Inference of demographic histories of natural populations using sequence data

Coalescence, Mutation and Recombination

Richard Durbin rd109@cam.ac.uk

Cesky Krumlov 24/1/18

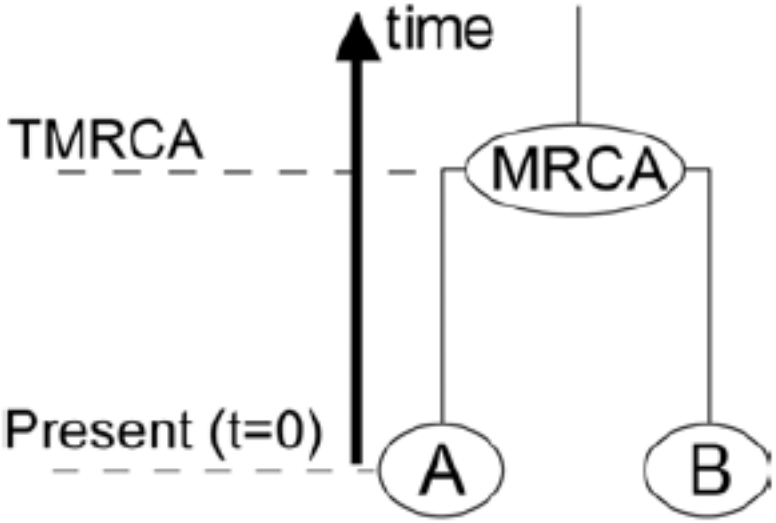
What I mean by demography

1. Population size going back in time
   - Actually "effective population size" N_e(t)
     - We will come back to what this means
   - Approximate time range 10k – 1M years ago
     - Again we will see why
2. Population structure
   - Subpopulations and when they split (and merged?)

- Based on explicit evolutionary models
  - Relate patterns of (shared) genetic variation accumulated since a common ancestor to history

Tree on two sequences

- Gustave Malécot (1940s)



- *Coalescence* is joining together, in our case going backwards in time
- Chance of coalescence per generation is 1/N
- TMRCA is exponentially distributed with mean N

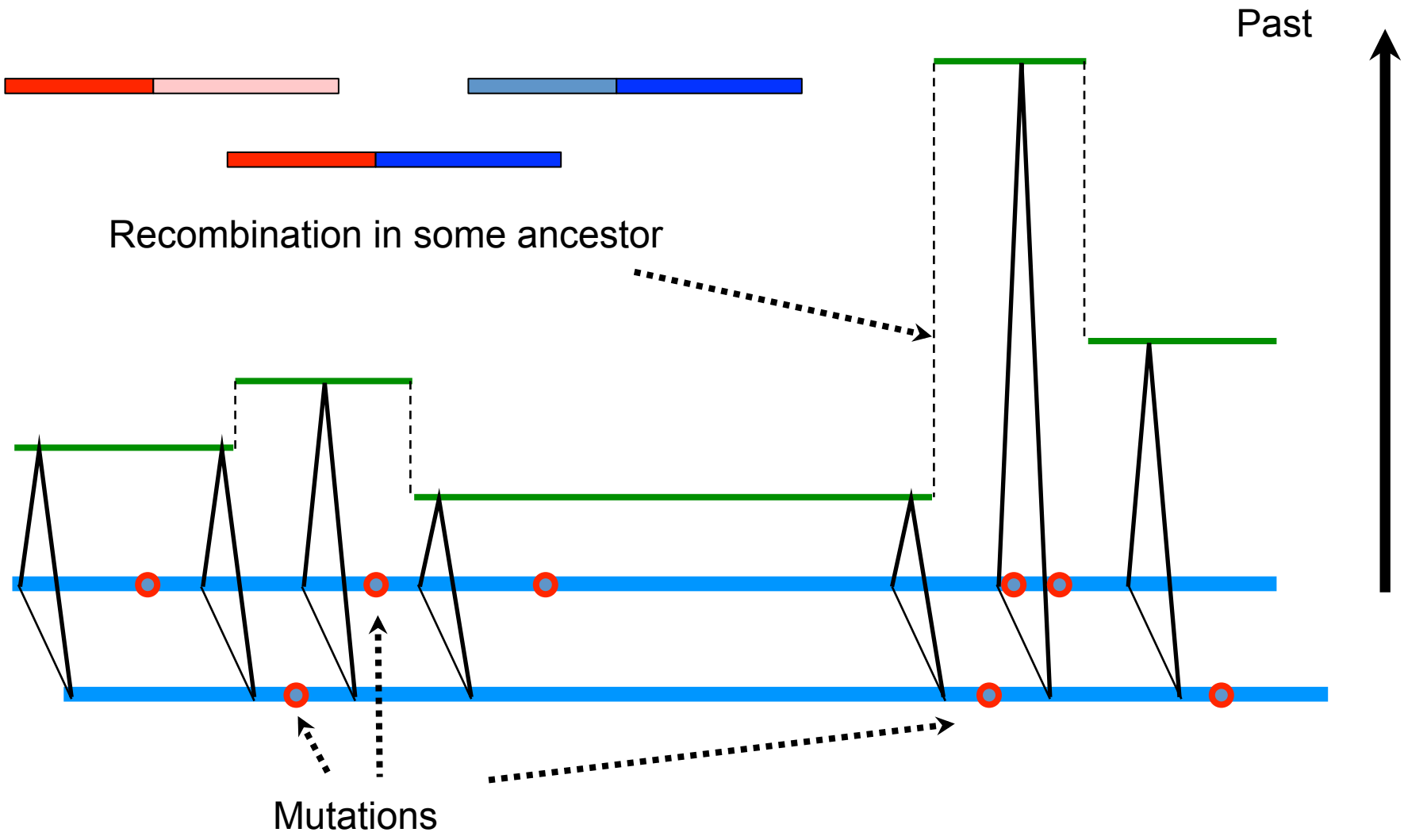
Probability of observing a mutation

- To see a mutation, it must have happened on one of the branches since the common ancestor
- P(observed mutation) = $2T\mu$
- E(observed difference rate) = $\theta_\pi = 2N\mu$
- Humans are diploid, so $\theta = 4N_e\mu$, where N_e is the *effective population size*
- For humans, θ_π =~ 0.001
  - 1/800 – 1/1200 depending on population
- Hard to measure N_e and μ independently…

Effective population size

- Lots of mystique/angst about this
  - Our definition is arguably at the core of the concept
    - the reciprocal of the probability of sharing a parent in the previous generation
    - = 1 / coalescence rate
- Why this is different from census population size:
  - Many consequences occur over large numbers (often order of N_e generations) – long term averaging
  - Structure generates non-random patterns of coalescence, and non-independence between generations
  - Maybe only a small percentage of individuals breed
  - Selection favours some individuals over others
- But is always something of this form that we get at by population genetic analysis

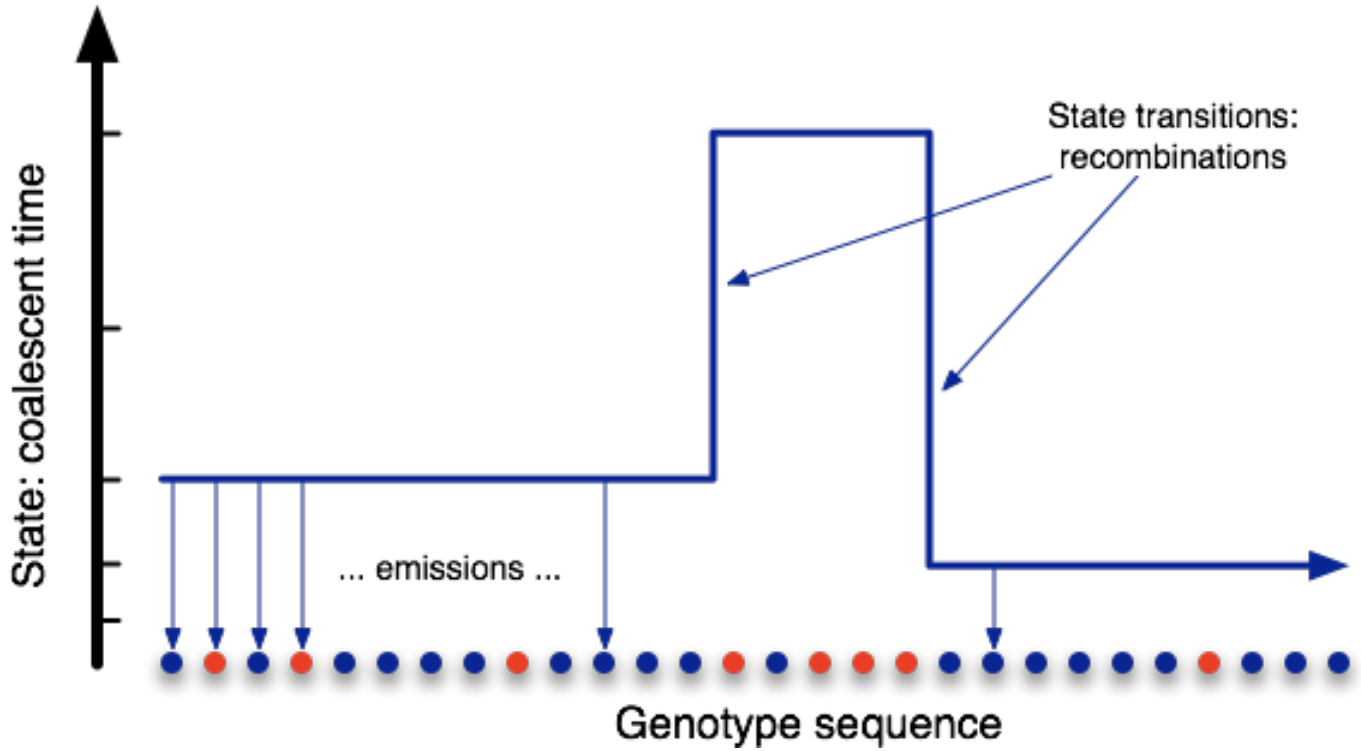
Segments of fixed TMRCA are separated by recombination



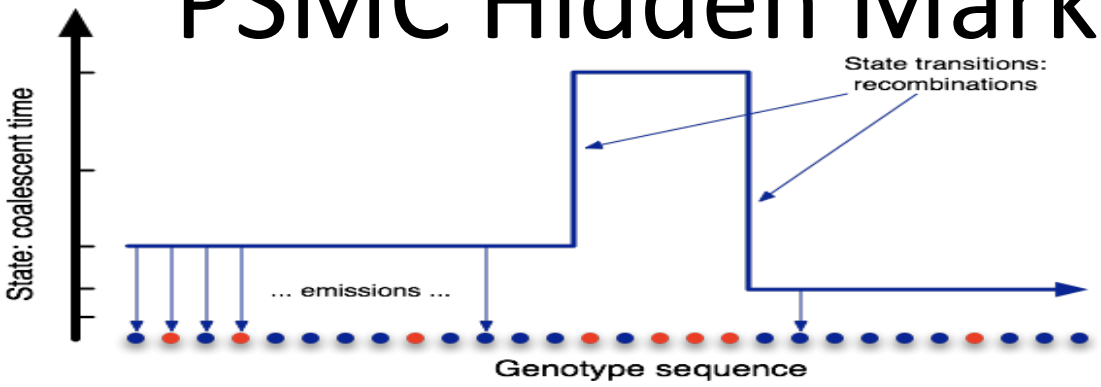
Pairwise Sequentially Markovian Coalescent

Li and Durbin (2010): Inference of human population history from individual genome sequences

Hidden Markov Model



PSMC Hidden Markov Model



- Move from left to right in the genome
  - Let P($x|t$) = prob(data up to x|TMRCA at $x = t$)
  - Calculate P($x+1|t$) = ($\sum_s$ P($x|s$) $r(t|s)$) $e(x)$
- $e(x)$ = "emission at x" = $2\mu t$ if a het, else $(1\text{-}2\mu t)$
- $r(t|s)$ = prob(recombination from TMRCA s to t)

  = $2\rho s$ prob (coalesce back to t) ← Depends on $N(t')$ $t' < s,t$

  $+ (1\text{-}2\rho s)$ if $t = s$

Markov assumption

- This model assumes that

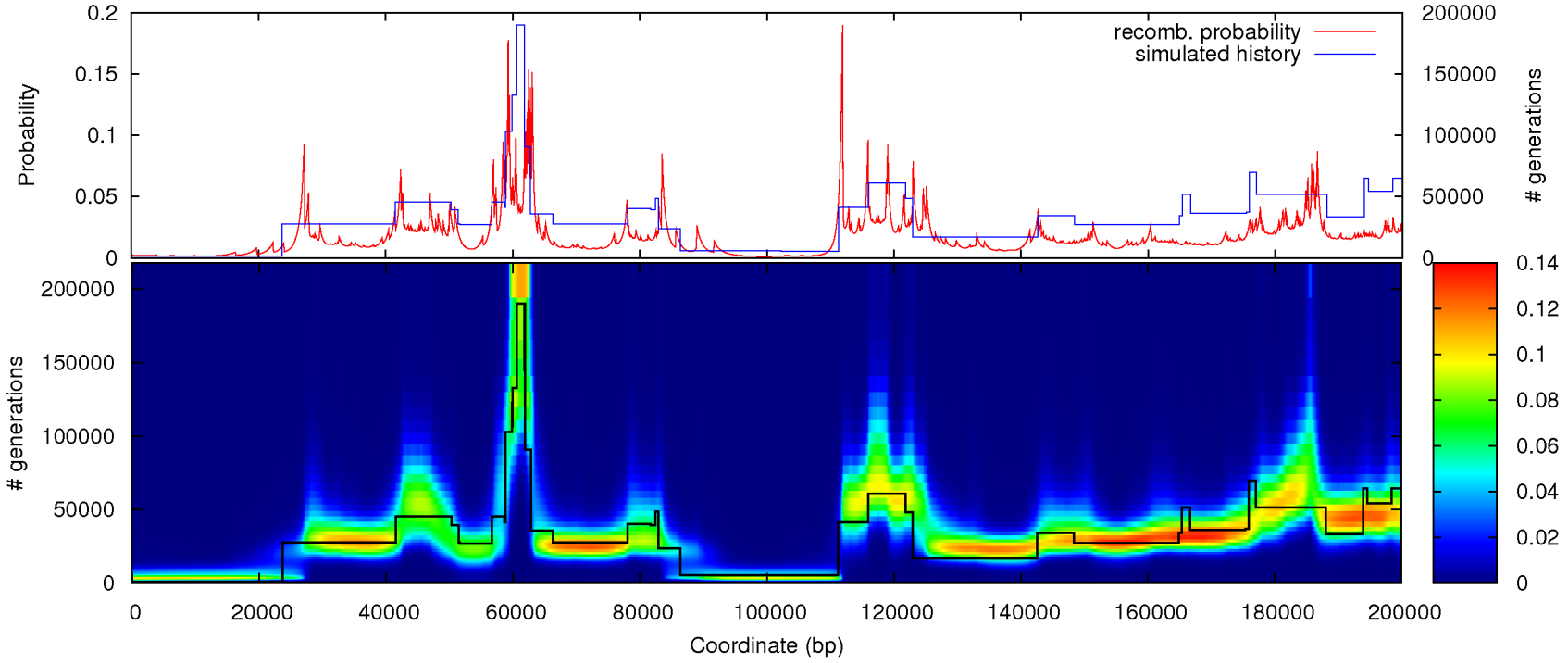
  data to the left of x|TMRCA at $x = t$

  is independent of

  data to the right of x|TMRCA at $x = t$

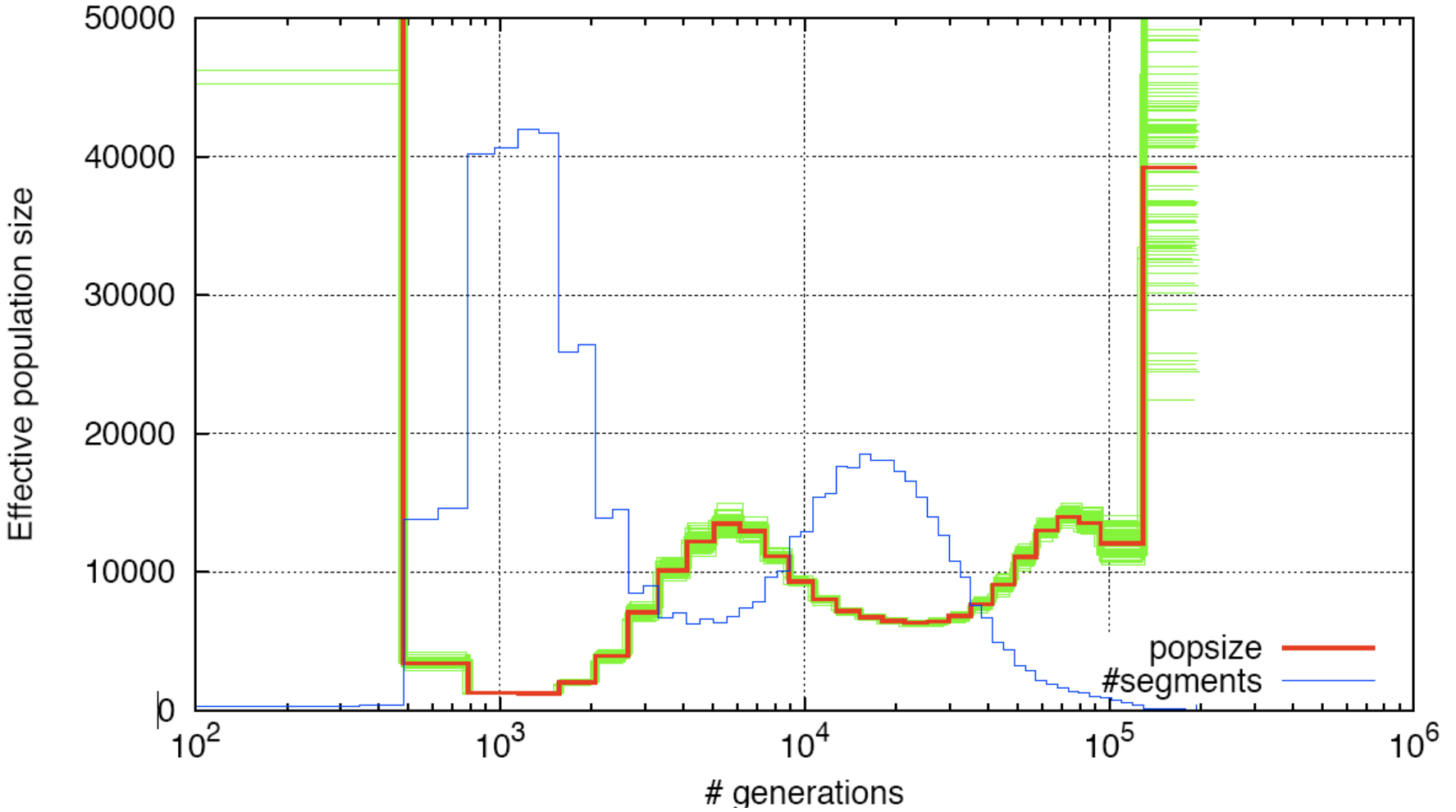
- For standard mixing populations this is a very good assumption
  - Sequentially Markovian Coalescent approximation, McVean & Cardin 2005

PSMC-HMM reconstructs individual history

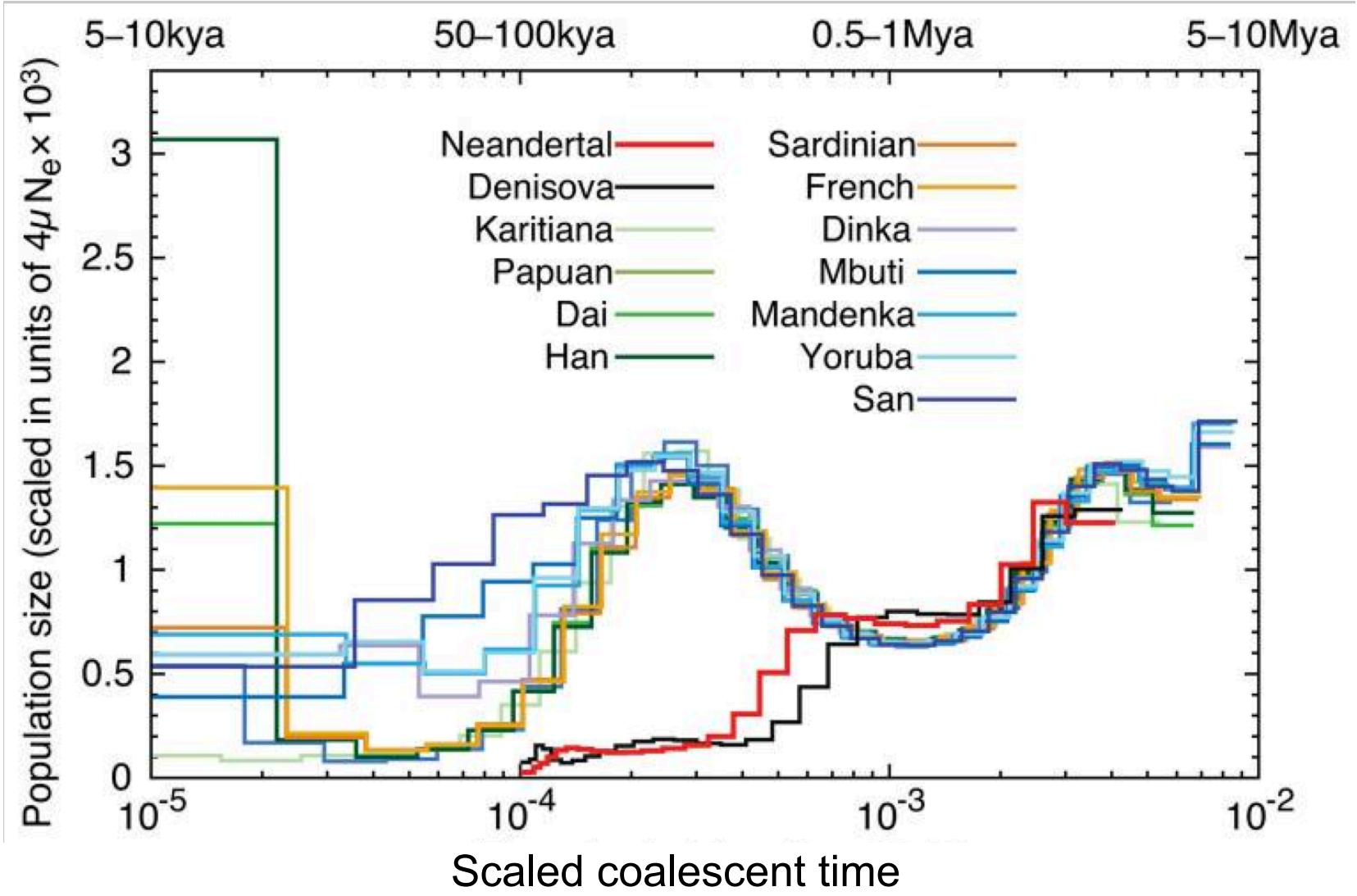


- Pairwise Sequentially Markovian Coalescent – Hidden Markov Model
- Data simulated using ms (Hudson)
- Model the coalescent time *t* by e.g. 50 discrete bins, spread logarithmically

Single human genome with bootstrap



Human population history, with Neanderthals



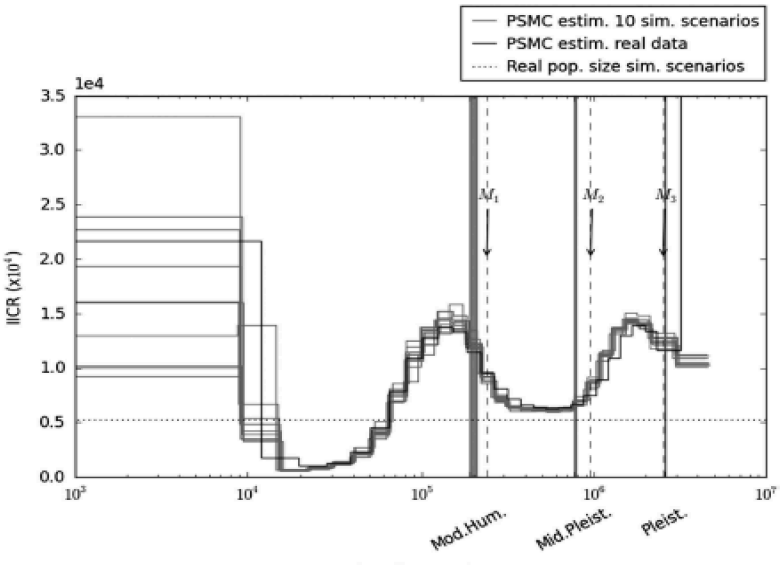
Heng Li

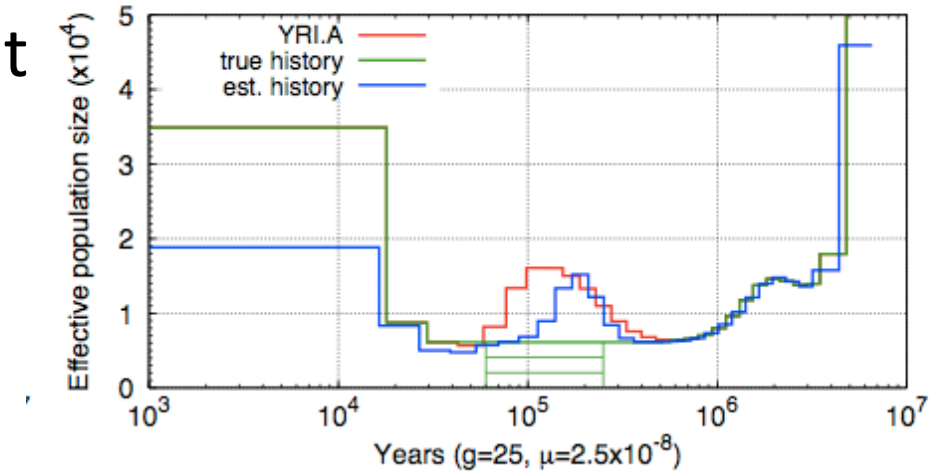
Advances since the original PSMC

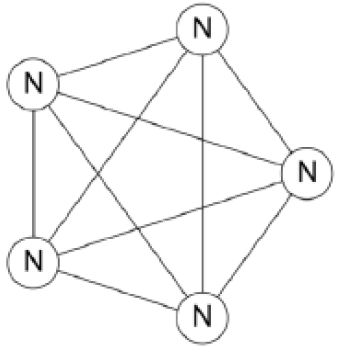
1. Use SMC' model which correctly handles recombinations coalescing back to the same ancestor (Schiffels, …)
   - Minor tweak to equations, but significant
   - Can now fit recombination:mutation ratio
   - Implemented in MSMC/MSMC2
2. Time speedup: linear not quadratic in number of time slices (Harris, ... Song, 2014)

Coalesecent $N_e(t)$ reflects ancestral structure as well as population size

- PSMC actually measures $\lambda = 1/$*coalescence rate*
- Structure can also change coalescence rate
  - Li & Durbin supplement
  - Olivier Mazet…Chikhi

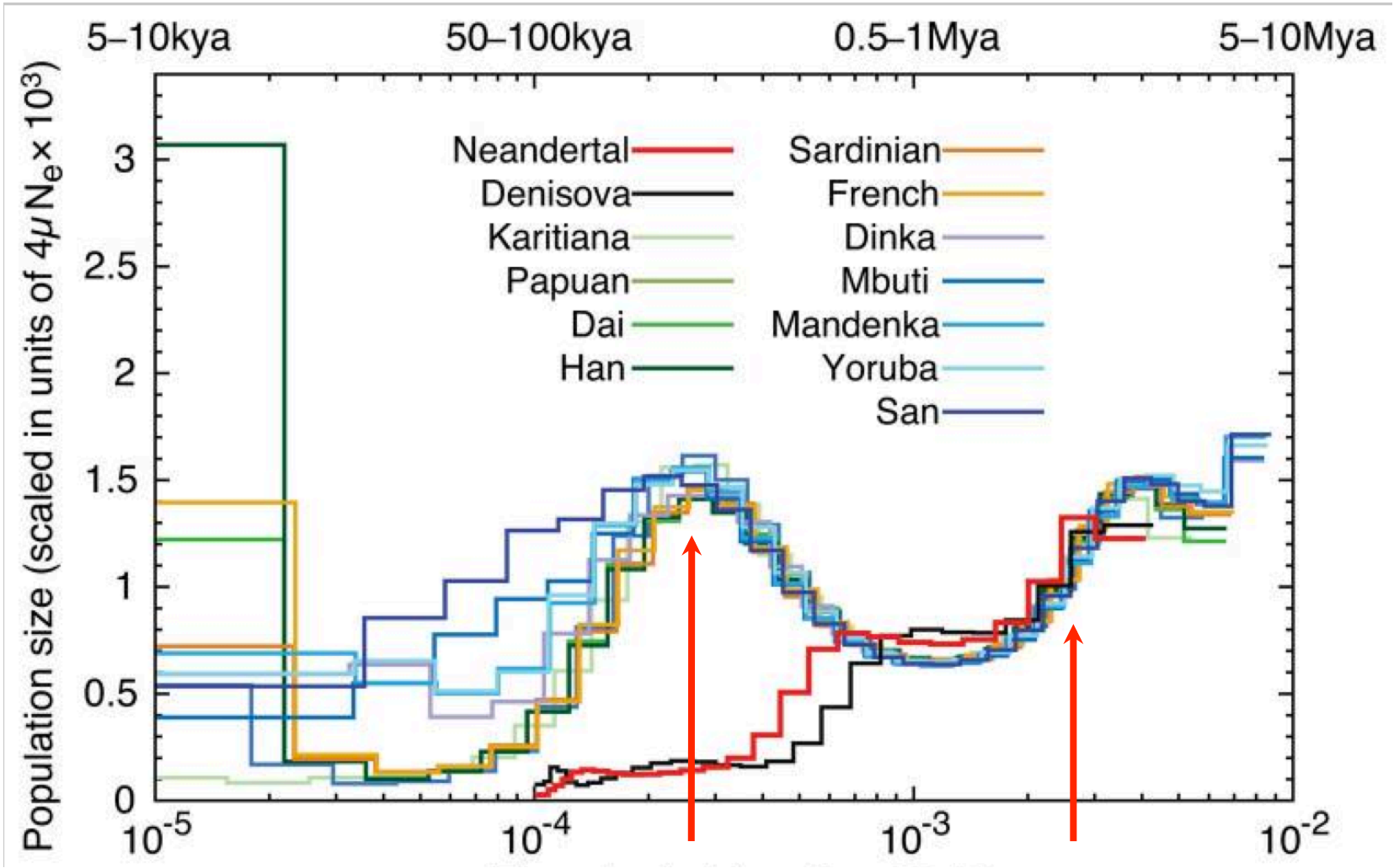






N-island model
Migration between islands controls coalescent rate

Human population history, with Neanderthals

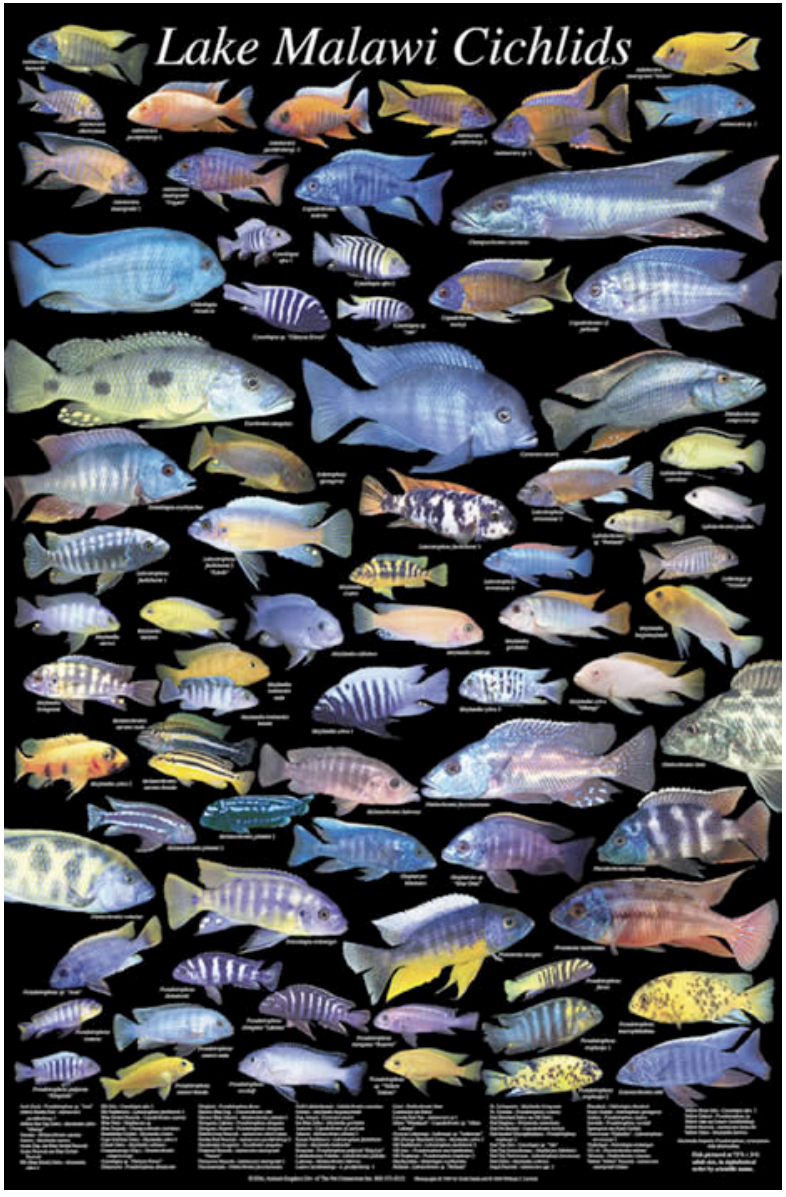


rise of anatomical modern humans 1-200kya

origin of *Homo* 1.5-2Mya

Heng Li

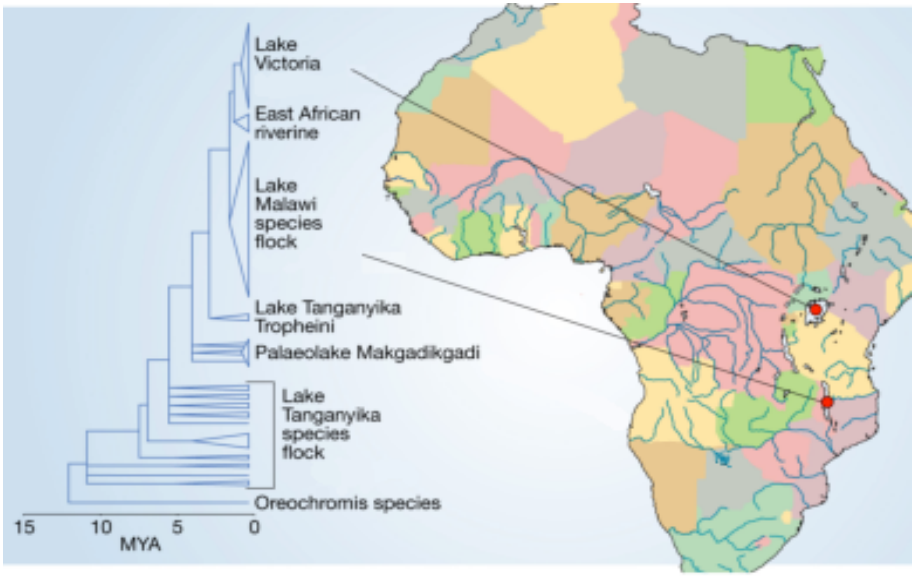
Brief introduction to another system



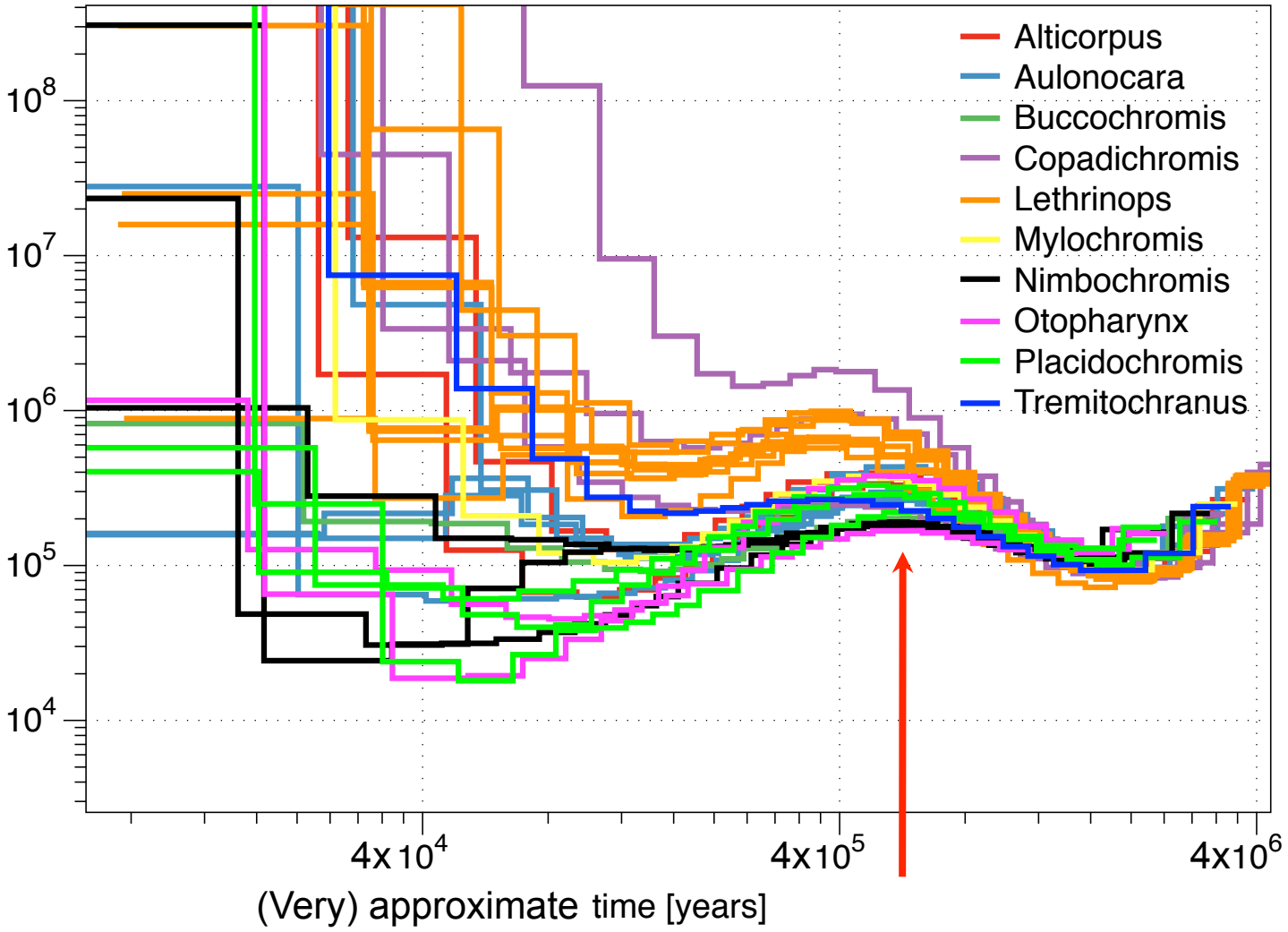
Dramatic recent radiations of haplochromime cichlids in the African rift valley great lakes

- Lake Malawi ~500 species within last 1M years

So far we have sequenced ~80 species at 15-20x coverage



Lake Malawi cichlid PSMC



Is structure associated with speciation?

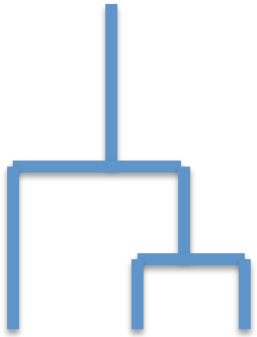
- Perhaps
  - Ideas of hybrid speciation, reuse of alleles selected in different environments, hybrid swarms and gene flow
- But this is another talk…

Might structure be (partly) identifiable in the PSMC model?

- The inferred values N(t) have dimension T, the number of time bins
- But the transition matrix M has dimension T^2
- Currently we derive M from N by theory assuming panmixia
  - Is there a richer theory for structured populations?
  - How to parameterise structural complexity S(t) at time t, with associated theory for M(N,S)
- Or can we fit the transition matrix M unconstrained?
  - Then search for evidence of structure within it
  - And or do goodness of fit?

Adding another sequence

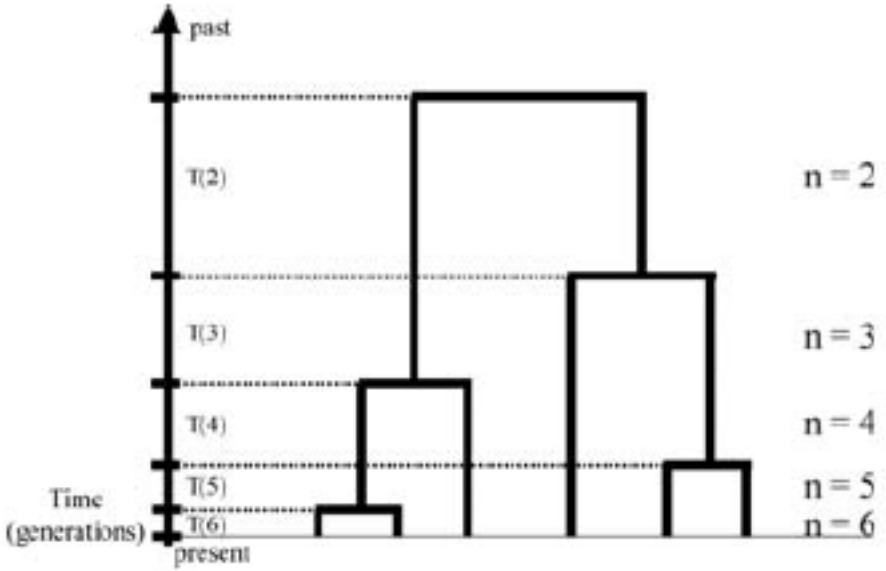
- Chance of coalescence per generation from three sequences is 3/N



- Once we have a coalescence we are back to the situation with two sequences

- From *i* sequences chance is *i*(*i*-1)/2N

Digression: Coalescent model (Kingman, 1980) A distribution on trees



- T(i) ~ exponential with mean 2N/i(i-1)

	E[*T*(*i*)]			E[*T*(*i*)/*N*]		
i	*N*=100	*N*=200	*N*=1000	*N*=100	*N*=200	*N*=1000
6	6.7	13	67	0.07	0.07	0.07
5	10	20	100	0.10	0.10	0.10
4	17	33	167	0.17	0.17	0.17
3	33	67	333	0.33	0.33	0.33
2	100	200	1000	1.00	1.00	1.00

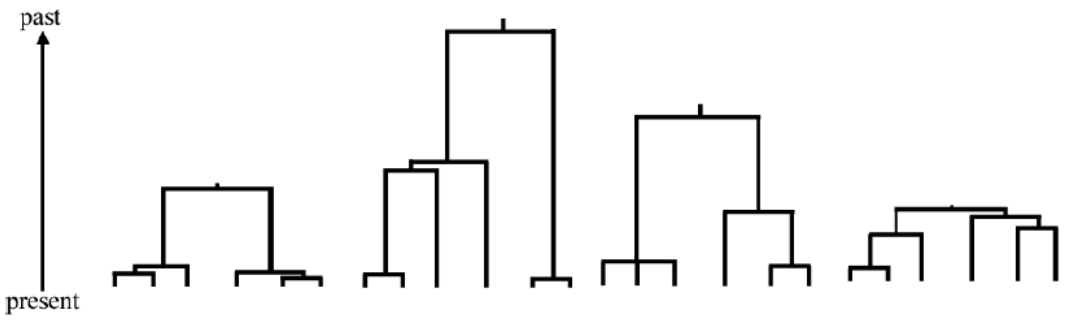
Properties of the coalescent

- As we add extra sequences, they are increasingly likely to coalesce very fast, and increasingly unlikely to affect the full TMRCA

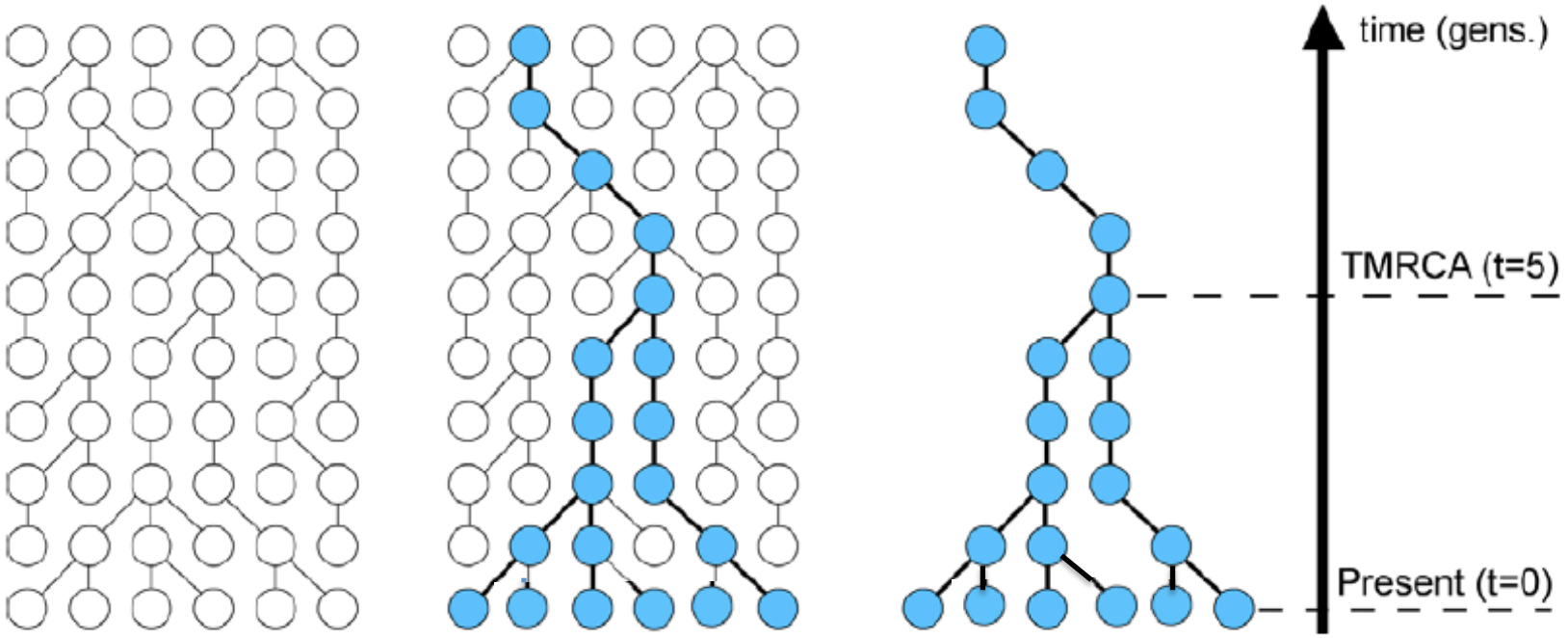
$$E[TMRCA] = \sum_{i=2}^{n} E[T(i)] = 2\left(1 - \frac{1}{n}\right)$$

The expected height of the tree for many samples is only twice that with two samples

- Trees are very variable
  - E.g. 4 samples on 6 leaves



Relationship between forwards in time (Wright-Fisher) and backwards in time (Coalescent) models



Population evolution forwards

Coalescent tree backwards

The coalescent tree describes a *sample* from the forward process

Kingman coalescent generates an “exact” sample from Wright-Fisher

Genetic variation in a sample

- Mutations occur at random on the tree
  - Separation of sources of randomness
    - Random *demography* tree structure from coalescent
    - Random *sampling* of mutations on the tree

Let S be the number of mutations = segregating sites

$$E[S] = 2\mu \sum_{i=2}^{n} iT(i)$$

$$E[S] = \frac{\theta}{2} \sum_{i=2}^{n} iT(i)$$

$$E[S] = \frac{\theta}{2} \sum_{i=2}^{n} i \frac{2}{i(i-1)}$$

$$E[S] = \theta \sum_{i=1}^{n-1} \frac{1}{i}$$

Watterson's theta

$$\hat{\theta}_S = \frac{S}{\sum_{i=1}^{n-1} \frac{1}{i}}$$

$E(S) \sim \theta \log n$

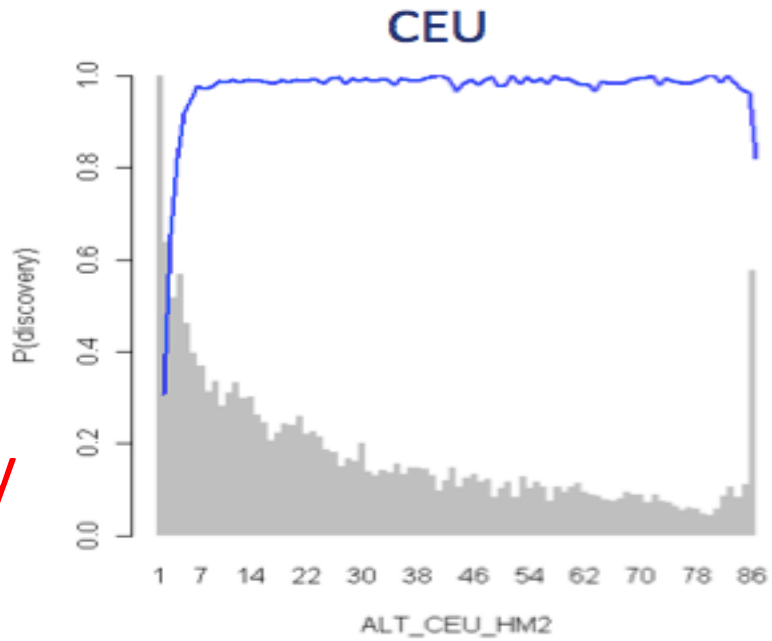
Distribution of variant allele frequencies

- Density of mutations with frequency *i* in a sample of *n* is θ/i

$$E[S] = \theta \sum_{i=1}^{n-1} \frac{1}{i}$$

- *1/f* distribution of population allele frequencies

site frequency spectrum SFS

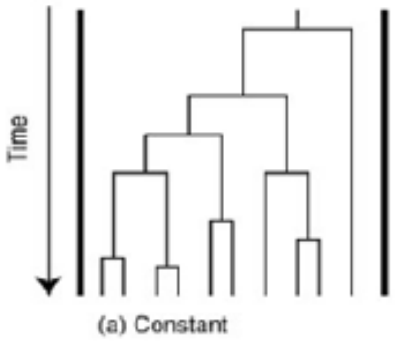


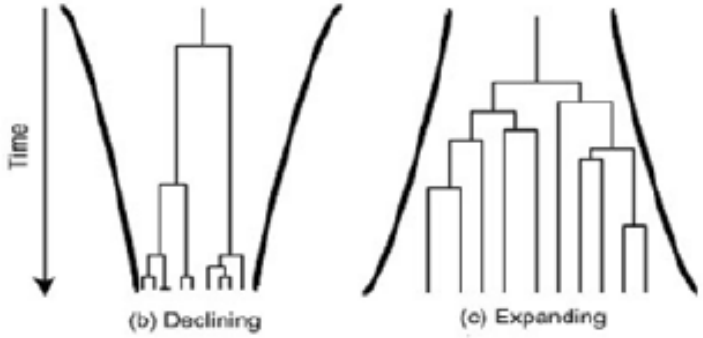
- Population minor allele frequency distribution of a difference observed between two sequences is flat
  - Probability $(1/f).2f(1-f) = 2(1-f)$, folded at ½ is 2

Relaxation of assumptions (1)

- E.g. change in population size changes the site frequency spectrum

  This is the basis of SFS-based demography inference





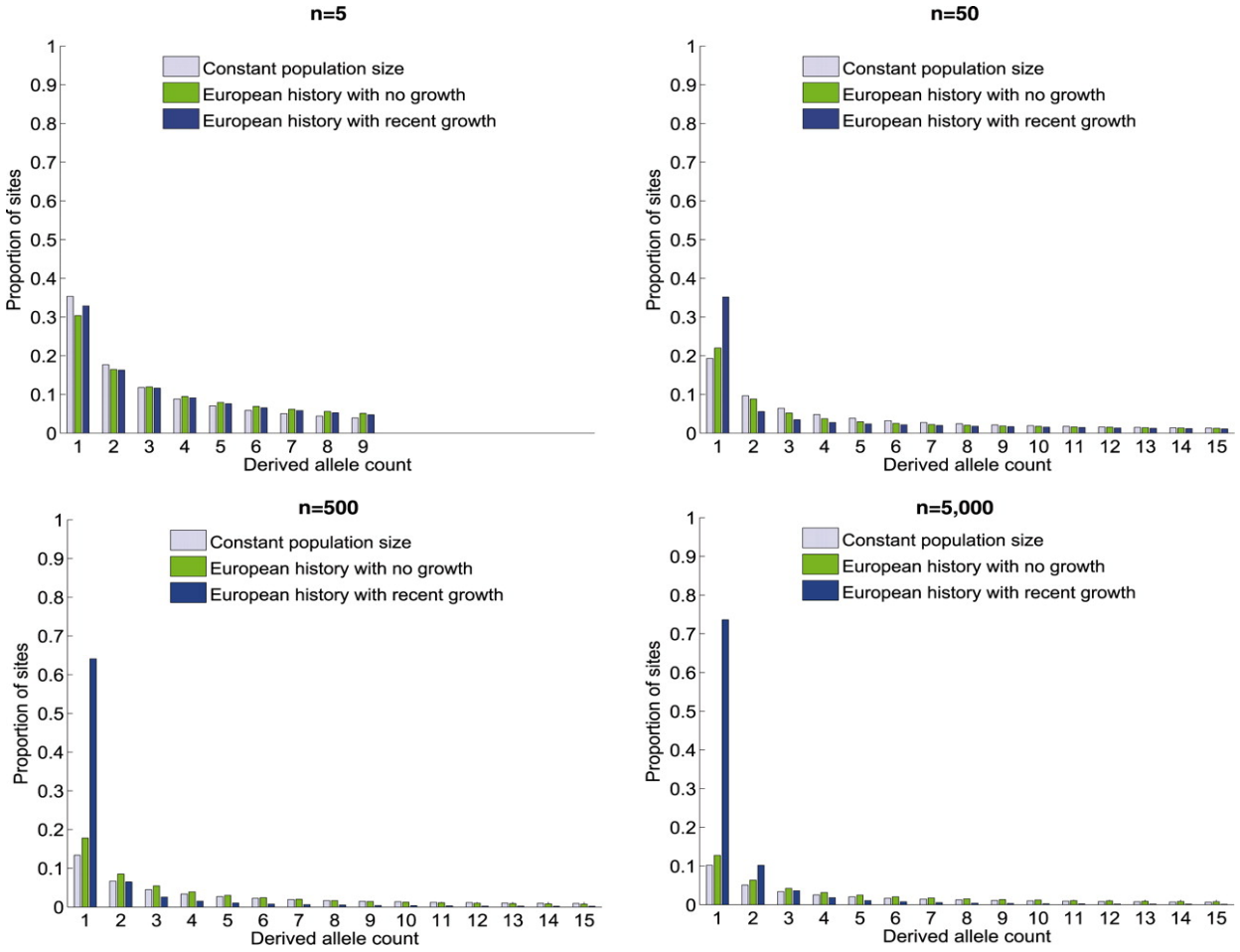
D > 0 D < 0

- Tajima's D

$$D = \frac{\hat{\theta}_\pi - \hat{\theta}_S}{\sqrt{Var(\hat{\theta}_\pi - \hat{\theta}_S)}}$$

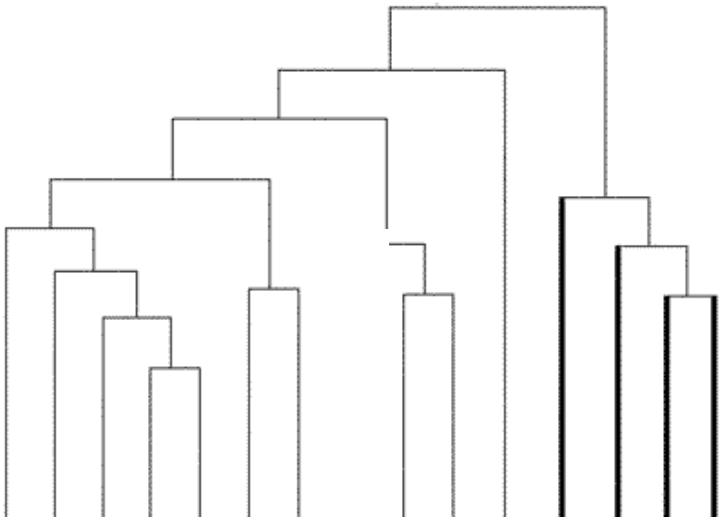
  - Sensitive to number of rare mutations, so change in N_e
  - If D is positive there is a deficiency of rare mutations
    - Excess recent coalescences, recent small N_e - *selection*

Fig. 2 The expected site frequency spectrum (SFS) of the derived allele (the new mutation arisen in the population) for three different demographic models: (i) a population that has been of constant size throughout history; (ii) a model previously fit to the derived allele frequency spectrum of Europeans, which includes an out-of-Africa population bottleneck and a second, more recent, population bottleneck (21); and (iii) the same two-bottleneck model of European history with the addition of recent exponential growth from a population size of 10,000 at the advent of agriculture to an extant effective population size of 10,000,000, which amounts to 1.7% growth per generation during the last 400 generations.



A Keinan, A G Clark Science 2012;336:740-743

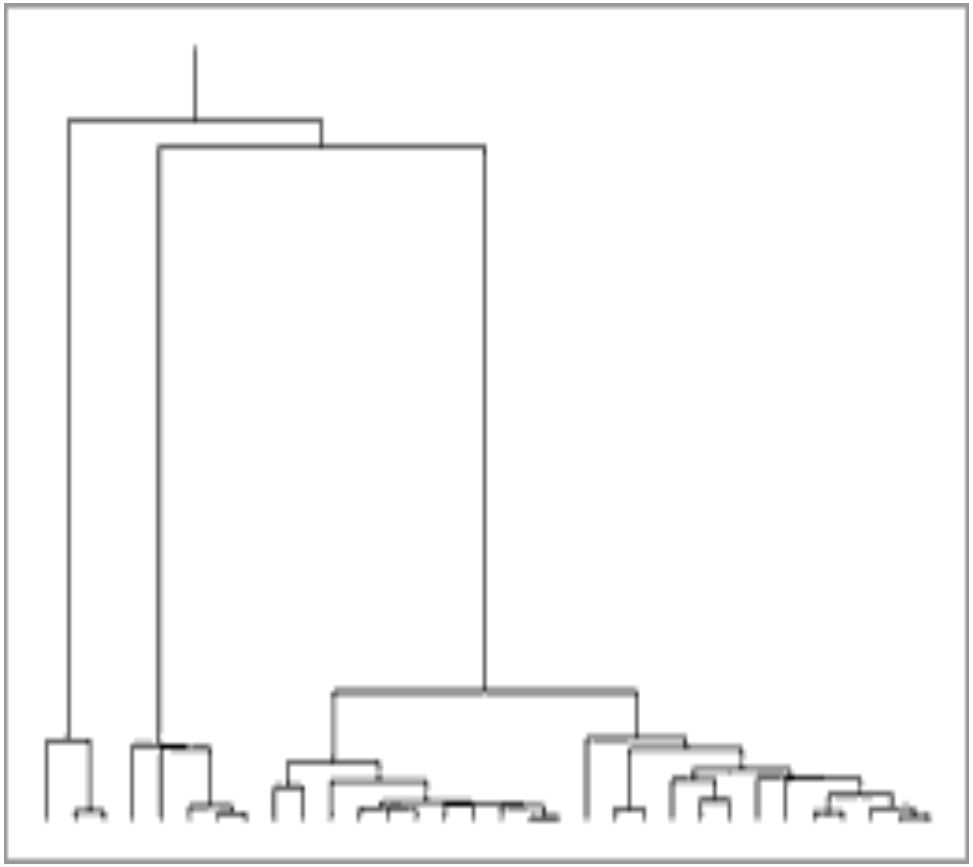
Individuals in human outbred populations still carry many variants not in the large sequence data sets (1000 Genomes etc.)



- Exponential population growth in last 10,000 years gives long tips to the tree
- In “big” populations, tips are hundreds of generations long, so tens of thousands of private variants per sample, hundreds functional

This behaviour is very dependent on population structure.

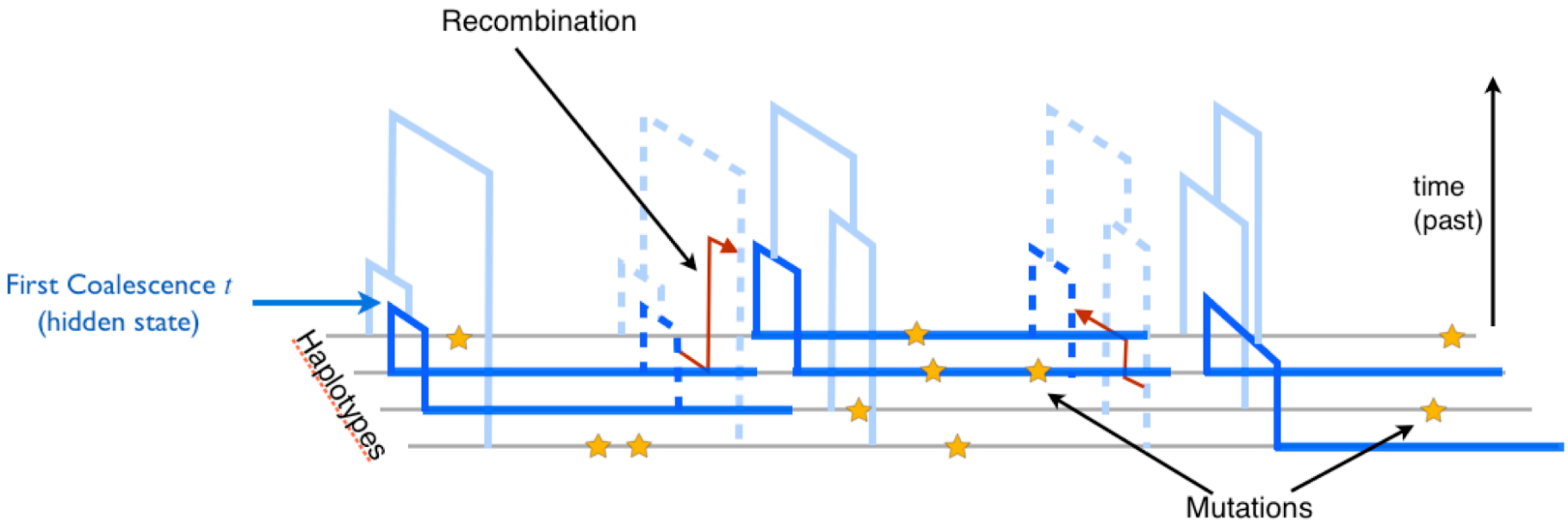
In genetic isolates the recent effective population size is smaller, and the tips are shorter



What about recombination?

- If points on the genome are very close, e.g. adjacent, they share the same tree
- If points are very far, their trees are sampled from the coalescent independently
- What happens in between?
- A recombination in the ancestor of a modern sequence made it out of two separate sequences, one contributing to the left and one to the right

Recombination changes the tree as you move along the sequence



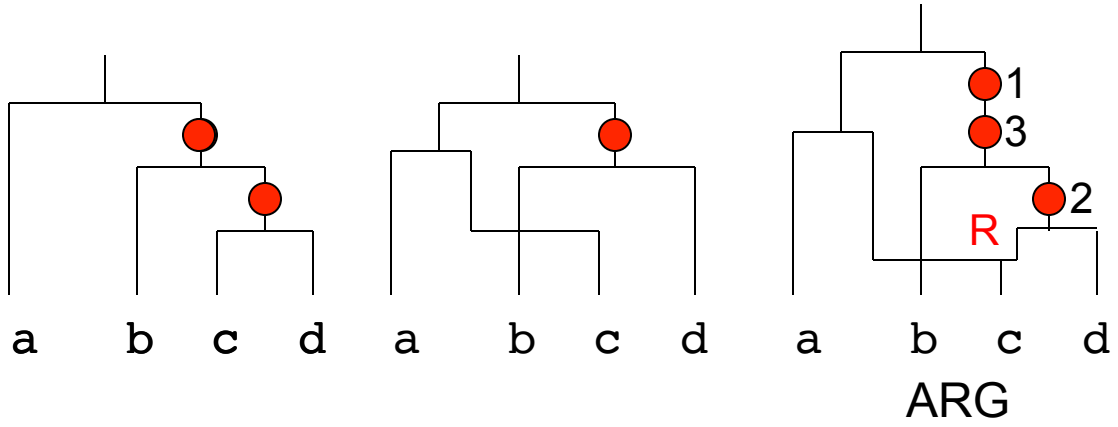
Typically recombination rate is comparable to or larger than the mutation rate: both ~10^{-8} /bp /gen in human

So "gene tree" varies every site in mixing populations

Ancestral Recombination Graph (ARG)

- The *Ancestral Recombination Graph* describes the way that individual sequences in a population are related
  - At a locus, sequences are related by a tree
  - Ancestral recombinations change the tree as you move along the chromosome

```
a  ..C..G..A..   0 0 0
b  ..T..G..C..   1 0 1
c  ..T..A..A..   1 1 0
d  ..T..A..C..   1 1 1
```



"Prune and graft" operation going left to right

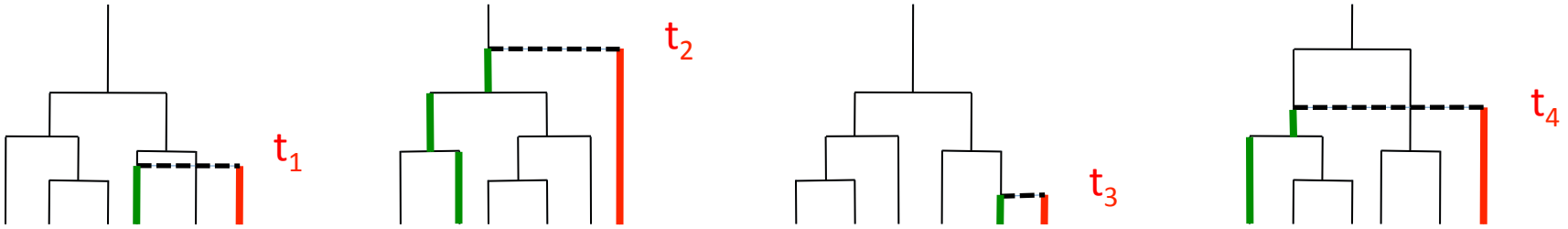
Coalescent with recombination

- ARG is a structure (data type)
- The probability distribution over ARGs that arises when recombination is added to the standard (Wright-Fisher) model is called the *Coalescent with Recombination*
  - Hudson's *ms* software is the classic simulator
  - New *msprime* from Jerome Kelleher MUCH faster
- Now two possible events going backwards in time
  - Coalescence: which merges two sequences
    - For i sequences, rate is $i(i\text{-}1)/2N$
  - Recombination: which splits a sequence into two
    - For i sequences, rate is $iL\rho$

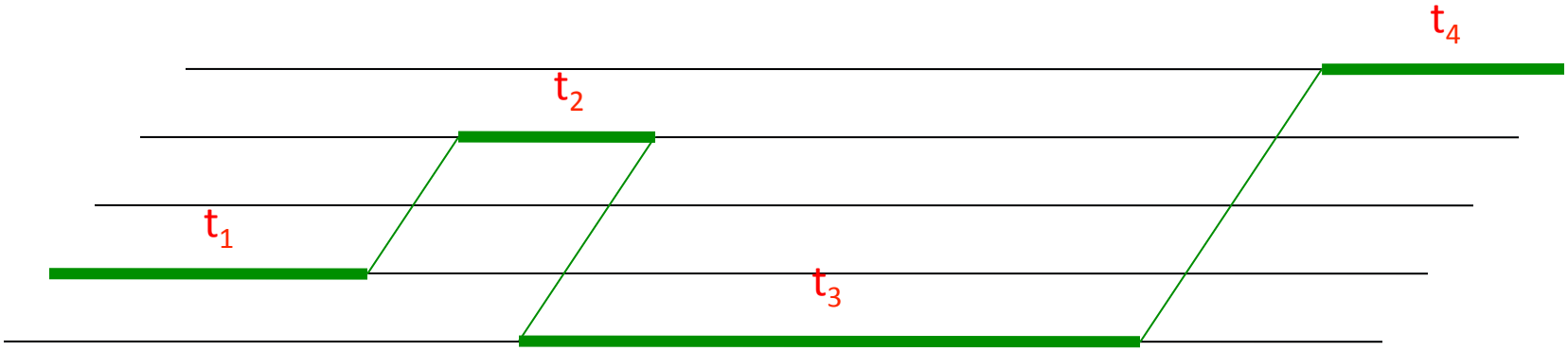
Extending to multiple sequences

- The recent time limit of ~20kya for PSMC is set because we run out of recent coalescences between two haplotypes
- If we add more haplotypes, then there are more recent coalescences and we could look at more recent history
- But, … the hidden state is then a tree (with branch lengths): impractical to model fully
  - MCMC is notoriously difficult

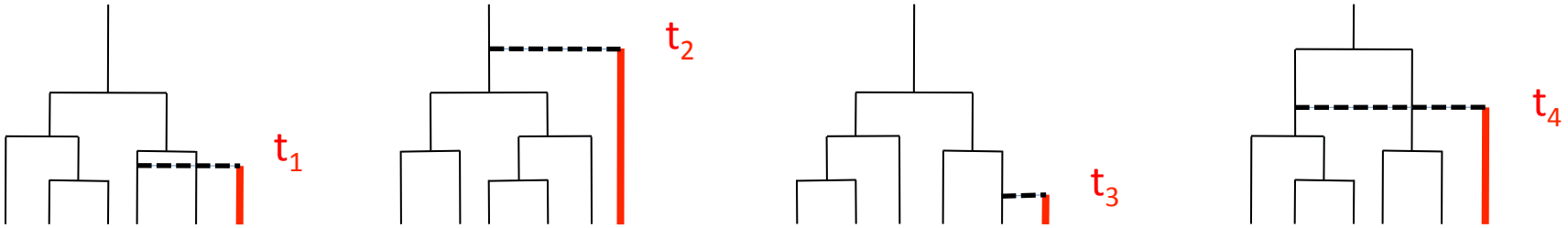
Option 1: First coalescence of one sequence to the tree of the others



- This is related to the Li and Stephens model (or Stephens and Donnelly) – chromopainter



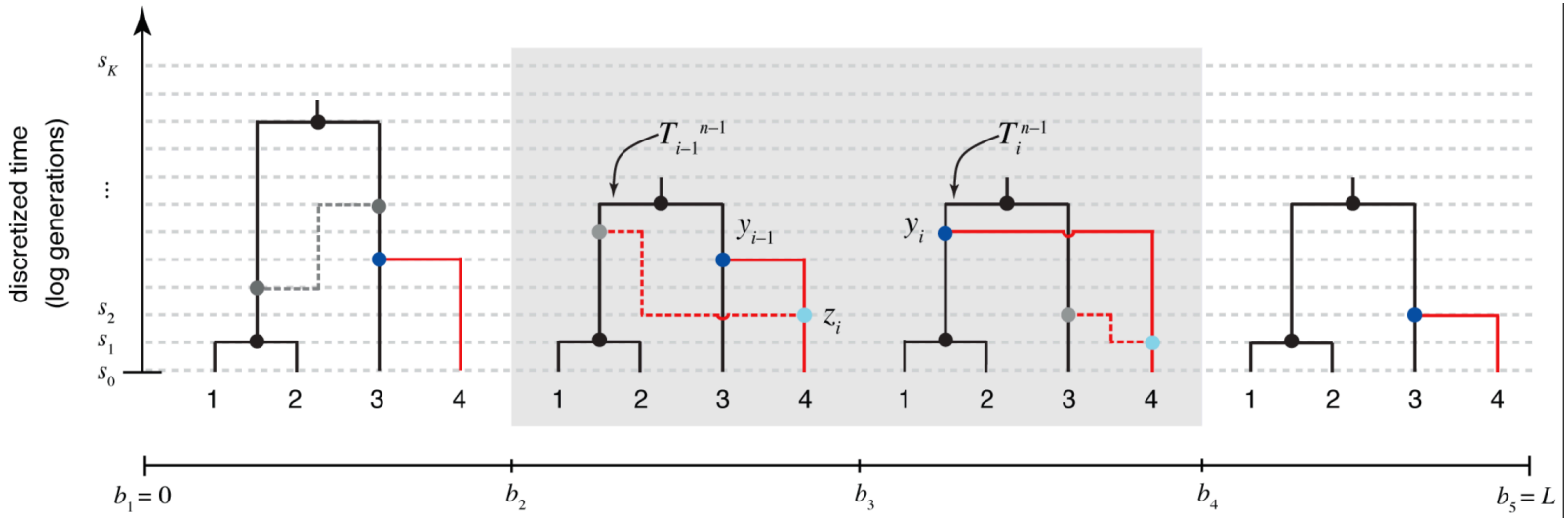
Problem: Coalescence of chosen sequence to the others depends on the number of lineages M(t) remaining at time t



- M(t) is a random variable, and we need the entire history of M(t) to calculate transition probabilities q
- Huge increase in state space and/or this breaks Markov assumptions

MCMC approach: ARGweaver

- Repeatedly remove a sequence* and add it back, sampling conditional on remaining ARG
- HMM: sample with forward-backward algorithm



- Costly – use for inference given history

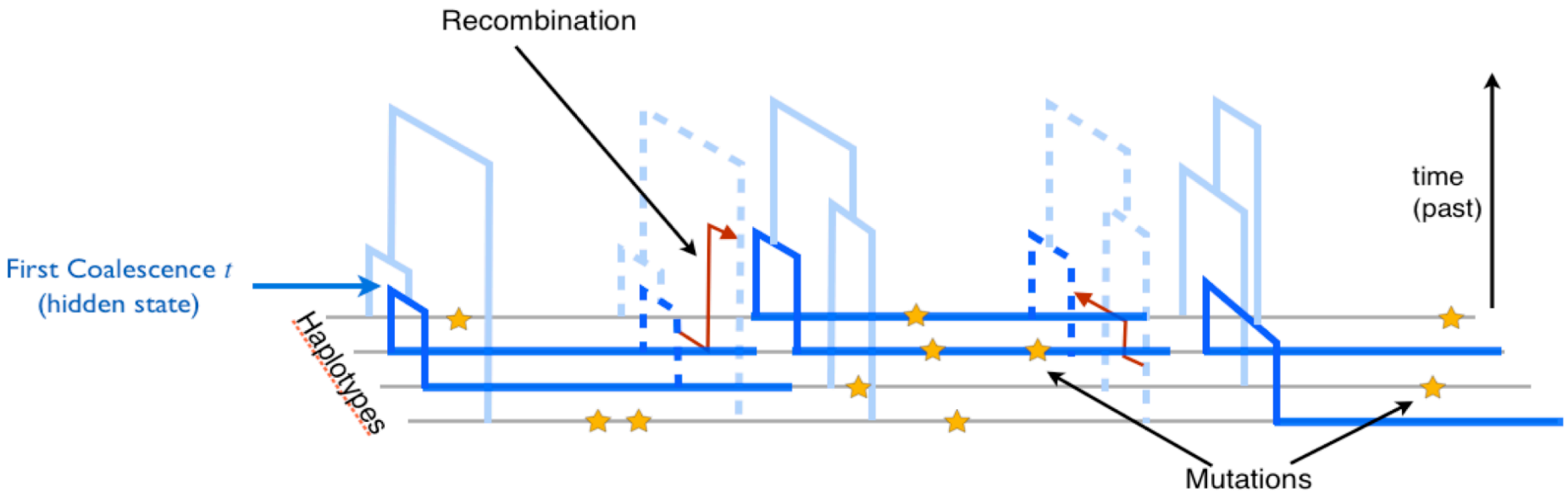
Genome-wide inference of ancestral recombination graphs

Rasmussen MD, Hubisz MJ, Gronau I, Siepel A. *PLoS Genet.* 10:e1004342 (2014)

Option 2: first coalescence between any pair

- This remains (approximately) Markov
- State space is $O(M^2T)$ – pair of states and time they coalesce
  - But transition updates are only $O(M^2T^2)$, because transitions are memoryless
- Emissions from X_{ij} are singletons on *i* or *j*
  - Non-singletons that are discrepant between *i* and *j* wipe out density at X_{ij}

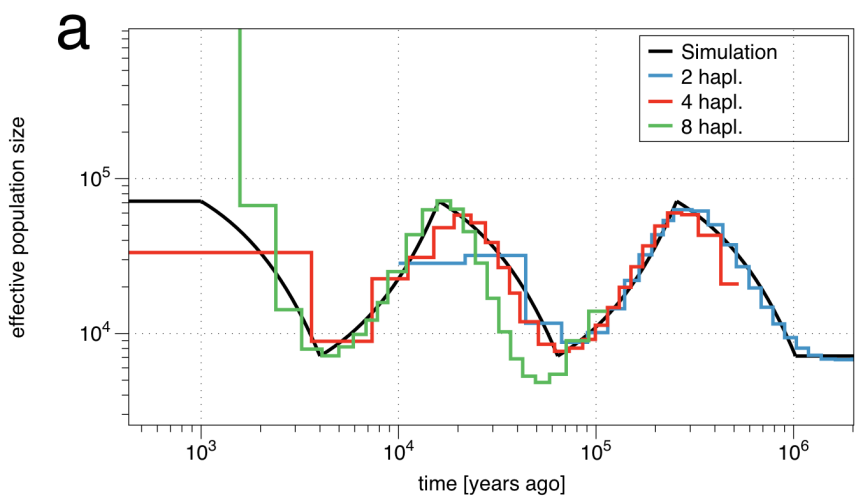
MSMC

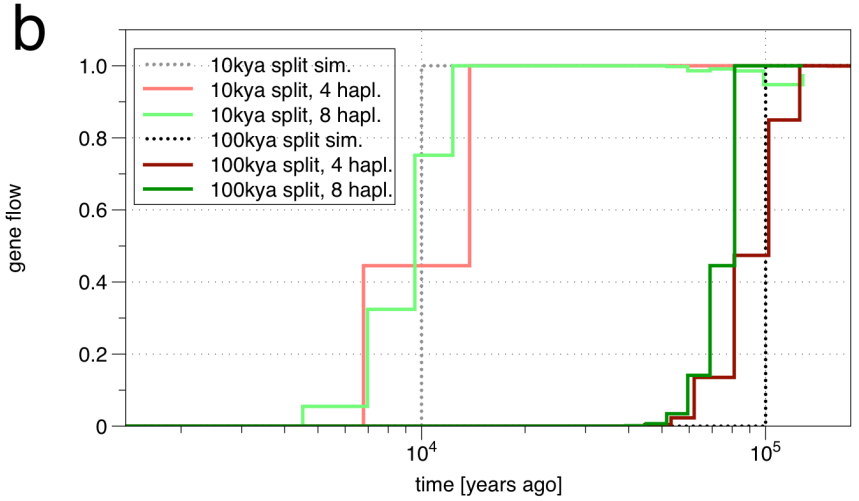


Stephan Schiffels and Durbin (Nature Genetics, 2015)

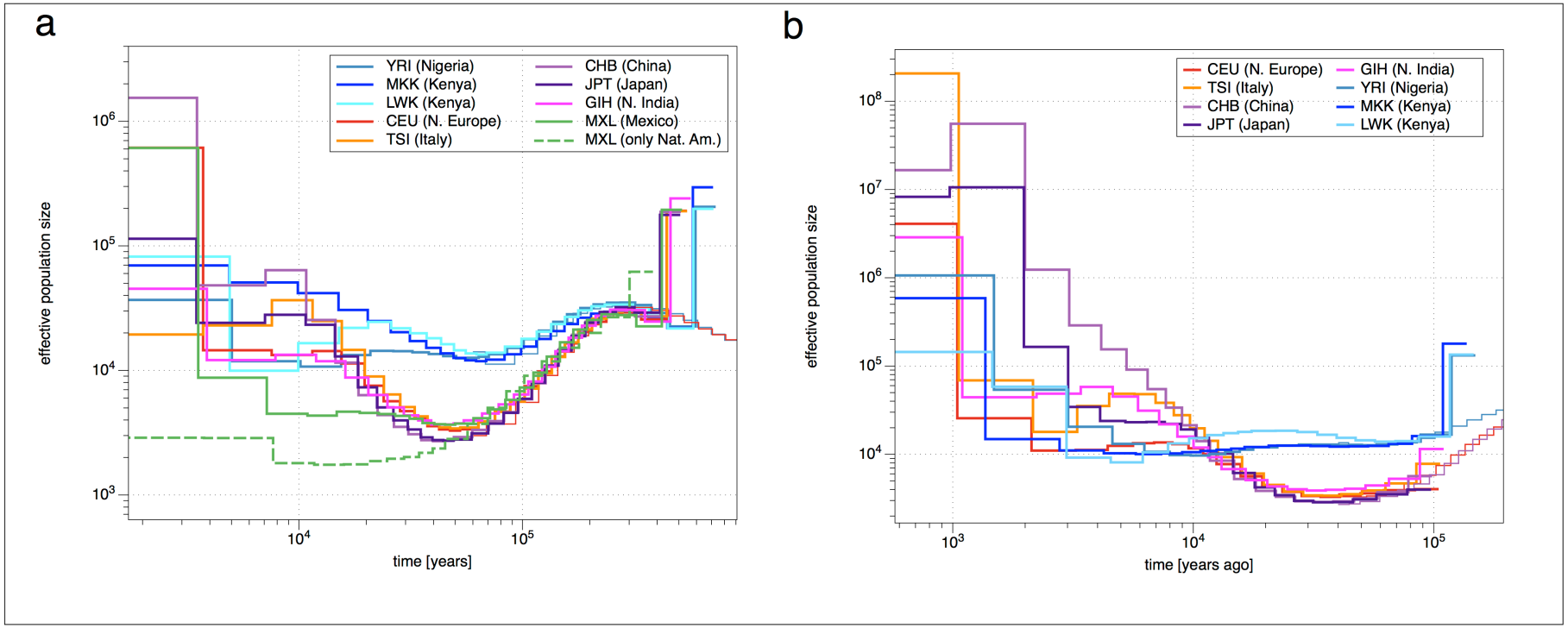
MSMC can fit both population size history and separation history

- Separation via the (scaled) ratio of coalescence between and within populations





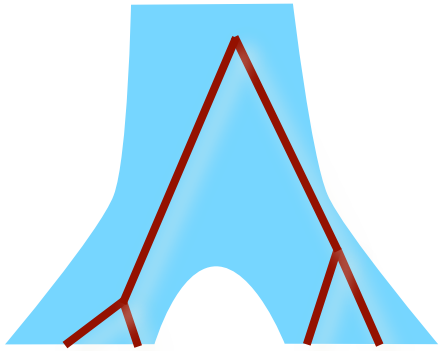
Access more recent history



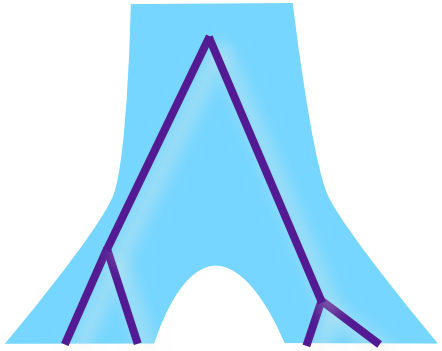
Use lower mutation rate here ~ 0.5×10^{-9}/year

Divergence between populations

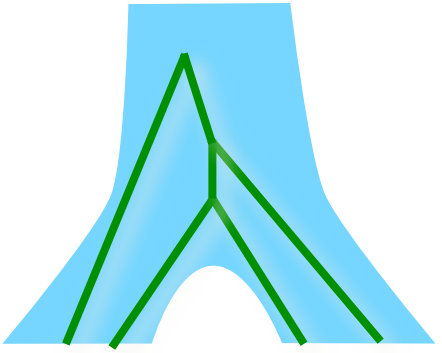
- Idea: Infer separate coalescence rates within and between populations:



First Coalescence within Population 1



First Coalescence within Population 2

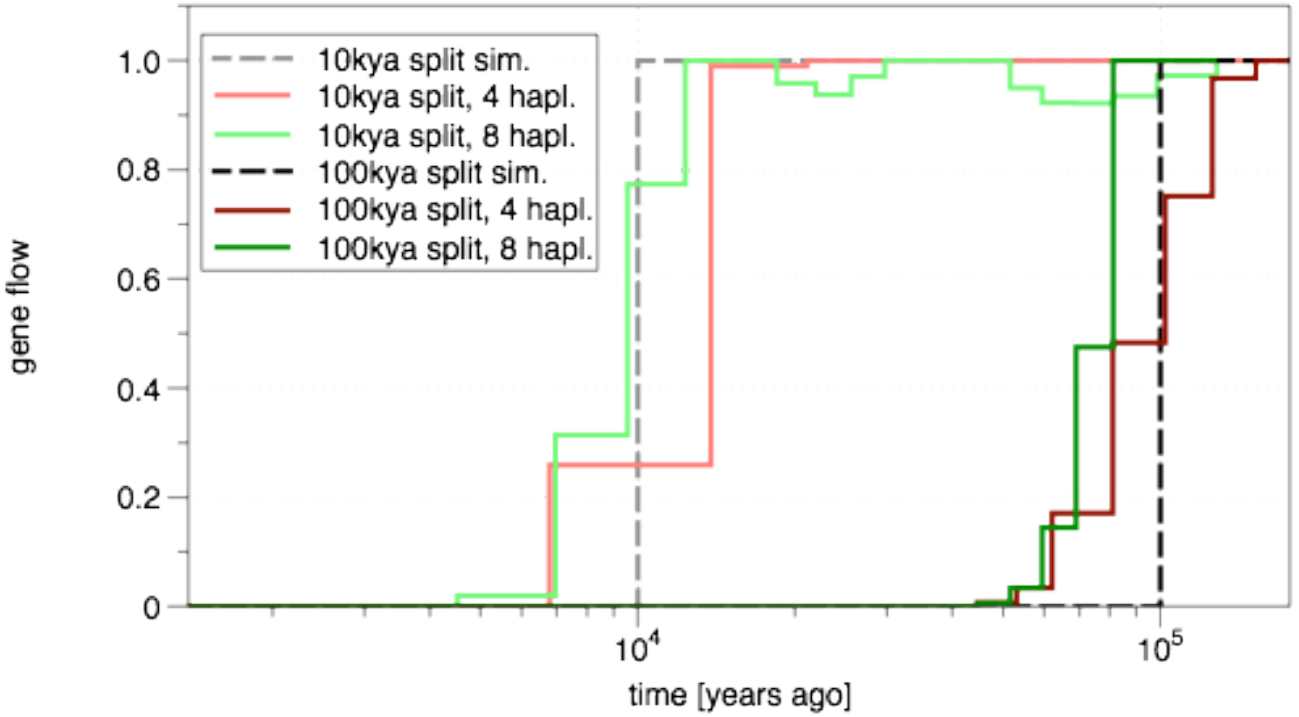


First Coalescence across both populations

- MSMC can infer separate coalescence rates within and between populations
- Given rates within populations, $\lambda_{11}(t)$ and $\lambda_{22}(t)$, and across populations, $\lambda_{12}(t)$, compute relative gene flow as ratio

$$m(t) = \frac{\lambda_{12}(t)}{[\lambda_{11}(t) + \lambda_{22}(t)] / 2}$$

Testing gene flow inference with simulated split



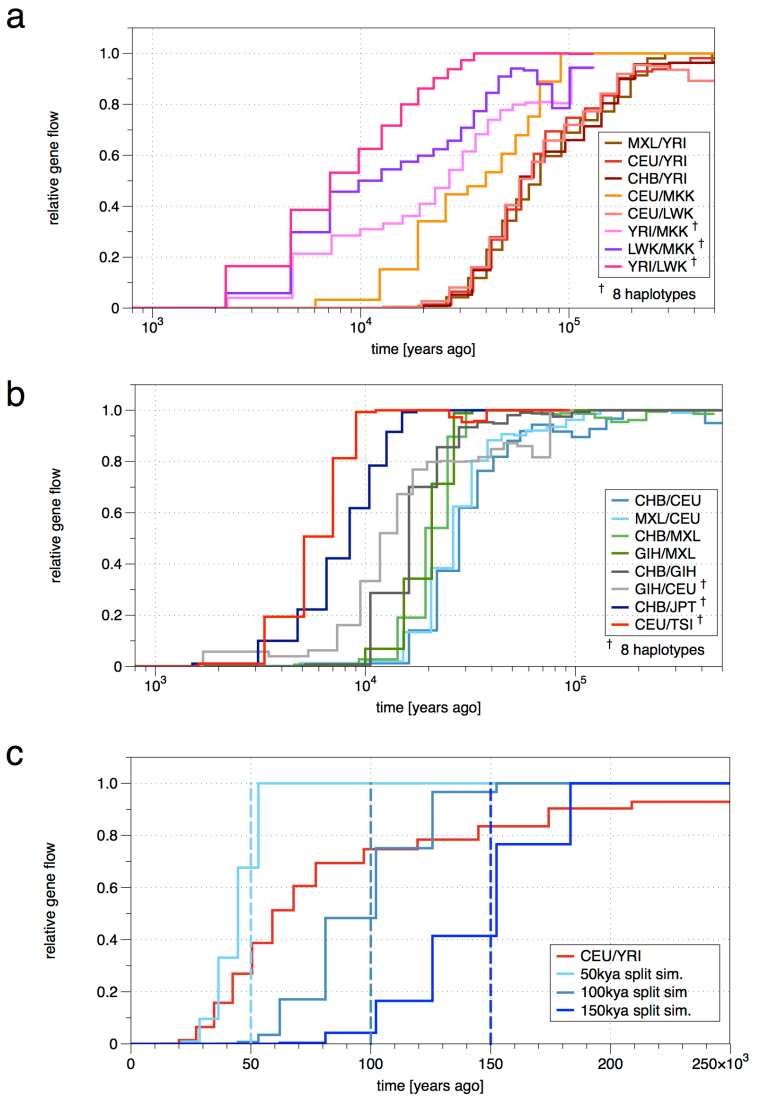
☜ m(t)=1: perfectly mixed

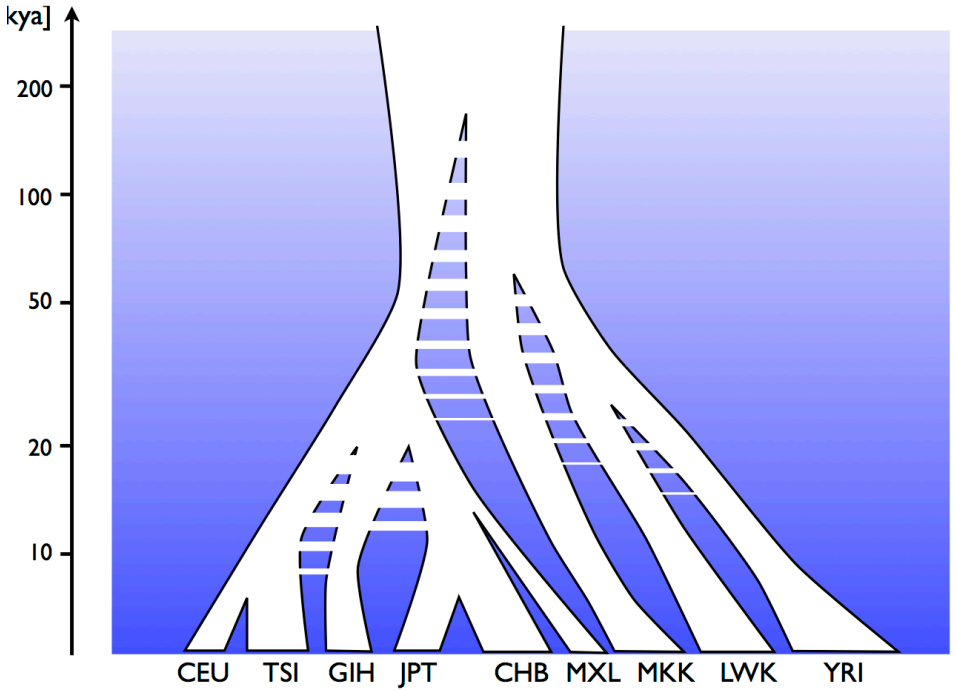
☜ m(t)=0: perfectly split

4 haplotypes: good for splits 50-200kya.

8 haplotypes: good for splits 5-50kya.

Separation history





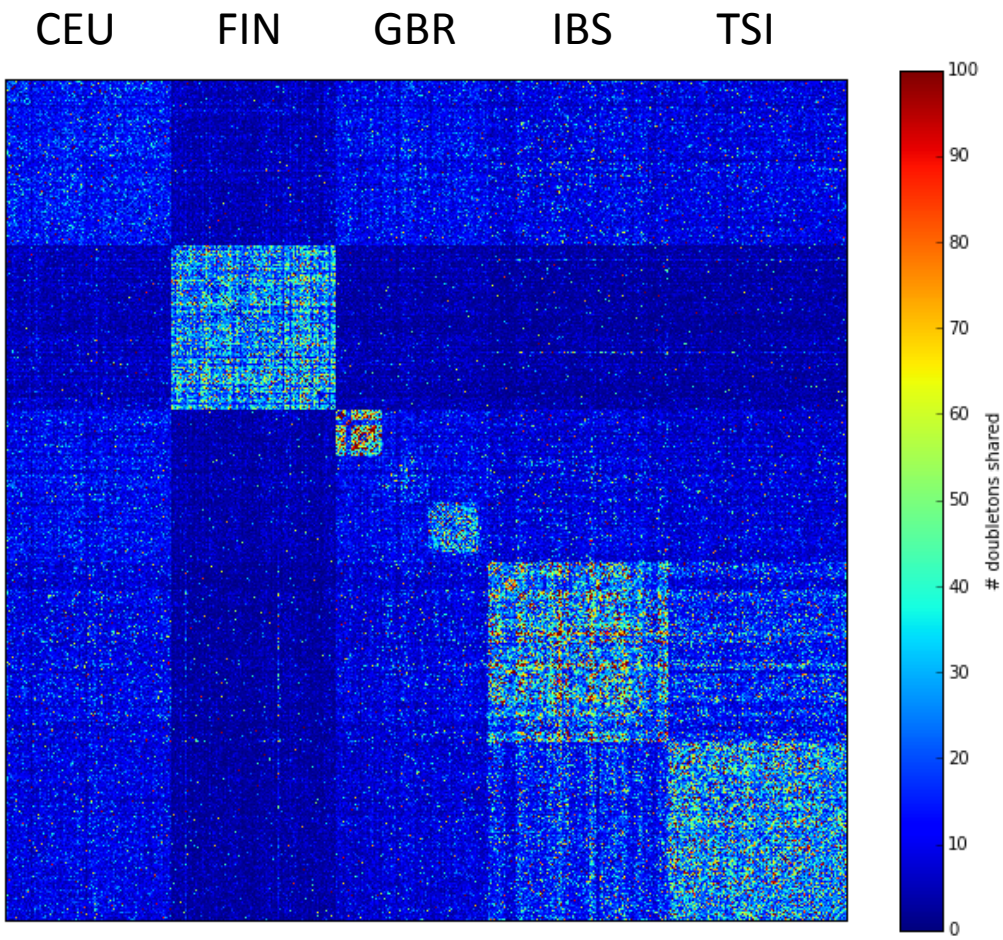
Alternatives to MSMC

- MSMC2 (Schiffels: in Malaspinas 2016/unpub.)
  - Run PSMC' on all pairs of sequences independently
  - Multiply the likelihoods – **Composite likelihood**
    - Assumes the pairs are independent, which is false
    - But gives unbiased estimation (though overconfident)
- SMC++ (Terhorst, Kamm, Song: Nat Gen 2017)
  - Pair, with p(het | **other sequences**)
  - Very cool – works even on genotype data!
  - But there are approximation problems analogous to those in MSMC – not a panacea

Using rare variants to infer demographic history

- Rare variants contain information about recent population history and structure
- Shown here: number of doubletons shared among European samples
- We would like to estimate population split times and population sizes from the frequency of rare variants

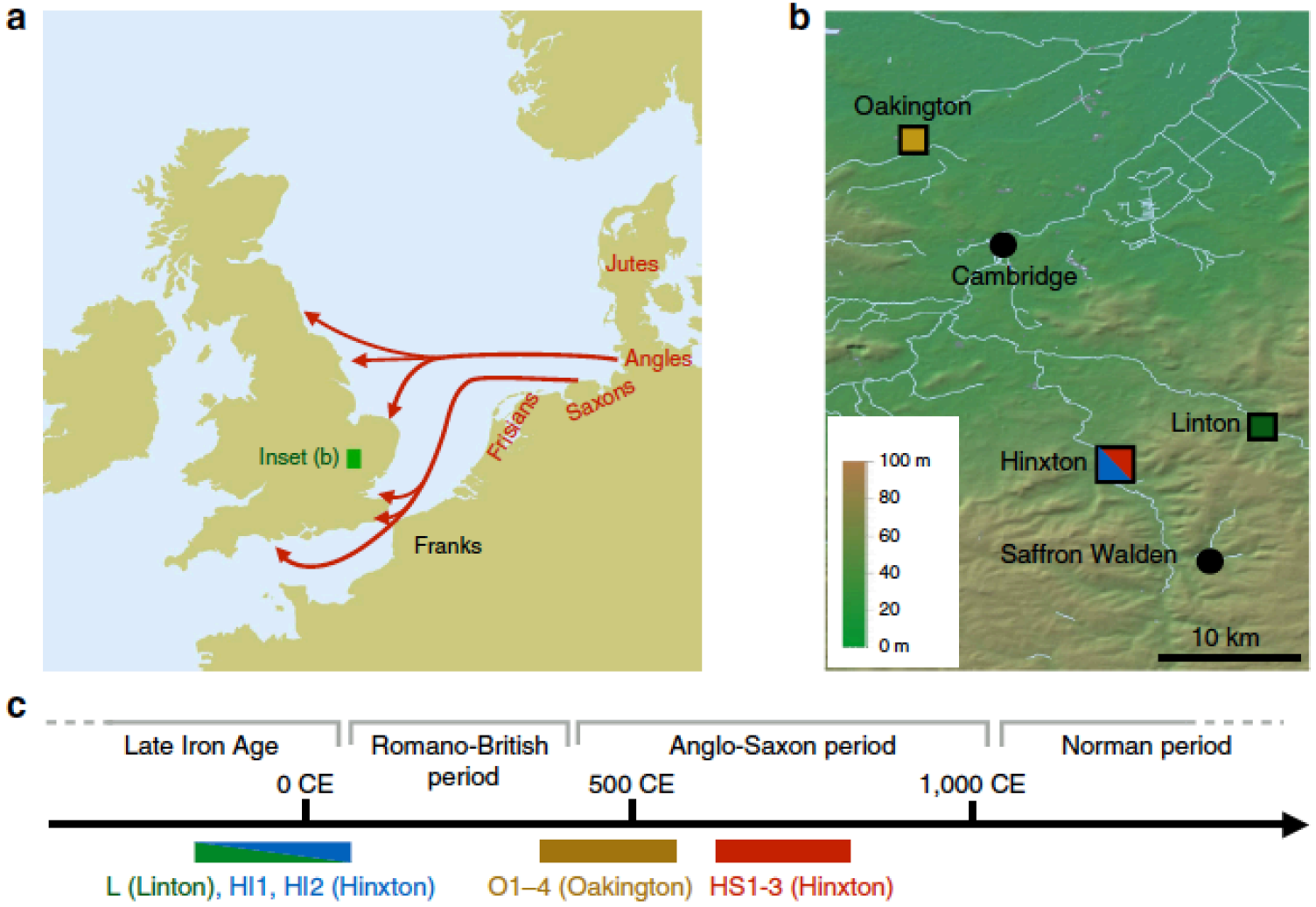
Compare to ChromoPainter data



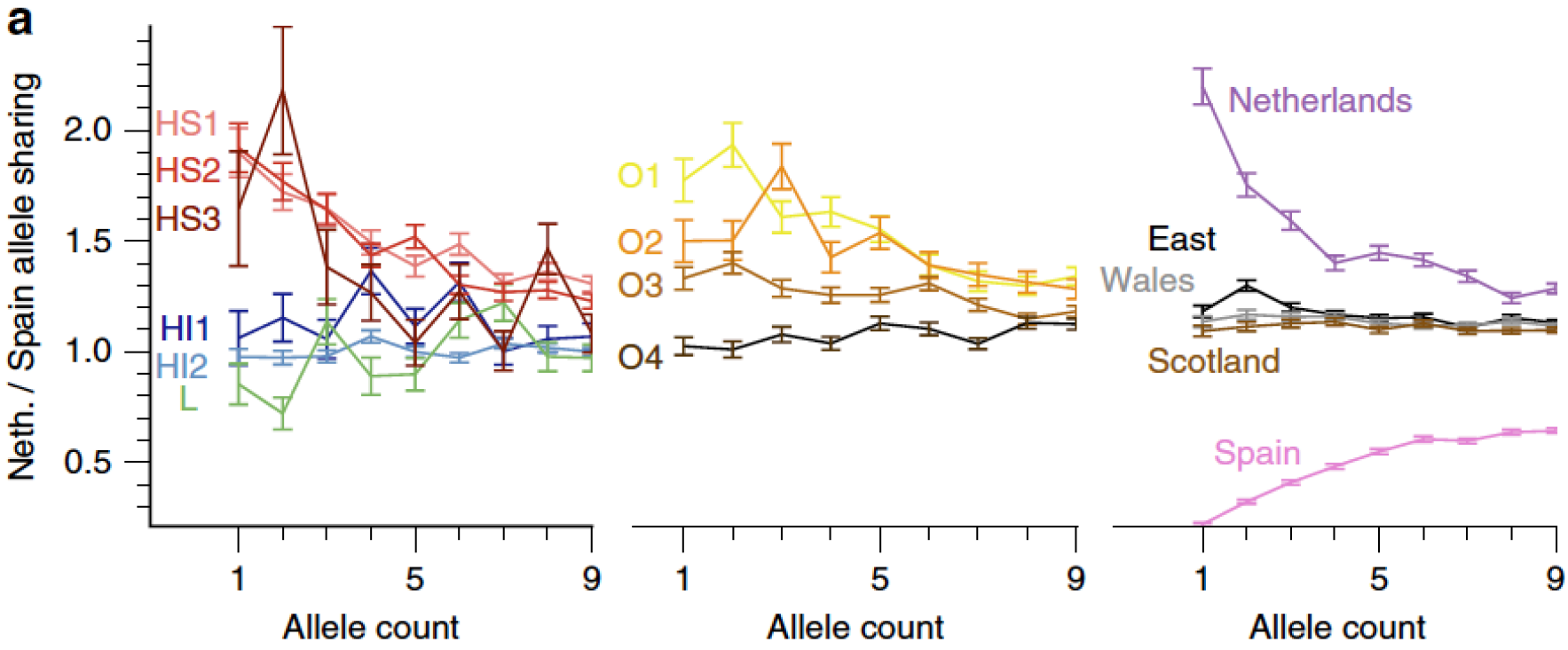
[1000 Genomes Project, Phase3]

Ancient samples from Hinxton

More samples from Linton/Oakington

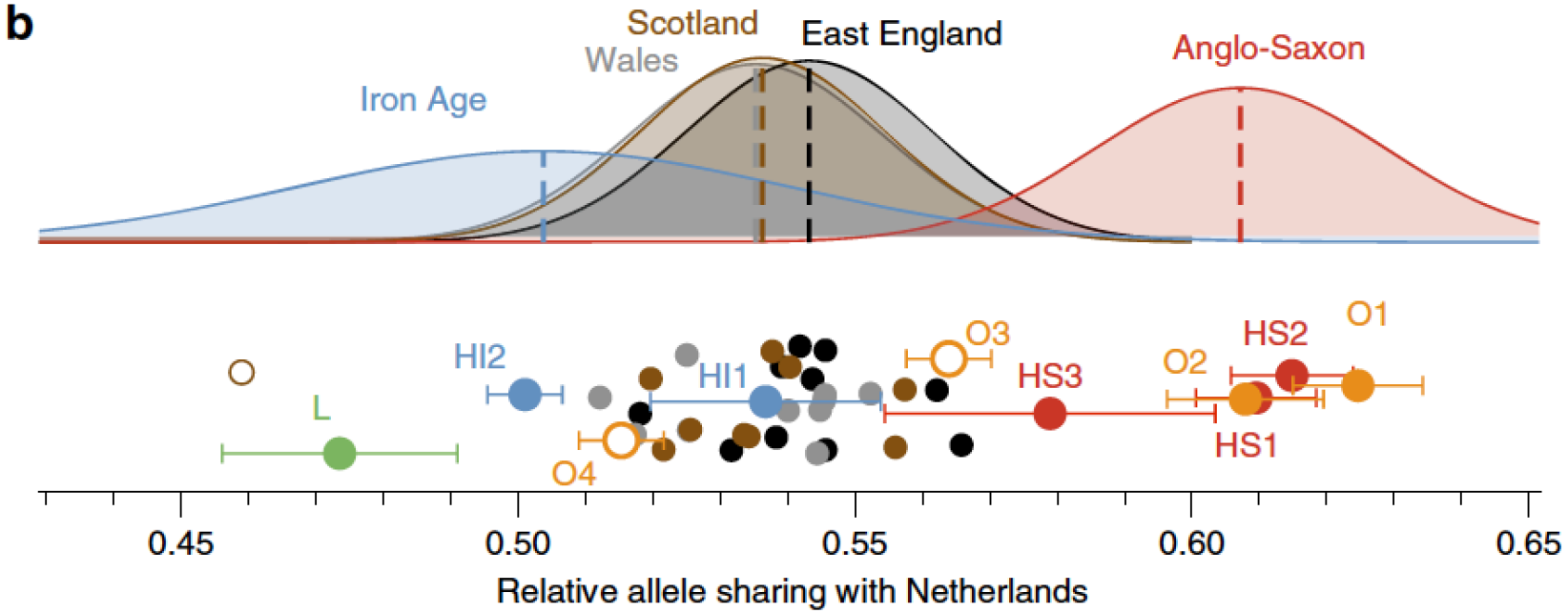


Sharing patterns between ancient and modern samples



- Small but significant differences also within modern Britain (UK10K): Samples from Wales and Scotland share fewer rare variants with Dutch people

Estimates of Anglo-Saxon contribution to modern British genomes



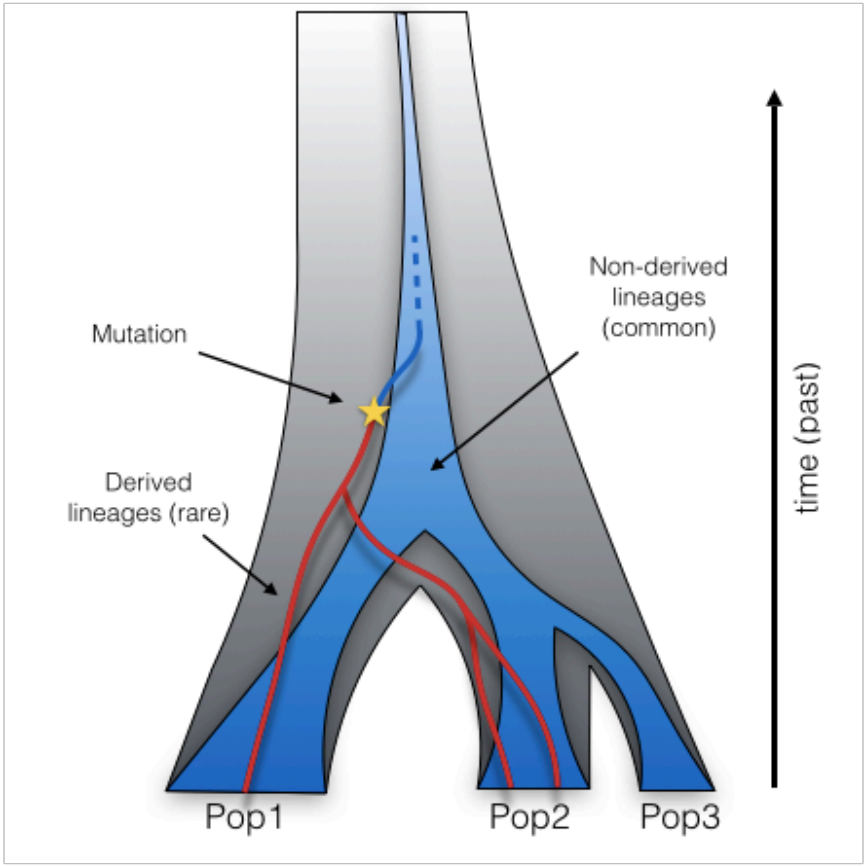
- Suggests ~30% Saxon contribution to samples in East of England, and ~20% to UK10K samples from Wales and Scotland

- Consistent with 20-40% indirect estimate from POBI (Peoples of the British Isles) study

The rare allele coalescent

- Goal: Estimate demographic history (population sizes and split times) from rare variants

- Compute likelihood of demographic model given a distribution of rare variants



RareCoal model

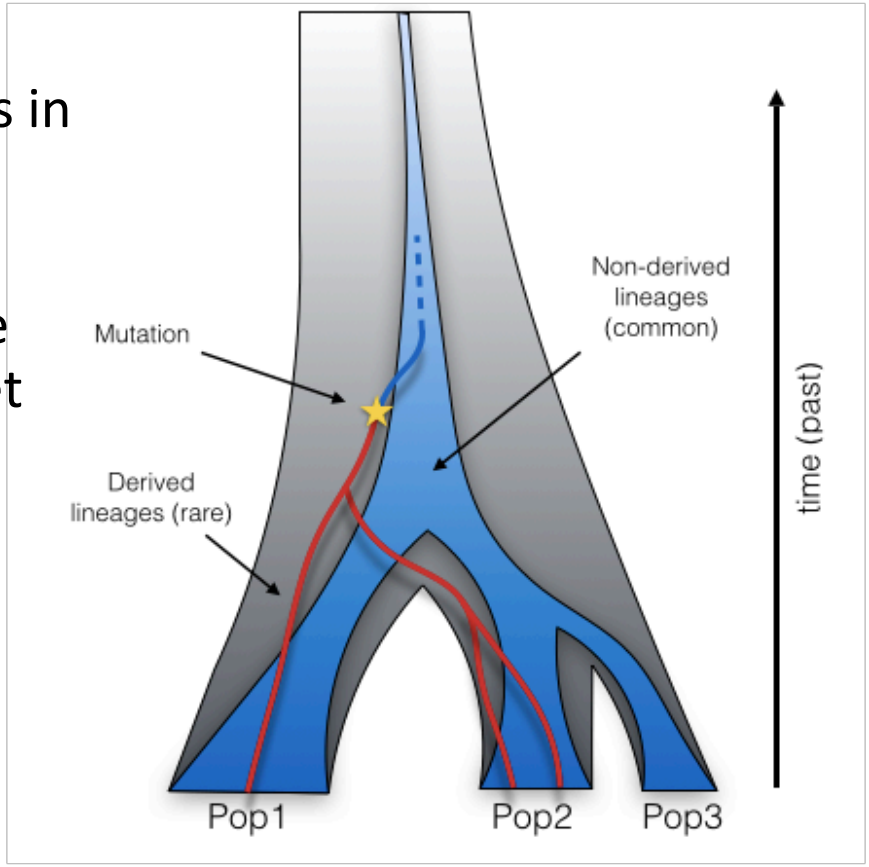
- Idea: Define recursion equations for probability of observing ***i*** derived alleles in population ***k***:

$$b_k^i(t)$$

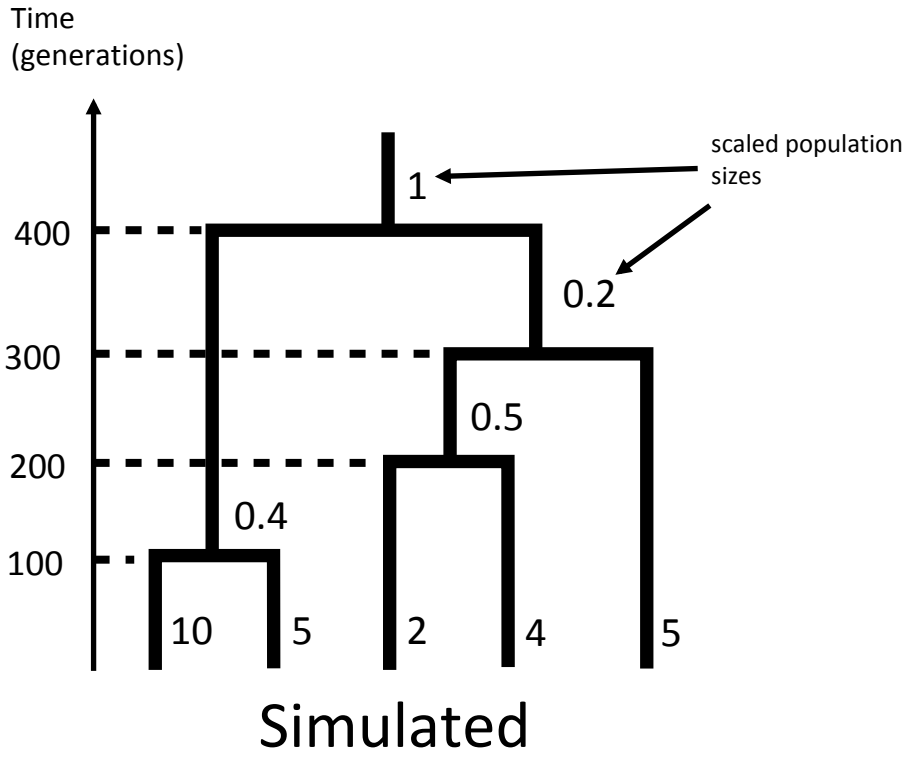
- Given a demographic model, propagate this probability backwards in time to get full likelihood of the data.

- Key simplification: Treat number of ancestral alleles over time as average (mean-field approximation):

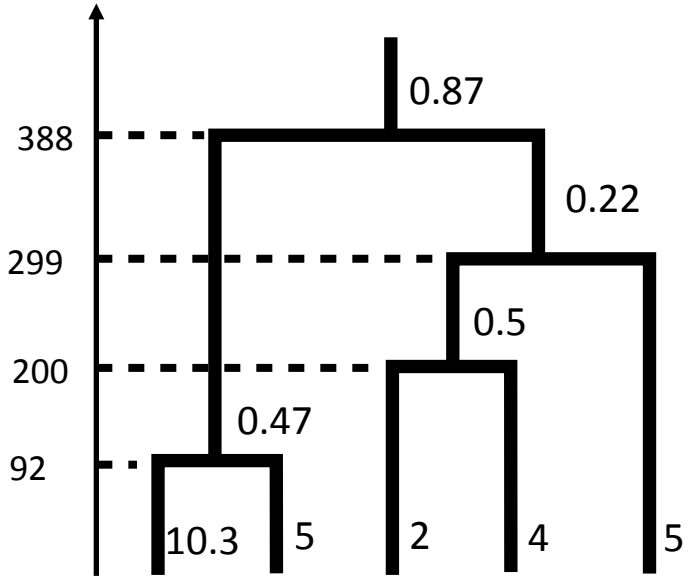
$$a_k(t)$$



Test inference with simulated data



Simulated



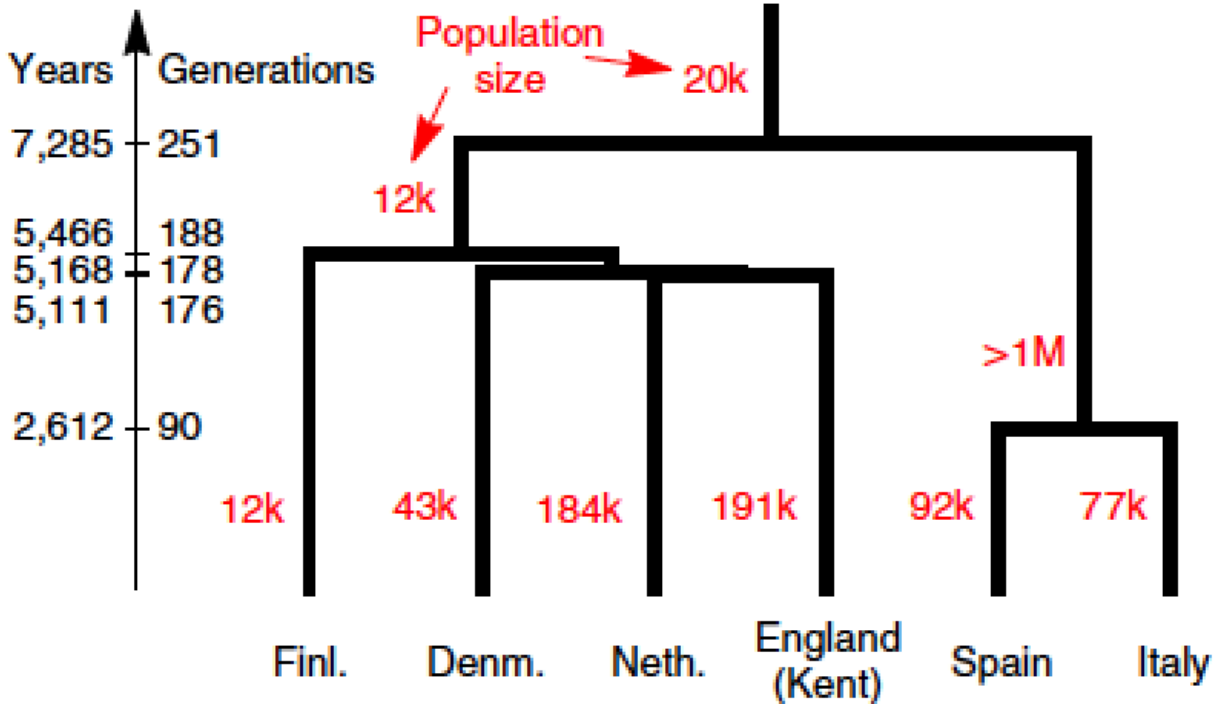
Estimated

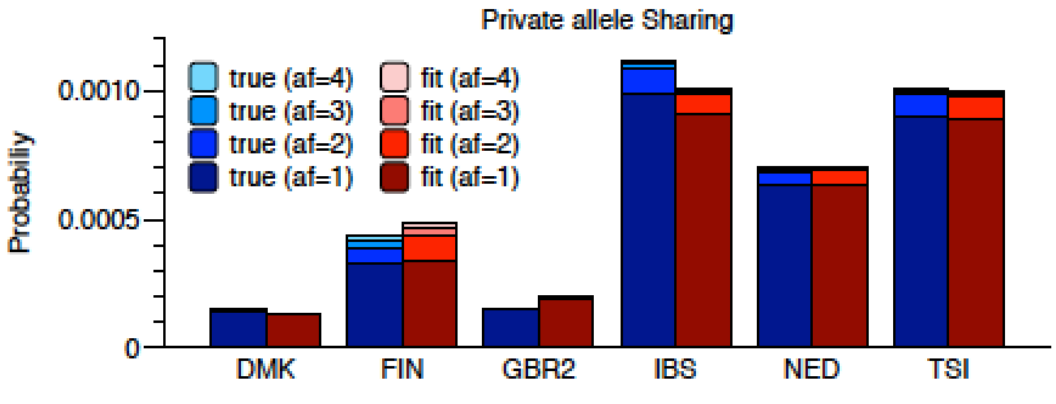
Fits (100 samples per pop.):

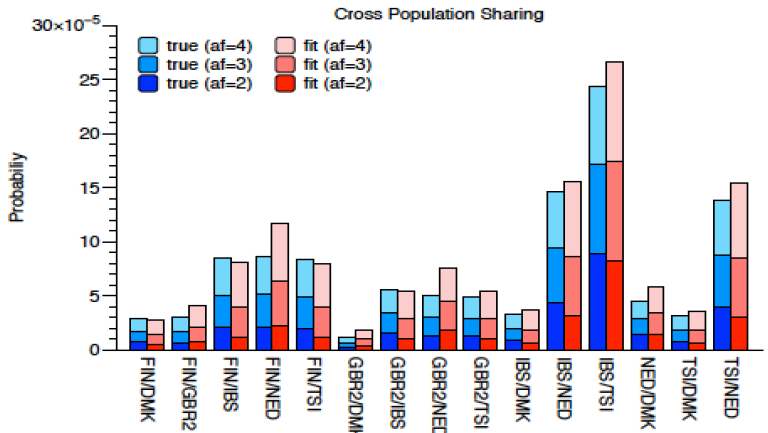
0,1,0,2,1	1114	1159
2,1,0,0,0	140585	139657
1,0,2,0,0	1138	1205
thousands of rows …		

Fitting population sizes and split times separates **drift** from **divergence -> different from Treemix, qpGraph etc.**

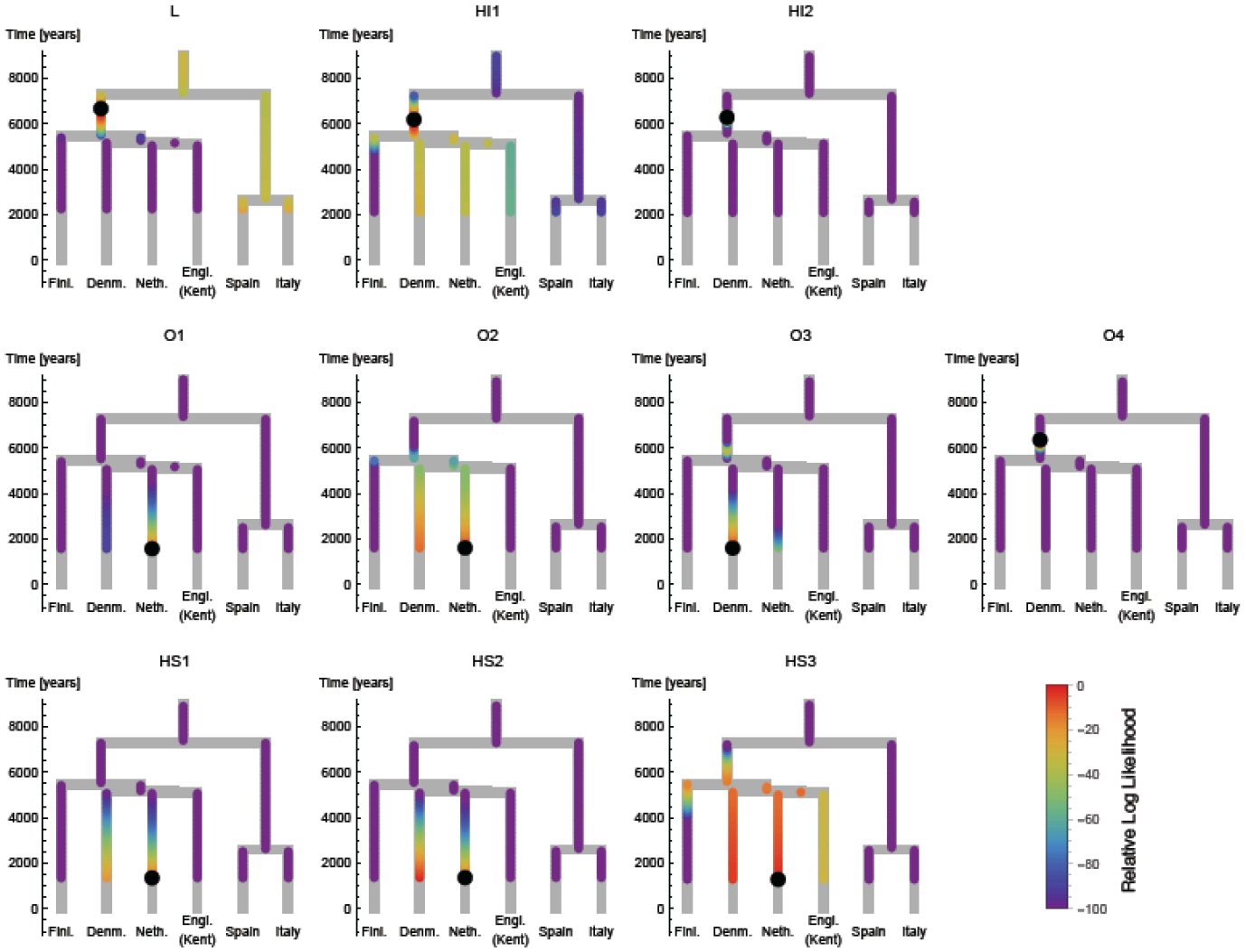
European Tree (Fits)





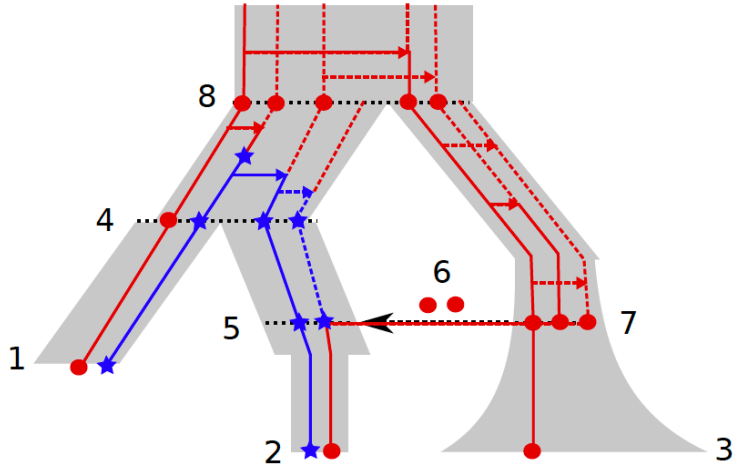


Placing ancient samples on the tree



- Plots show the likelihood for merging the population N=1 sample onto the tree as a heat map

More direct calculation of the likelihood of the joint site frequency spectrum with **momi**



- Complexity of ancestral allele state is reduced by using Moran model
- Use Automatic Differentiation to calculate gradients to maximise likelihood over demography with (limited) gene flow

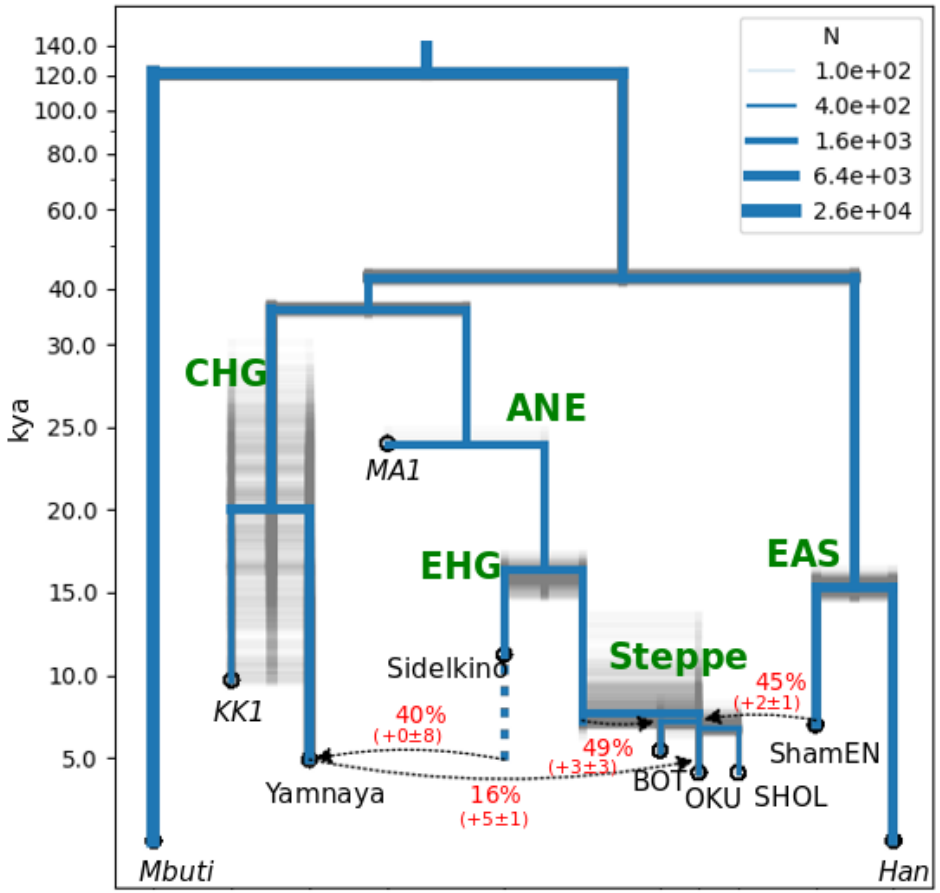
momi

Compute SFS using Moran model & Bayesian graph

Jack Kamm … Song 2016, and unpublished

Momi applied to central Asian data

- Include ancient samples
  - Condition ascertainment on modern/deep samples
    - Total branch length on these
  - Random allele sampling for low coverage samples
- Estimates split times
- Bootstrap for confidence intervals
  - But beware model misspecification



with Peter de Barros Damgaard, Rui Martiniano, Martin Sikora, Eske Willerslev

Momi calculations

- To calculate $P(x_1,x_2,x_3,\ldots)$
  - Set leaves to $\mathrm{Indicator}(x_i)$, e.g. $[0,0,1,0\ldots0]$ for $x_i=2$
  - Propagate likelihoods up tree ("tree-peeling")
- Can correspondingly calculate the expectation of any multi-linear function of allele counts
  - $\mathbb{E}[f_1(x_1)f_2(x_2)f_3(x_3)\ldots]$
- by setting leaf i to $[f_i(0), f_i(1), \ldots, f_i(n_1)]$
  - Works because propagation is linear

Examples

- Total branch length $\propto$ chance of any mutation
  - $f_i(j) = 1$, vector is $[1,1,1\ldots1]$
- TMRCA for pop i (i arbitrary unless ancient model)
  - $f_i(j) = j/n_i$, vector is $[0,0.2,0.4,0.6,0.8,1]$ for n_i=5
  - $f_k(j) = 1,\ k \neq i$
- $f_3 = \mathbb{E}[(X_1\text{-}X_3)(X_2\text{-}X_3)]$, $f_4 = \mathbb{E}[(X_1\text{-}X_2)(X_3\text{-}X_4)]$
  - Requires terms such as $\mathbb{E}[X_1X_2]$ for which
  - $f_1(j) = j/n_1$, $f_2(j) = j/n_2$, $f_k(j) = 1,\ k > 2$
- Also numerators, denominators of F_{ST}, Tajima's D

Summary

- PSMC(‘) estimates demography from a single pair of sequences
  - Sample size is in length not number
  - Quite a clean model
  - Major issue is population structure
- MSMC, MSMC2, SMC++ use additional samples to get at more recent times
- RareCoal/Momi use coalescent modelling of the SFS on more samples to estimate trees
  - With limited modelled gene flow for Momi

Experimental design

- (Sequence) data collection costs money
- We always need to make decisions in how to sample and sequence
  - Number of samples
  - Number of populations
  - Depth of sequencing
  - Whole Genome Shotgun or RADseq or Exomes…

1000 Genomes Project

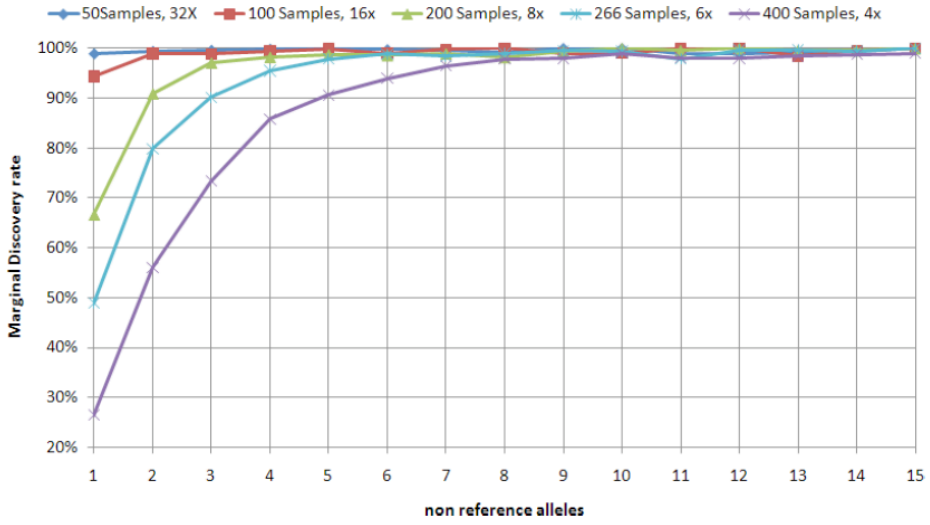
- Pilot (a **very** long time ago!)
  - 2 trios at high depth 30x
    - Phasing, accurate single-sample genotype calling, mutation rates
  - 3 populations x 60 samples at low depth 2-4x + exomes
- Main project
  - 26 populations of ~100 (2504 total) at 6-8x (+exomes)
  - (150 trios at high depth – but who remembers them?)

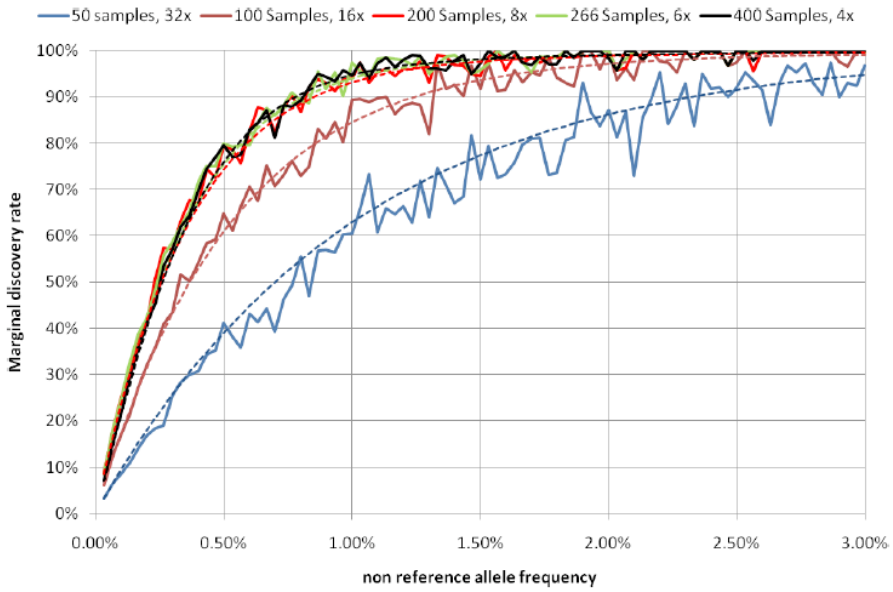
Malawi cichlid sequencing

- Phase 1
  - Three trios at 30x: mutation rate estimation, controls
  - ~70 species at 15-20x, additional samples for some at 8-12x
- Phase 2
  - 7 sets of 20 at 15x
  - More species
  - Some sets of 24 or 48 to address specific questions
- Massoko GWAS (Turner)
  - 200 samples at 4x + 100 samples for replication

Low coverage sequencing strategy

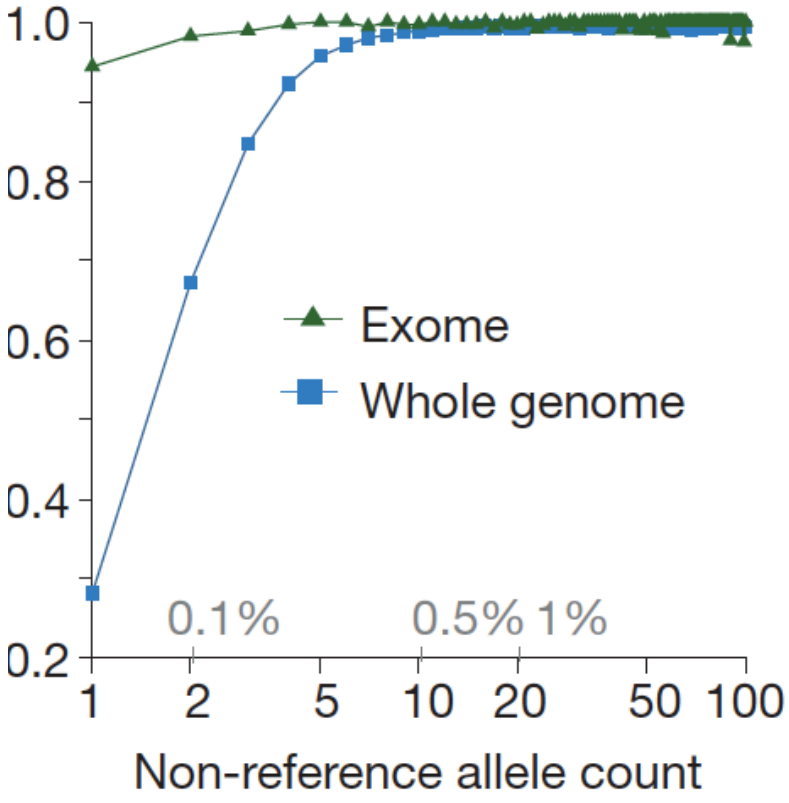
- Typically one needs to sequence at ~30x depth to find (almost) all variants in a sample
- To find low frequency variants we want to sequence many samples
- Spread sequence across more samples



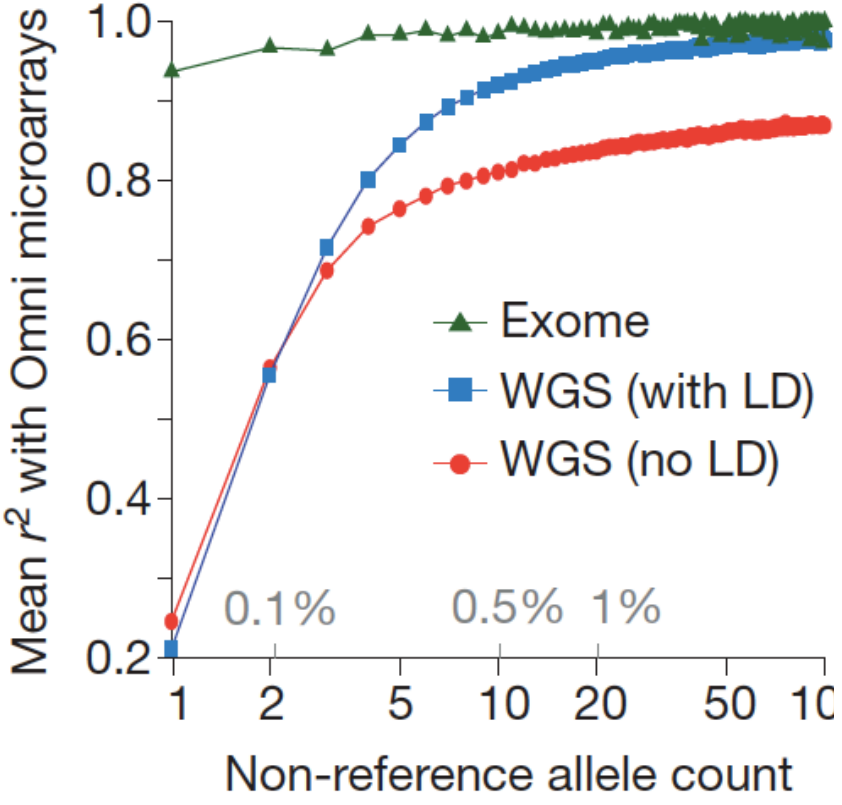


Phase 1 power and genotyping accuracy

SNP detection



Genotyping accuracy



Hyun Min-Kang (U Michigan)

Calling from low coverage sequence

- Multi-sample call **sites** with samtools or GATK
- Obtain **genotype likelihoods** at each site in each same (also samtools or GATK)
  - Likelihood = P(data | genotype)
- Combine in an imputation framework using BEAGLE (Browning), or MINIMAC (Abecasis), or perhaps STITCH (Mott)?
- Phase using SHAPEIT2 (Marchini) or EAGLE2 (Loh)

Sequencing depth

- 30x is standard for near-complete accuracy
  - Sufficient to estimate mutation rates in trios (need several trios for most species)
- 15x is good enough for SNPs (~97%), not quite so good for indels (perhaps 90-95%)
- 4-8x gives good low coverage imputation as in previous slides
- People have used 1-2x, but this is hard work…
- 60x + is necessary for subclonal structure, e.g. cancer, high ploidy
- In a cross, sequence the founders to high depth, and the F2/F3 to low depth (1x or less is fine) and impute using STITCH or other Richard Mott tools