IS EARTH TILTED
WHY IS SPACE BLACK
WHY IS OUTER SPACE SO COLD
WHY ARE THERE PYRAMIDS ON THE MOON
WHY IS NASA SHUTTING DOWN
WHY ARE THERE FEMALE MR MIMES

WHY ARE THERE GHOSTS

WHY IS THERE AN OWL IN MY BACKYARD
WHY IS THERE AN OWL OUTSIDE MY WINDOW
WHY IS THERE AN OWL ON THE DOLLAR BILL
WHY DO OWLS ATTACK PEOPLE
WHY ARE AK 47s SO EXPENSIVE
WHY ARE THERE HELICOPTERS CIRCLING MY HOUSE
WHY ARE THERE GODS
WHY ARE THERE TWO SPOCKS
WHY IS MT VESUVIUS THERE
WHY DO THEY SAY T MINUS
WHY ARE THERE OBELISKS
WHY ARE WRESTLERS ALWAYS WET
WHY ARE OCEANS BECOMING MORE ACIDIC
WHY IS ARWEN DYING
WHY AREN'T MY QUAIL LAYING EGGS
WHY AREN'T MY QUAIL EGGS HATCHING
WHY AREN'T THERE ANY FOREIGN MILITARY BASES IN AMERICA

WHY ARE DOGS AFRAID OF FIREWORKS
WHY IS THERE NO KING IN ENGLAND
WHY IS LIFE SO BORING

WHY ARE CIGARETTES LEGAL
WHY ARE THERE DUCKS IN MY POOL
WHY IS JESUS WHITE
WHY IS THERE LIQUID IN MY EAR
WHY DO Q TIPS FEEL GOOD
WHY DO GOOD PEOPLE DIE

WHY AREN'T THERE GUNS IN HARRY POTTER

WHY ARE ULTRASOUNDS IMPORTANT
WHY ARE ULTRASOUND MACHINES EXPENSIVE
WHY IS STEALING WRONG

A gallery of useful
discrete probability distributions

Geometric Distribution

- A series of Bernoulli trials with probability of success =*p*. continued **<u>until the first success</u>**. X is the number of trials.
- Compare to: Binomial distribution has:
  - Fixed number of trials =n.
  - Random number of successes = x.

$$P(X=x)=C_x^n p^x (1-p)^{n-x}$$

- Geometric distribution has reversed roles:
  - Random number of trials, *x*
  - Fixed number of successes, in this case 1.
  - Success always comes in the end: so no combinatorial factor C_x^n
  - $P(X=x) = p(1-p)^{x-1}$ where:

    $x-1$ = 0, 1, 2, … , the number of failures until the 1st success.
- NOTE OF CAUTION: Matlab, Mathematica, and many other sources use x to denote the number of failures until the first success. We stick with Montgomery-Runger notation

Geometric Mean & Variance

- If *X* is a geometric random variable (according to Montgomery-Bulmer) with parameter *p*,

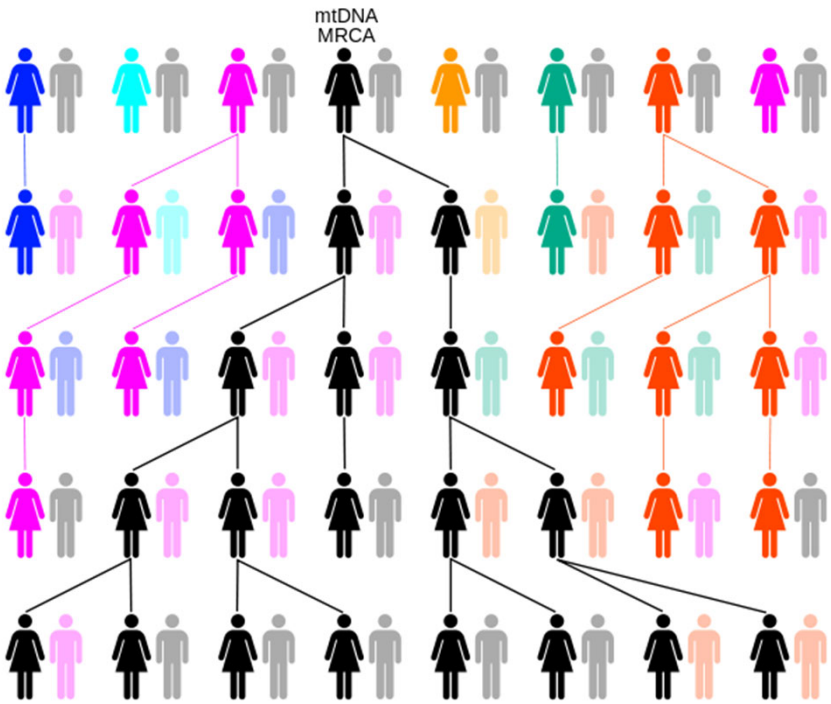
$$\mu = E(X) = \frac{1}{p} \quad \text{and} \quad \sigma^2 = V(X) = \frac{(1-p)}{p^2} \qquad (3\text{-}10)$$

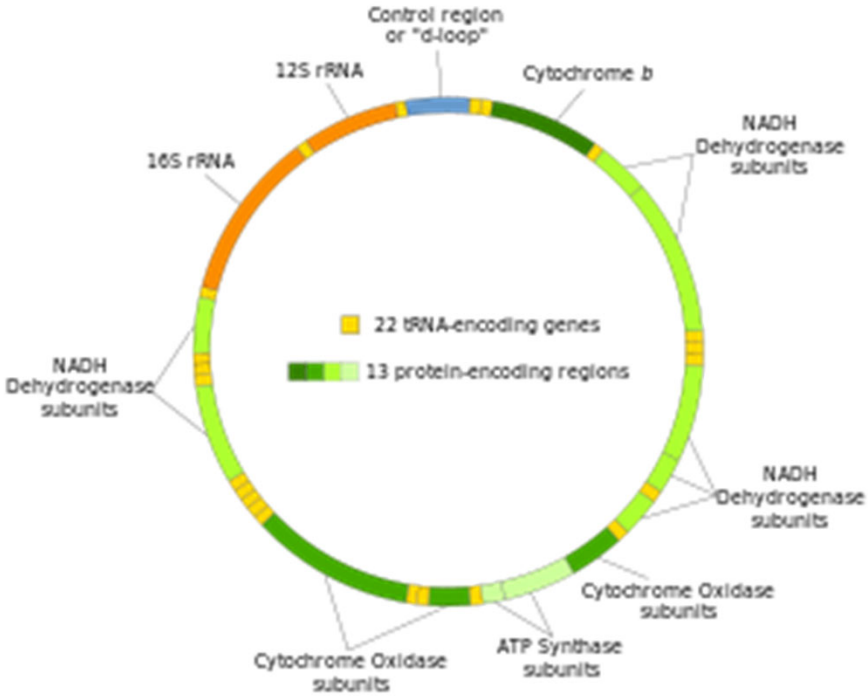
- For small *p* the standard deviation $=(1-p)^{0.5}/p$ ~= mean=1/p

- Very different from Binomial and Poisson, where

variance = mean and standard deviation = $\text{mean}^{1/2}$

Geometric distribution in biology

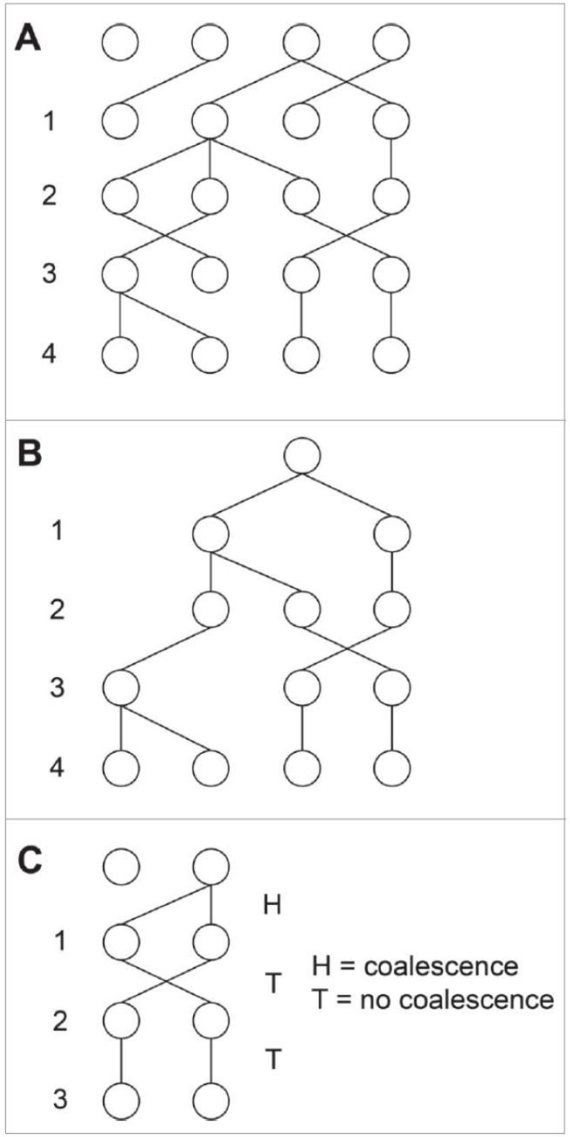




- Each of our cells has mitochondria with 16.5kb of mtDNA **inherited only from our mother**
- Human mtDNA has 37 genes encoding 13 proteins, 22+2 tRNA & rRNA
- Mitochondria appeared 1.5-2 billion years ago as a symbiosis between an alpha-proteobacterium (1000s of genes) and an archaeaon (of UIUC's Carl R. Woese fame)
- Since that time most mitochondrial genes were transferred into the nucleus
- Plants also have plastids with genomes related to cyanobacteria

Time to the last common (maternal) ancestor follows geometric distribution

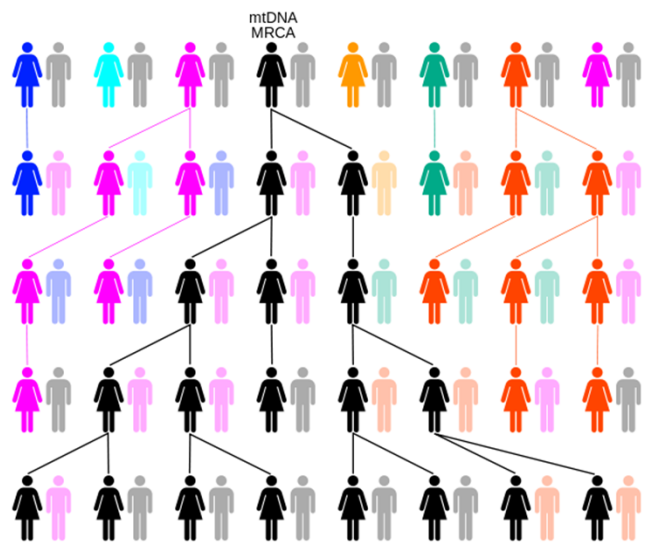
- Constant population of N women
- Random number of (female) offsprings. Average is 1 (but can be 0 or 2)
- Randomly pick two women.
  Question: how many generations T since their last maternal ancestor?
- T is a random variable What is its PMF: P(T=t)?
  Answer: P(T=t) follows a geometric distribution
- Do these two women have the same mother? Yes: "success" in finding their last common ancestor (p=1/N). P(T=1)=1/N.
- No? "failure" (1-p=1-1/N). Go to their mothers and repeat the same question.
- $P(T=t)=(1-1/N)^{t-1}(1/N) \approx (1/N)\exp(-(t-1)/N)$
- t can be inferred from the density of differences on mtDNA =2μt



Maddamsetti R, MOBILE GENETIC ELEMENTS, 6, e1137380(2016)

Most Recent Common Ancestor (MRCA)

- Start with N individuals. Unit of time is N generations (time for one pair to merge) since $E(T)=\sum_{t=1}^{\infty} t \cdot (1/N)\, exp(-t/N)=N$
- Any of $\frac{N(N-1)}{2}$ pairs can merge first. The average time for the first pair to merge is $\frac{2}{N(N-1)}$
- After merger $N \to N-1$,
- so time until the next
- merger is $\frac{2}{(N-1)(N-2)}$



Most Recent Common Ancestor (MRCA)

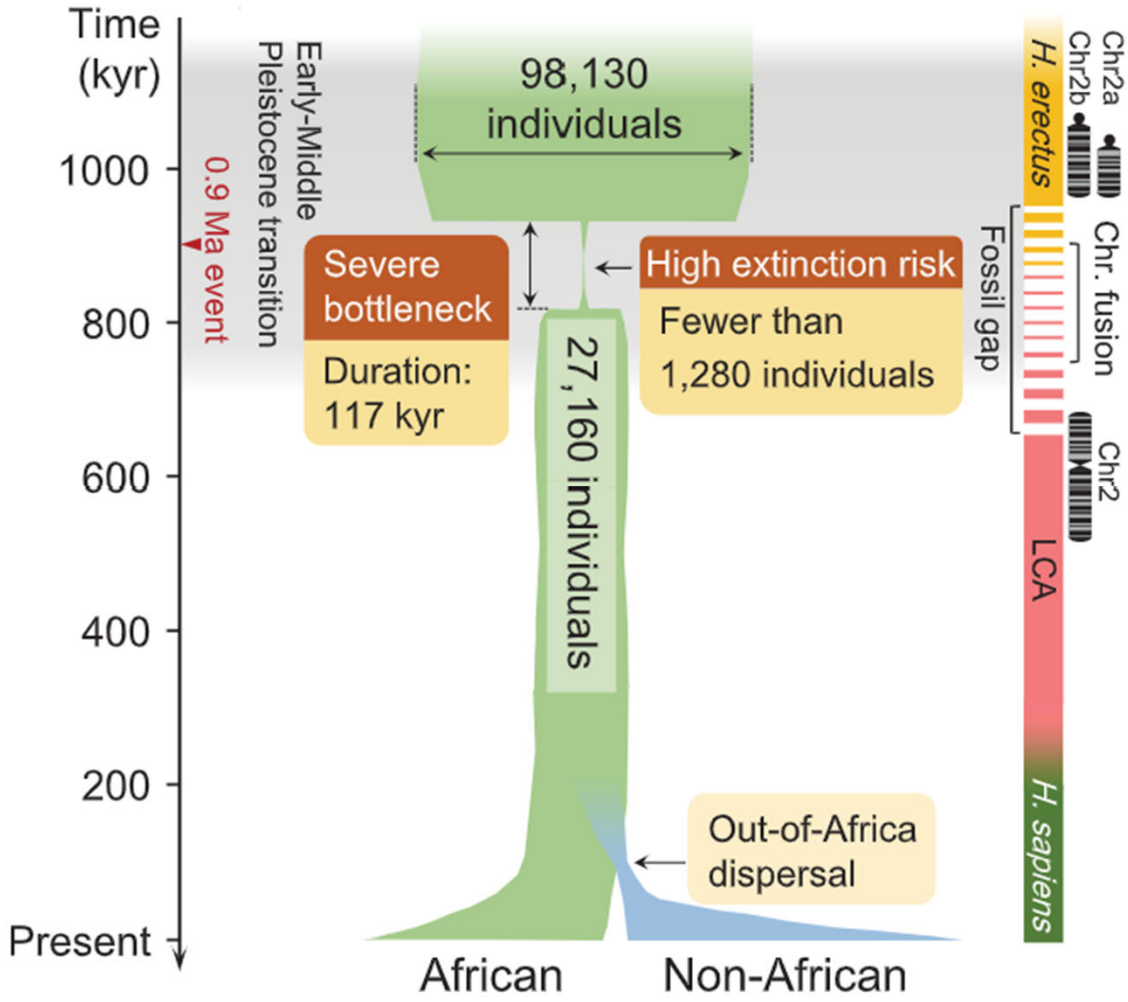
Total time until the MRCA

$$T_{MRCA} = N \cdot \sum_{k=2}^{N} \frac{2}{k(k-1)}$$

$$= 2N \sum_{k=2}^{N} \left(\frac{1}{k-1} - \frac{1}{k} \right) = 2N \left(1 - \frac{1}{N} \right) \approx 2N$$

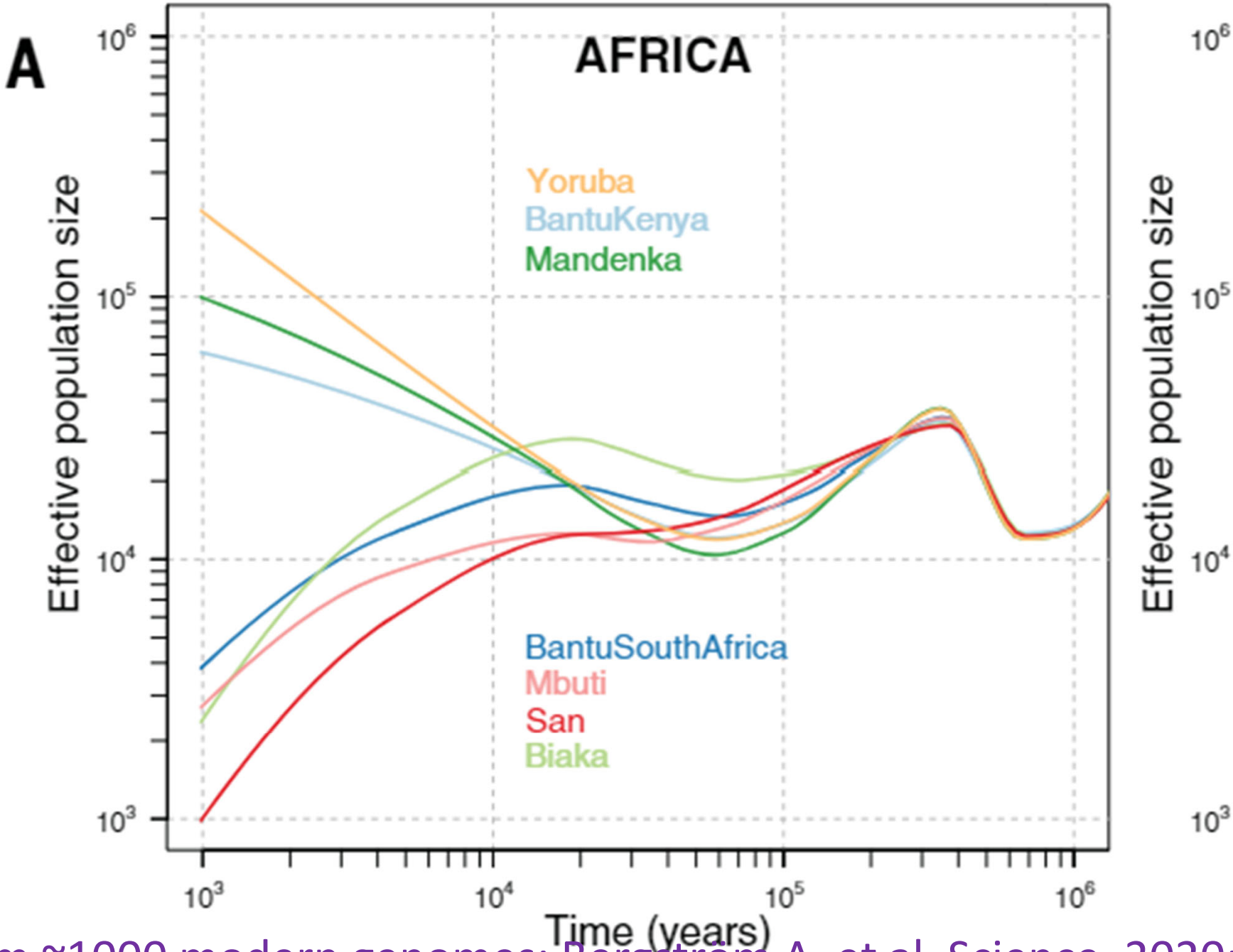
- There are about $N=3.5x10^{9}$ women living today
- Most Recent maternal Common Ancestor (MRCA)
  of all people living today lived $T_{MRCA}=2N$ generations ago
- $T_{MRCA} = 2 \cdot 3.5x10^{9}$ generations
- If the generation time 20 years it is 140 billion years > 10 times the time since the Big Bang.
- Something is wrong here!

Hot off the press: human ancestors almost got extinct about 1M years ago



Hu W, et al. Science. 2023;381: 979–984

Effective human population size ~10,000



From ~1000 modern genomes: Bergström A, et al. Science. 2020;367

- Population is not constant and for a long time was very low
- Change N to the "effective" size N_e
- Current thinking is that for all of us including people of African ancestry N_e~10,000 people
- For humans of European + Asian ancestry N_e~ 3000 people
- Mito Eve lived in Africa ~2*(Ne/2)*20 years=10,000*20 years= 200,000 years ago