

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

Courtesy of [Ben Langmead](http://www.langmead-lab.org/teaching-materials/). Used with permission.

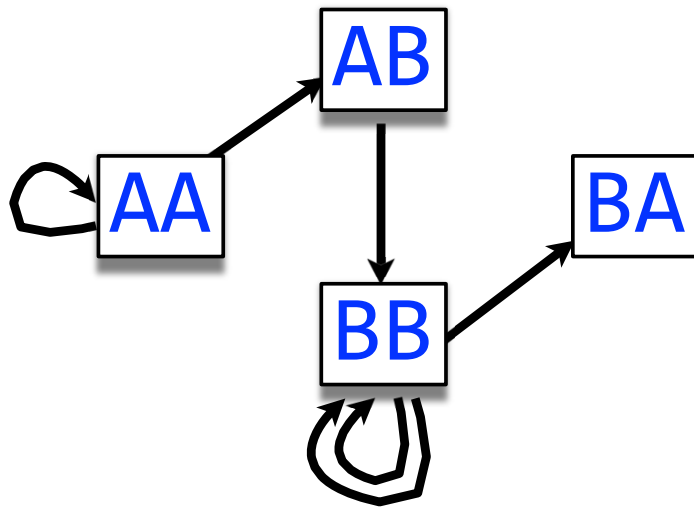
<http://www.langmead-lab.org/teaching-materials/>

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**



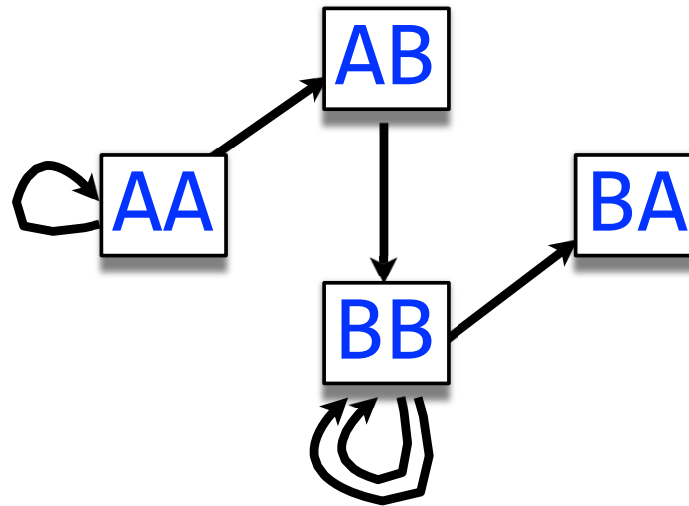
One edge per **every** k -mer

One node per **distinct** $k-1$ -mer

Courtesy of [Ben Langmead](http://www.langmead-lab.org/teaching-materials/). Used with permission.

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De Bruijn graph

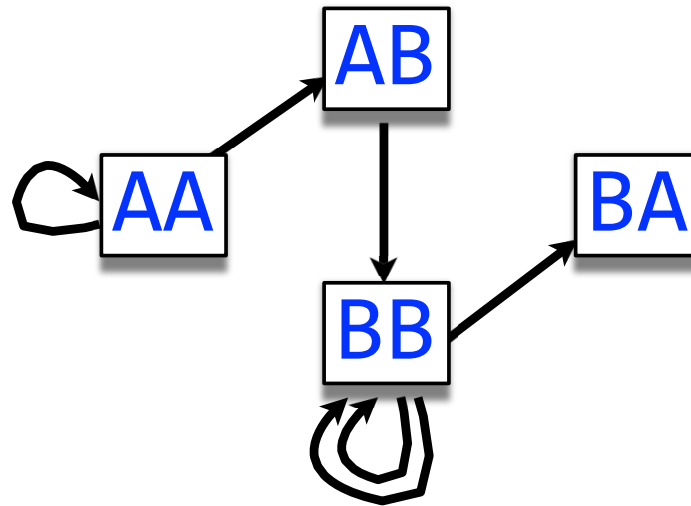


Walk crossing each edge exactly once gives a reconstruction of the genome

Courtesy of [Ben Langmead](http://www.langmead-lab.org/teaching-materials/). Used with permission.

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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**, $k=2$

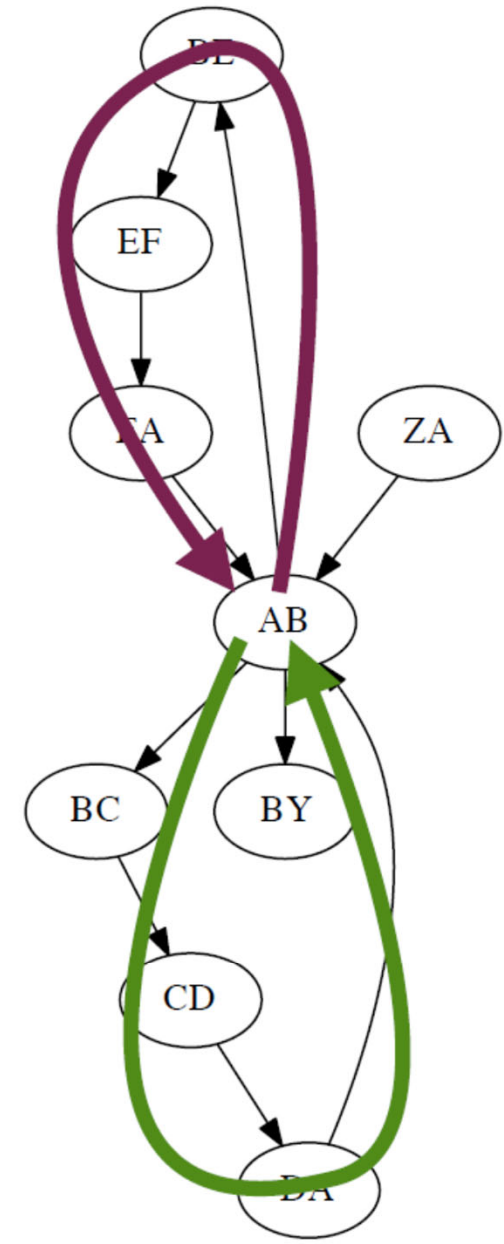
Alternative Eulerian walks:

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**

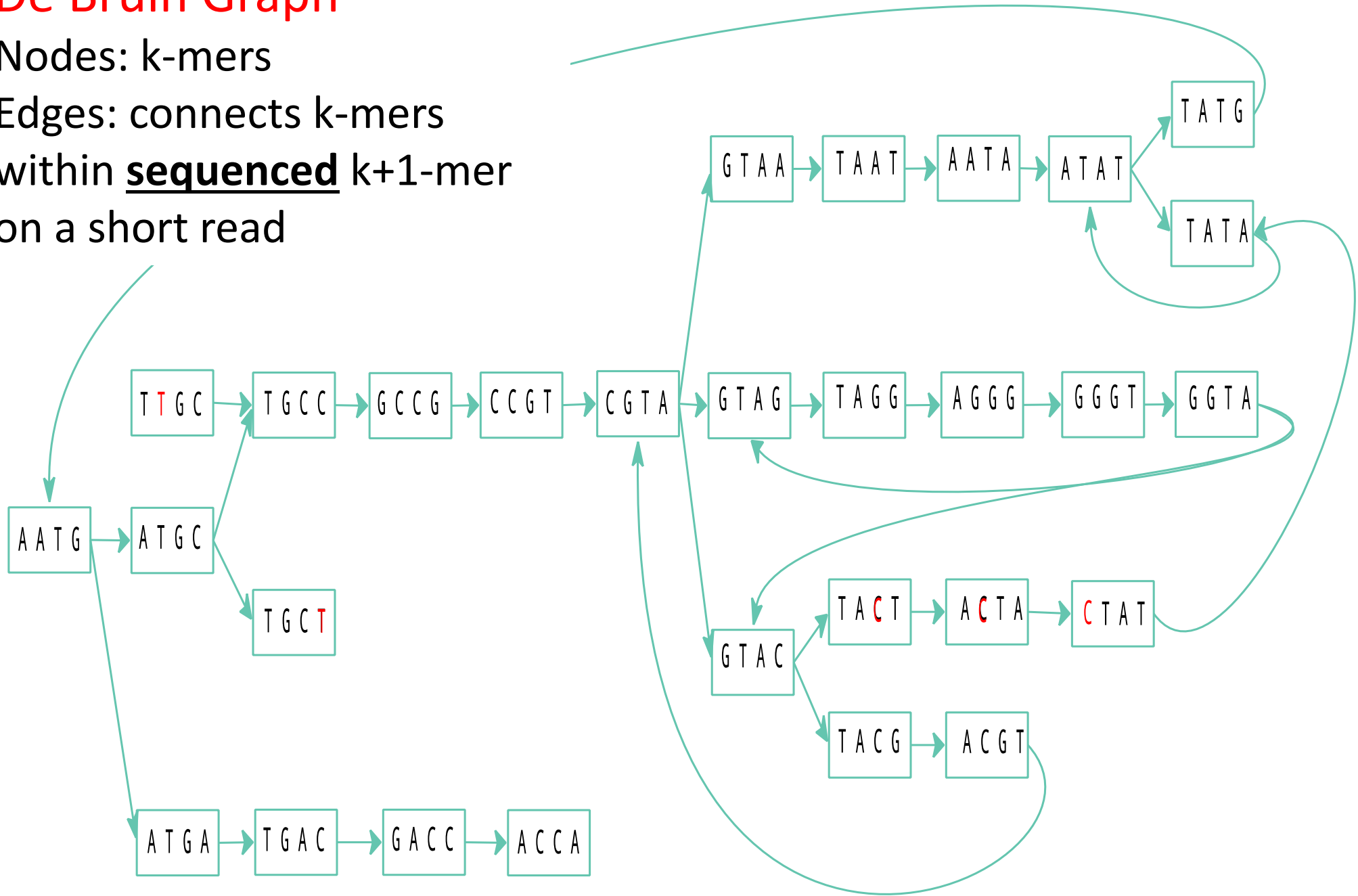


Adapted from a slide by Ben Langmead, Johns Hopkins U.

De Bruin Graph

Nodes: k-mers

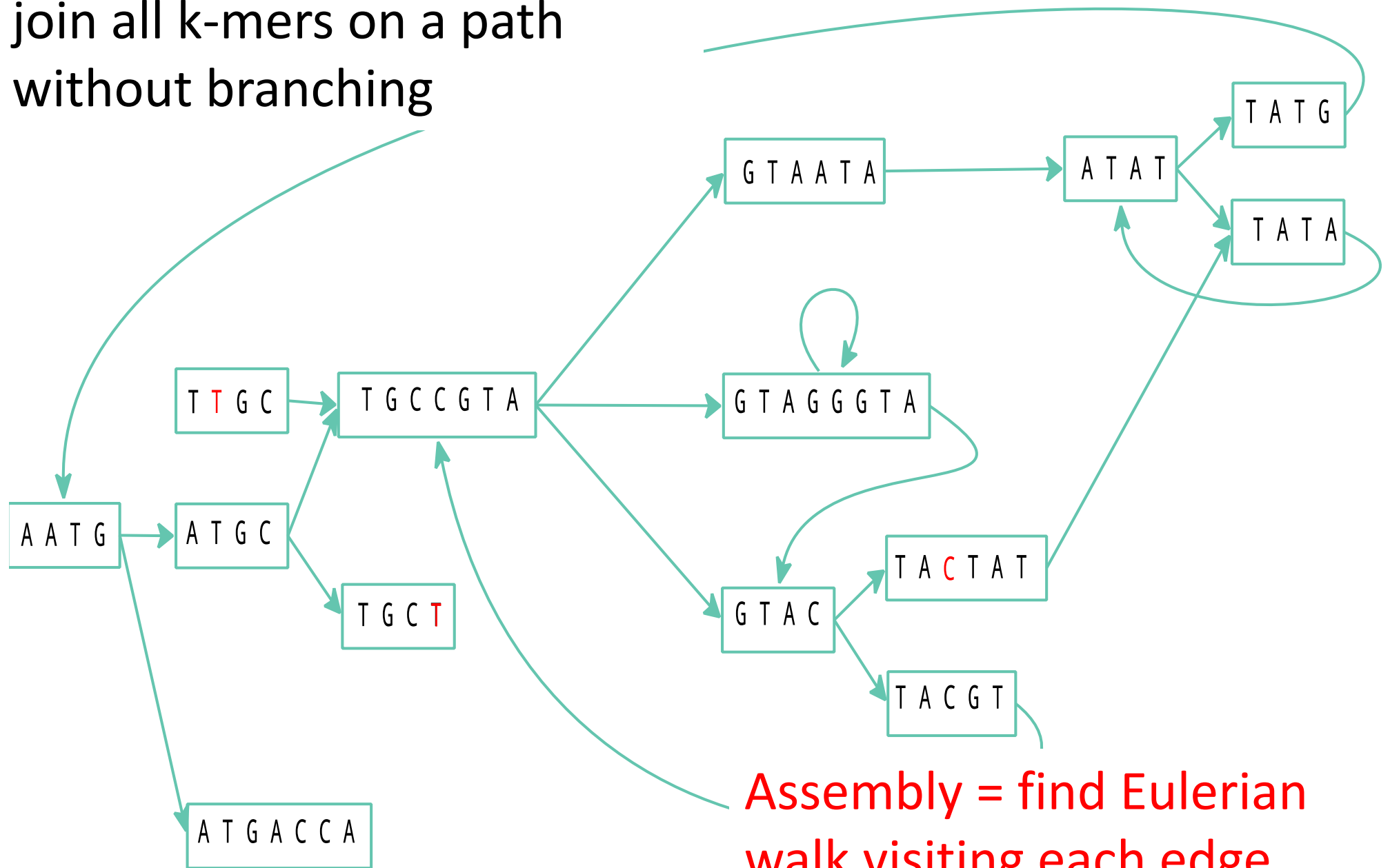
Edges: connects k-mers
within **sequenced** k+1-mer
on a short read



Slide by Sorin Istrail, Brown U.

Simplified De Bruin Graph

join all k-mers on a path
without branching



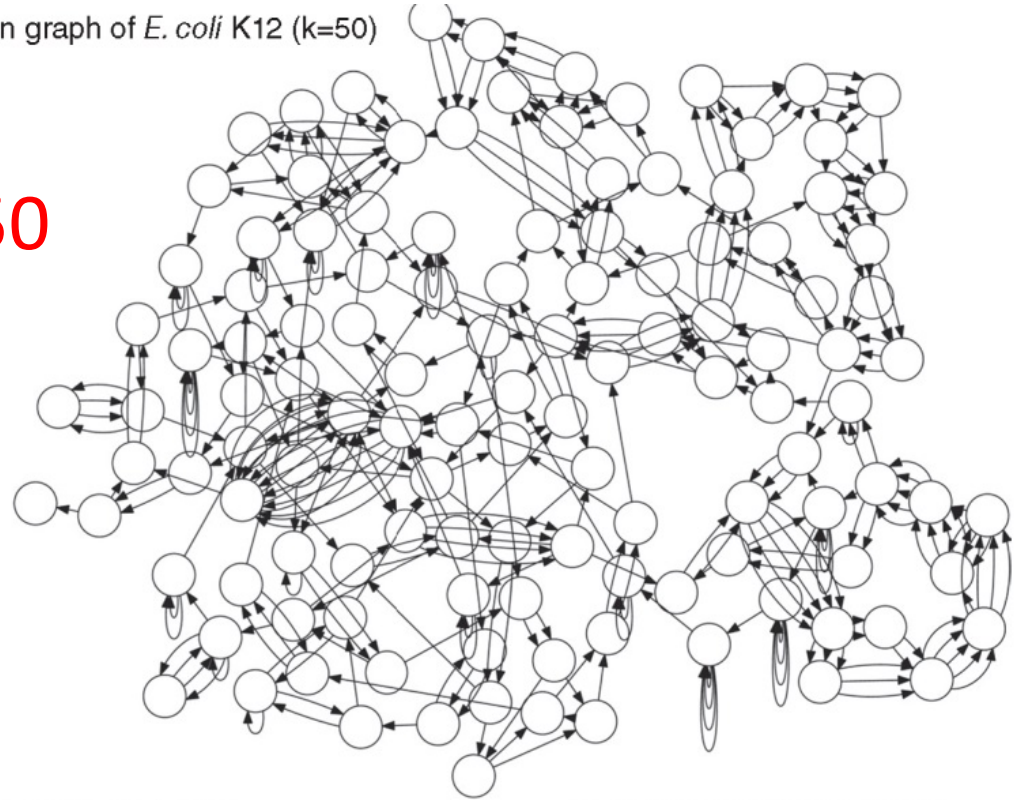
Assembly = find Eulerian
walk visiting each edge
once

How to assemble a genome with repeats?

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

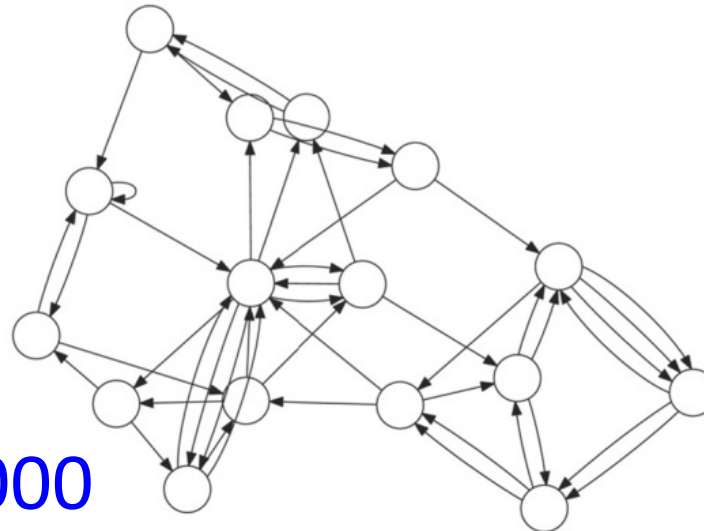
(a) de Bruijn graph of *E. coli* K12 ($k=50$)

$k=50$



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

$k=1000$



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

$k=5000$



| Technology | Read length (bp) |
|---------------------|------------------|
| Roche 454 | 700 |
| Illumina | 50–250 |
| SOLiD | 50 |
| Ion Torrent | 400 |
| Pacific Biosciences | >10,000 |

A gallery of useful discrete probability distributions

Geometric Distribution

- A series of **Bernoulli trials** with **probability of success = p** . continued **until the first success**. X is the number of trials.
- Compare to: Binomial distribution has:
 - Fixed number of trials = n . $P(X = x) = C_x^n p^x (1 - p)^{n-x}$
 - Random number of successes = 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:
 $x-1 = 0, 1, 2, \dots$, 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**

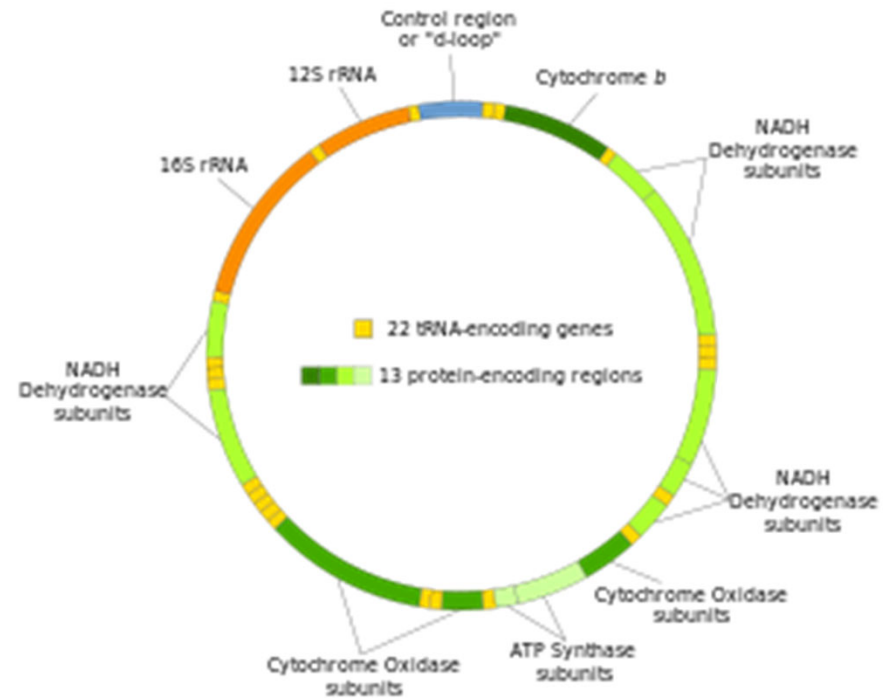
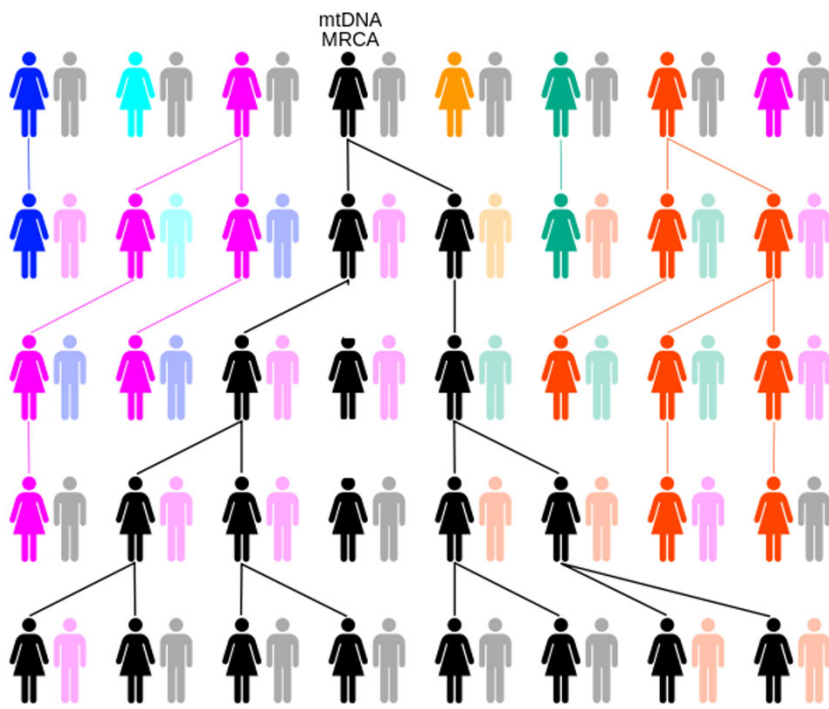
Geometric Mean & Variance

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

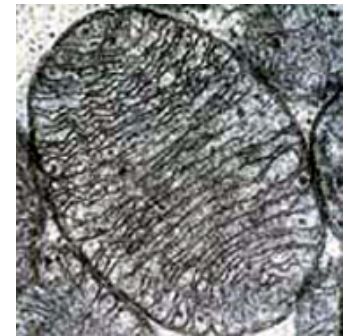
$$\mu = E(X) = \frac{1}{p} \quad \text{and} \quad \sigma^2 = V(X) = \frac{(1-p)}{p^2} \quad (3-10)$$

- For small p the **standard deviation** $= (1-p)^{0.5}/p \approx$
mean $= 1/p$
- Very different from Binomial and Poisson, where
variance $=$ **mean** and **standard deviation** $=$ **mean**^{1/2}

Geometric distribution in biology

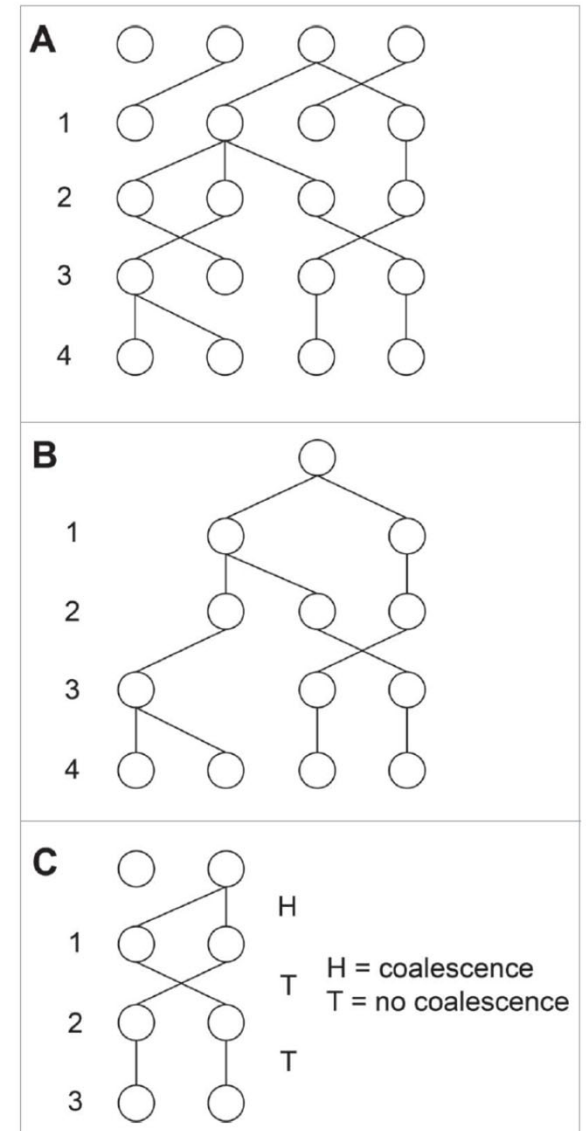


- Each of our cells has mitochondria with 16.5kb of mtDNA **inherited only from our mother**
- Human mtDNA has 37 genes encoding 13 proteins, 22+2 tRNA & rRNA
- Mitochondria appeared 1.5-2 billion years ago as a symbiosis between an alpha-proteobacterium (1000s of genes) and an archaeon (of UIUC's Carl 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** $=2\mu t$



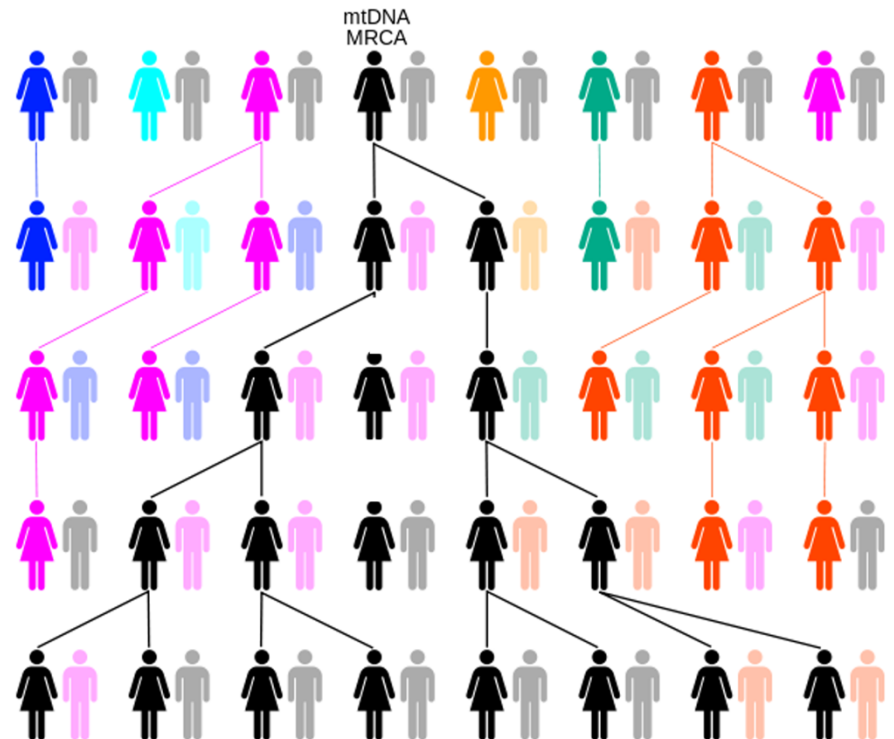
Most Recent Common Ancestor (MRCA)

- Consider N women living today. Let $U(t)$ be the number of maternal ancestors t generations before present connected to all modern women
- At $t=0$, $U(0) = N$. Any of $\frac{U(0)(U(0)-1)}{2}$ pairs of woman can coalesce with probability $\frac{1}{N}$

- We expect $\frac{1}{N} \frac{U(0)(U(0)-1)}{2}$ pairs to coalesce

$$U(1) = U(0) - \frac{1}{N} \frac{U(0)(U(0)-1)}{2}$$

- Now the expected number of coalescing pairs $\frac{1}{N} \frac{U(1)(U(1)-1)}{2}$



Most Recent Common Ancestor (MRCA)

$$\frac{\frac{dU(t)}{dt}}{\frac{U(t)^2}{2N}} = - \overset{\substack{\text{probability} \\ \text{of coalescence}}}{\underbrace{\frac{1}{N}}} \times \overset{\substack{\text{number of pairs}}}{\underbrace{\frac{U(t)(U(t)-1)}{2}}} \approx$$

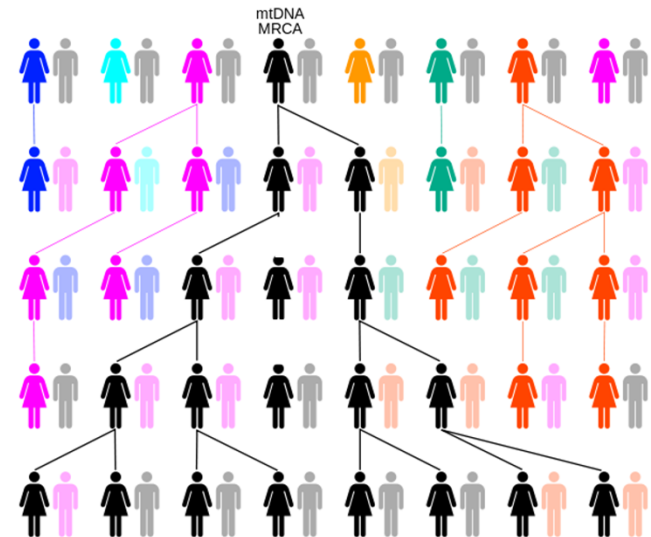
$$\text{Solution: } U(t) = \frac{C}{t+2}$$

$$\text{Let's check: } \frac{dU}{dt} = -\frac{C}{(t+2)^2} = \frac{U^2}{C} \rightarrow C = 2N$$

$$T_{MRCA} = 2(N - 1) \approx 2N$$

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 $N \rightarrow N - 1$,
- So, the time until the next merger is longer $\frac{2}{(N-1)(N-2)}$



Most Recent Common Ancestor (MRCA)

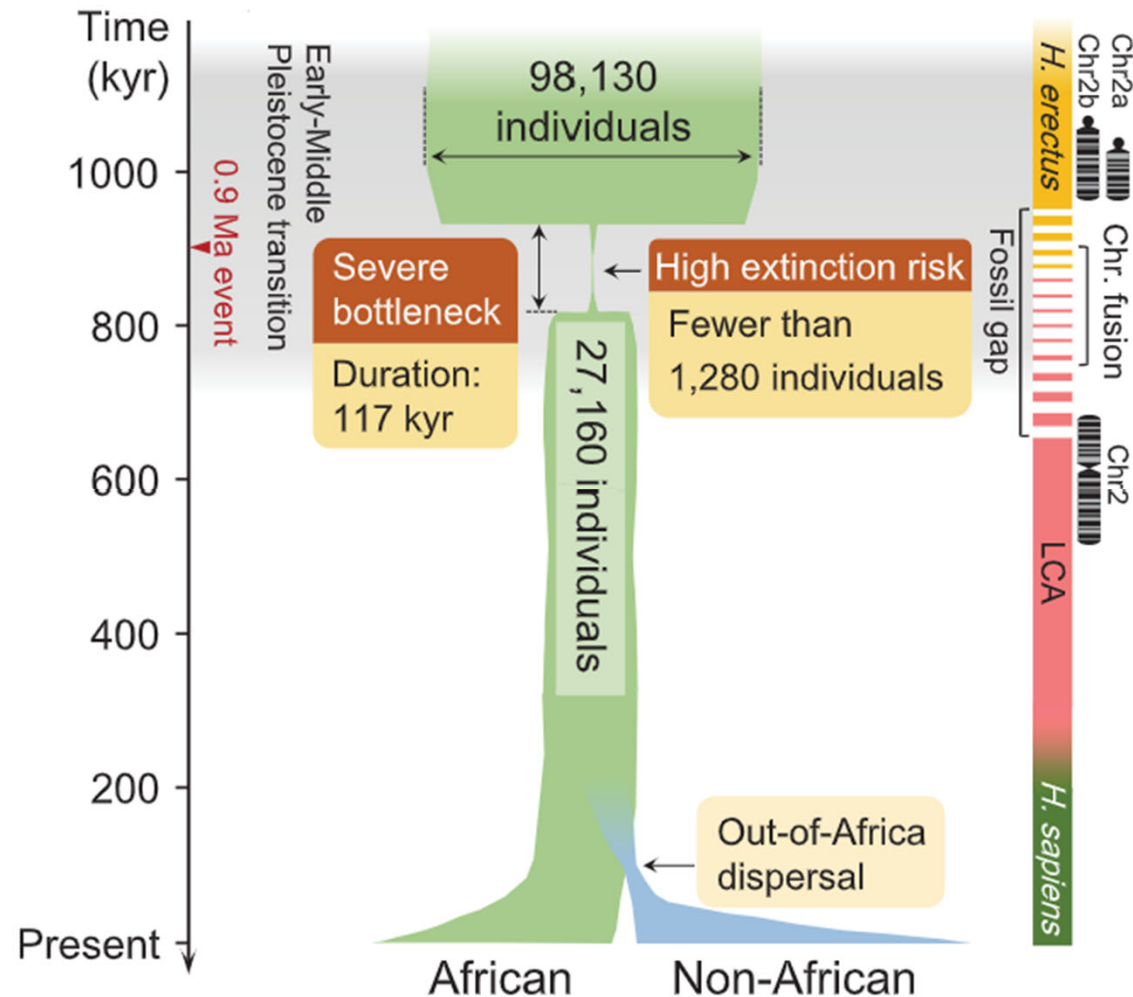
Total time until the MRCA

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

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

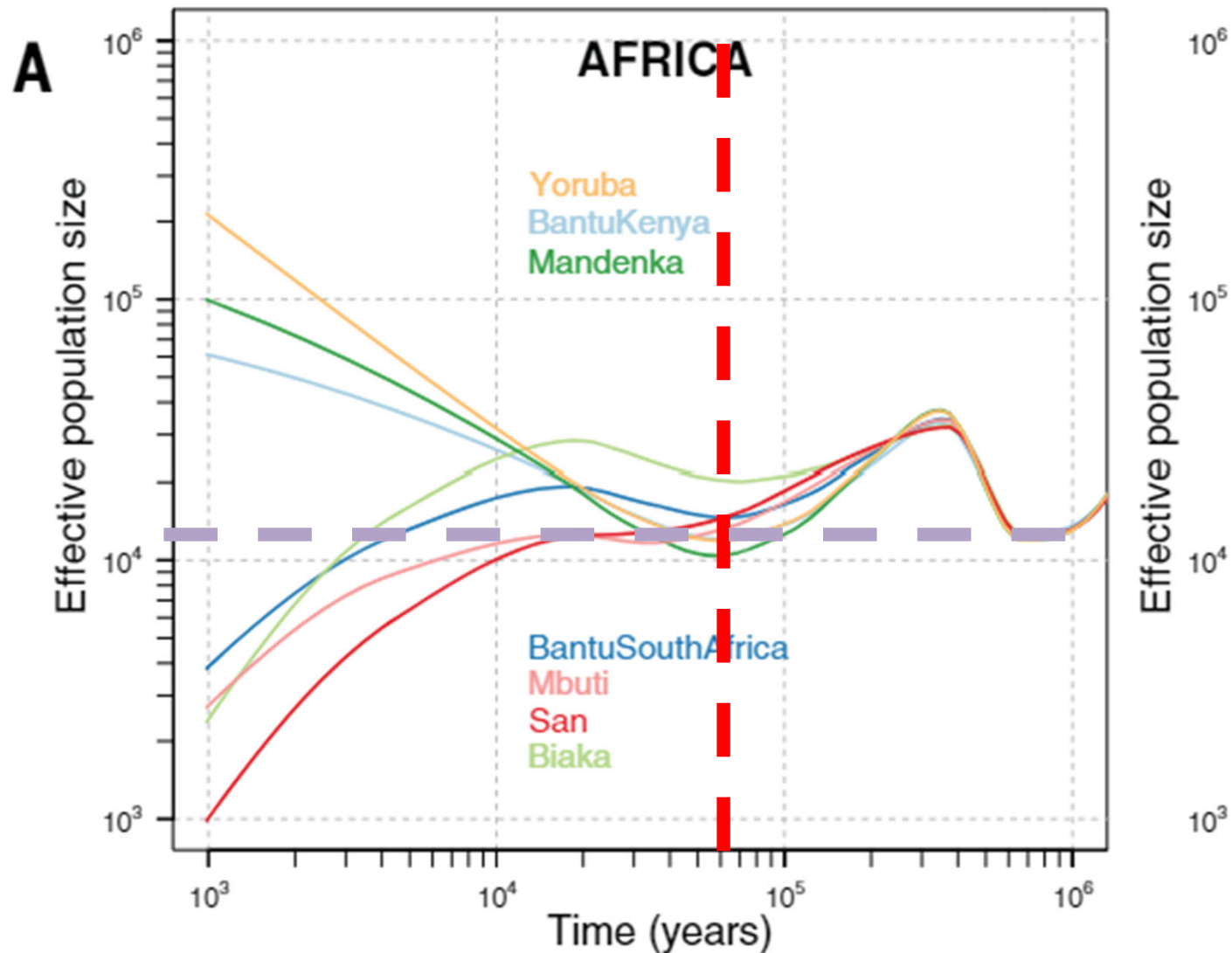
- There are about $N = 8 \times 10^9 / 2 = 4 \times 10^9$ women living today
- **M**ost **R**ecent maternal **C**ommon **A**ncessor (**MRCA**)
of all people living today lived $T_{MRCA} = 2N$ generations ago
- $T_{MRCA} = 2 \cdot 4 \times 10^9$ generations
- If the generation time 20 years it is 160 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

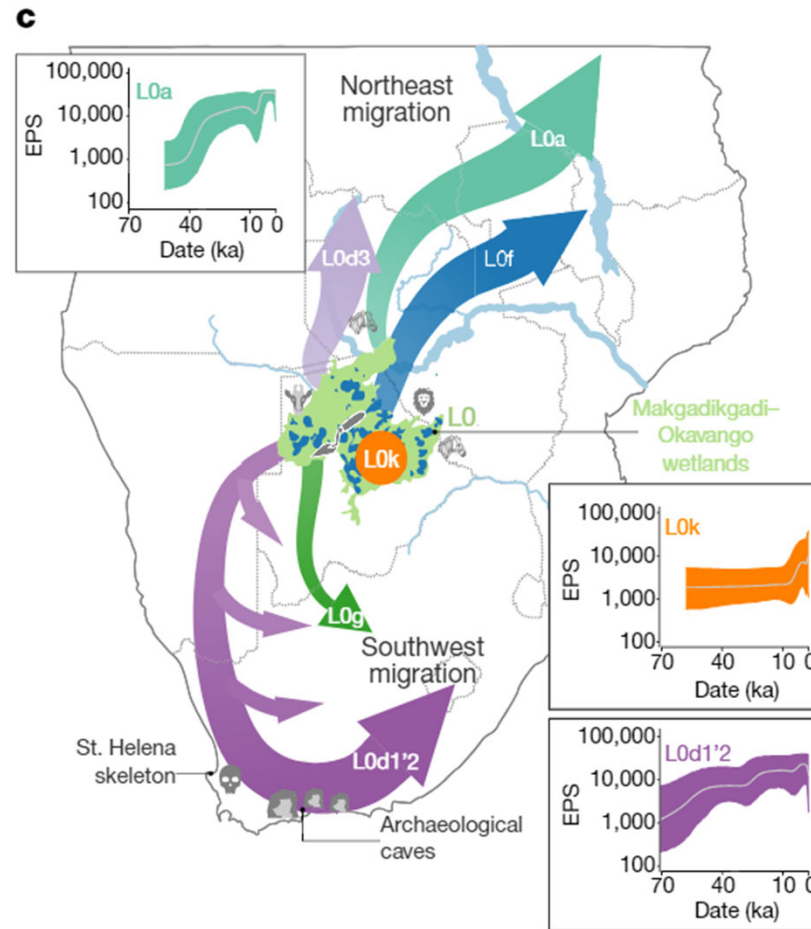
Bottleneck for human population in Africa around 10,000 individuals reached around 60,000 years ago



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 reached during **the bottleneck**
- Current thinking is that for all of humankind $N_e \sim 10,000$ people
- **Mito Eve lived in Africa** $\sim 2 * (N_e / 2) * 20$
years = $10,000 * 20$ years = **200,000 years ago**

“Mitochondrial Eve” lived in Africa



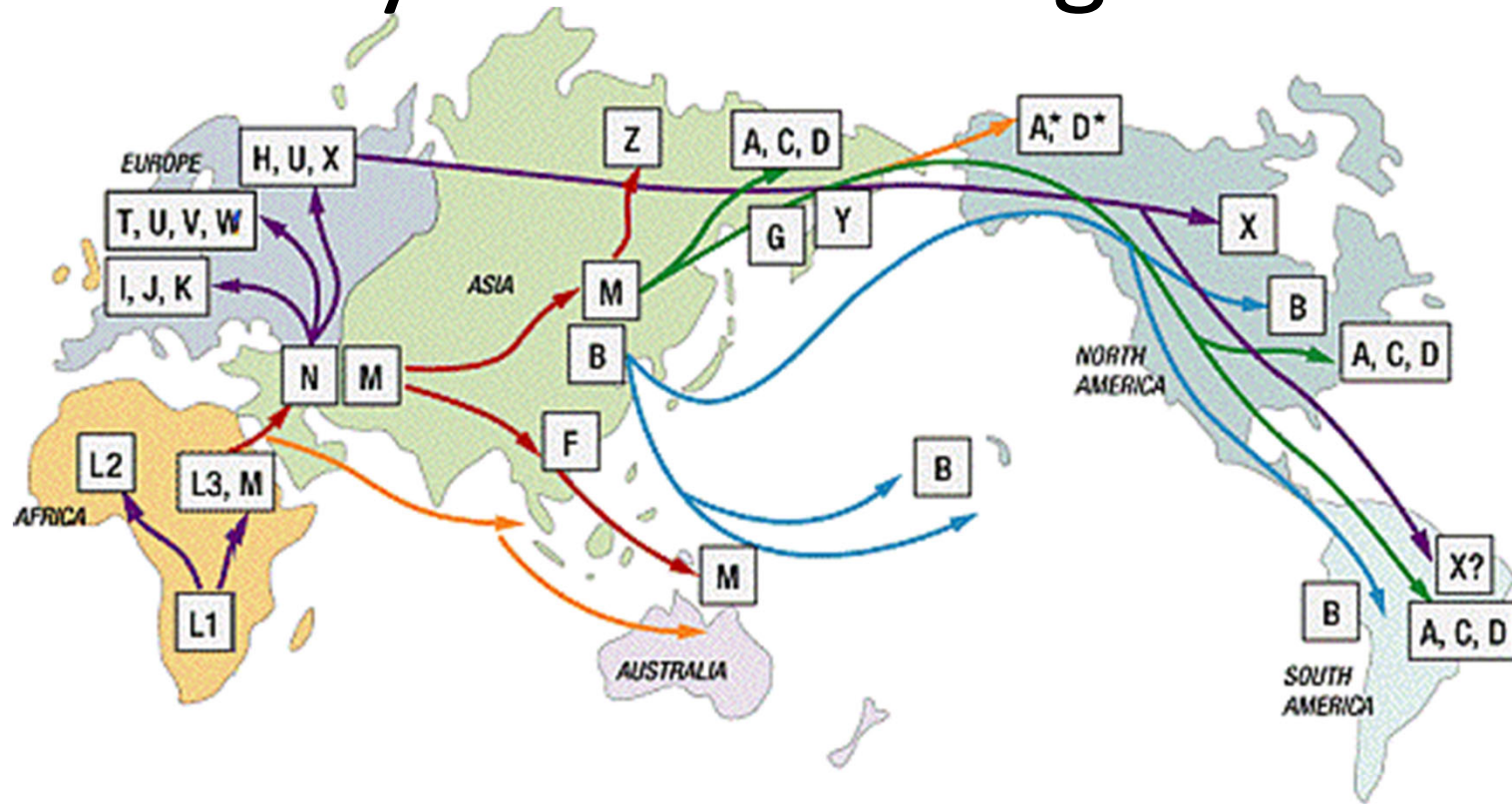
“Mitochondrial Eve” lived in Makgadikgadi–Okavango paleo-wetland of southern Africa ~200,000 years ago (between 165,000 and 240,000 years ago)

Chan EKF, et al. Nature. 2019; 575: 185–189.

Okavango Delta now



Modern mitochondrial DNA contains history of human migrations

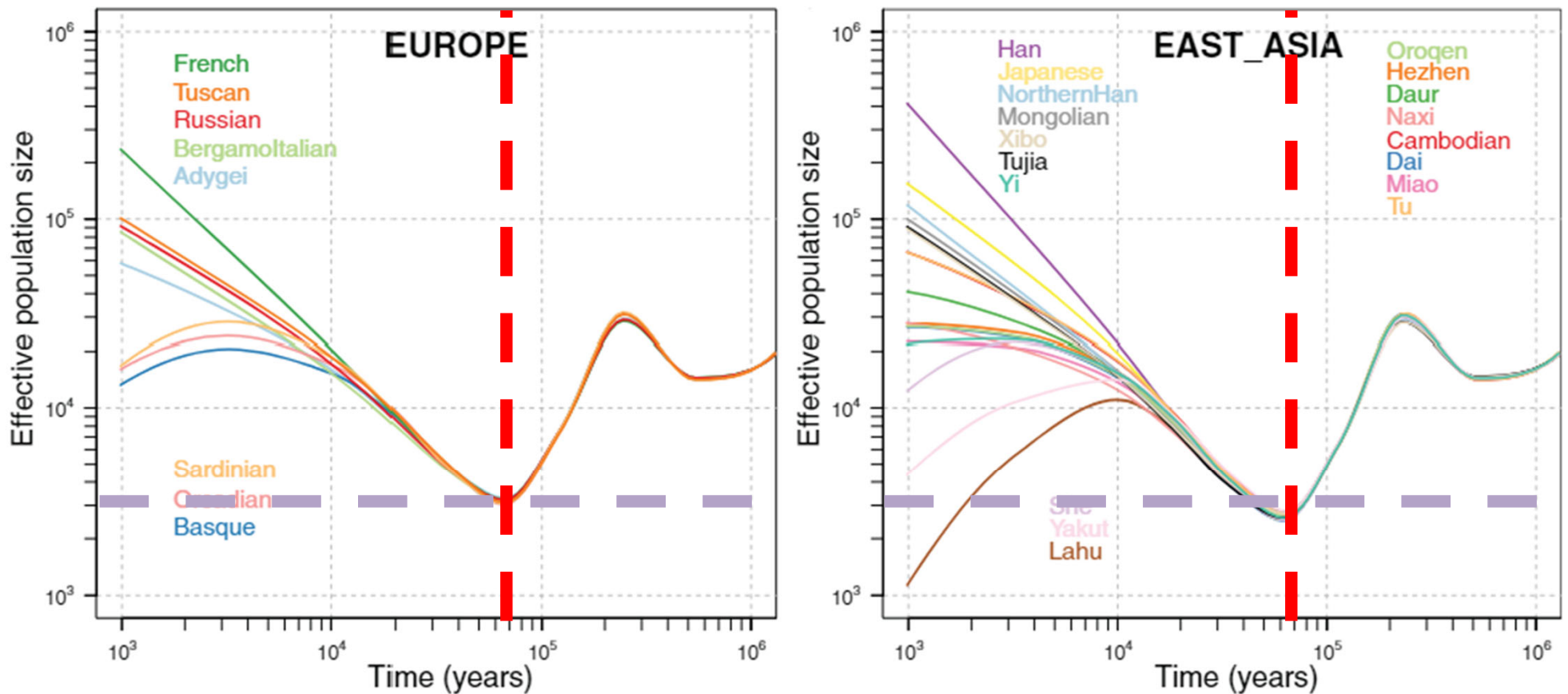


| EXPANSION TIMES (years ago) | |
|-----------------------------|-------------------|
| Africa | 120,000 - 150,000 |
| Out of Africa | 55,000 - 75,000 |
| Asia | 40,000 - 70,000 |
| Australia/PNG | 40,000 - 60,000 |
| Europe | 35,000 - 50,000 |
| Americas | 15,000 - 35,000 |
| Na-Dene/Esk/Aleuts | 8,000 - 10,000 |



Poznik GD, et al (Carlos Bustamante lab in Stanford), *Science* **341**: 562 (August 2013).

- Bottleneck for human population in Europe and Asia was around 3,000 individuals reached around 70,000 years ago
- Non-African Eve lived ~60,000 years ago



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

What about men?

- Y-chromosome is transferred from father to son
- Like mitochondria it can be used to trace ancestry of all men to the “Y-chromosome Adam”
- Where did “Adam” live? Did he meet the “mitochondrial Eve”?

Y-chromosomal Adam also lived in Africa

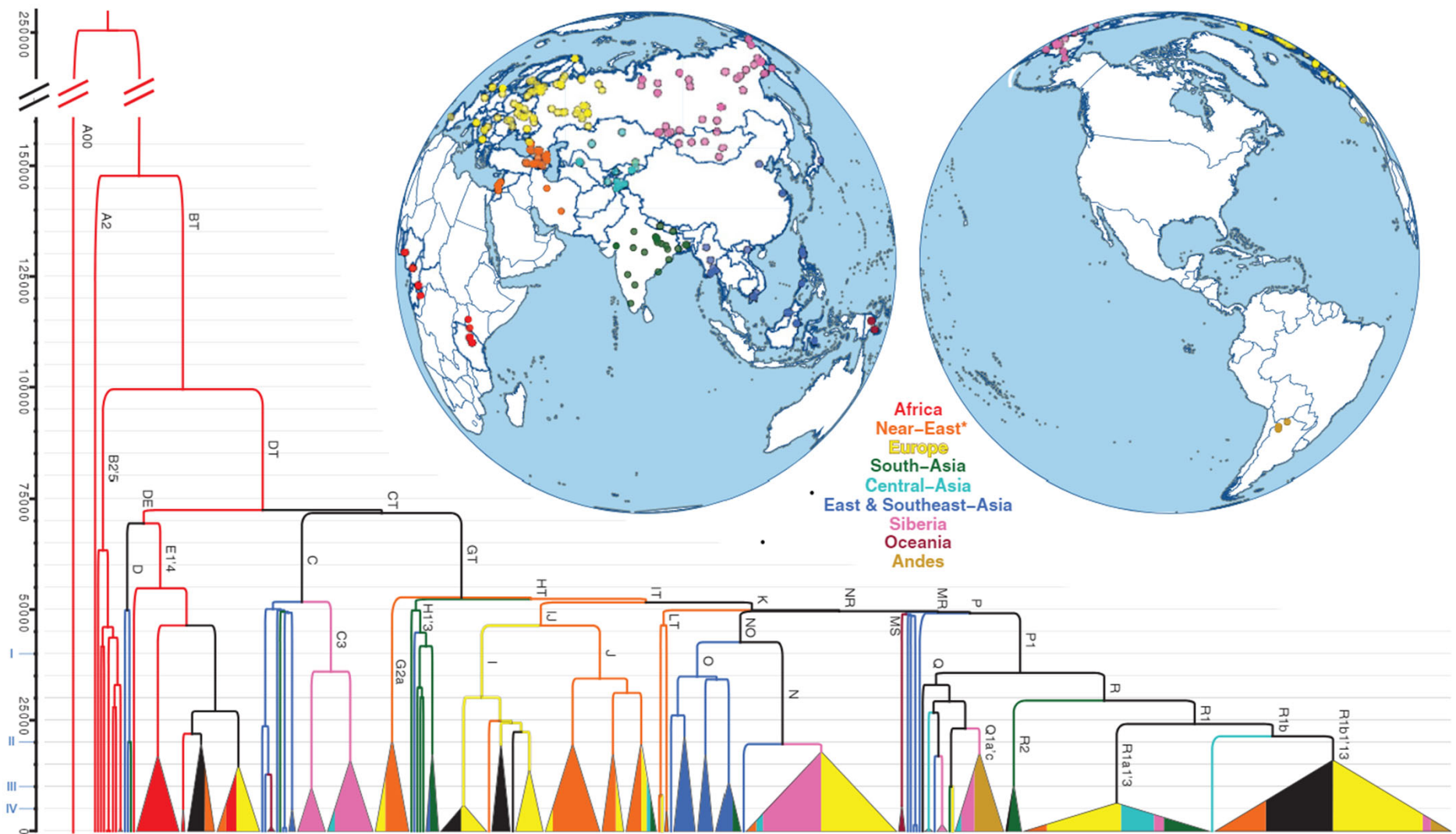
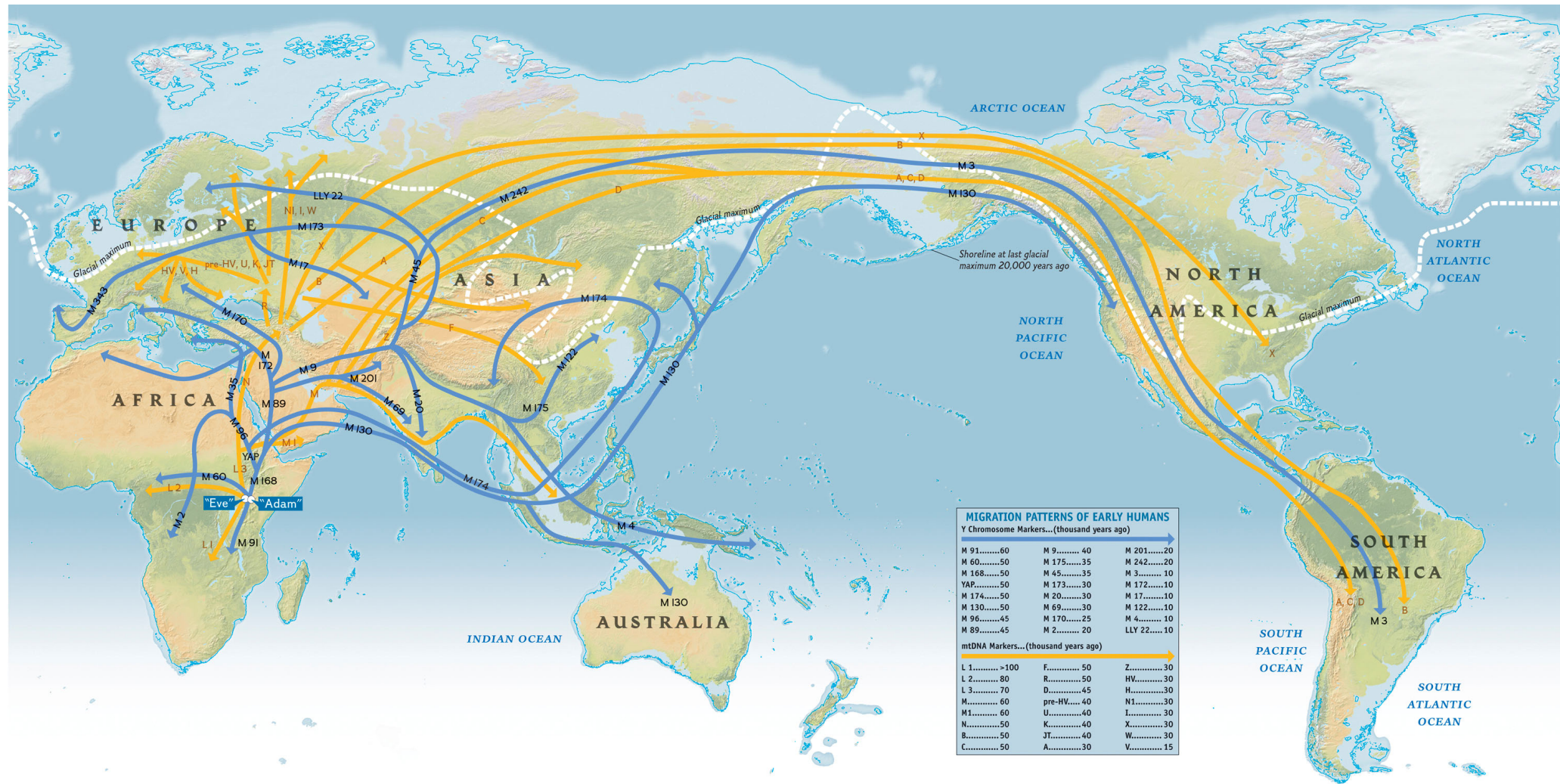


Figure 1. The phylogenetic tree of 456 whole Y chromosome sequences and a map of sampling locations. The phylogenetic tree is reconstructed using BEAST. Clades coalescing within 10% of the overall depth of the tree have been collapsed. Only main haplogroup labels are shown (details are provided in Supplemental Information 6). Colors indicate geographic origin of samples (Supplemental Table S1), and fill proportions of the collapsed clades represent the proportion of samples from a given region. Asterisk (*) marks the inclusion of samples from Caucasus area. Personal Genomes Project (<http://www.personalgenomes.org>) samples of unknown and mixed geographic/ethnic origin are shown in black. The proposed structure of Y chromosome haplogroup naming (Supplemental Table S5) is given in Roman numbers on the y-axis.

Karmin M, Saag L, Vicente M, Sayres MAW, Järve M, Talas UG, et al. *Genome Res.* 2015;25: 459–466.

“Adam” and “Eve” both lived in Africa



- “Mitochondrial Eve” lived in Africa between 100,000 and 240,000 years ago
- “Y-chromosome Adam” also lived in Africa between 120,000 and 160,000 years ago
- Poznik GD, et al (Carlos Bustamante lab in Stanford), *Science* **341**: 562 (August 2013).

Mitochondrial Eve (maternally transmitted ancestry)
Y-chromosome Adam (paternally transmitted ancestry)
lived ~200,000 years ago.

**When lived the latest common ancestor
shared by all of us based on nuclear DNA?**

- A. 1 million years ago
- B. 200,000 years ago
- C. 3400 years ago
- D. 1320 years ago
- E. Yesterday, I really have no clue

Get your i-clickers

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Get your i-clickers

Last common ancestor in nuclear (non Y-chr) DNA is another matter

- Unlike Mito or Y-chromosome, **nuclear DNA gets mixed with every generation**
 - Each of us gets 1/2 of nuclear DNA from the father and 1/2 from the mother
 - Each of us has 2 parents, 4 grandparents, 8 great-grand parents ...
- If one assumes:
 - Well-mixed marriages (not true: mostly local marriages)
 - Constant size population (not true: much smaller in the past)
 - In 33 generations the number of ancestors:
 $2^{33} = 8 \text{ billion}$ = 8 billion people living today
- Every pair of us living today should have at least one shared ancestor who lived
 - 33 generations * 20 years/generation = 660 years ago ~1360 AD
- **Assuming $T_{MRCA} = 2 T_{\text{average pairwise ancestor}} = 2 \cdot 660 \text{ years ago} = 700 \text{ AD}$**

Corrected for (mostly) local marriages and rare migrations

562

NATURE | VOL 431 | 30 SEPTEMBER 2004 |

Modelling the recent common ancestry of all living humans

Douglas L. T. Rohde¹, Steve Olson² & Joseph T. Chang³

¹Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

²7609 Seaboard Road, Bethesda, Maryland 20817, USA

³Department of Statistics, Yale University, New Haven, Connecticut 06520, USA

With 5% of individuals migrating out of their home town, 0.05% migrating out of their home country, and 95% of port users born in the country from which the port emanates, the simulations produce a mean **MRCA date of 1,415 BC** and a mean **IA date of 5,353 BC**.

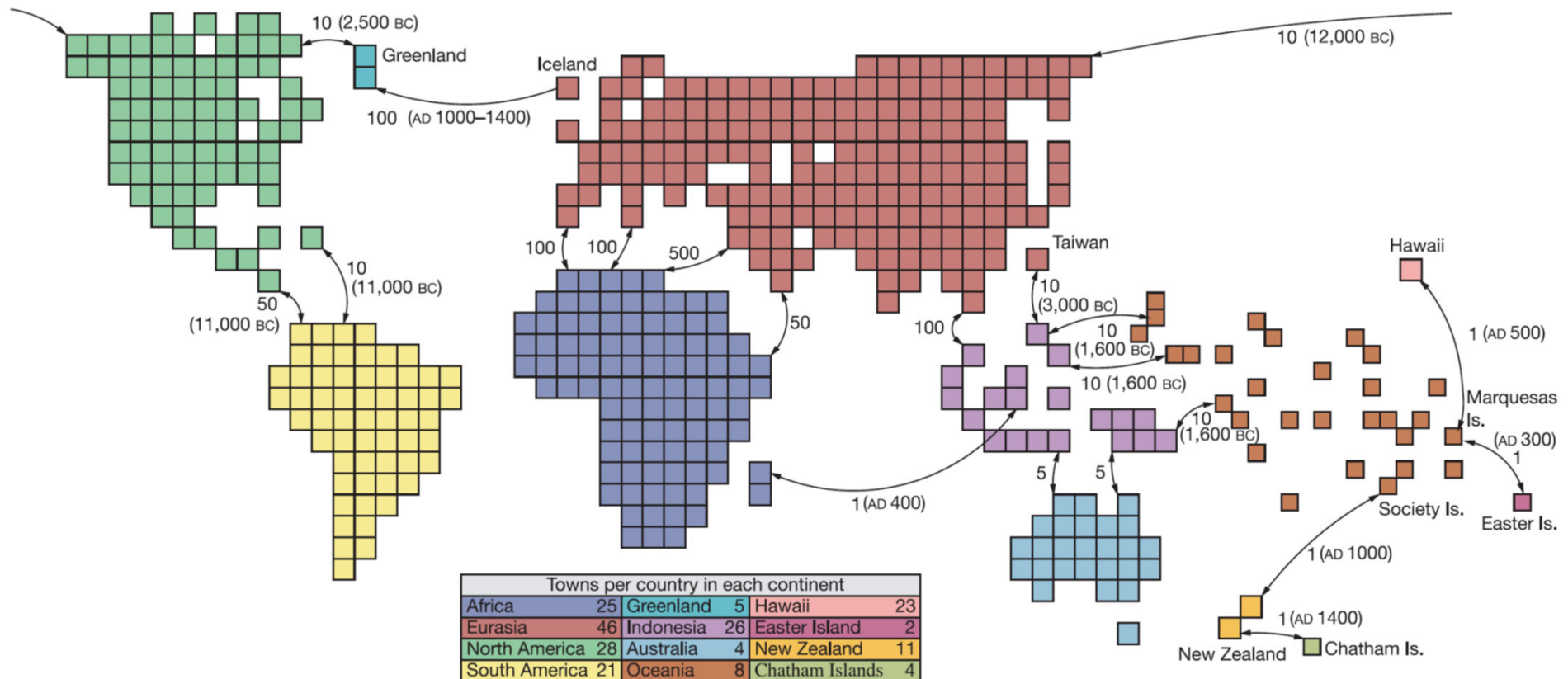
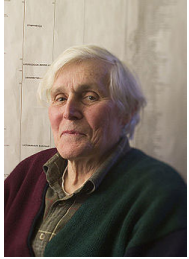


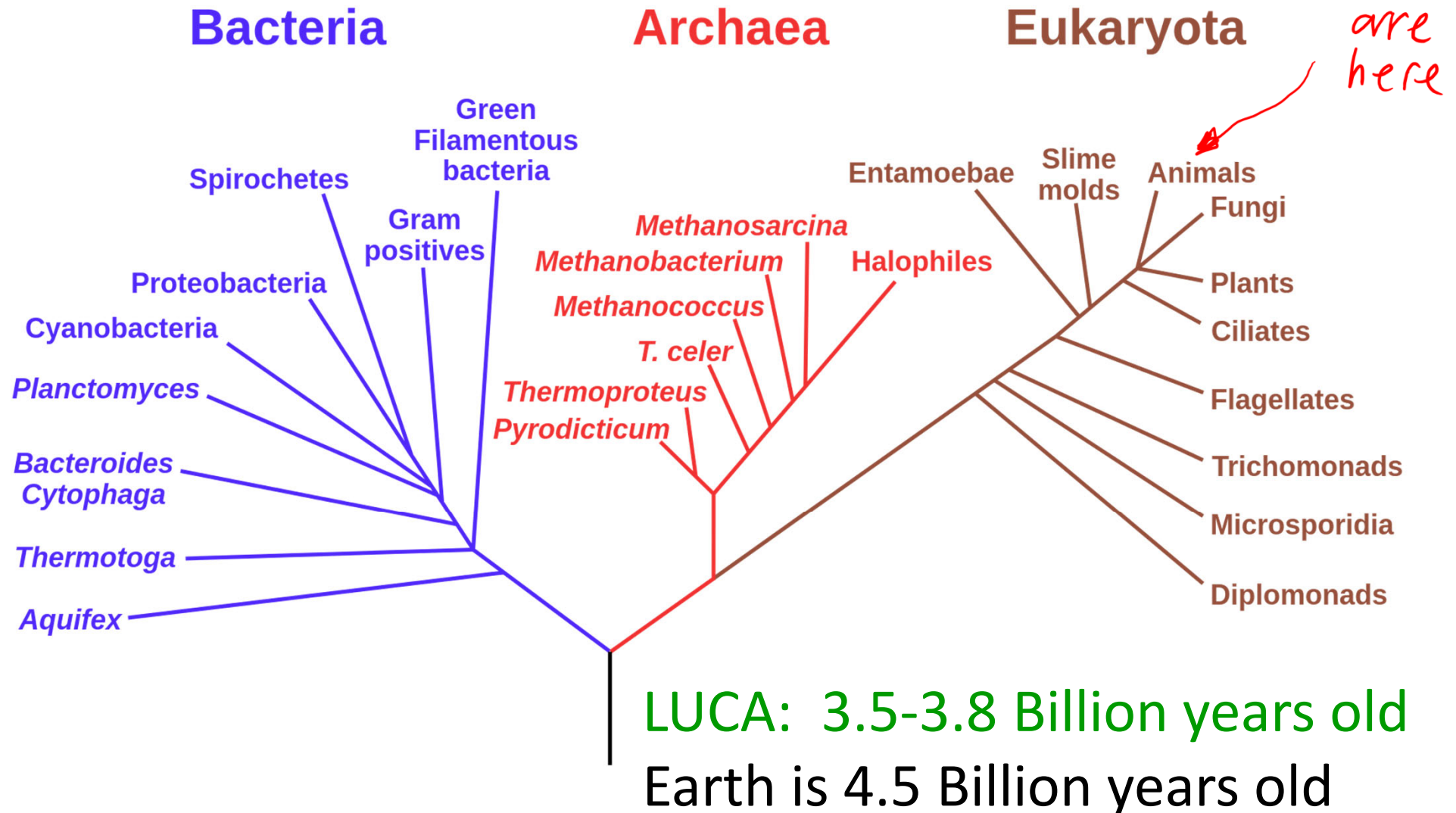
Figure 2 Geography and migration routes of the simulated model. Arrows denote ports and the adjacent numbers are their steady migration rates, in individuals per generation. If

given, the date in parentheses indicates when the port opens. Upon opening, there is usually a first-wave migration burst at a higher rate, lasting one generation.

Last Universal Common Ancestor (LUCA)



Archaea were discovered here at **UIUC** in 1977
by **Carl R. Woese (1928-2012)** and **George E. Fox**



Credit: XKCD
comics

WHY ARE THERE SLAVES IN THE BIBLE

WHY DO TWINS HAVE DIFFERENT FINGERPRINTS
WHY ARE AMERICANS AFRAID OF DRAGONS

QUESTIONS

FOUND IN GOOGLE AUTOCOMPLETE

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 AREN'T MY
ARMS GROWING



WHY ARE THERE WEEKS
WHY DO I FEEL DIZZY

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 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 MALE AND FEMALE BIKES

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 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 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

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 KINGDOMS 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 ARE THERE SO MANY SPIDERS IN MY ROOM

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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 ARE THERE TINY SPIDERS IN MY HOUSE

WHY DO SPIDERS COME INSIDE

WHY ARE THERE HUGE SPIDERS IN MY HOUSE

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