Guest lectures

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- Office hours?
- I teach this course in even-numbered years
- I will give 3 lectures on coalescent theory and 1 on chromosome evolution

Coalescent theory

- Basic idea–backwards in time genetics
- Gray whale example
- Deriving the coalescent from the Wright-Fisher model
- Uses and extensions of the coalescent:
  - Population size (genetic drift)
  - Population growth/shrinkage
  - Gene flow (migration)
  - Divergence of populations
  - Recombination
  - Selection
- Red drum example

Basic idea of coalescent theory

- All term long you've looked at problems posed like this:
  - Given a starting situation–
  - And a given set of evolutionary forces–
  - What are the likely outcomes?

- This is a "forward time" approach to population genetics

Basic idea of coalescent theory

- Suppose that I have data from an existing population and want to know how it got that way
- I don't know the starting situation
- Running forward from hypothetical starting situations may or may not ever give me my current data
- I would be better off with a "backward time" approach that started from what I know (current data)

Basic idea of coalescent theory

- Consider the ancestry of my current gene copies backward in time:
  - Which ones are more or less closely related?
  - How big are the chunks that have the same common ancestor?
  - How long ago are their common ancestors?

- The answers to these questions contain a surprising amount of information about past evolutionary forces:
  - Population size (drift)
  - Gene flow
  - Selection
  - Recombination

What was the long-term population size of gray whales?

Alter, Rynes and Palumbi (2007) DNA evidence for historic population size and past ecosystem impacts of gray whales. PNAS 104: 15162-15167.

What was the long-term population size of gray whales?

- How many gray whales pre-whaling?
- Whaling ship records not conclusive
- Recent slowing of the observed growth rate may suggest recovery
- Molecular data an alternative source of information

What was the long-term population size of gray whales?

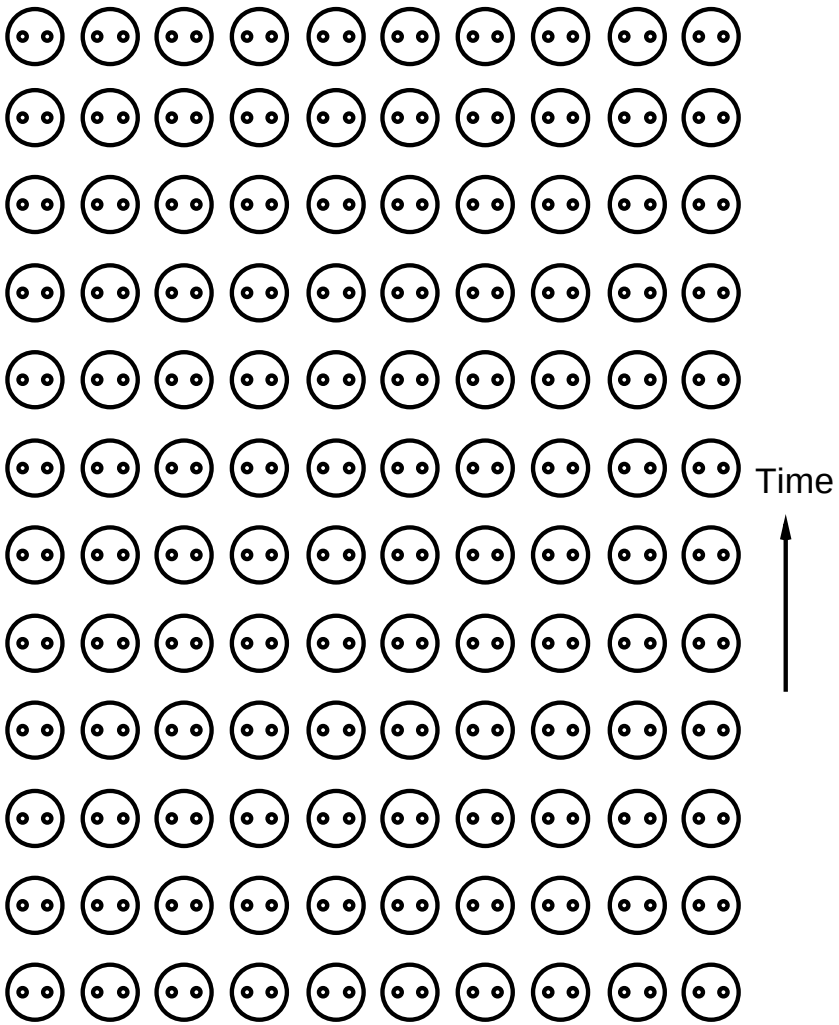
- 10 loci:
  - 7 autosomal
  - 2 X-linked
  - 1 mtDNA
- Complex mutational model with rate variation among loci
- Complex population model with subdivision and copy number
- Complex demographic model relating N_{census} to N_e

What was the long-term population size of gray whales?

	Locus	n	Estimated N
Aut	ACTA	72	162,625
	BTN	72	76,369
	CP	76	77,319
	ESO	72	272,320
	FGG	72	180,730
	LACTAL	72	44,410
	WT1	80	51,972
X	G6PD	30	2,769
	PLP	52	92,655
mtDNA	Cytb	42	107,778
	All data		96,400 (78,500-117,700)
	Current census		18,000-29,000
	Previous models		19,480-35,430

What was the long-term population size of gray whales?

- Important conservation implications
- Effect on ecosystem significant:
  - Resuspension of up to 700 million cubic meters sediment
  - (12 Yukon Rivers worth)
  - Food for 1 million sea birds
- If accepted, result suggests halving gray whale kill rate
- Broadly similar results for minke, humpback, and fin whales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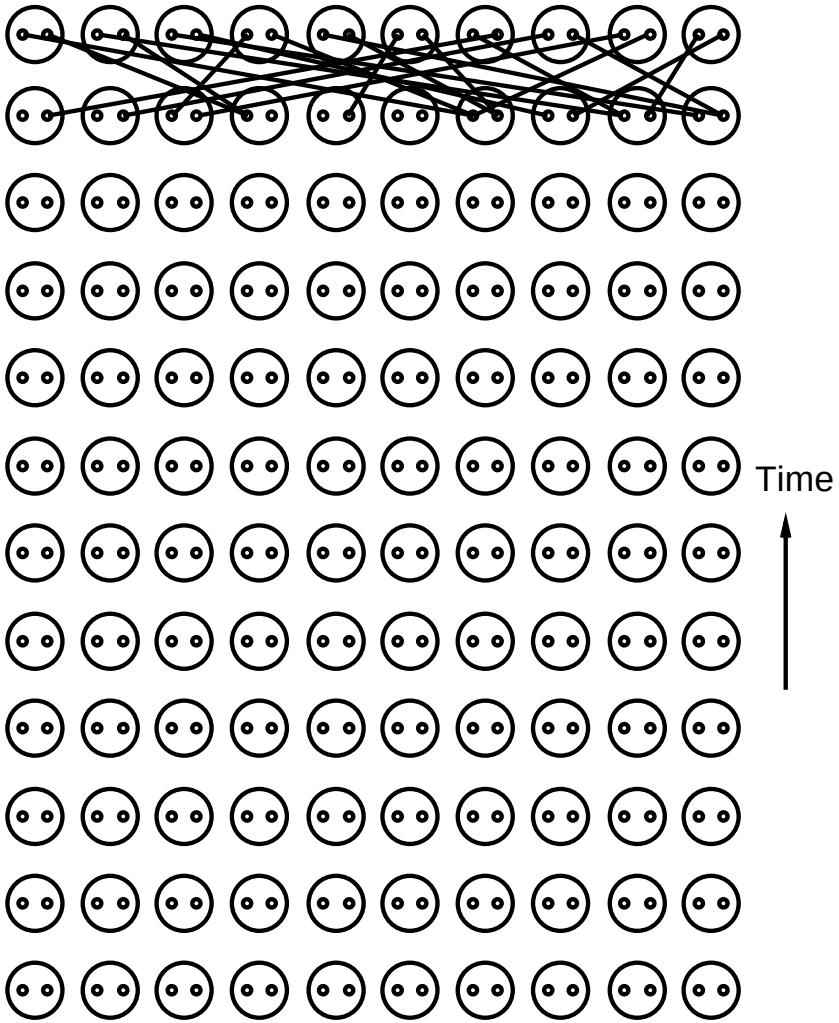
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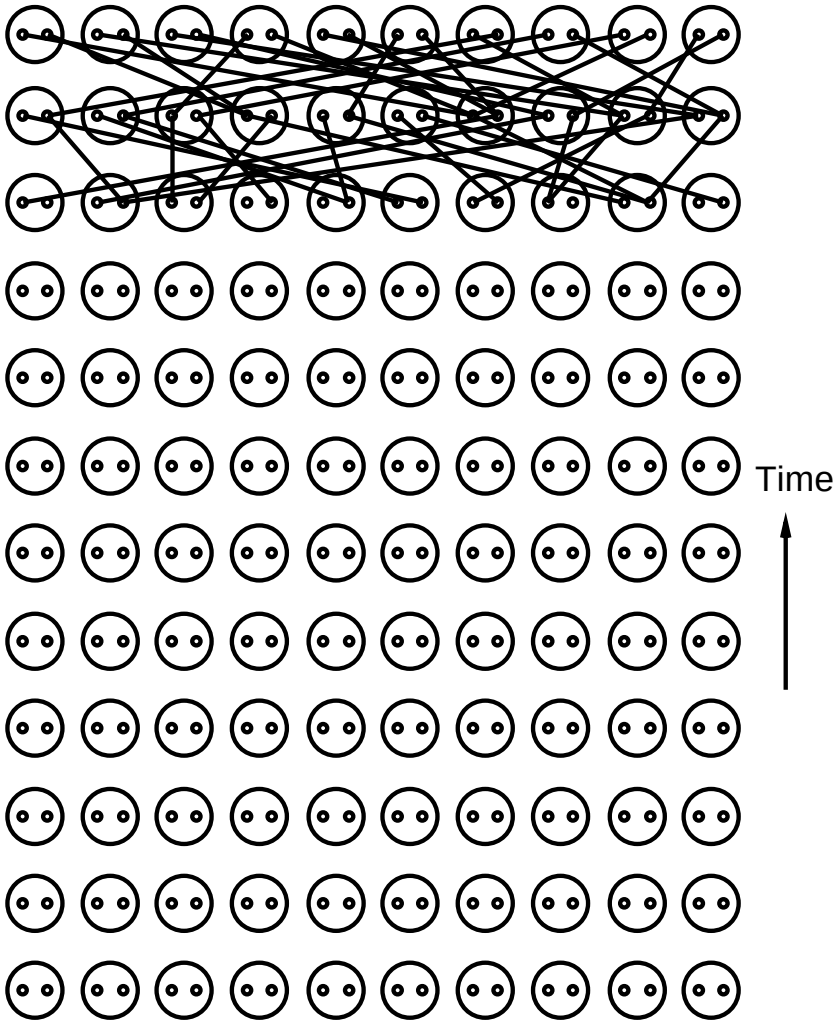
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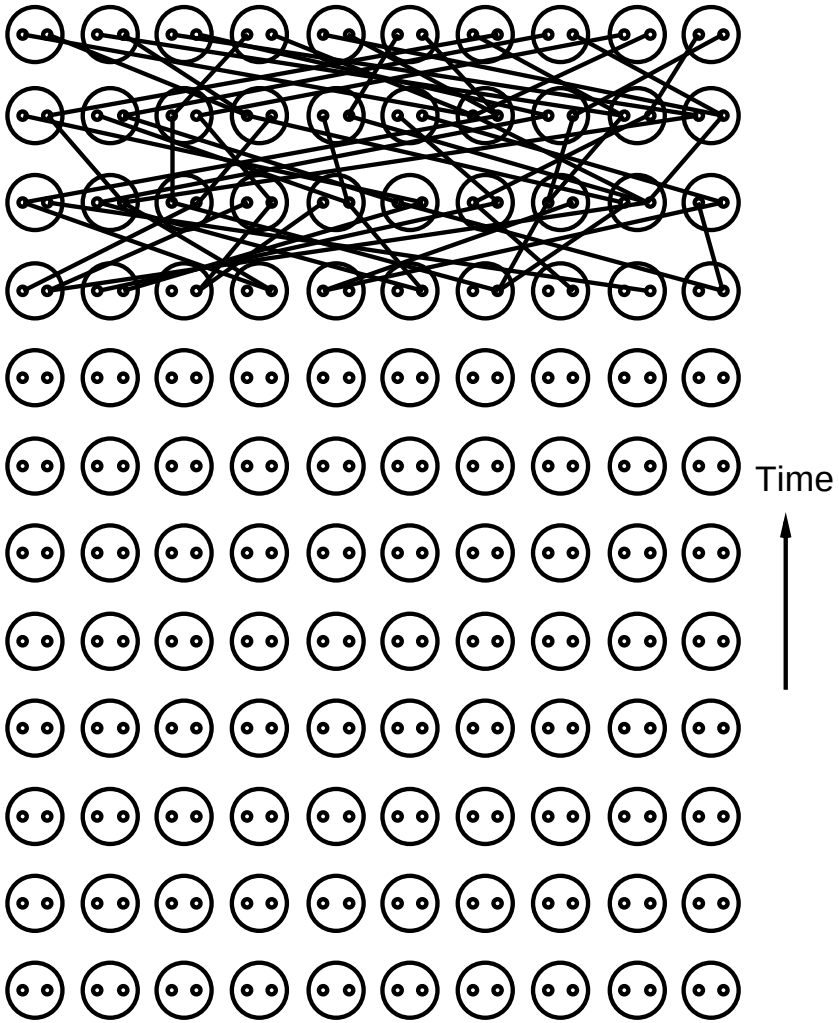
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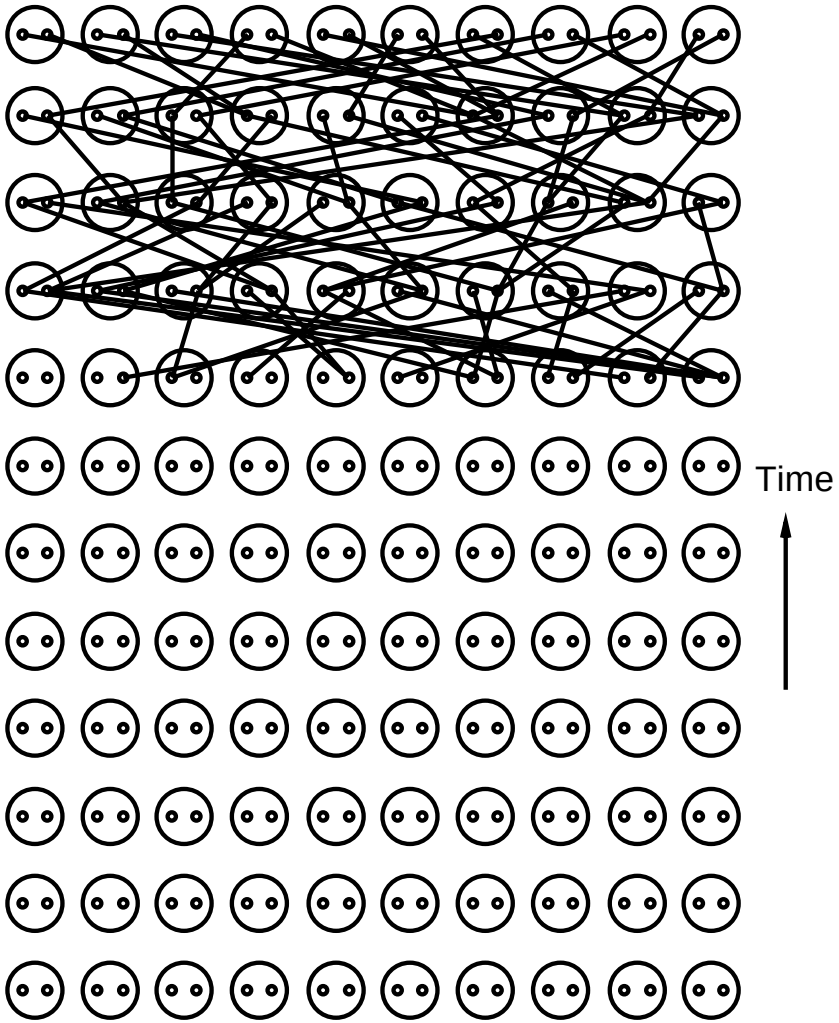
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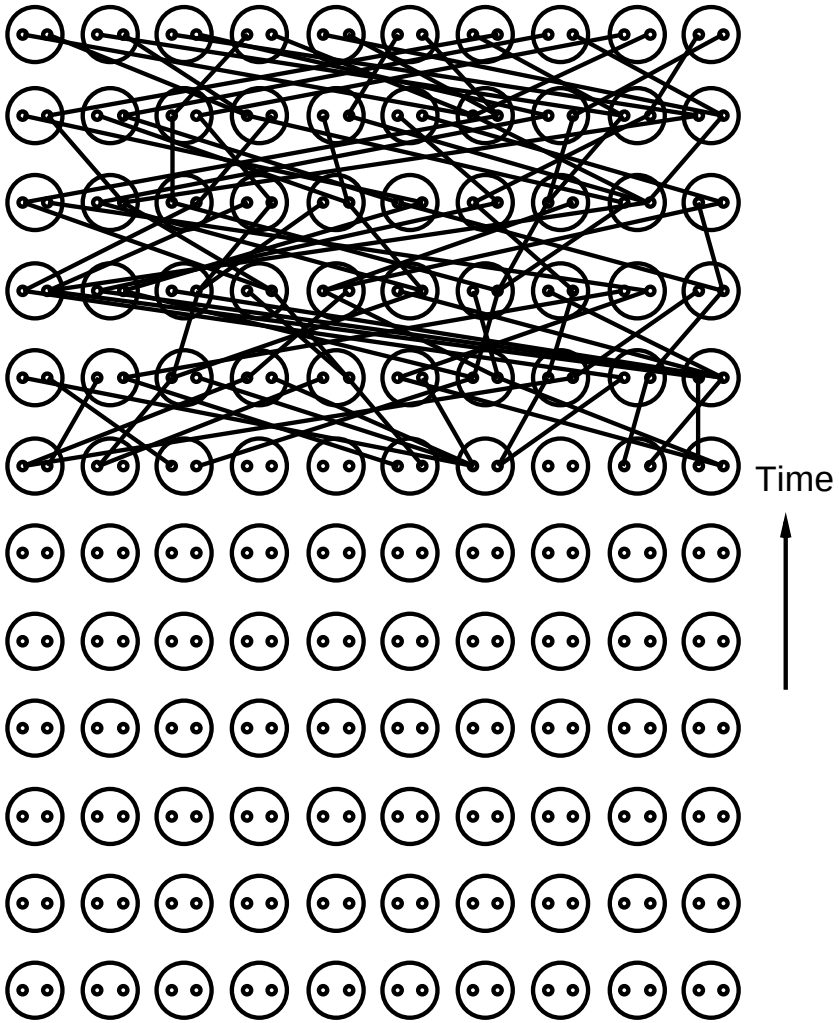
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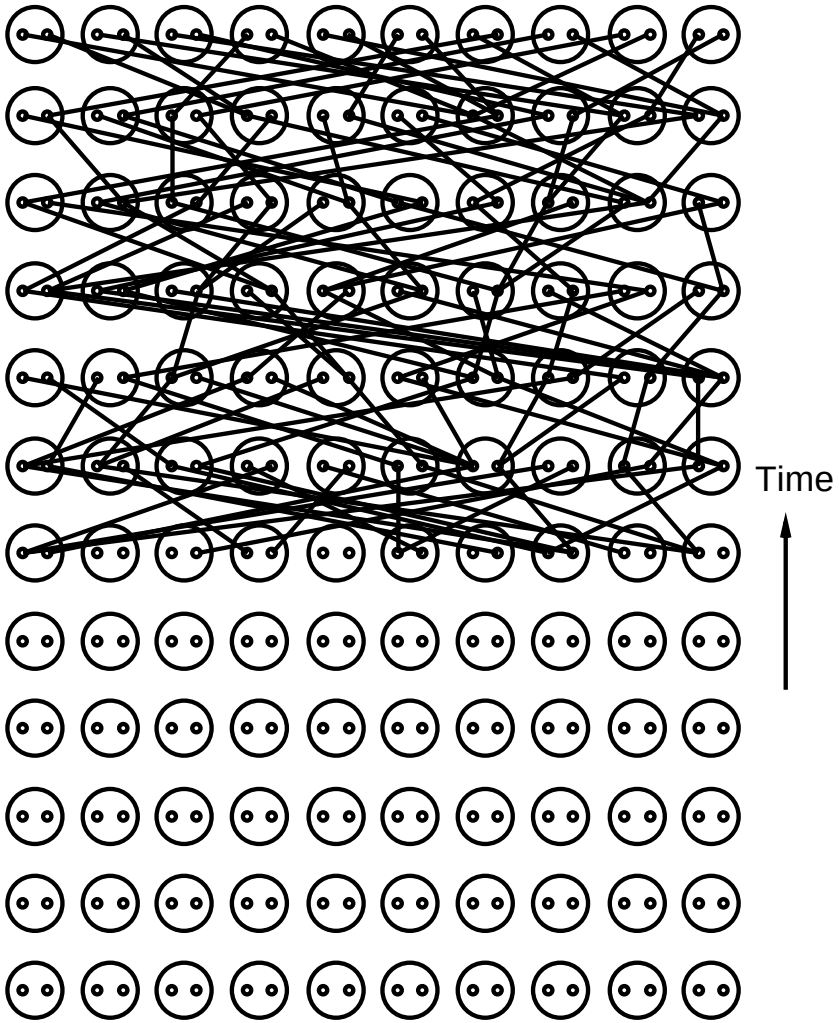
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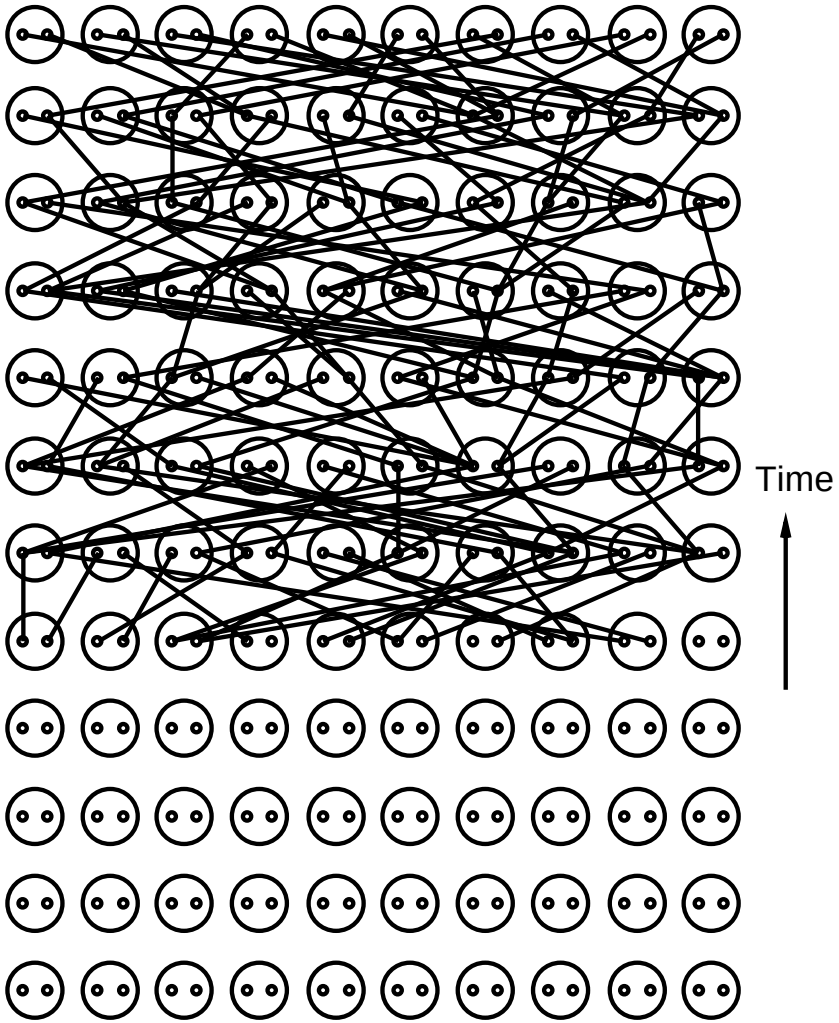
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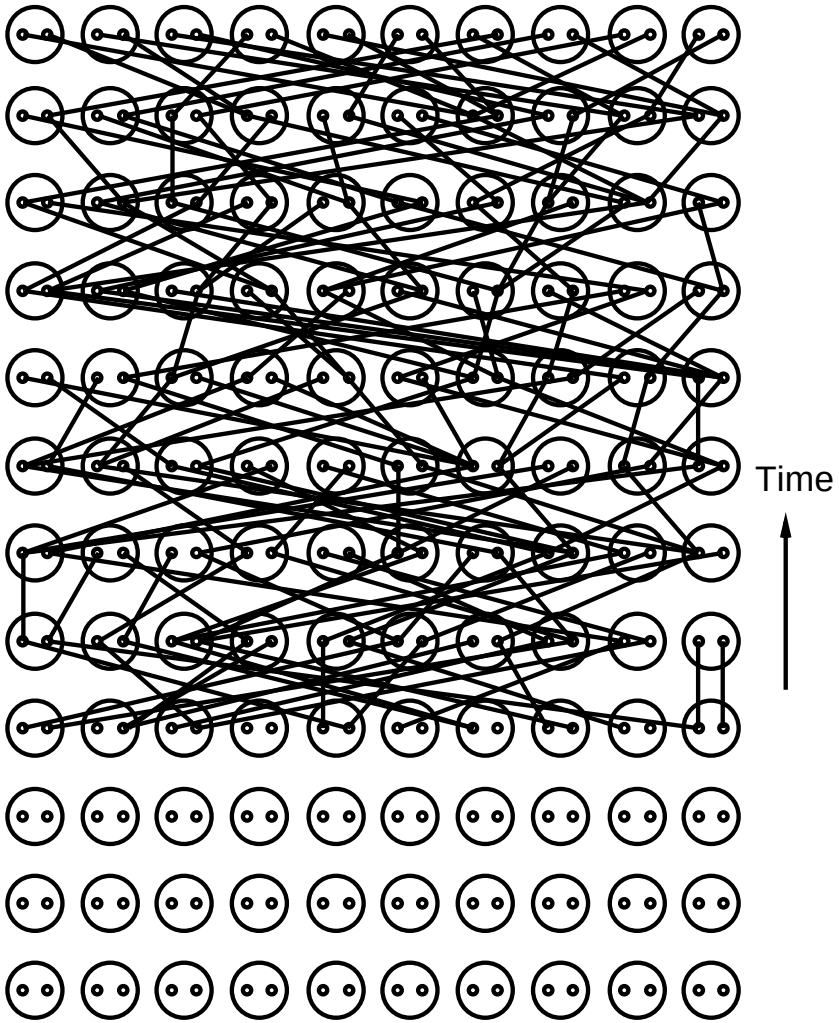
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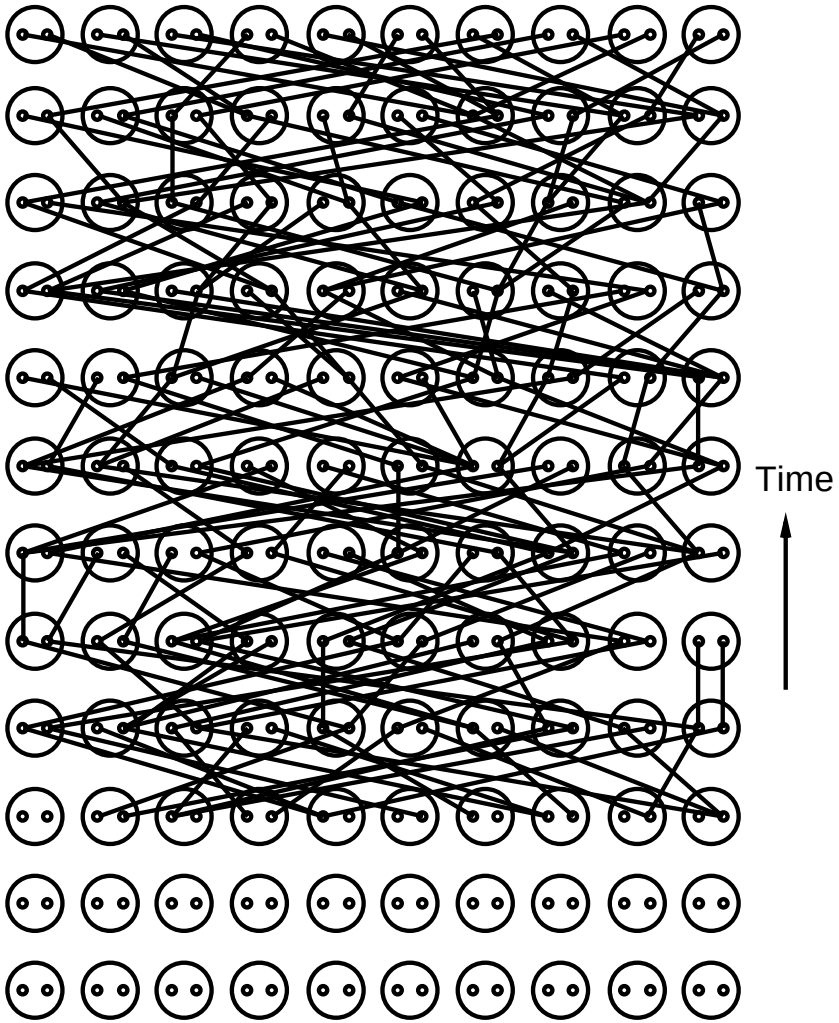
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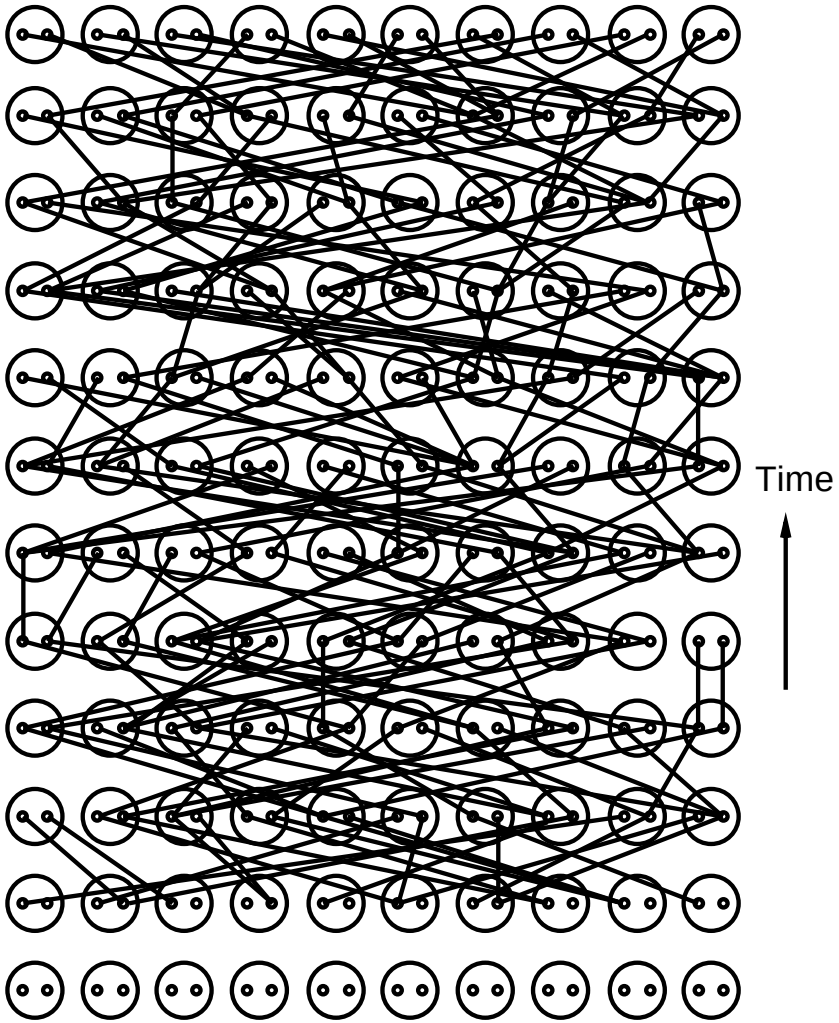
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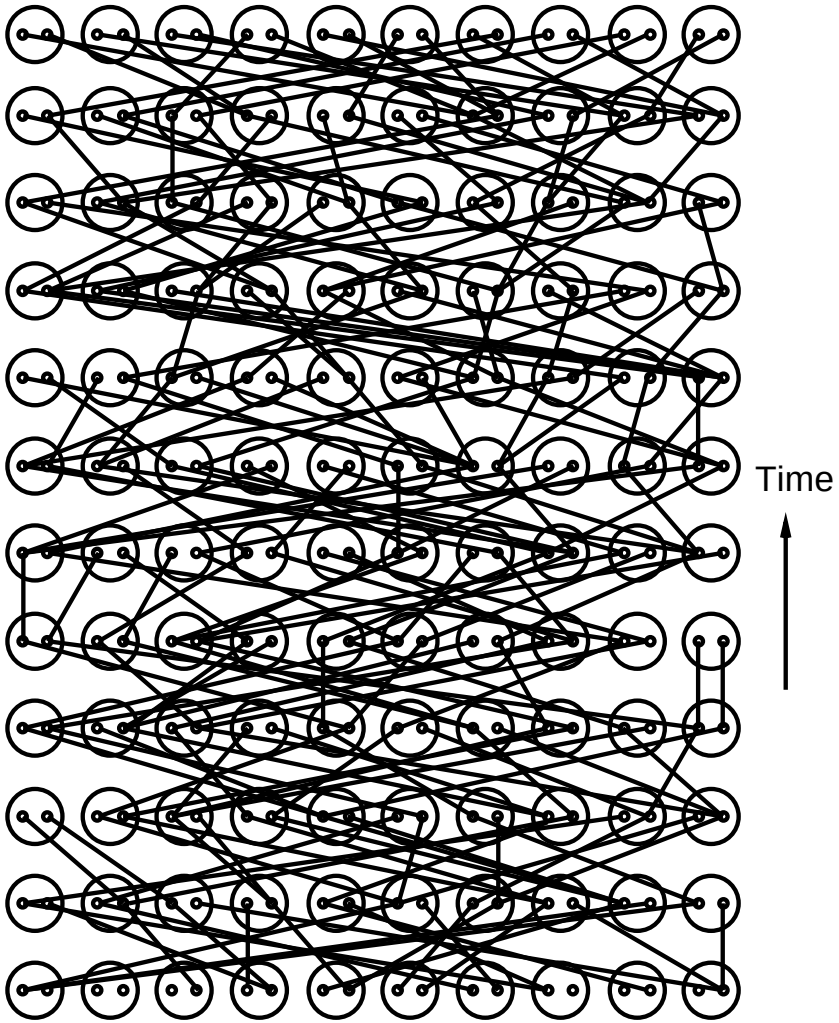
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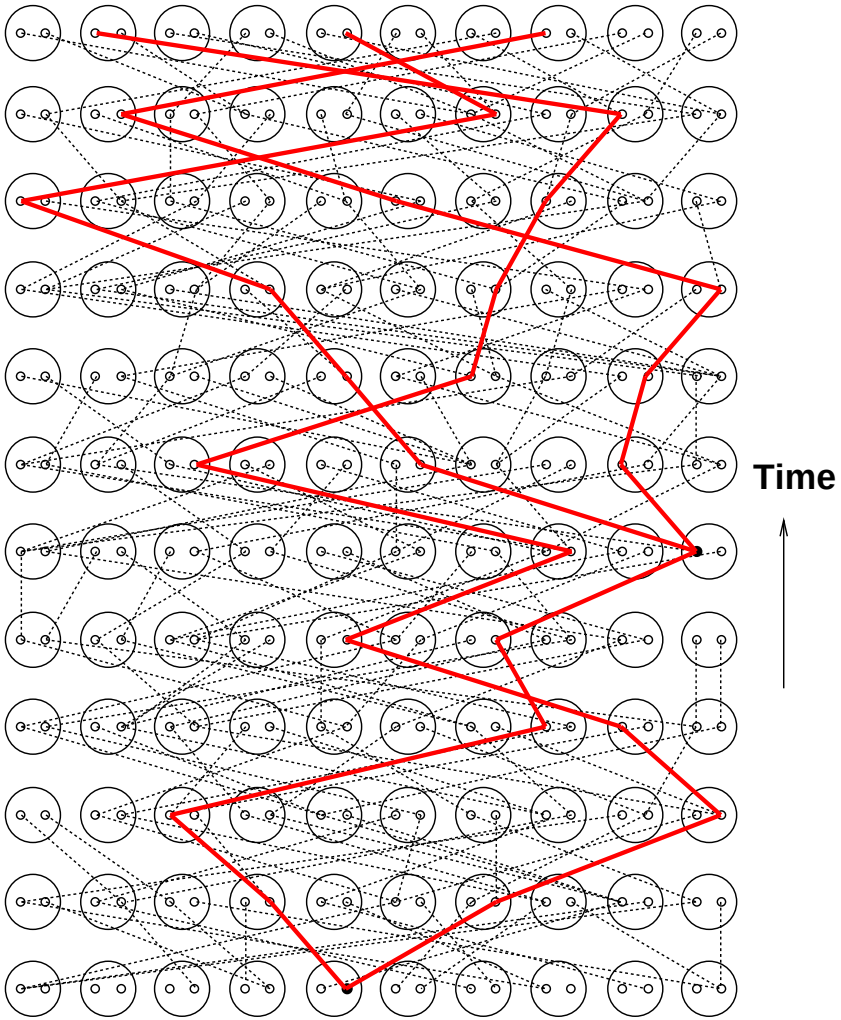
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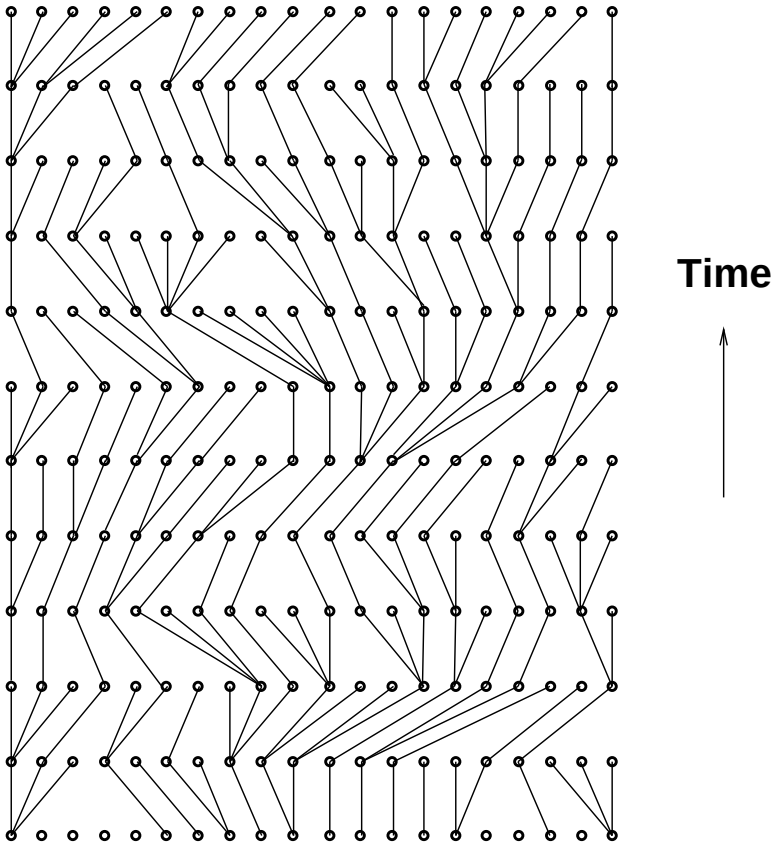
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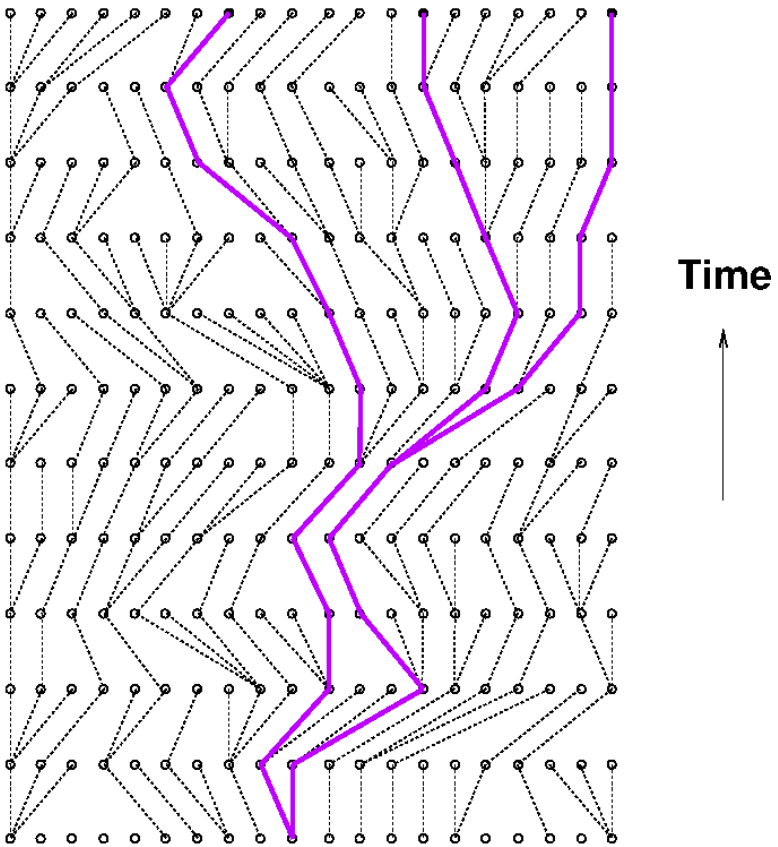
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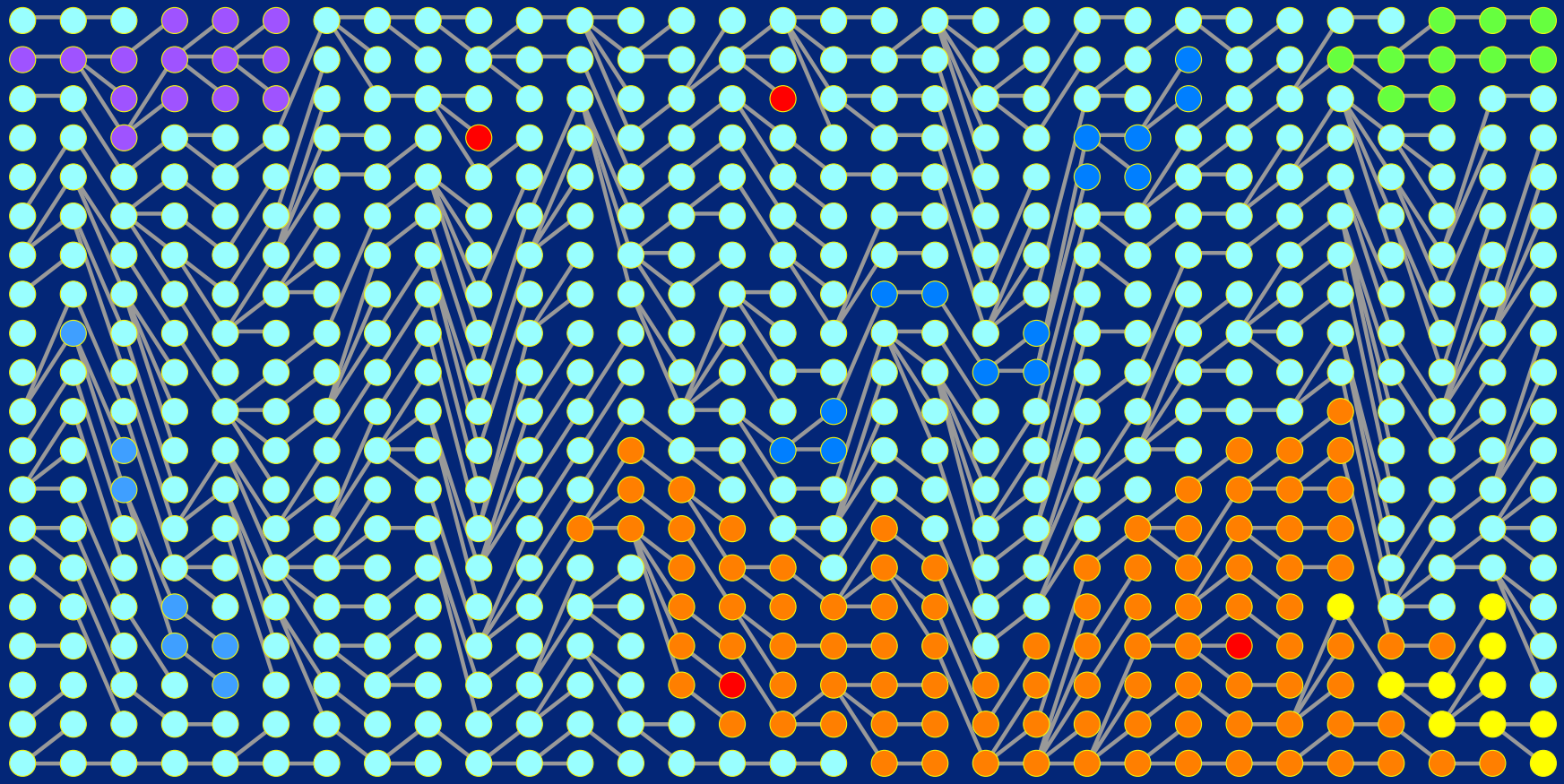
Wright-Fisher population model



Wright-Fisher population model

- Population size N is constant through time.
- Each individual gets replaced every generation.
- Next generation is drawn randomly from a large gamete pool.
- Only genetic drift affects the allele frequencies.

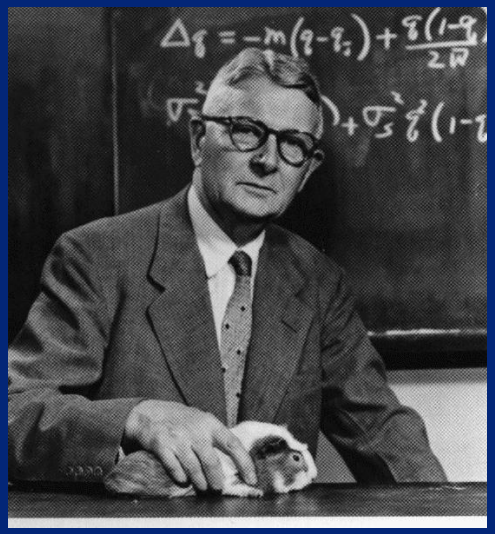
Other population models

- Other population models can often be equated to Wright-Fisher
- The N parameter becomes the effective population size N_e
- For example, cyclic populations have an N_e that is the harmonic mean of the various sizes
- Honeybees have an N_e that is the number of breeding individuals, not the vastly larger number of total bees

From Wright-Fisher to the coalescent

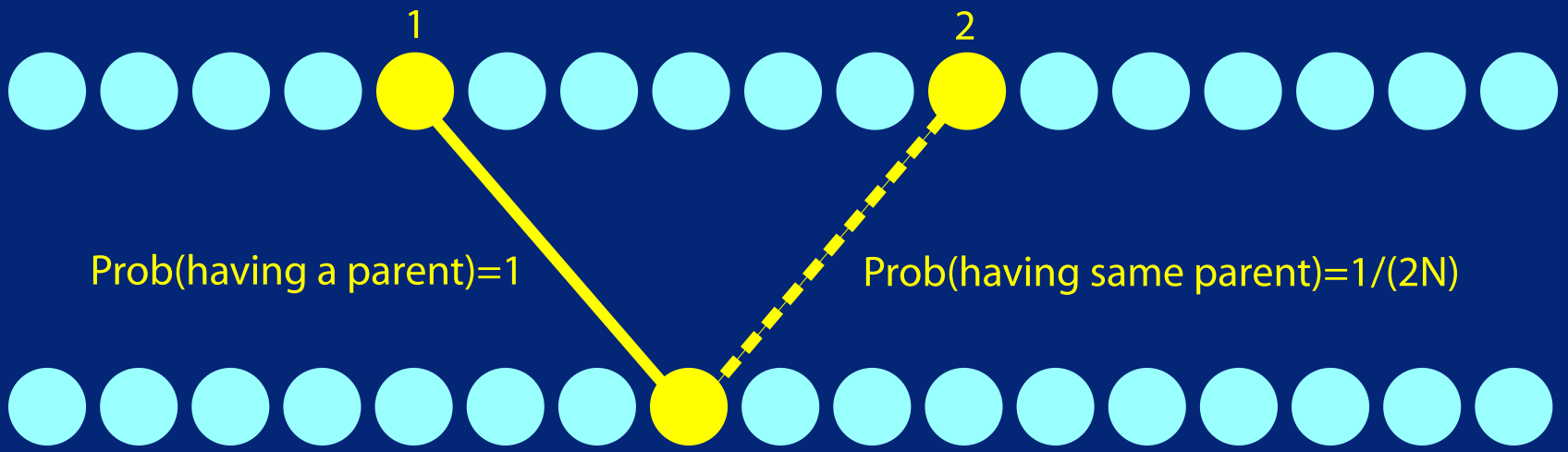
- Key to coalescent theory: think BACKWARDS
- Each individual "chooses" its parent randomly from the previous generation
- When two lines come from the same parent, they "coalesce"
- As you look further back, eventually all lines must coalesce
- If there is no recombination, this is a within-population tree (genealogy)

The Coalescent

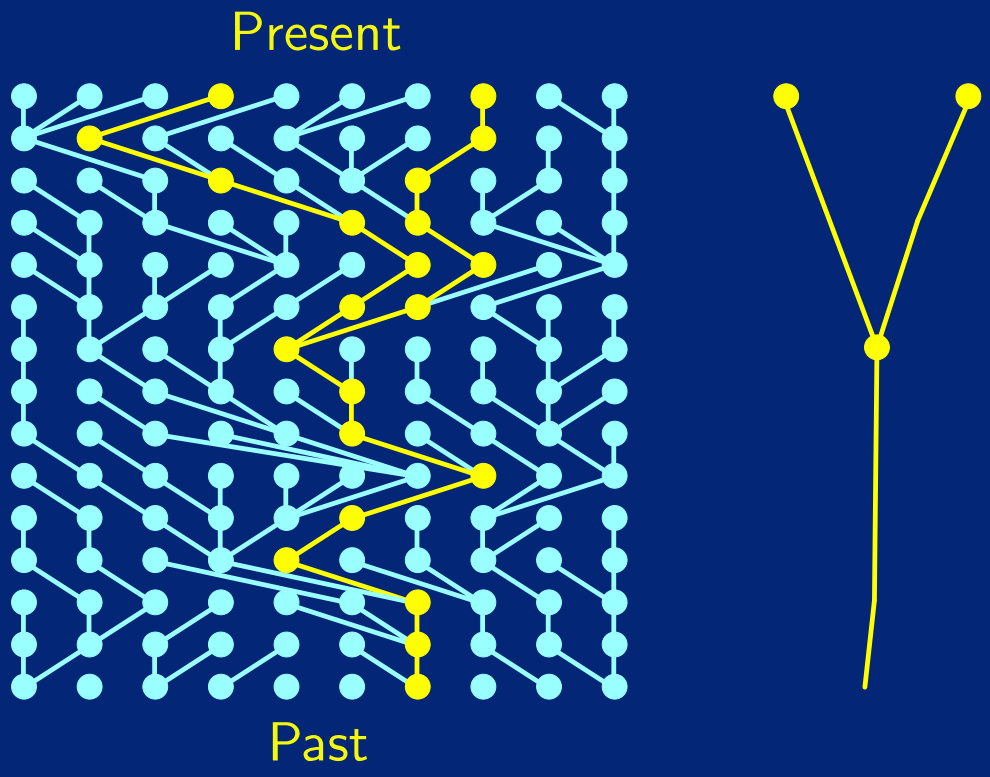


Sewall Wright showed that the probability that 2 gene copies come from the same gene copy in the preceding generation is

$$\text{Prob (two genes share a parent)} = \frac{1}{2N}$$



The Coalescent



In every generation, there is a chance of $1/2N$ to coalesce. Following the sampled lineages through generations backwards in time we realize that it follows a geometric distribution with

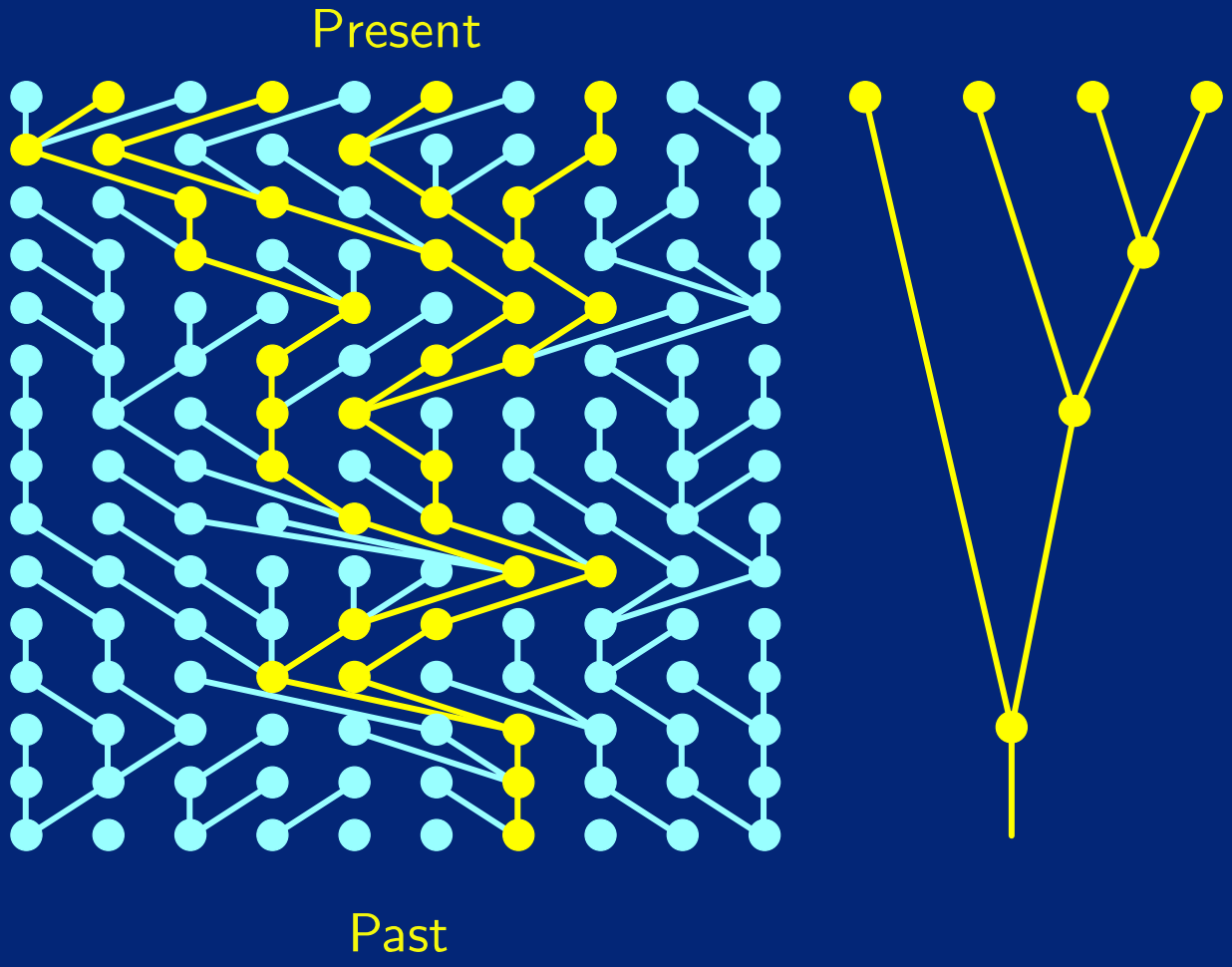
$\mathbb{E}(u) = 2N$ [the expectation of the time of coalescence u of **two** tips is $2N$]

The Coalescent

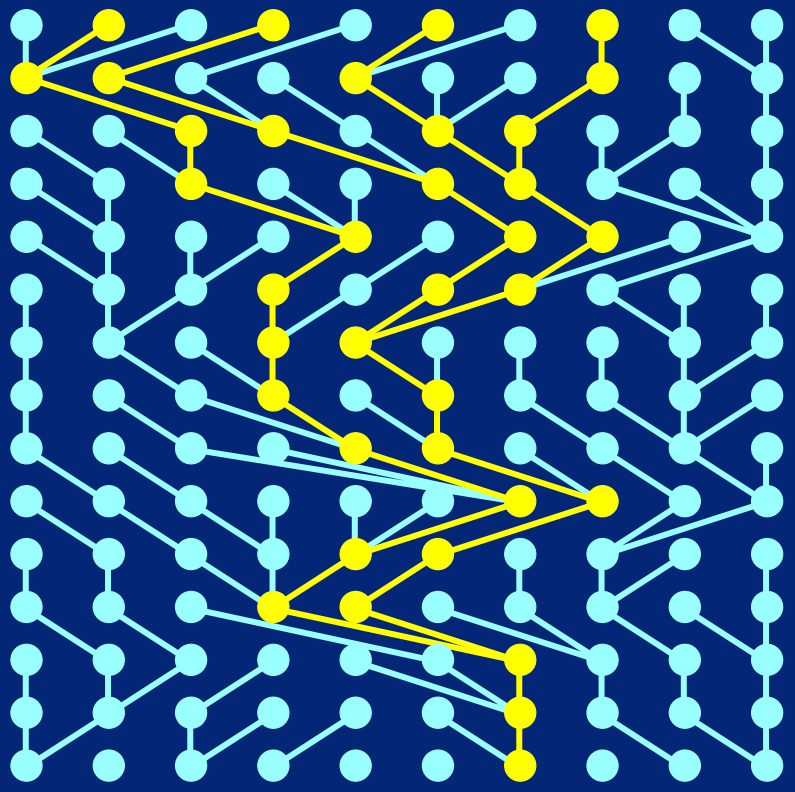
JFC Kingman generalized this for k gene copies.

$$\text{Prob } (k \text{ copies are reduced to } k-1 \text{ copies}) = \frac{k(k-1)}{4N}$$

Kingman's n-coalescent

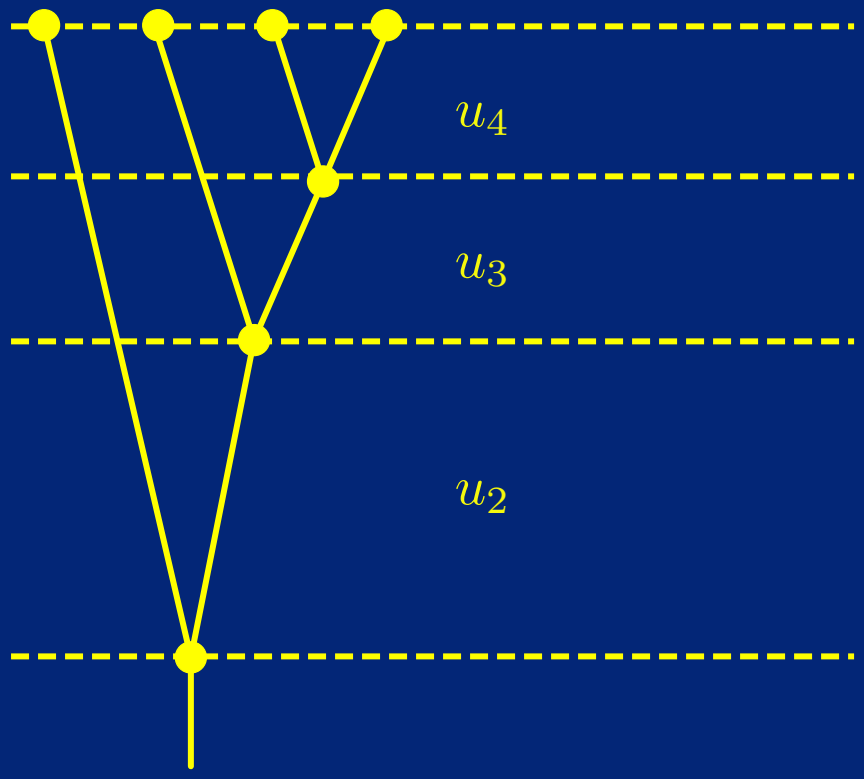


Kingman's n-coalescent



The expectation for the time interval u_k is

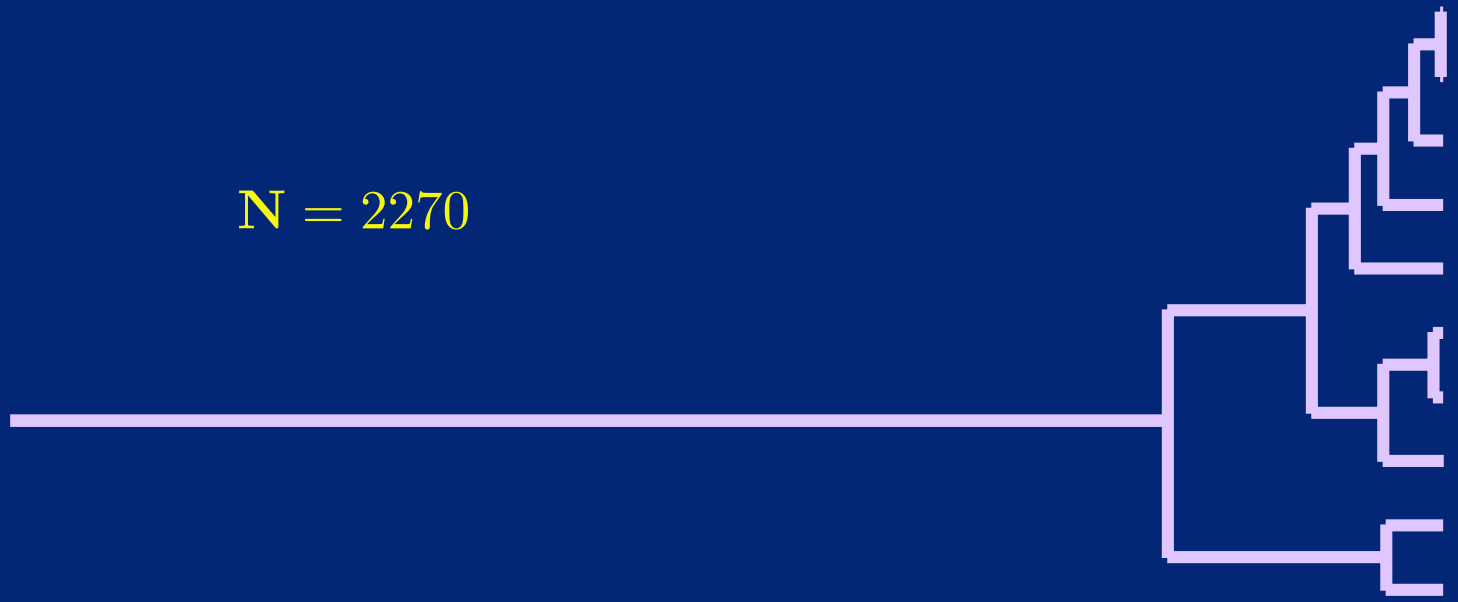
$$\mathbb{E}(u_k) = \frac{4N}{k(k-1)}$$



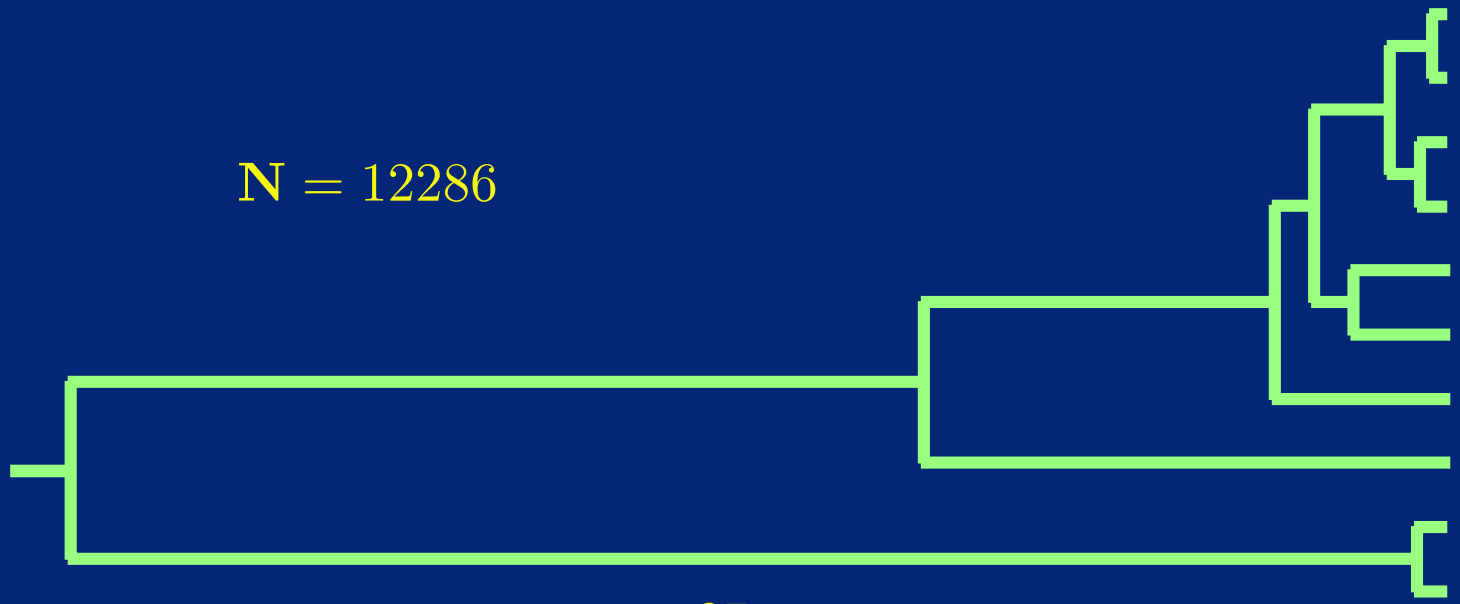
$$\mathrm{p(G|N)} = \prod_{\mathrm{i}} \exp(-\mathrm{u_i}\frac{\mathrm{k(k-1)}}{\mathrm{4N}})\frac{1}{\mathrm{2N}}$$

Coalescence time depends on population size

$\mathbf{N} = 2270$



$\mathbf{N} = 12286$



This would be great if....

- If we knew the tree, including its times, we would have a powerful estimator of population size
- Unfortunately this is difficult to infer
- Within-population variation is usually too low for really accurate phylogeny estimation
- We also have a problem with observing time directly

The variable Θ

- Goal: Estimate $2N_e$, the effective number of gene copies
- Problem: to estimate this, we need to know coalescence times
- We do not observe times (except in viruses and fossil DNA), only numbers of differences (mutations)

The variable Θ

- The number of differences is proportional to the product of mutation rate μ and time
- We can only estimate the compound parameter $4N_e\mu$ also called Θ
- One factor of 2 comes from each individual having two gene copies (so the number of gene copies is $2N$)
- The other comes from mutations accumulating on both branches of the tree, so in 1 unit of time we accumulate 2 units of mutations

The variable Θ

- It is disappointing not to get N_e directly
- If we can measure μ experimentally we can convert Θ to N_e
- Even if we can't, Θ is interesting:
  - Comparing populations with similar mutation rate
  - Expected "carrying capacity" of genetic diversity

The coalescent without recombination: "Mitochondrial Eve"

- Cann, Stoneking and Wilson analyzed 149 human mtDNA sequences
- mtDNA (mitochondrial DNA) is:
  - Inherited only from the mother
  - Essentially haploid: the child receives only 1 genotype from the mother
  - Higher mutation rate than the nuclear genome
- Question: what is the population size of an mtDNA sequence relative to a nuclear sequence?

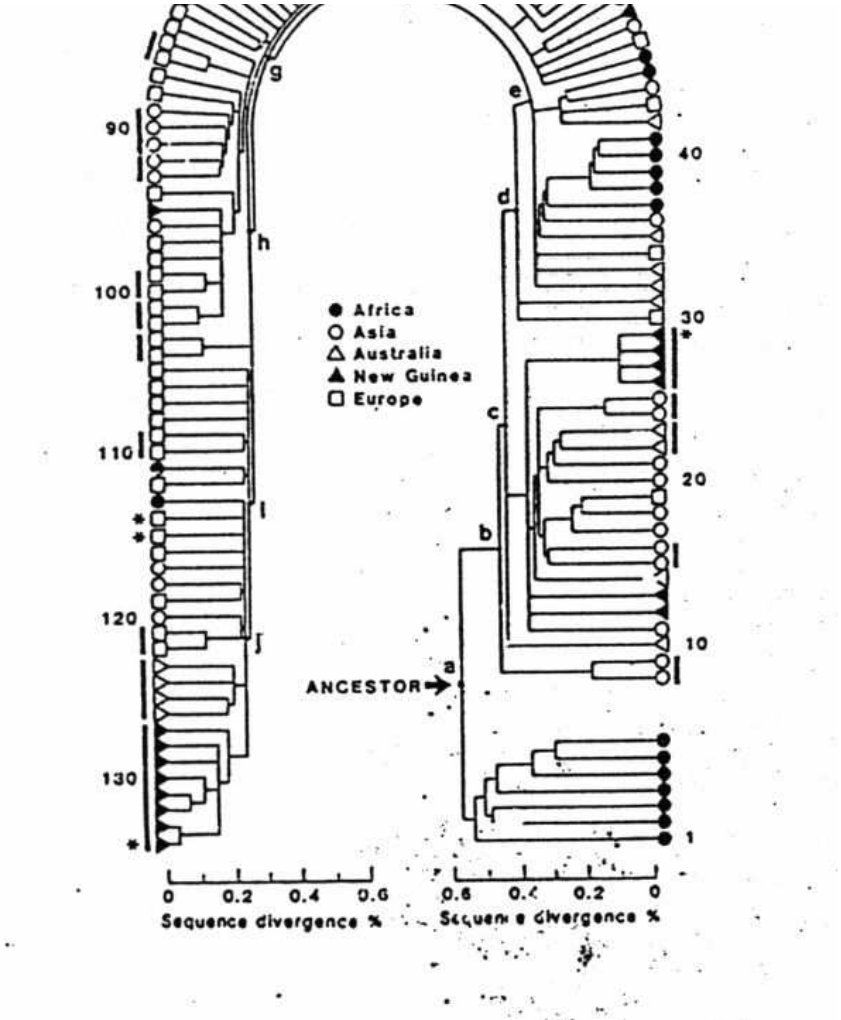


Fig. 3 *a*, Genealogical tree for 134 types of human mtDNA (133 rest
restriction sites used. The tree accounts for the site differences obs

The coalescent without recombination: "Mitochondrial Eve"

Three observations from Cann et al.

- All of their mtDNAs traced to a single ancestor
- That ancestor was dated to approx. 200,000 years ago
- She apparently lived in Africa

Let's take them in turn.

All human mtDNA has a common ancestor

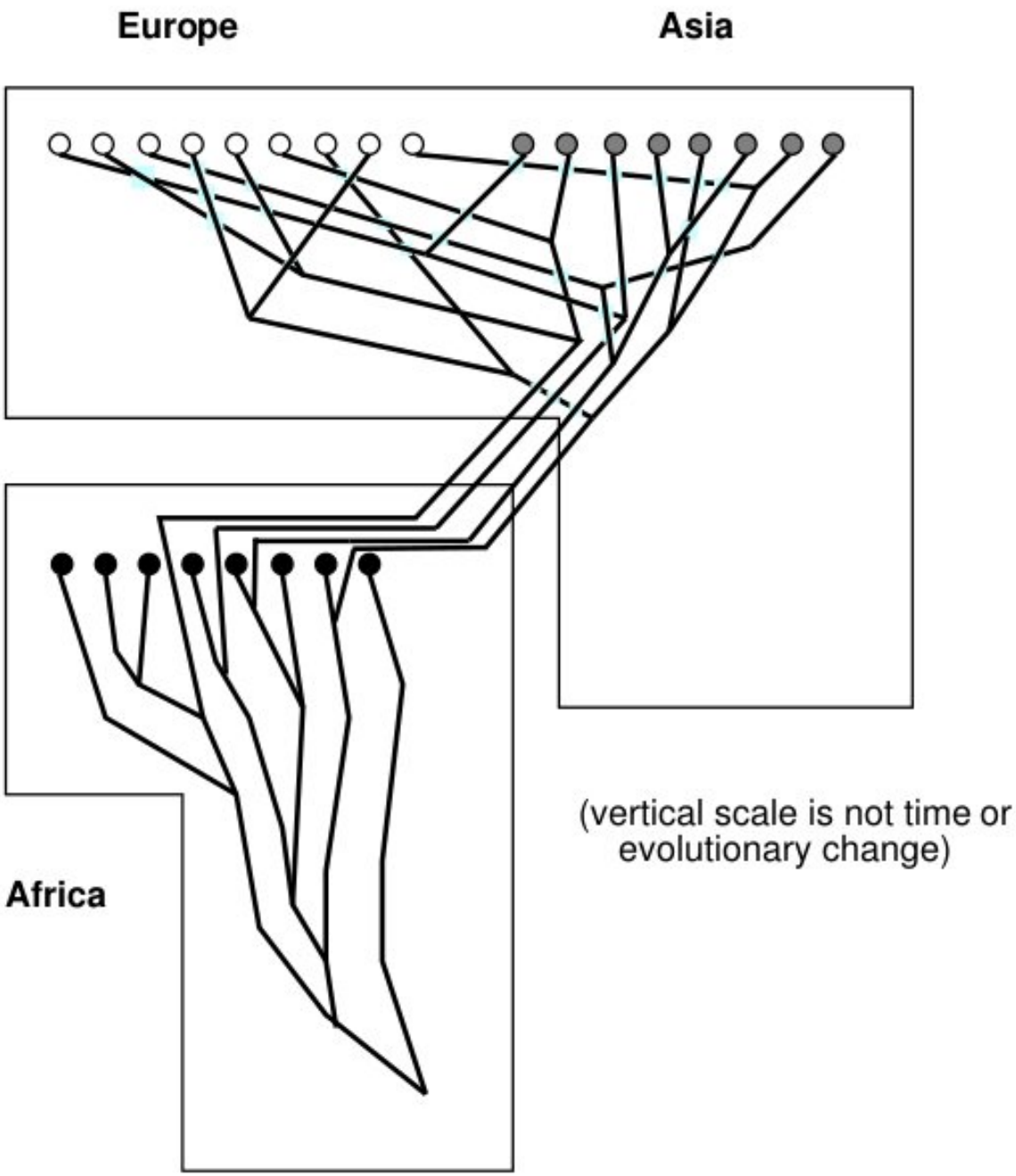
- The popular press found this the most exciting result
- It's actually a logical necessity
  - Start with some finite number of matrilines
  - Every generation some fail to reproduce
  - Given enough generations, only one will remain

"Eve" dates to roughly 200,000 years ago

- We can't do a straight population size calculation because the population size is not constant
- But if it were:
  - Expected time to Eve is $2N_f$ generations
  - (Why not $4N_e$?)
  - 25 years/generation suggests N_f around 4000
- That doesn't sound like the widespread Eurasian $Homo\ erectus$ population

The oldest splits lead to African lineages

- Two hypotheses for modern human origins:
  - Widespread Eurasian and African populations evolved into modern humans ("multiregional hypothesis")
  - Modern humans evolved in Africa and displaced previous Eurasian populations ("out-of-Africa hypothesis")


Europe
Asia
Africa
(vertical scale is not time or evolutionary change)

Testing these hypotheses

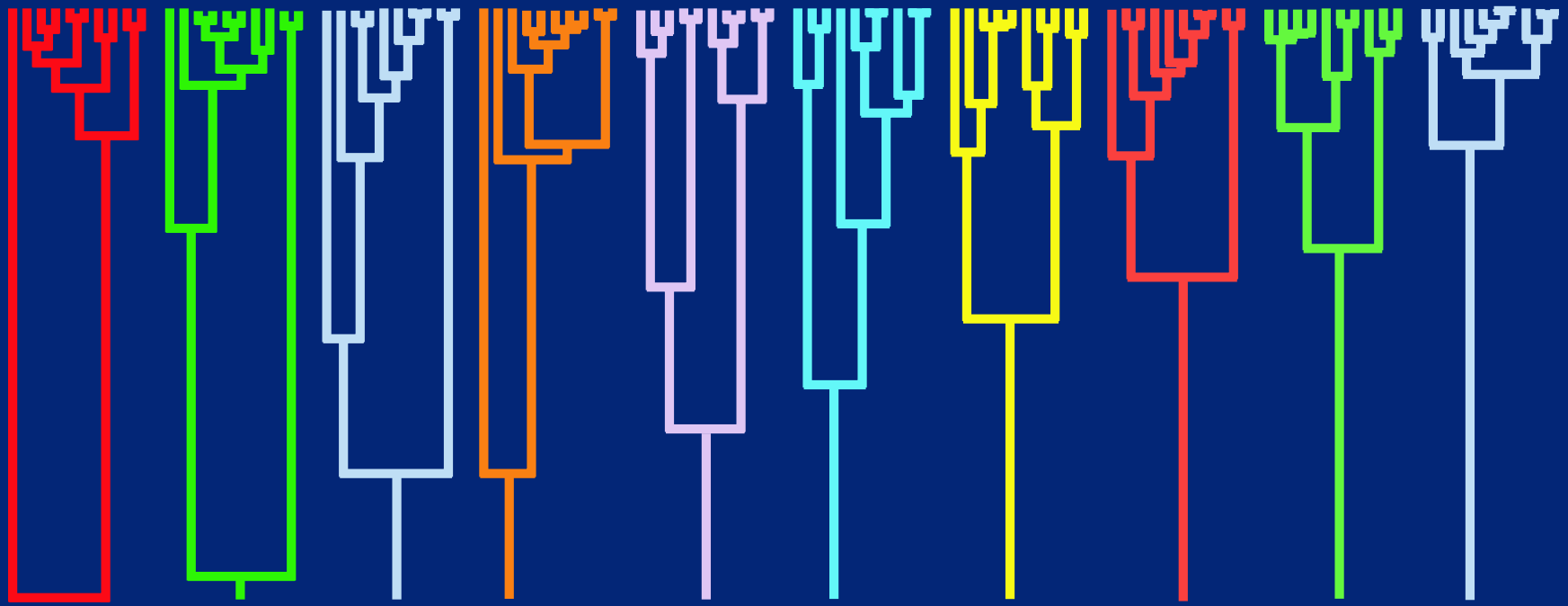
- When Eve lived there were tool-using hominoids all over Eurasia
- Their mtDNA does not seem to have survived
- Descendants of Eve have gotten all over the globe, at the expense of matrilines elsewhere
- This supports (doesn't prove) out-of-Africa hypothesis
- It at least proves that no population has been completely isolated for more than 200,000 years

Three reality checks about Eve

- Was Eve the only female in her generation?
- Is our nuclear genome likely to be descended from Eve?
- Could part of our nuclear genome come from those widespread Eurasian populations even if mtDNA did not?

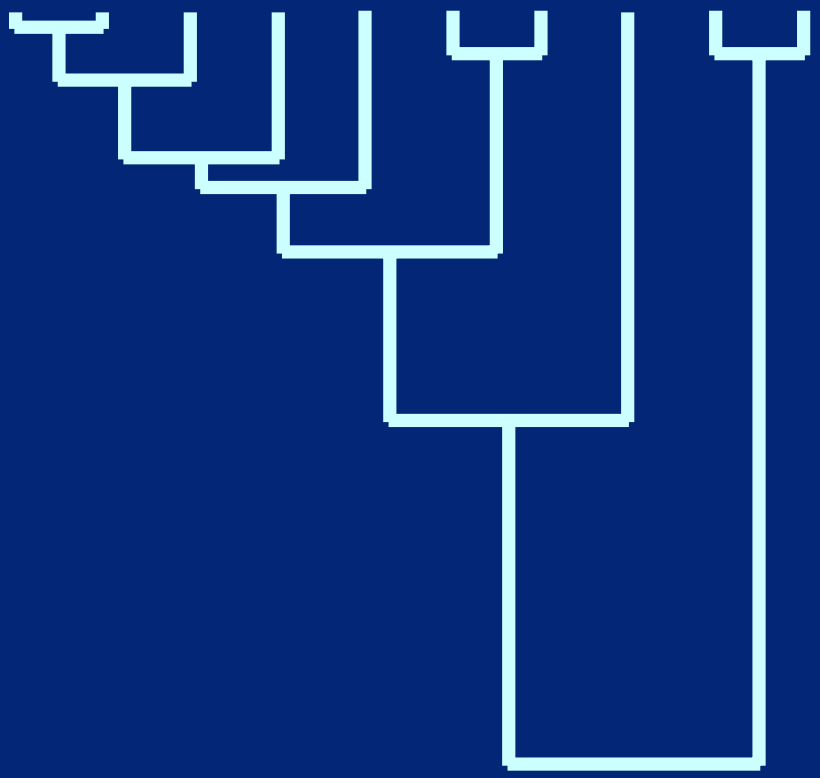
Variability of the coalescent

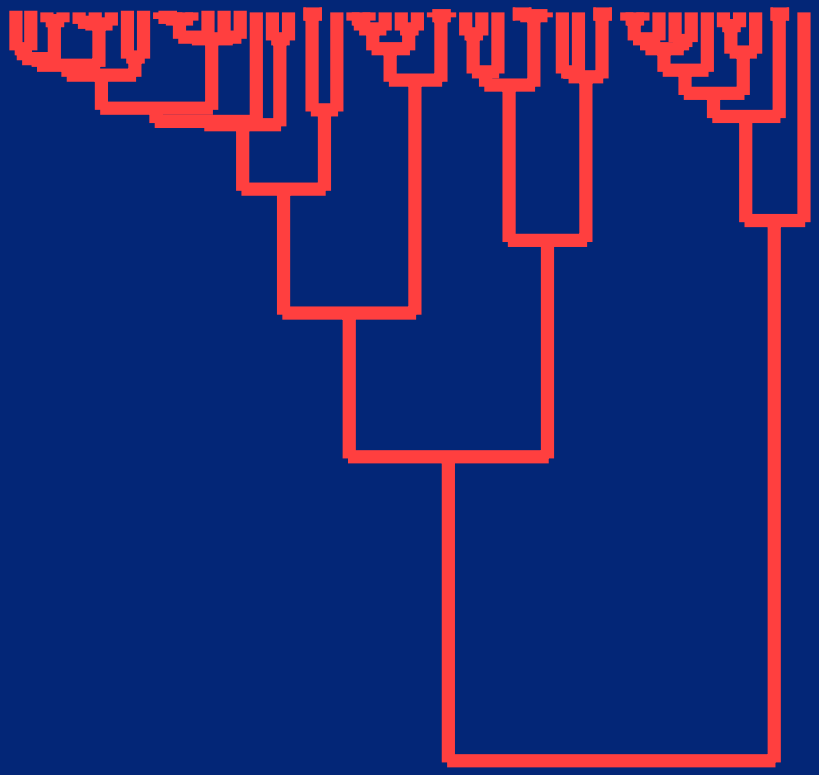
A single gene can give a misleading answer, because the coalescent is a highly variable distribution.



10 coalescent trees generated with the same population size, $N = 10,000$

Does sampling more individuals help? (No)





The origins of the rest of the genome

- Neanderthal and Denisovan genomes sequenced
- Several labs have searched human genomes for sequences which:
  - Have a rare haplotype very different from the common ones
  - That rare haplotype is also in Neanderthals or Denisovans
- They found a lot of hits:
  - Europeans and Asians appear to have some Neanderthal haplotypes
  - Asians appear to have some Denisovan haplotypes
  - Africans show few hits for either one
- Estimates of 4-5% admixture

Why the different results?

- No Neanderthal or Denisovan mtDNA – initial conclusion was no admixture
- Reasonable amount of apparent Neanderthal and Denisovan nuclear DNA
- Could be:
  - Chance? (With 5% admixture, no surprise mtDNA doesn't show it)
  - Lower effective population size for mtDNA? (Lineages are lost faster)
  - Different fertility for male and female hybrids? (Common in other animals)
  - Negative selection for foreign mtDNA?
  - Positive selection for foreign nuclear DNA?

Approaches to using the coalescent

- Summary-statistics approaches
- Many-tree approaches

Summary-statistic approaches

- Summary statistics look at the bulk properties of coalescent trees.
- They often require a simplified model of mutation.
- Watterson developed an estimator of Θ based on counting the number of variable sites
- We know how many variable sites to expect for various values of Θ, sequence length and number of sequences
- This approach discards much of the information in the data

Many-tree approaches

- Many groups, including mine, develop computer algorithms to to estimate Θ by considering many possible trees
- There are too many trees to consider all possibilities
- We write sampling algorithms which visit mainly the most likely trees
- While developed independently, this is similar to the Bayesian phylogenetic algorithm

What do I mean by too many trees?

Tips	Topologies
3	3
4	18
5	180
6	2700
7	56700
8	1587600
9	57153600
10	2571912000
15	6958057668962400000
20	564480989588730591336960000000
30	436846661310306951246468019862076389144064000000000000000
40	3027333829948007356546303364551457200042939432053862501707888721920000000000000000000
50	3.28632×10^{112}
100	1.37416×10^{284}

Uses and extension of the coalescent

- Genetic drift/population size estimation
- Population growth/shrinkage over time
- Migration between populations
- Recombination
- Divergence of populations
- Selection

Genetic drift ($Theta$)

- With one time point, we estimate $\Theta = 4N_e\mu$ in diploids
- The number estimated is $2N_e\mu$ in haploids or $N_e\mu$ in mtDNA
- Two ways to separate N_e and μ:
  - Dated historical data (ancient DNA, etc.)
  - External estimate of mutation rate
- For most organisms, N_e is less than N
- Demographic models can help resolve this

Why N_e differs from N

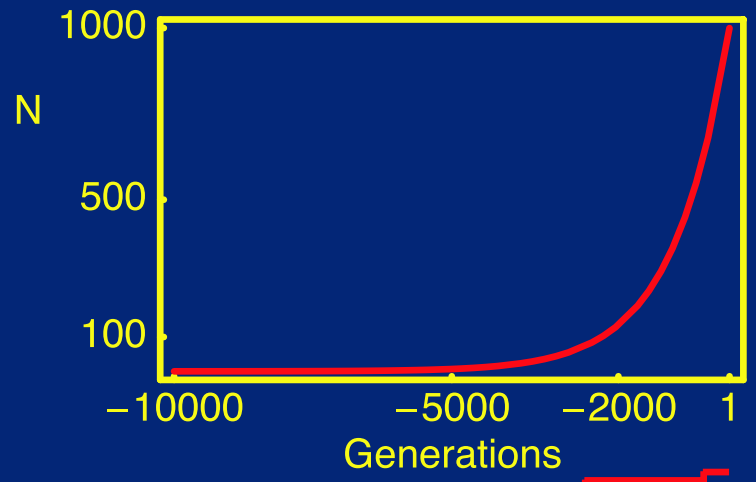
- N_e less than N
  - Non-reproductive individuals (bees)
  - Cyclic population size (snowshoe hares)
  - Overlapping generations (redwoods)
  - Highly unequal reproductive success (cattle)
- N_e greater than N (rare)
  - Negative assortative mating (mice?)
  - Equalized reproductive success (lab mice)

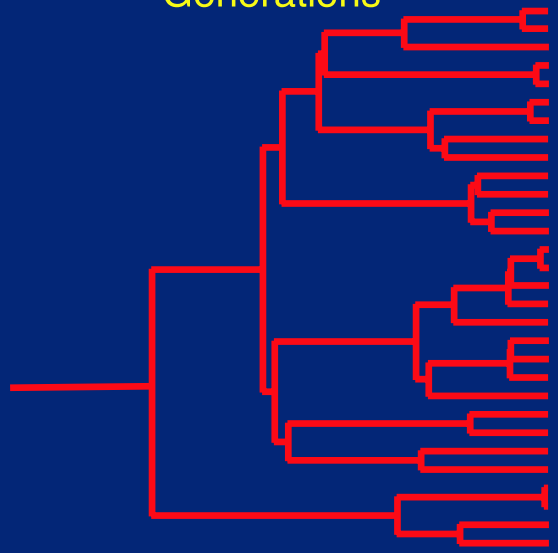
Variable population size

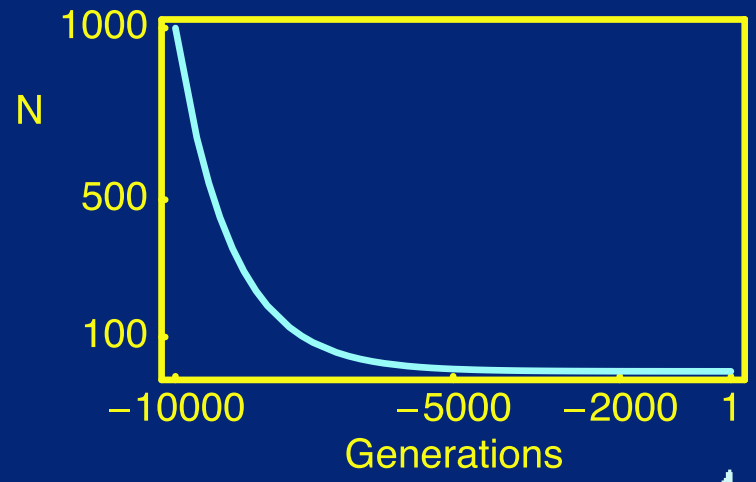
- In a small population lineages coalesce quickly
- In a large population lineages coalesce slowly

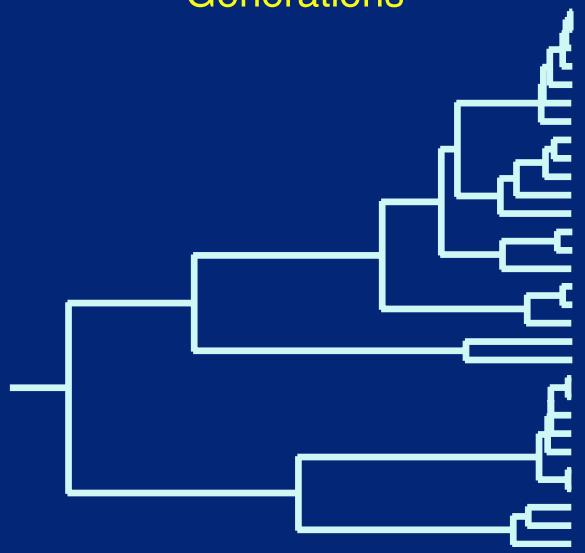
This leaves a signature in the data. We can exploit this and estimate the population growth rate g jointly with the current population size Θ.

Exponential population size expansion or shrinkage

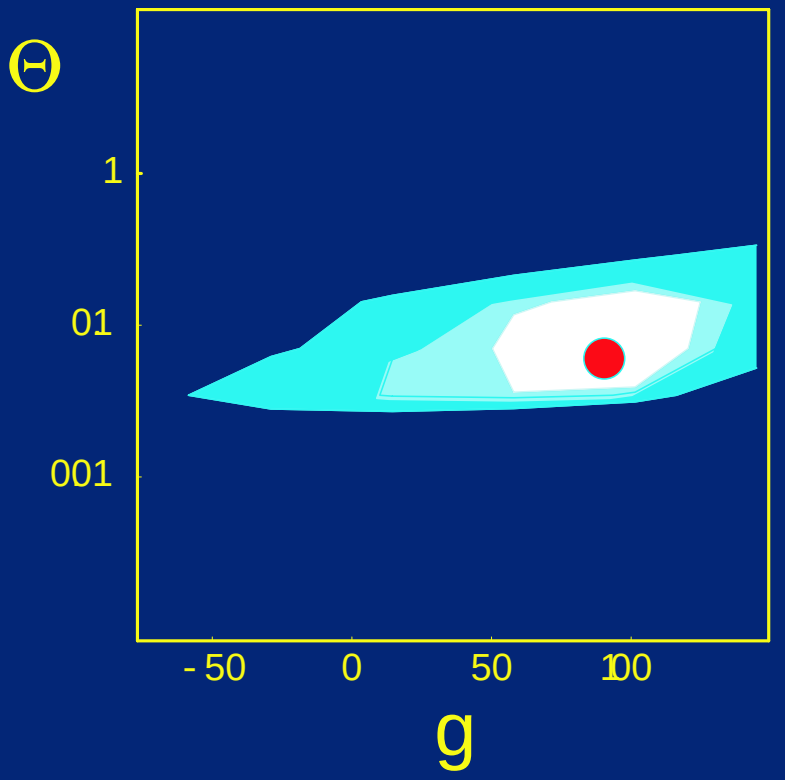






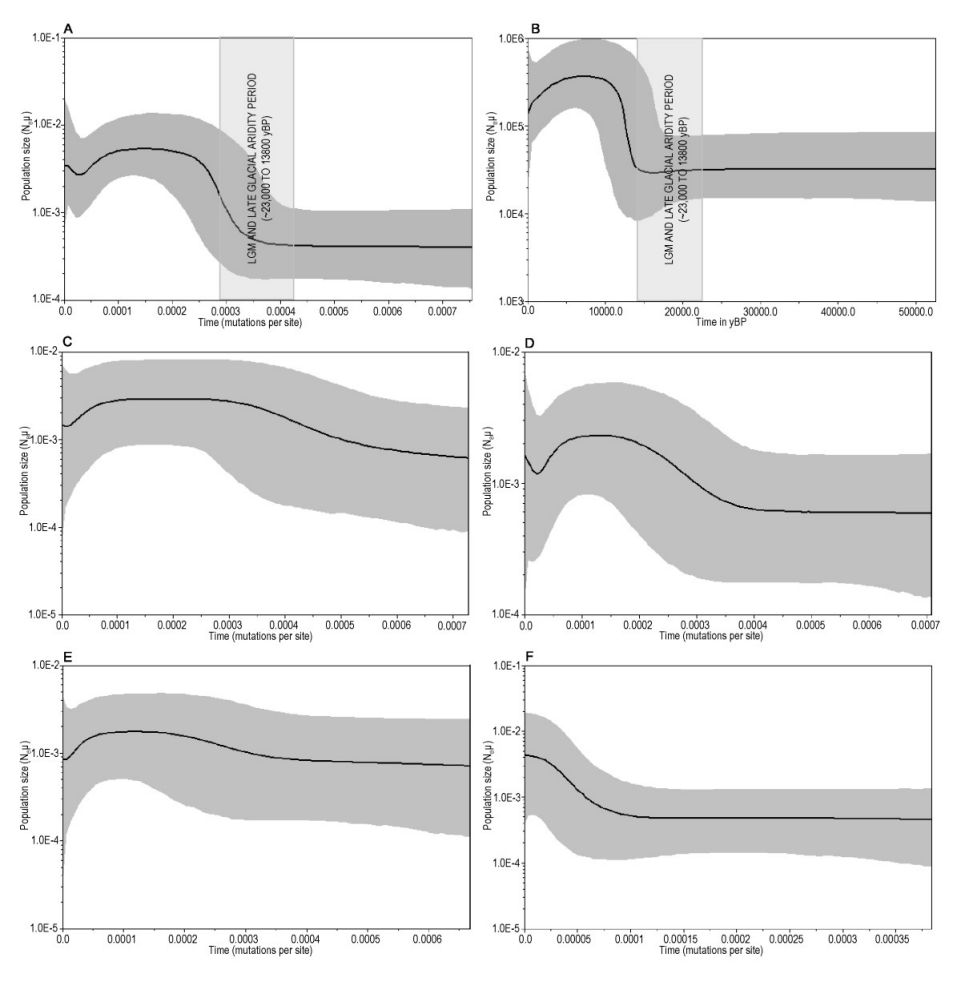


Grow a frog

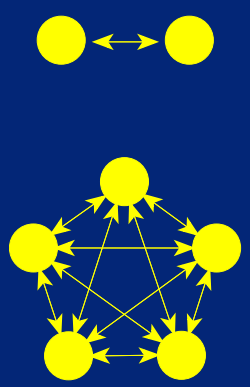


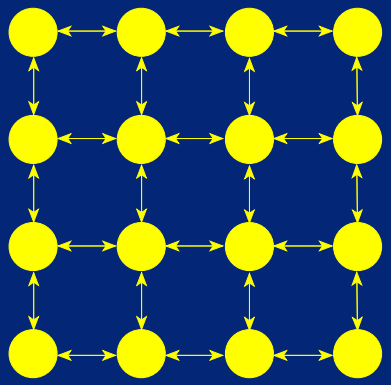
Mutation Rate	Population sizes	
	-10000 generations	Present
10^{-8}	$8,300,000$	$8,360,000$
10^{-7}	$780,000$	$836,000$
10^{-6}	$40,500$	$83,600$

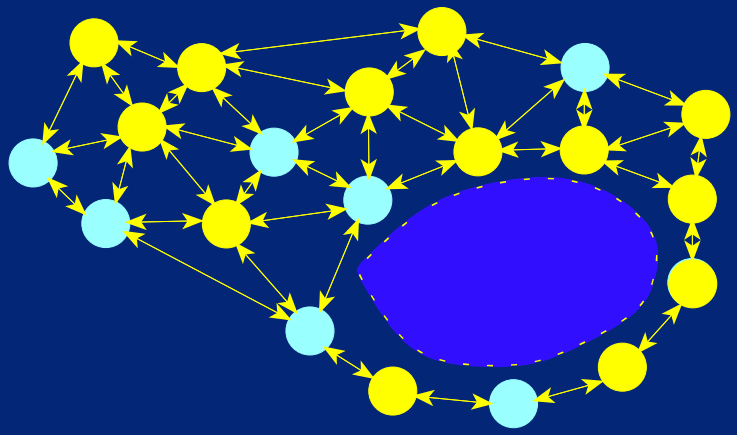
Bayesian skyline plots



Gene flow



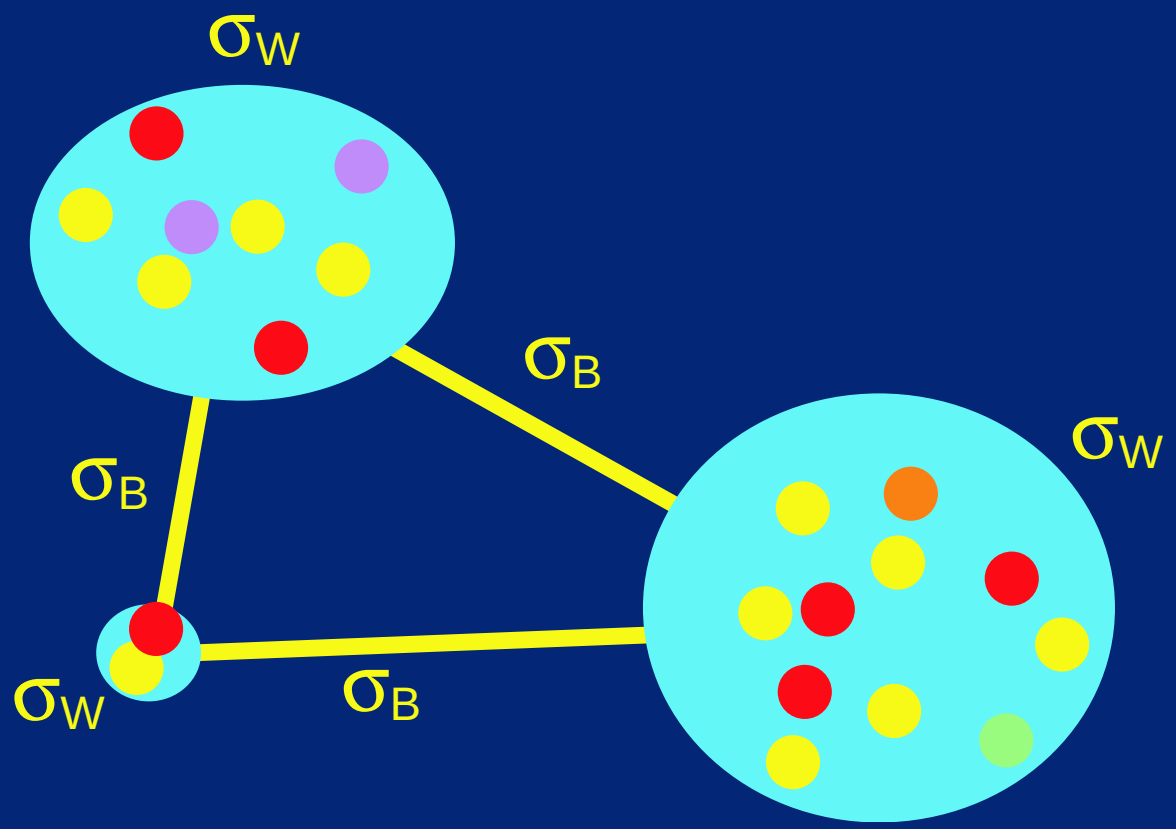




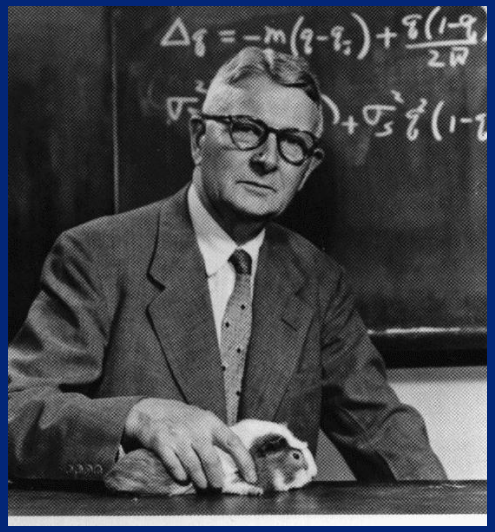
$$\mathrm{p}(\mathrm{G}|\boldsymbol{\Theta},\mathbf{M}) = \prod_{\mathrm{u_j}} \left(\prod_{\mathrm{i}}^{\text{pop.}} \mathrm{g}(\Theta_{\mathrm{i}}, \mathbf{M}_{\cdot \mathrm{i}}) \right) \begin{cases} \frac{2}{\Theta} & \text{if event is a coalescence,} \\ M_{ji} & \text{if event is a migration from } j \text{ to } i. \end{cases}$$

Gene flow: What researchers used (and still use)

F_{ST}



What researchers used (and still use)



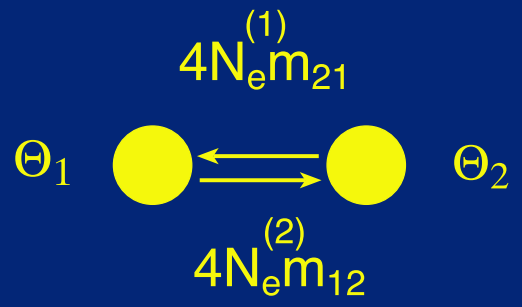
Sewall Wright showed that

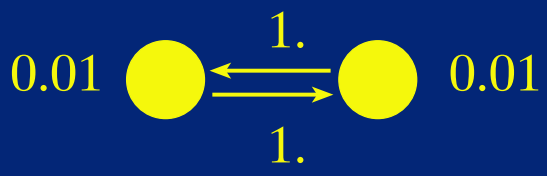
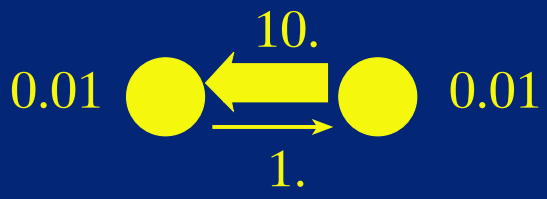
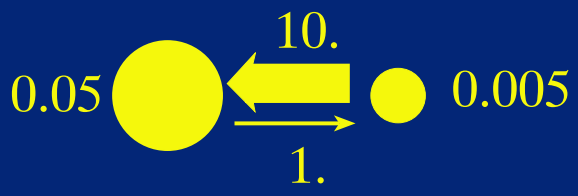
$$F_{ST} = \frac{1}{1 + 4Nm}$$

and that it assumes

- migration into all subpopulation is the same
- population size of each island is the same

Simulated data and Wright's formula



True values	Estimated values
	1.14±0.77
	7.80±22.20
	11.46±18.54

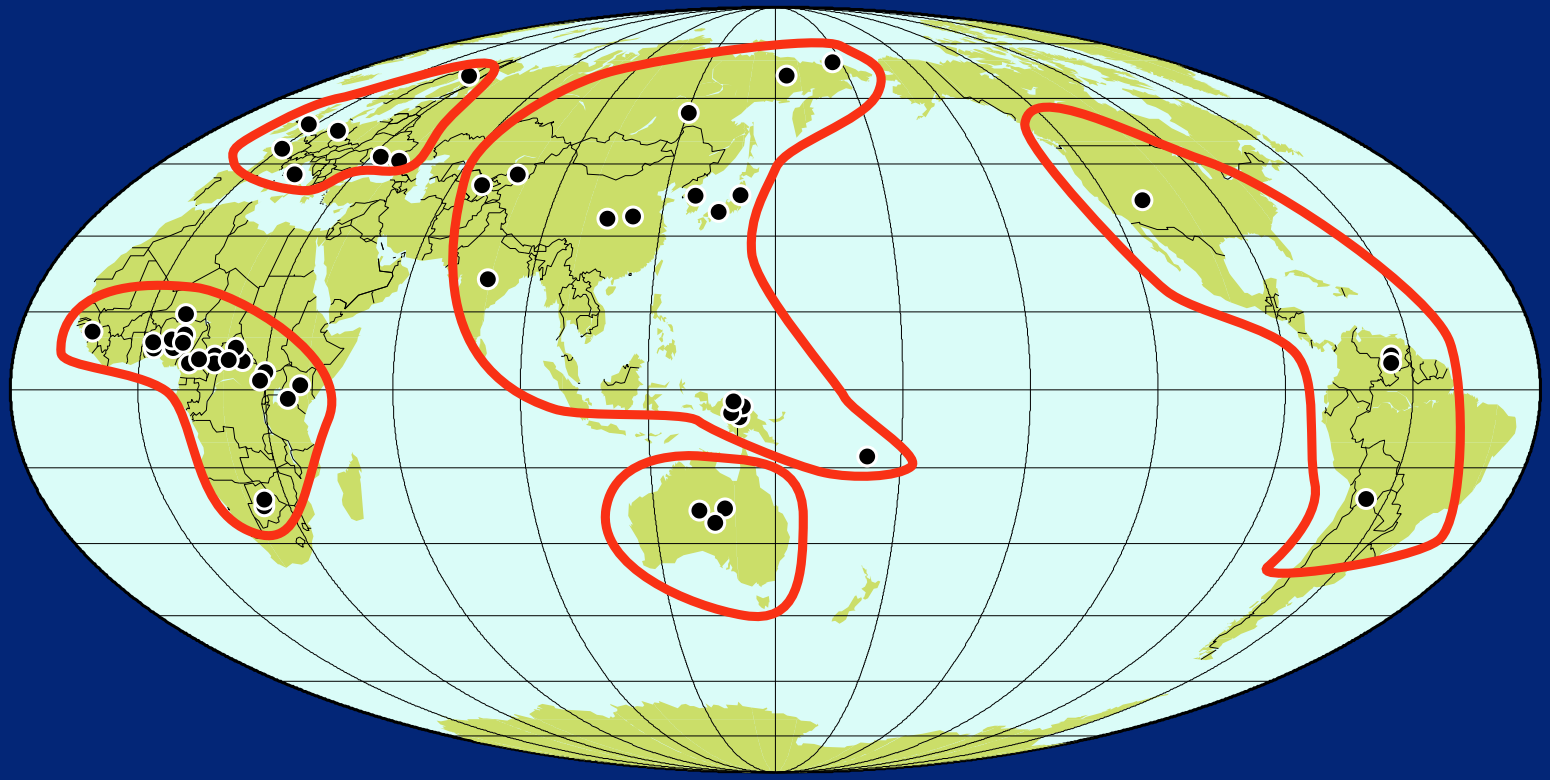
Maximum Likelihood method to estimate gene flow parameters

(Beerli and Felsenstein 1999)

100 two-locus datasets with 25 sampled individuals for each of 2 populations and 500 base pairs (bp) per locus.

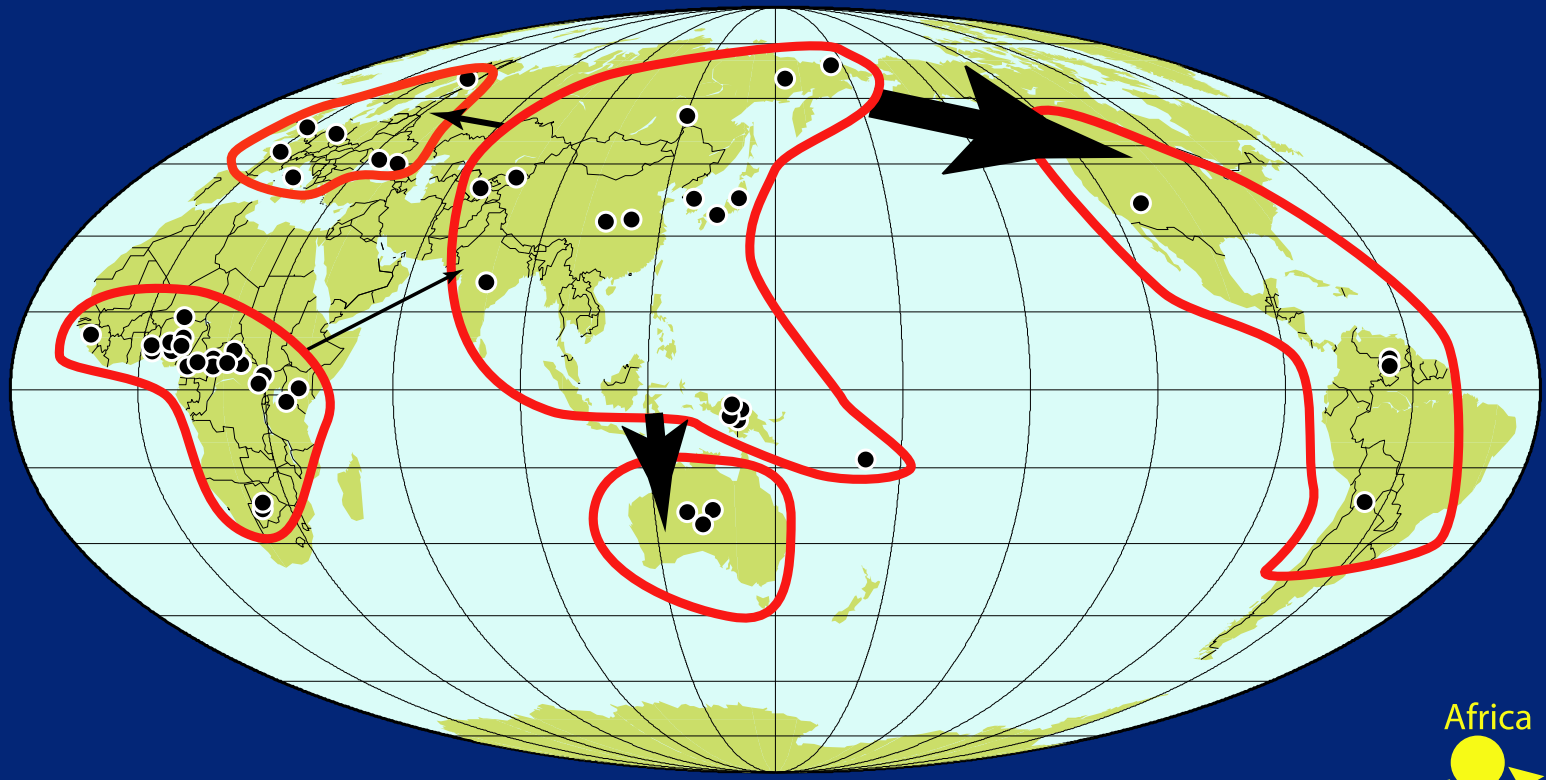
	Population 1		Population 2	
	Θ	$4N_e^{(1)}m_1$	Θ	$4N_e^{(2)}m_2$
Truth	0.0500	10.00	0.0050	1.00
Mean	0.0476	8.35	0.0048	1.21
Std. dev.	0.0052	1.09	0.0005	0.15

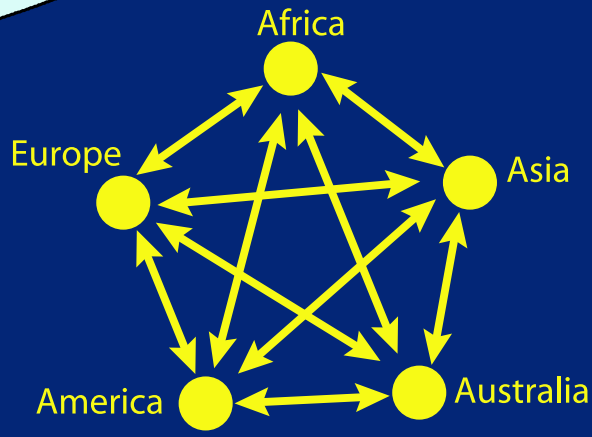
Complete mtDNA from 5 human "populations"



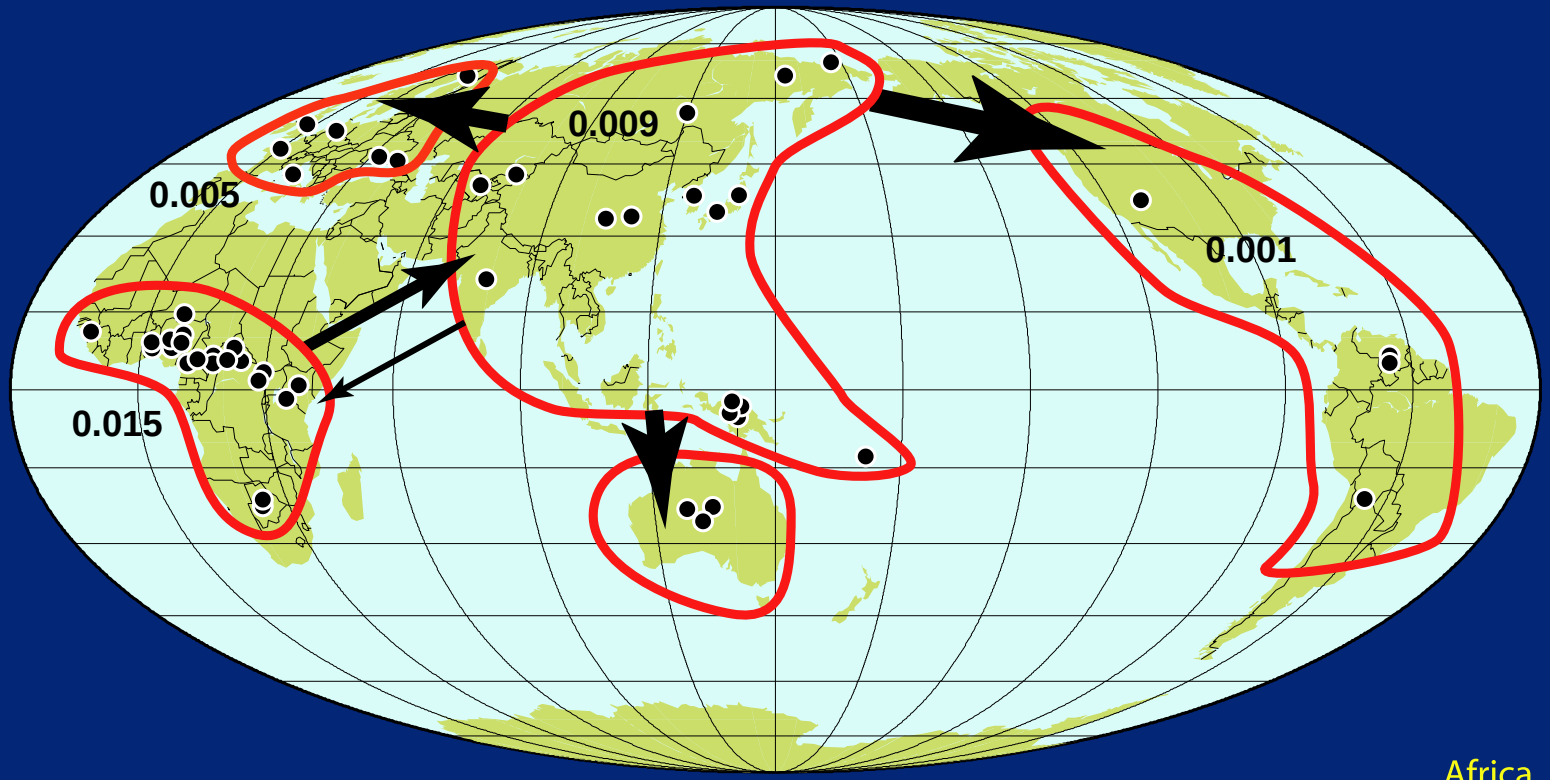
A total of 53 complete mtDNA sequences ($\sim$ 16 kb):
Africa: 22, Asia: 17, Australia: 3, America: 4, Europe: 7.
Assumed mutation model: F84+Γ

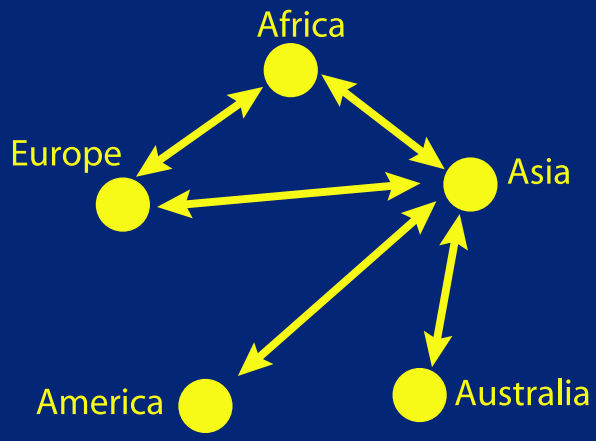
Full model: 5 population sizes + 20 migration rates





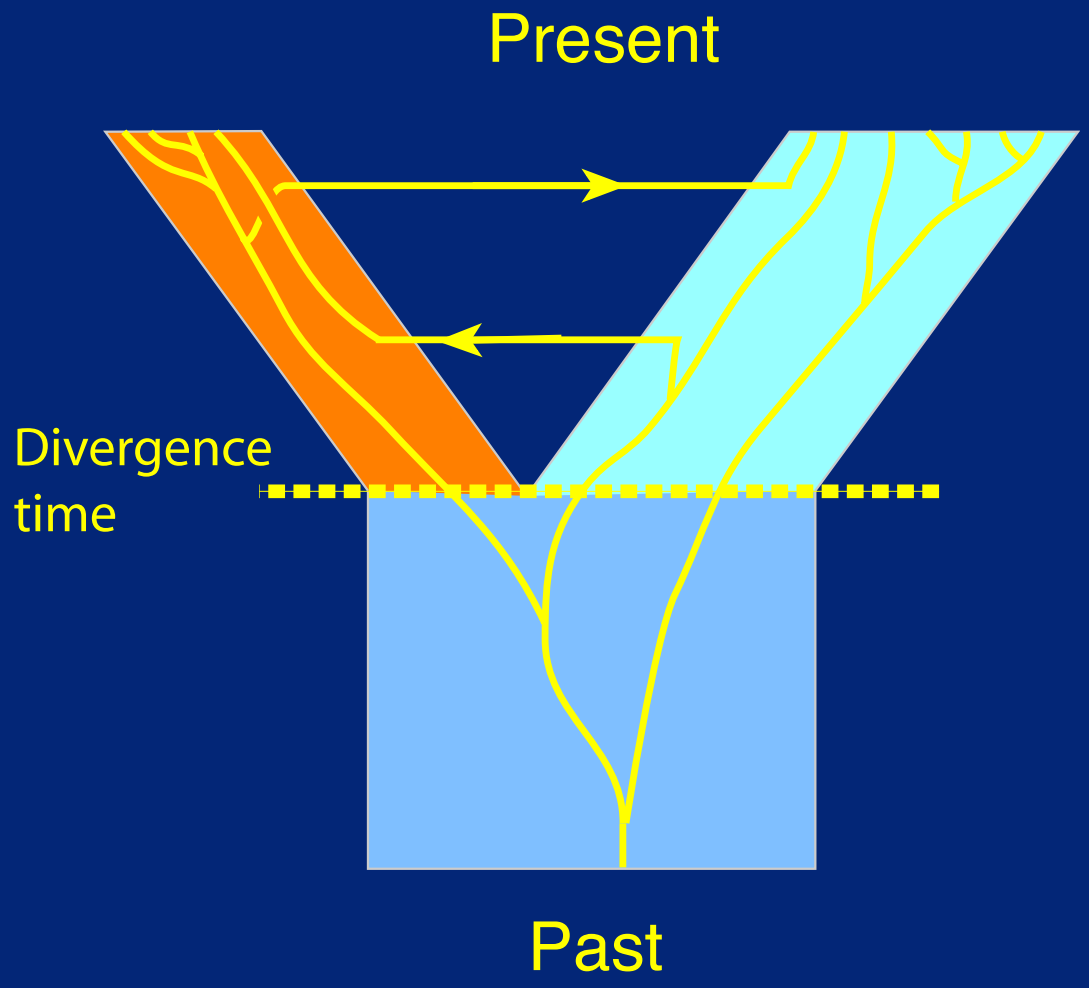
Restricted model: only migration into neighbors allowed





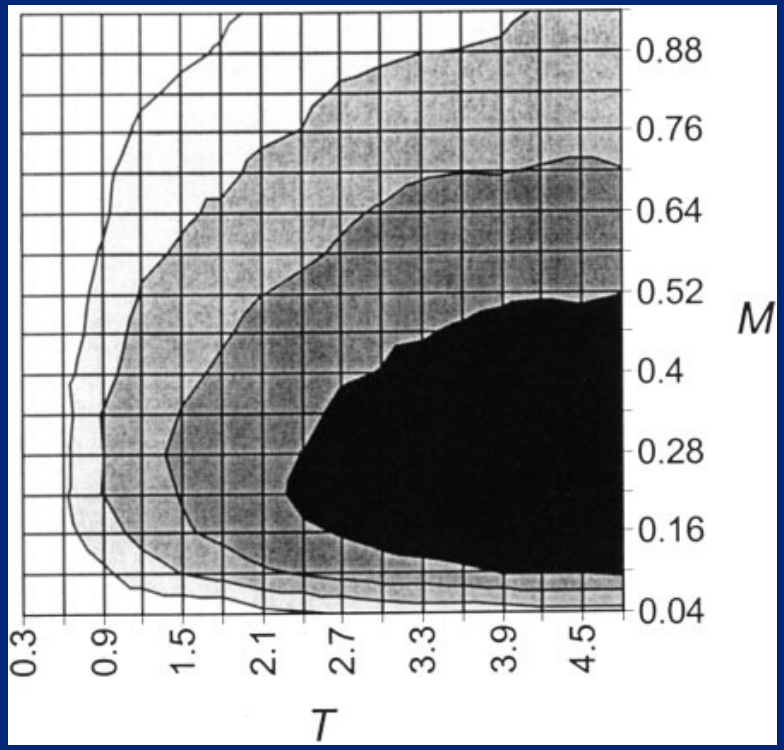
Estimation of divergence time

Wakeley and Nielsen (2001)



Estimation of divergence time

Wakeley and Nielsen (2001) Figure 7. The joint integrated likelihood surface for T and M estimated from the data by Orti et al. (1994). Darker values indicate higher likelihood.

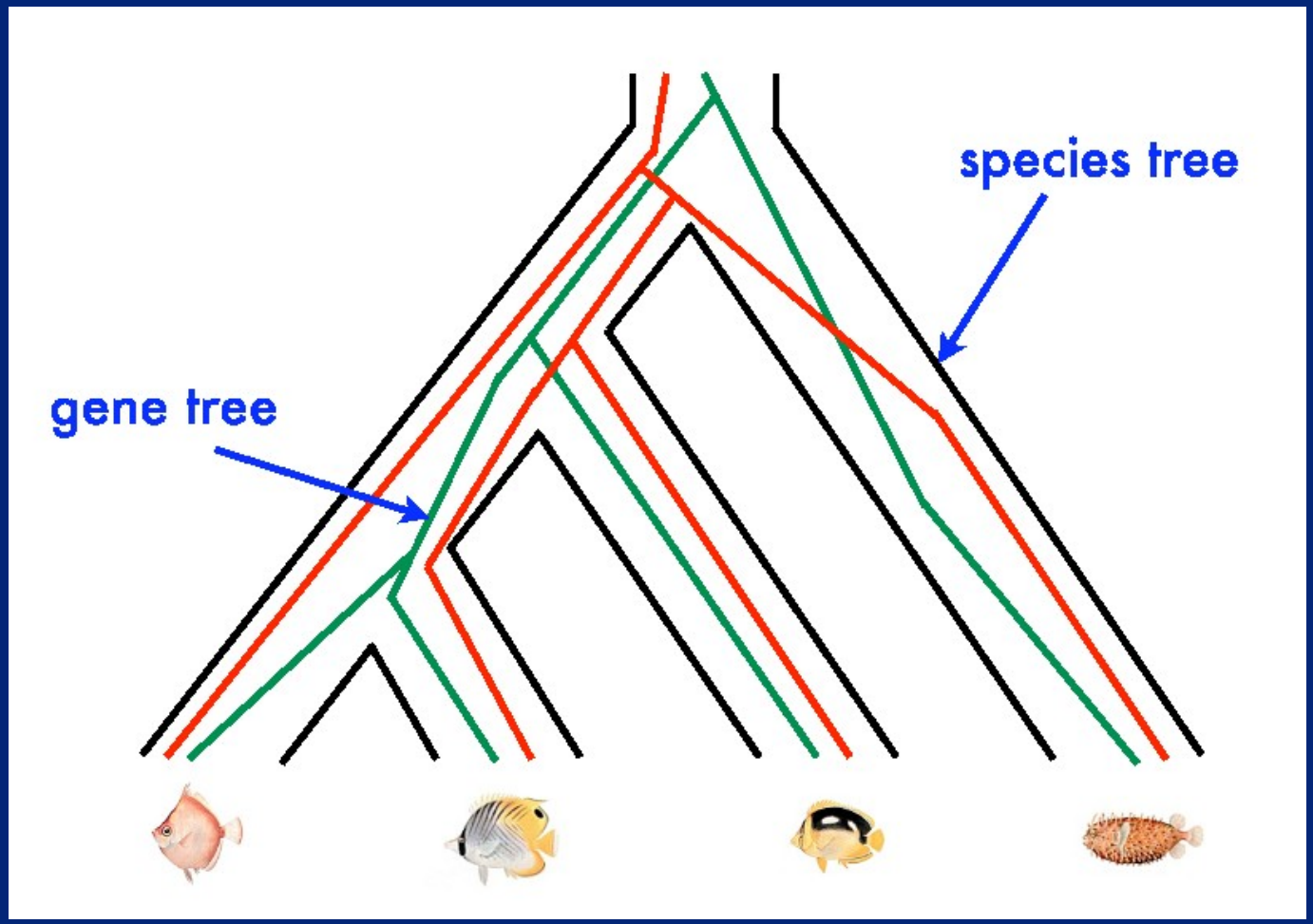


Highland and lowland ducks

- Several duck species in South America have highland and lowland forms
- Highland populations have hemoglobin mutations needed to fly at high altitude
- Unanswered questions about these ducks:
  - How do hemoglobin mutations remain sorted even when nuclear genomes are well mixed?
  - Did hemoglobin mutations happen independently five times or was there cross-species gene flow?
  - Can a species split into highland/lowland evolve into two new split species? Is that even possible?
  - What is the phenotype of the hemoglobin heterozygote? (Lab question!)


Puna Teal (highland) and Silver Teal (lowland). Images from Wikipedia

Coalescence at the boundary of speciation



Coalescence at the boundary of speciation

- When we draw species phylogenies we hope the gene tree reflects the species tree

- For a given gene this may not be true:
  - More than one lineage survives from the ancestral species
  - They have a random coalescent back there
  - Their coalescence pattern won't reflect the speciation pattern as they coalesce before the species formed

Coalescence at the boundary of speciation

- This is called "ancestral polymorphism" or "incomplete lineage sorting"
- It is mainly a problem for recent species
- Modern species tree methods need to take it into account
- Two major approaches:
  - Concatenate the genes and hope that the true signal wins out
  - Make a separate tree for each gene and try to reconcile them

Multiple time points

- Ancient DNA or historical samples of fast-evolving organisms
- Points must be:
  - Dated
  - Far enough apart for measurable evolution
- Advantages:
  - Separation of Θ into N_e and μ
  - Much better resolution of growth rates
  - Can look back past most recent bottleneck

The coalescent in nuclear genes

- mtDNA has a single coalescent tree
- The nuclear genome has multiple trees due to recombination and assortment
- Say that my 17q comes from my maternal grandfather and my 17p comes from my maternal grandmother
- They will have totally different coalescent ancestries

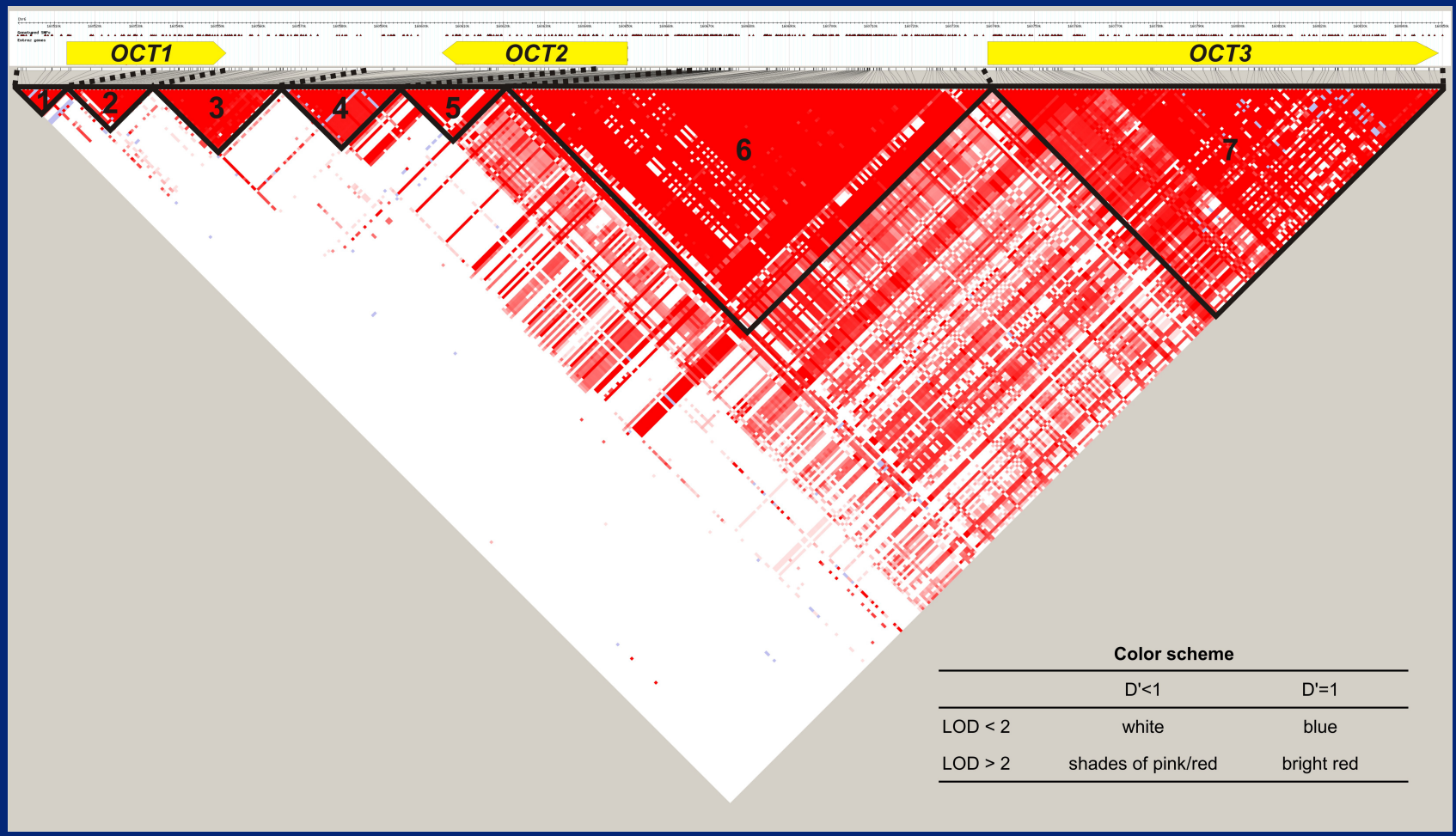
The coalescent in nuclear genes

- "Haplotype blocks" have the same common ancestor
- Length of blocks depends on:
  - Frequency of recombination (breaks up blocks)
  - Rate of coalescences (determines how distant common ancestors are)
  - Coalescence rate depends on population size–large populations have shorter blocks of linkage disquilibrium
  - Presence of hotspots
- The HapMap project tries to capitalize on this block structure

Ancestral recombination graph (ARG)

- The history of the nuclear genome is a tangled graph
- I wrote a program (LAMARC) to search over such graphs using MCMC
- Much remains to be done in this field
- Many current approaches use very short sequences to try to avoid recombination
- More information is available from a full analysis, but it's hard

Disequilibrium patterns arise from the ARG

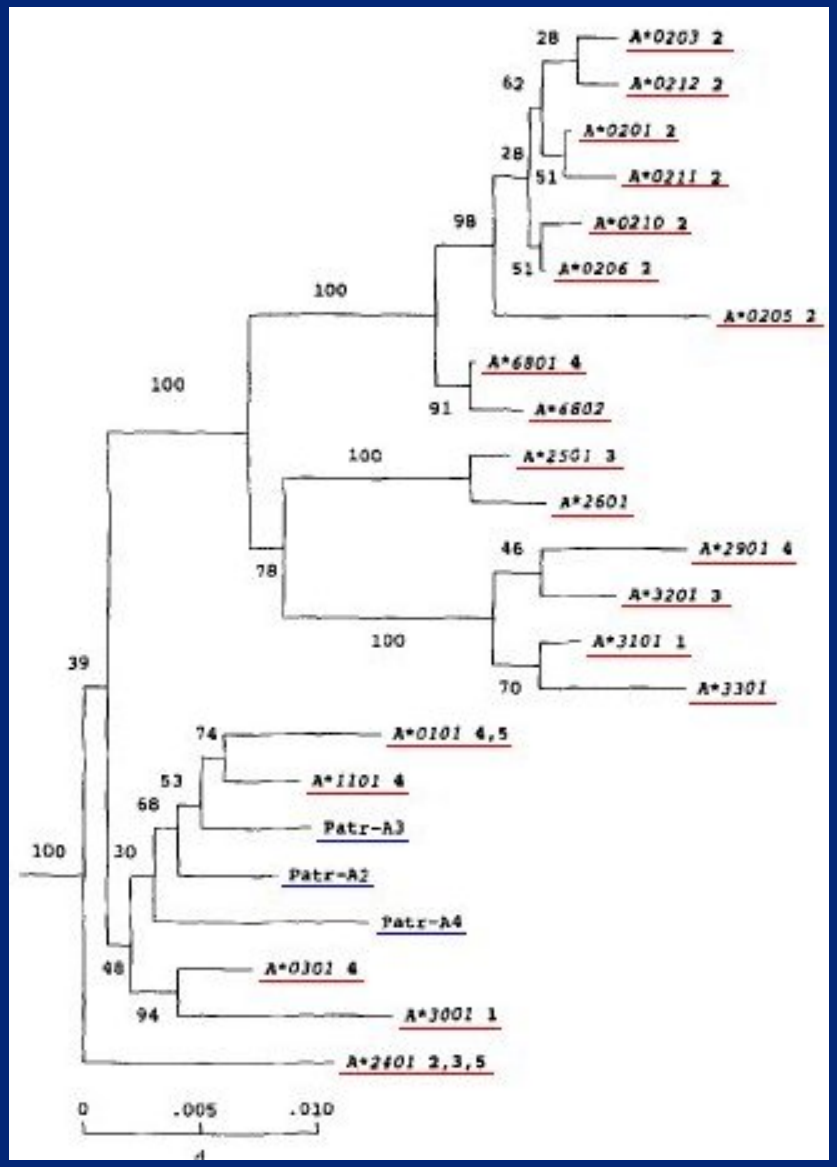


LD in the OCT1-OCT3 loci. From Tzvetkov et al. (2009).

Some lessons from the coalescent with recombination

MRCA = Most Recent Common Ancestor

- Different parts of the genome have:
  - different times back to MRCA
  - different amounts of variation
  - different amounts of LD
- Recombinations between unlike sequences can cause dramatic changes in time to MRCA and in variability
- This is true *even without natural selection*



Phylogeny of human and chimpanzee ("Patr") alleles at the HLA-A locus. From Hughes and Nei 1988. Note intermixing of human and chimp lineages.

Selection distorts the coalescent

- HLA-A has a MUCH older MRCA than expected under neutrality
- This pattern can be produced by:
  - Heterozygote advantage
  - Frequency-dependent selection
  - Nonrandom mating
- All three of these might be involved
- The HLA genes are the most extreme examples of this known in humans

Selection distorts the coalescent

- At the Duffy (FY) blood group locus:
  - Africans are fixed for allele O
  - Non-Africans lack O but have alleles A and B
  - African haplotypes are almost identical near the Duffy locus
  - Non-African haplotypes are diverse
- This contradicts the pattern in the rest of the genome
- Normally Africans are much more diverse (larger effective population size–they did not go through a bottleneck)

Selection distorts the coalescent

- Duffy-O homozygotes do not get $Plasmodium\ vivax$ malaria
- Selective sweep of Duffy-O across Africa wiped out variation
- Charla Lambert (grad student here) estimated that this took about 3000 years (large error bars)
- $P.\ vivax$ does not occur in Africa–did we out-evolve it?

General patterns with selection

- Purifying selection has only a small impact on the coalescent (and is usually ignored)
- Diversifying selection (balancing, frequency-dependent):
  - Older MRCA
  - More neutral variation near selected locus
- Directional selection:
  - More recent MRCA
  - Less neutral variation near selected locus
- Hard to tell these from random variation in the coalescent unless they are extreme

What is the effective population size of red drum?

Red drum, *Sciaenops ocellatus*, are large fish found in the Gulf of Mexico.

Turner, Wares, and Gold
Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish
Genetics 162:1329-1339 (2002)

What is the effective population size of red drum?

- Census population size: 3,400,000
- Effective population size: ?
- Data set:
  - 8 microsatellite loci
  - 7 populations
  - 20 individuals per population

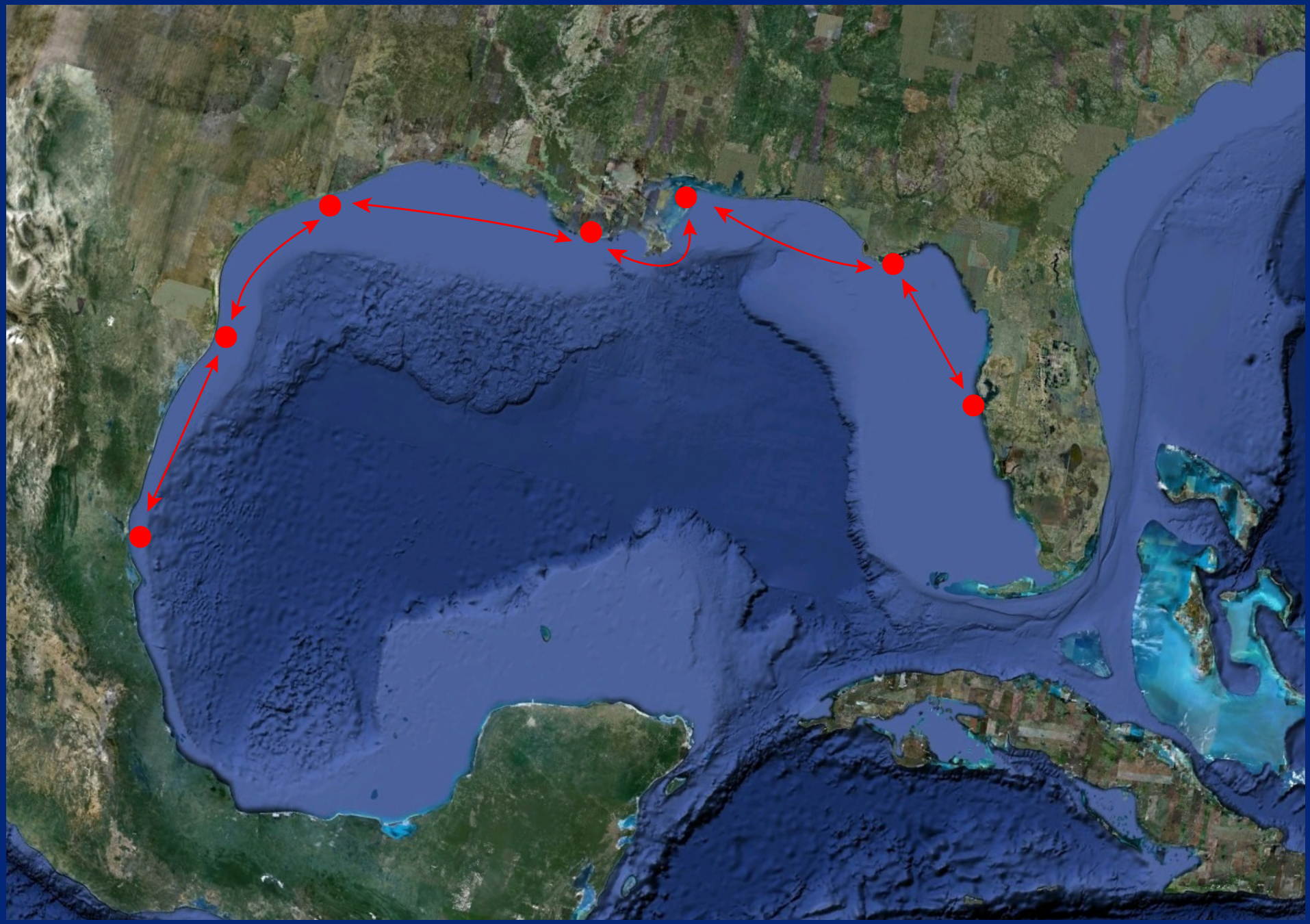
What is the effective population size of red drum?

Three approaches:

1. Allele frequency fluctuation from year to year
   - Measures current population size
   - May be sensitive to short-term fluctuations
2. Coalescent estimate from *Migrate*
   - Measures long-term harmonic mean of population size
   - May reflect past bottlenecks or other long-term effects
3. Demographic models
   - Attempt to infer genetic size from census size
   - Vulnerable to errors in demographic model
   - Not well established for long-lived species with high reproductive variability

Population model used for Migrate

- Multiple populations along Gulf coast
- Migration allowed only between adjacent populations
- Allowing for population structure should improve estimates of population size



What is the effective population size of red drum?

Estimates:

Census size (N):	3,400,000
Allele frequency method (N_e):	3,516 (1,785-18,148)
Coalescent method (N_e):	1,853 (317-7,226)

The demographic model can be made consistent with these only by assuming enormous variance in reproductive success among individuals.

What is the effective population size of red drum?

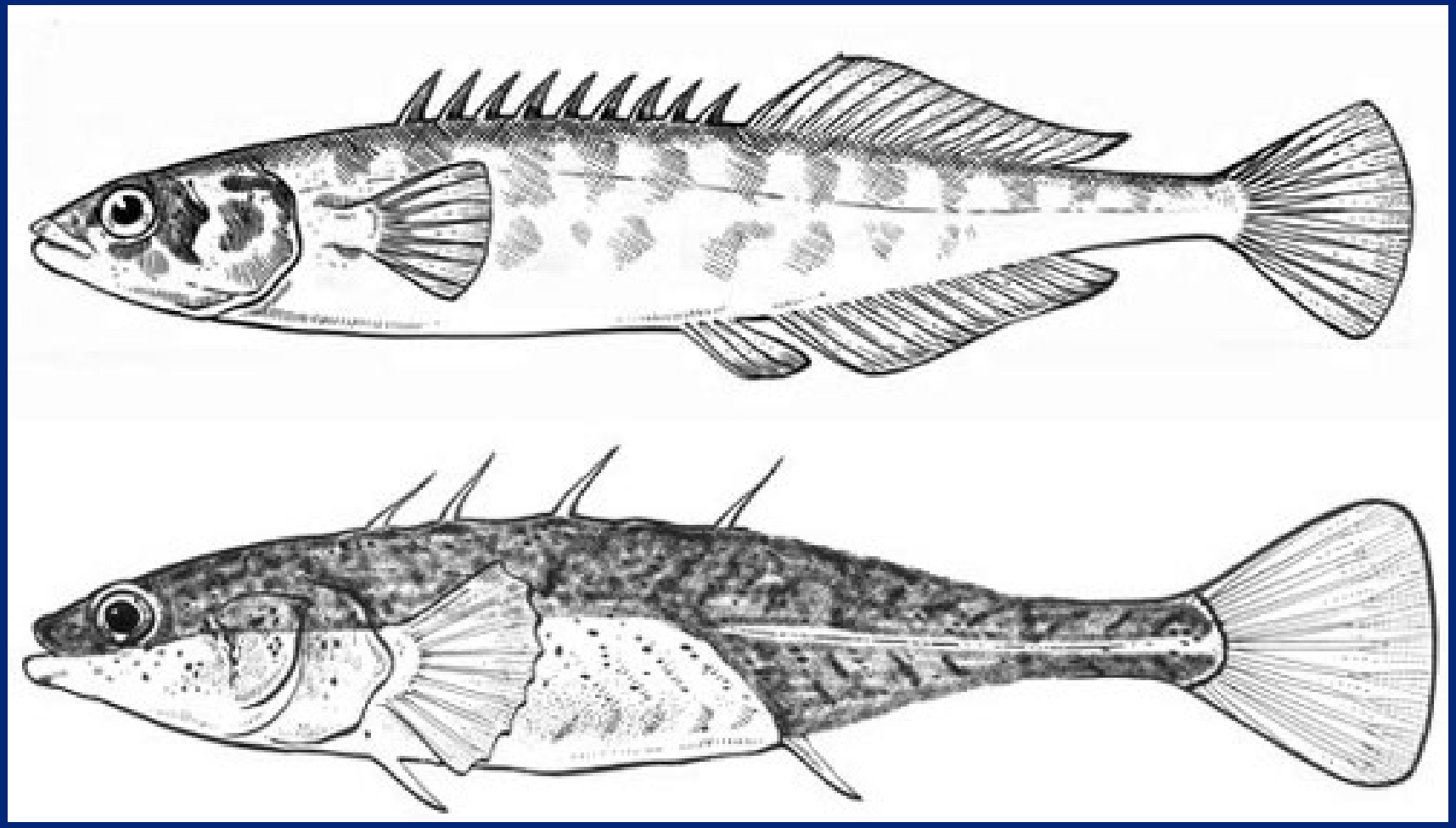
- Allele frequency estimators measure current size
- Coalescent estimators measure long-term size
- Conclusion: population size and structure have been stable

What is the effective population size of red drum?

- Effective population size at least 1000 times smaller than census
- This result was highly surprising
- Red drum has the genetic liabilities of a rare species
- Turner et al. hypothesize an "estuary lottery"
- Unless the eggs are in exactly the right place, they all die

Where do freshwater stickleback come from?

- Stickleback are small fish that live in either fresh or salt water
- The two kinds are morphologically different



Upper image is saltwater, lower is freshwater. From Smithsonian Magazine blog.

Where do freshwater stickleback come from?

- Dolph Lundgren and colleagues made coalescent trees of stickleback populations
- Freshwater populations do not cluster
- Each one is related to nearby saltwater populations

Where do freshwater stickleback come from?

- After the Park Service wiped out all fish in a lake, it was connected to the sea by a small creek
- Within 7 years there were FRESHWATER stickleback in the lake
- How can that happen?
- (Hatching and living in freshwater does not change the morphology; it really is genetic)

Where do freshwater stickleback come from?

- Saltwater populations have recessive traits suitable for freshwater
- Selection to remove recessives is very weak
- Saltwater populations are large, so drift is also weak
- In the lake:
  - High inbreeding pairs up recessives
  - Strong selection increases their frequency rapidly
- Lundgren called this the "Star Trek Transporter"