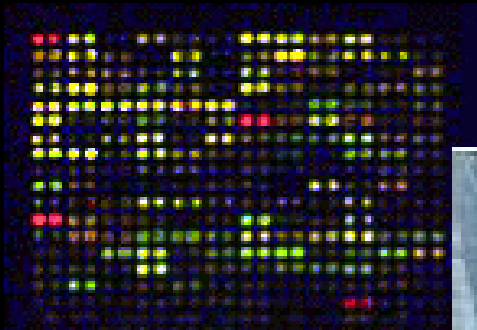


The utility of the HapMap reference samples for clinical populations

(the informatics of sequence variations and haplotypes)



VanBug Seminar
Vancouver, BC, Canada
September 9, 2004



Gabor T. Marth

Department of Biology, Boston College
marth@bc.edu

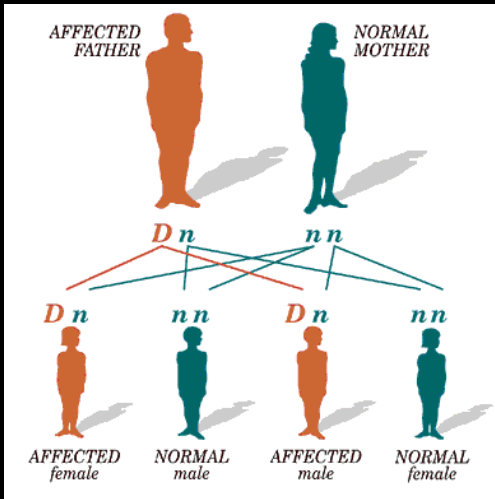


Why do we care about variations?

underlie phenotypic differences



cause inherited diseases



allow tracking ancestral human history





How do we find sequence variations?



- look at **multiple** sequences from the same genome region

```
TCTGACCAATCTAAAAATACCTGTGATTAA
TCTGACCAATCTAACAATACCTGTGATTAA
TCTGACCAATCTAACAATACCTGTGATTAA
TCTGACCAATCTAAAAATACCTGTGATTAA
tctgaccaatctaacaatcacctgtgattaa
```

- use base quality values to decide if mismatches are **true polymorphisms** or sequencing errors

```
TTGATCCCTGT
```

```
TTGATTCCTGT
```

```
TGAAAggAATT
```

```
TGAAAtGAATT
```



Automated polymorphism discovery

Netscape: PolyBayes Web site

File Edit View Go Communicator Help

Back Forward Reload Home Search Netscape Print Security Shop Stop

Location: <http://genome.wustl.edu/gsc/Informatics/polybayes/>

WebMail Radio People Yellow Pages Download Calendar Channels

Site map

Home About Software Availability Publication SNP training Authors Slide show Documentation Demo Links Contact

14	-	30
15	-	30
16	-	30
17	-	30
18	-	30
19	A	40
20	G	38

Evaluate Reset default values

Results

Description	Symbol	Value
Probability of SNP	P(SNP)	D.853076589574195
Most likely variation	VAR	A/G
Probability of variation	P(VAR)	D.853003076184499
Alignment depth	D	2

Comments to: Gabor Marth, gmarth@wustl.edu, Washington University Genome Sequencing Center
Last modified: Mon Feb 12 17:06:10 2001

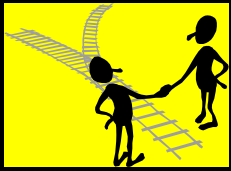
100%

```
TCTGACCAATCTA A A A ATACCTGTGATTAA
TCTGACCAATCTA A C A ATACCTGTGATTAA
TCTGACCAATCTA A C A ATACCTGTGATTAA
TCTGACCAATCTA A A A ATACCTGTGATTAA
tctgaccaatctaac a atacctgtgattaa
```



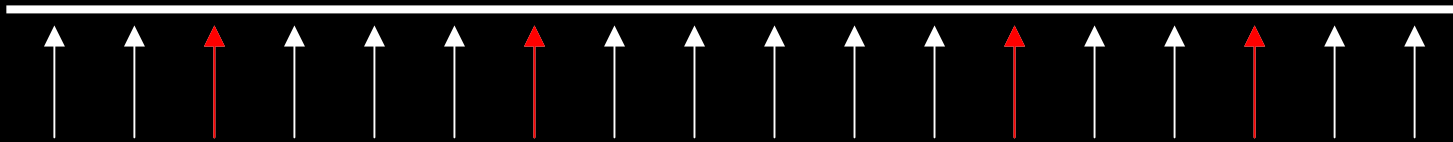
$$P(SNP) = \frac{\sum_{\text{all variable } s} \prod_{S_i \in \{A,C,G,T\}} \frac{P(S_i/R_i)}{P_{Prior}(S_i)} \dots \prod_{S_N \in \{A,C,G,T\}} \frac{P(S_N/R_N)}{P_{Prior}(S_N)} \cdot P_{Prior}(S_1, \dots, S_N)}{\sum_{S_1 \in \{A,C,G,T\}} \dots \sum_{S_N \in \{A,C,G,T\}} \frac{P(S_1/R_1)}{P_{Prior}(S_1)} \dots \frac{P(S_N/R_N)}{P_{Prior}(S_N)} \cdot P_{Prior}(S_1, \dots, S_N)}$$

Marth et al.
Nature Genetics 1999

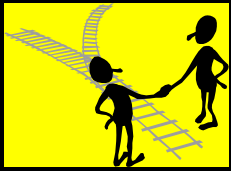


How to use markers to find disease?

genome-wide, dense SNP marker map

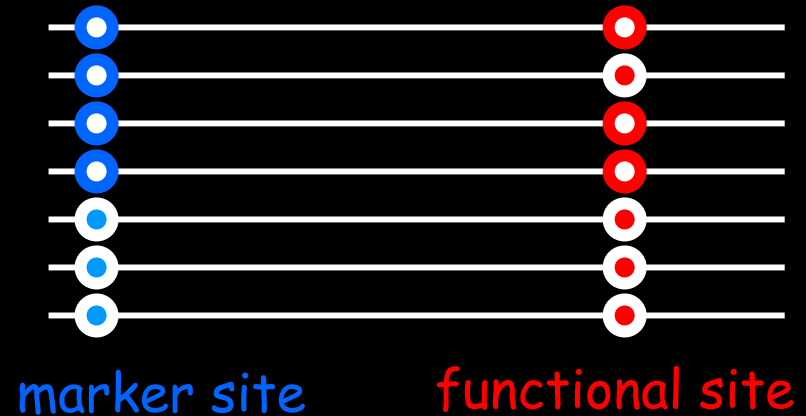


- problem: **genotyping cost** precludes using millions of markers simultaneously for an association study
- question: how to select from all available markers a **subset** that captures most mapping information (**marker selection**, marker prioritization)
- depends on the patterns of **allelic association** in the human genome

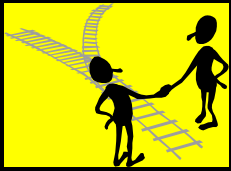


Allelic association

- allelic association is the **non-random assortment** between alleles i.e. it measures how well knowledge of the allele state at one site permits prediction at another

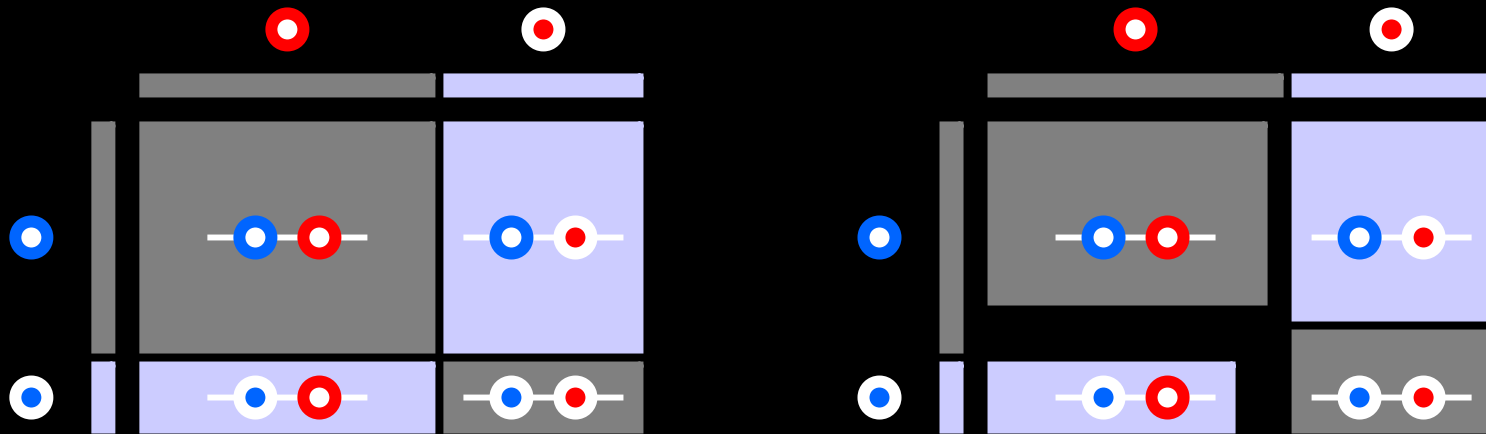


- significant allelic association between a marker and a functional site permits localization (**mapping**) even without having the functional site in our collection
- by necessity, the strength of allelic association is measured **between markers**
- there are **pair-wise** and **multi-locus** measures of association



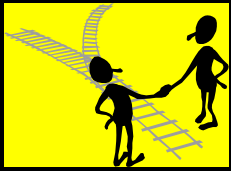
Linkage disequilibrium

- LD measures the deviation from random assortment of the alleles at a **pair** of polymorphic sites



$$D = f(\text{red-blue}) - f(\text{red}) \times f(\text{blue})$$

- other measures of LD are derived from D , by e.g. normalizing according to allele frequencies (r^2)

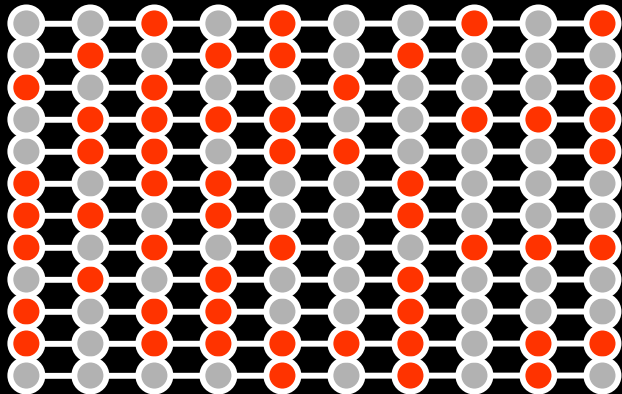


Haplotype diversity

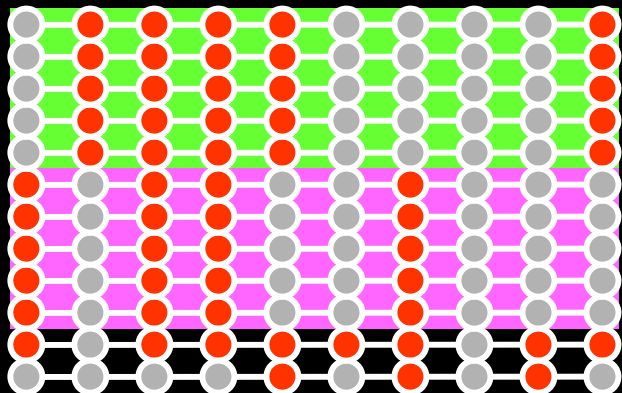
- the most useful multi-marker measures of associations are related to haplotype diversity

n markers

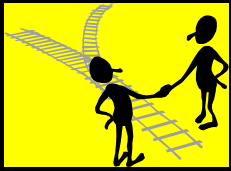
2^n possible haplotypes



random assortment of alleles at different sites

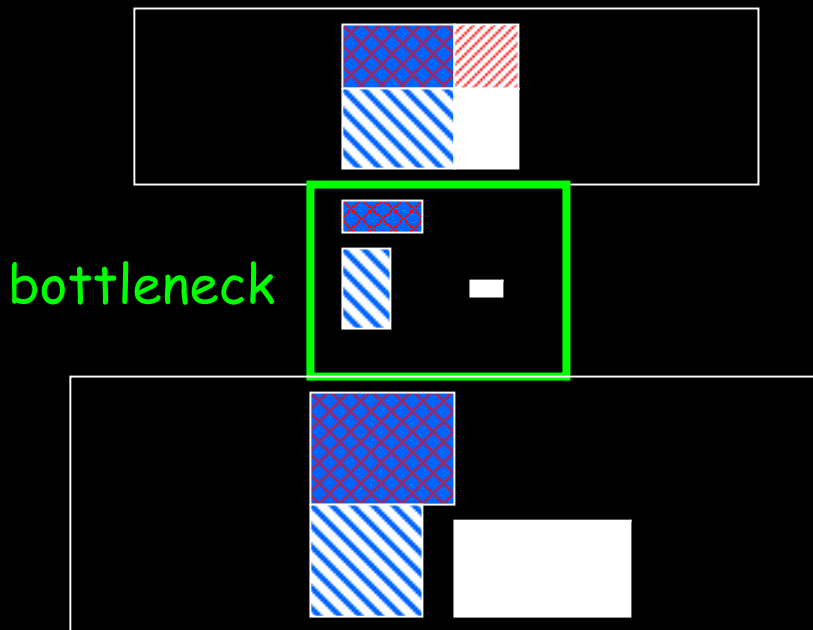
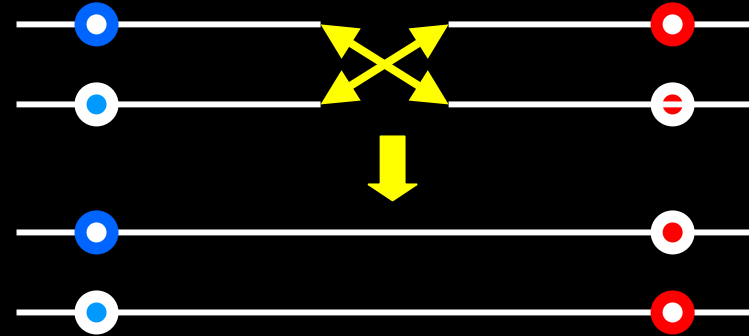


strong association: most chromosomes carry one of a few common haplotypes
- reduced haplotype diversity

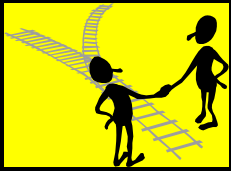


The determinants of allelic association

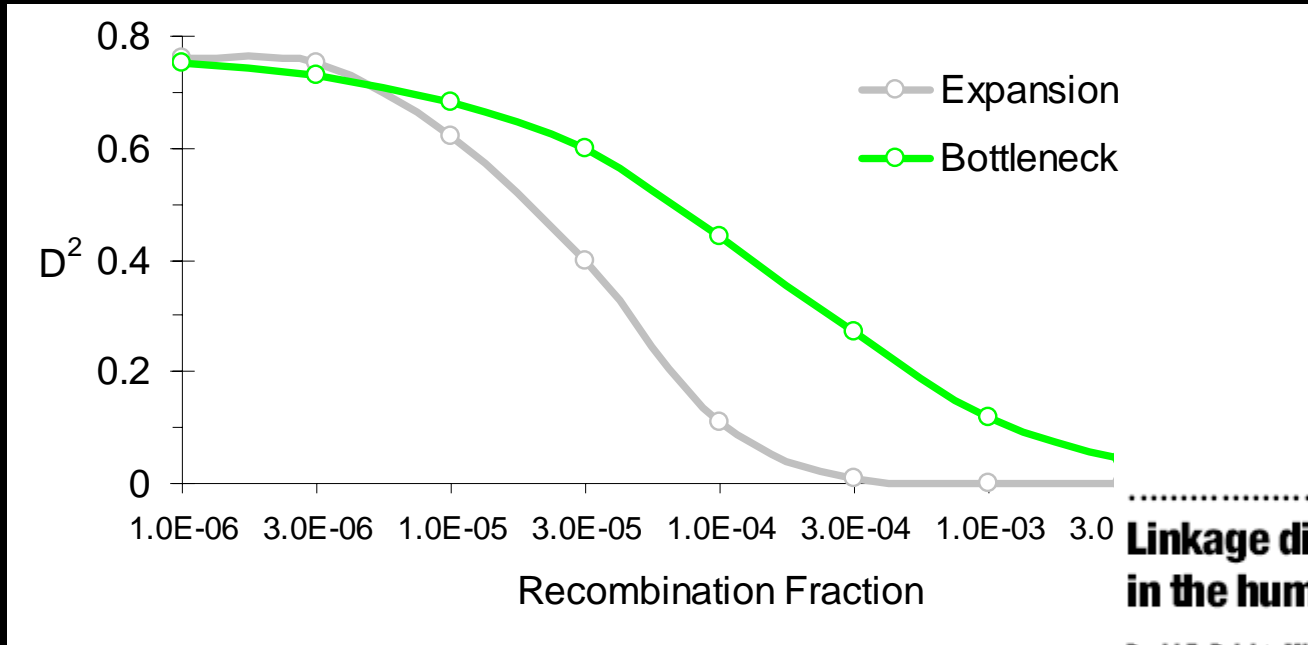
- **recombination**: breaks down allelic association by “randomizing” allele combinations



- **demographic history** of effective population size: bottlenecks increase allelic association by non-uniform re-sampling of allele combinations (haplotypes)



Strength of LD in the human genome

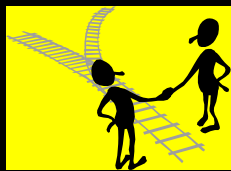


letters to nature

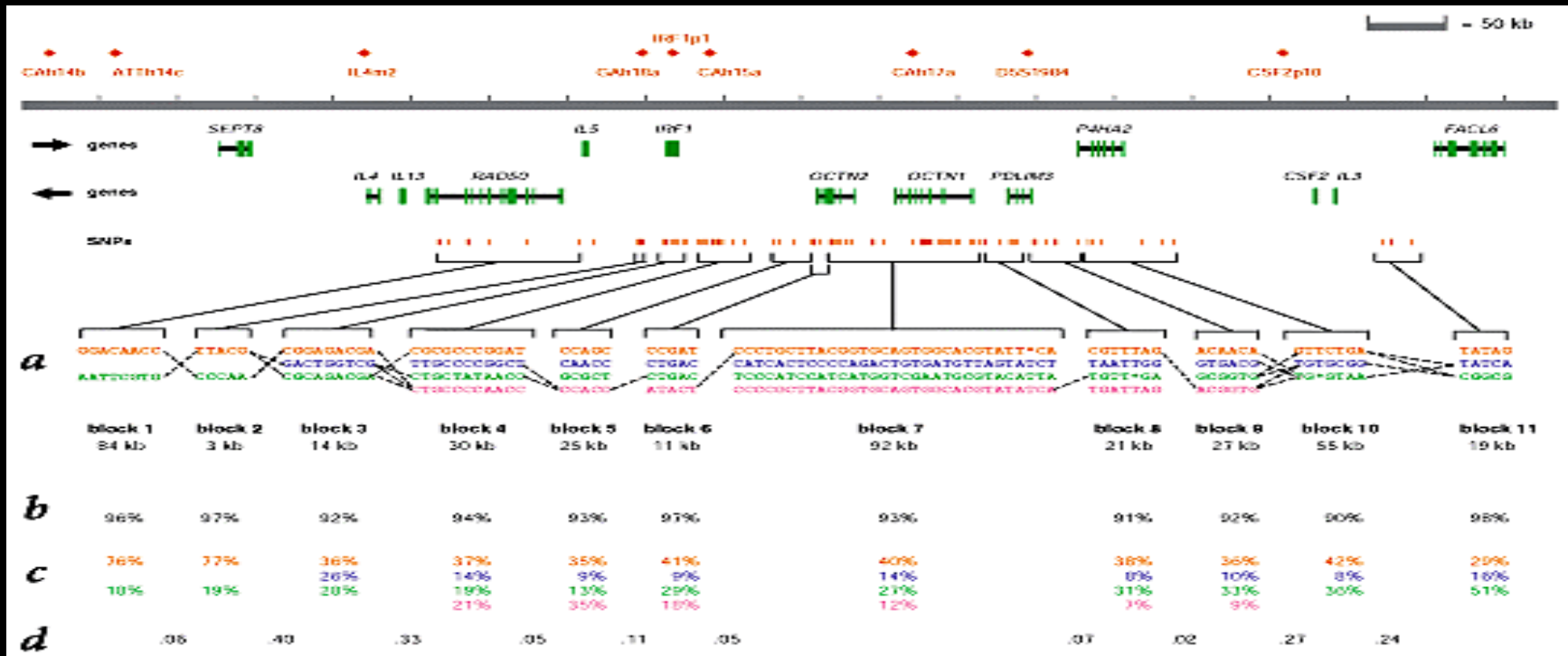
Linkage disequilibrium in the human genome

David E. Reich¹, Michele Cargill^{1,2}, Stacey Boulk¹, James Ireland¹, Pardis C. Sabeti^{1,2}, Daniel J. Richter¹, Thomas Lavery¹, Rose Kouyoumjian¹, Shelli F. Farhadian¹, Ryk Ward¹ & Eric S. Lander^{1,3}

- LD is **stronger, extends longer** than previously thought

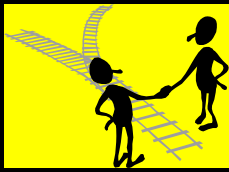


Haplotype blocks



Daly *et al.*
Nature Genetics 2001

- experimental evidence for **reduced haplotype diversity** (mainly in European samples)



The promise for medical genetics

C	A	C	T	A	C	C	G	A
C	A	C	G	A	C	T	A	T
T	T	G	G	C	G	T	A	T
↑	↑	↑	↑					

- within blocks a small number of SNPs are sufficient to distinguish the few common haplotypes → significant **marker reduction** is possible

-
- 
- if the block structure is a general feature of human variation structure, **whole-genome association studies** will be possible at a reduced genotyping cost

- this motivated the HapMap project

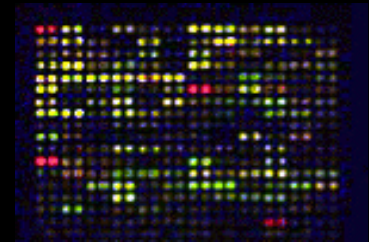
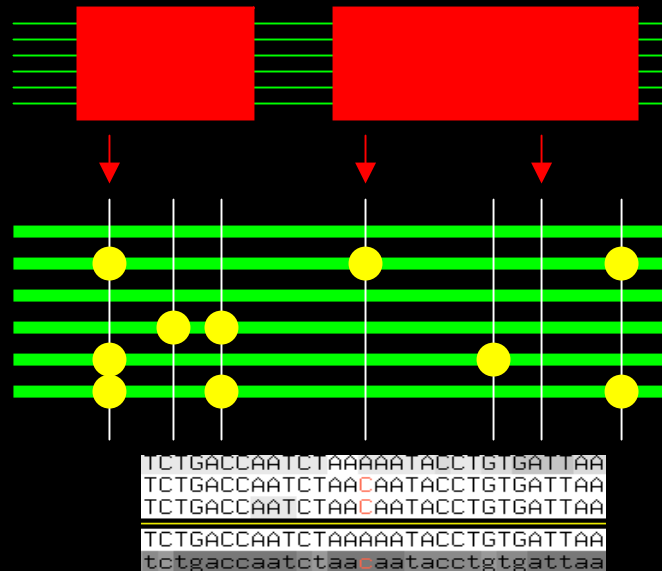
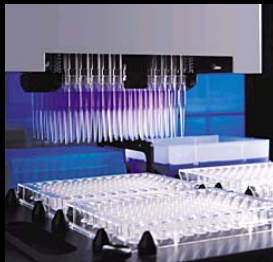
Gibbs *et al.*
Nature 2003





The HapMap initiative

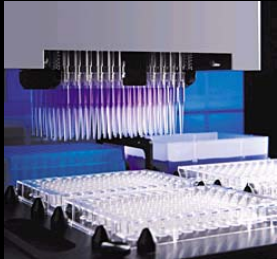
- goal: to map out human allele and association structure of at the kilobase scale
- deliverables: a set of **physical and informational reagents**





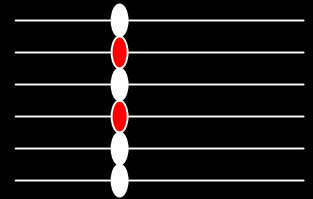
HapMap physical reagents

- **reference samples**: 4 world populations, ~100 independent chromosomes from each

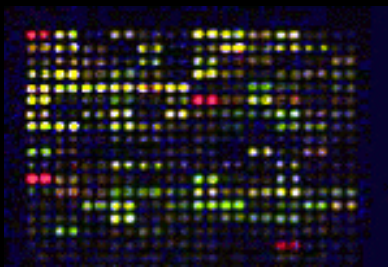


- **SNPs**: computational candidates where both alleles were seen in multiple chromosomes

```
TCTGACCAATCTAAAAATACCTGTGATTAA  
TCTGACCAATCTAAACAATACCTGTGATTAA  
TCTGACCAATCTAAAAATACCTGTGATTAA  
tctgaccaatctaa aataacctgtgattaa
```



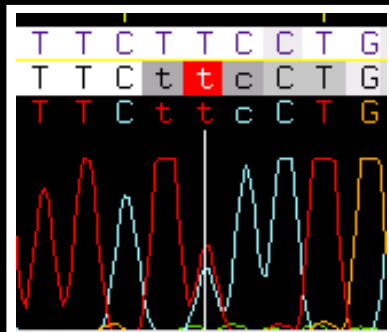
- **genotypes**: high-accuracy assays from various platforms; fast public data release





Informational reagents: haplotypes

- the problem: the substrate for genotyping is diploid, genomic DNA; **phasing of alleles** at multiple loci is in general not possible with certainty



- experimental methods of haplotype determination (single-chromosome isolation followed by whole-genome PCR amplification, radiation hybrids, somatic cell hybrids) are expensive and laborious



Computational haplotype inference

- Parsimony approach: **minimize the number of different haplotypes** that explains all diploid genotypes in the sample

Clark
Mol Biol Evol 1990

- Maximum likelihood approach: **estimate haplotype frequencies** that are most likely to produce observed diploid genotypes

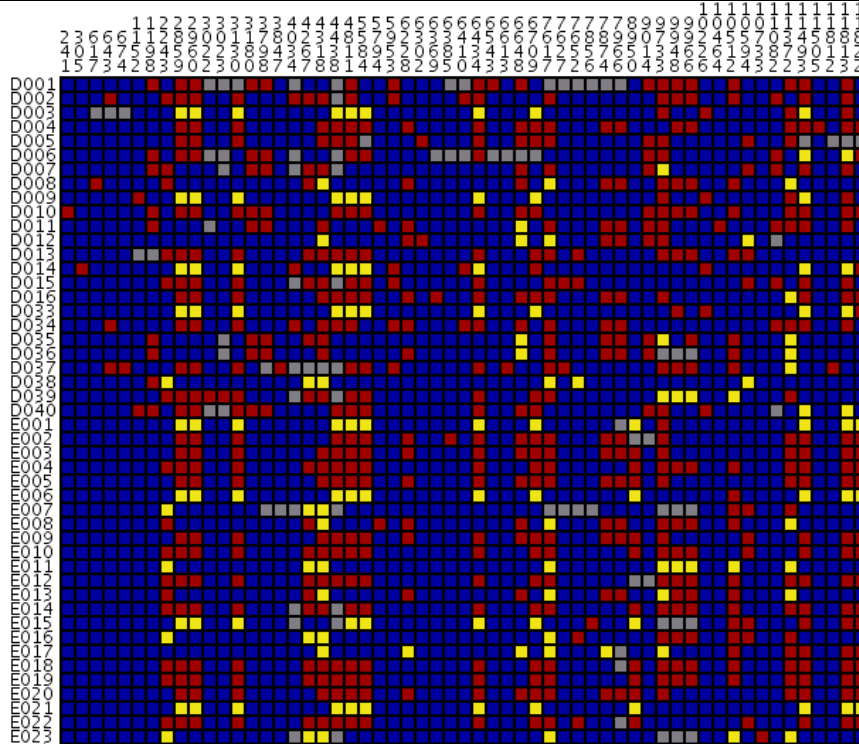
Excoffier & Slatkin
Mol Biol Evol 1995

- Bayesian methods: estimate haplotypes based on the observed diploid genotypes and the a priori expectation of **haplotype patterns** informed by Population Genetics

Stephens *et al.*
AJHG 2001



Haplotype inference



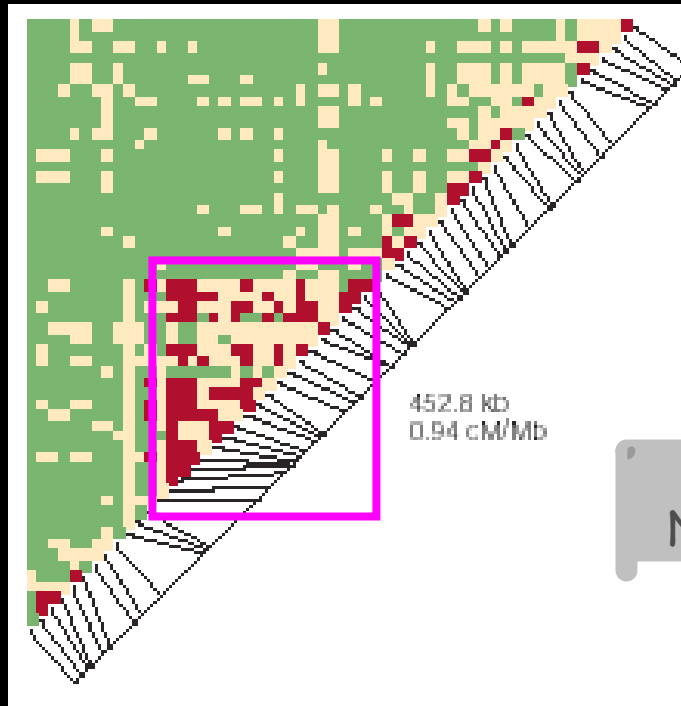
VG2 of: ikbbe.prettybase.txt

- Homozygote-Common Allele
 - Homozygote-Rare Allele
 - Heterozygote
 - Undetermined
- Rare Allele Percentage: 0.0
 Clustered By: NOTHING
 Population: null

<http://pga.gs.washington.edu/>



Haplotype annotations - LD based



Wall & Pritchard
Nature Rev Gen 2003

- Pair-wise LD-plots
- LD-based multi-marker **block** definitions requiring strong pair-wise LD between all pairs in block



Annotations - haplotype blocks

- Dynamic programming approach

Zhang *et al.*
AJHG 2001

B-1	1-1-1-1-1-1-1-1-1-1 1-0-1-0-0-0-0-0-1 1-1-1-1-1
B-2	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-1-1-1-0
B-3	1-1-1-1-1-1-1-1-1-1 1-0-1-0-0-0-0-0-1 1-1-1-1-1
B-4	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-1-1-1-0
B-5	0-0-0-0-0-0-0-0-0-0 0-0-0-1-0-1-1-0-1 0-0-0-0-0
B-6	1-1-1-1-1-1-1-1-1-1 1-0-1-0-0-0-0-0-1 1-1-1-1-1
B-7	1-1-1-0-1-1-1-0-1-1 0-0-0-1-0-1-1-0-1 0-0-0-0-0
B-8	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-1-1-1-0
B-9	1-1-1-0-0-0-0-0-0-0 0-1-0-0-1-0-0-0-0 1-0-1-1-0
B-10	0-0-0-0-0-0-0-0-0-0 0-0-0-1-0-1-1-0-1 0-0-0-0-0
B-11	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-0-1-1-0
B-12	0-0-0-0-0-0-0-0-0-0 0-1-0-0-1-0-0-0-0 0-0-0-0-0
B-13	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 0-0-0-0-0
B-14	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-1-1-1-0
B-15	1-1-1-0-0-0-0-0-0-0 0-1-0-0-1-0-0-0-0 1-0-1-1-0
B-16	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-0-1-1-0
B-17	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-1-1-1-0
B-18	1-1-1-0-1-1-1-0-1-1 1-0-1-0-0-0-0-0-1 1-1-1-1-1
B-19	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-1-1-1-0
B-20	1-1-1-1-1-1-1-1-1-1 1-0-1-0-0-0-0-0-1 1-1-1-1-1
B-21	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-0-1-1-0
B-22	1-1-1-1-1-1-1-1-1-1 1-0-1-0-0-0-0-0-1 1-1-1-1-1
B-23	1-1-1-0-1-1-1-0-1-1 0-0-1-0-0-0-0-0-0 1-1-1-1-0

1. meet block definition based on common haplotype requirements

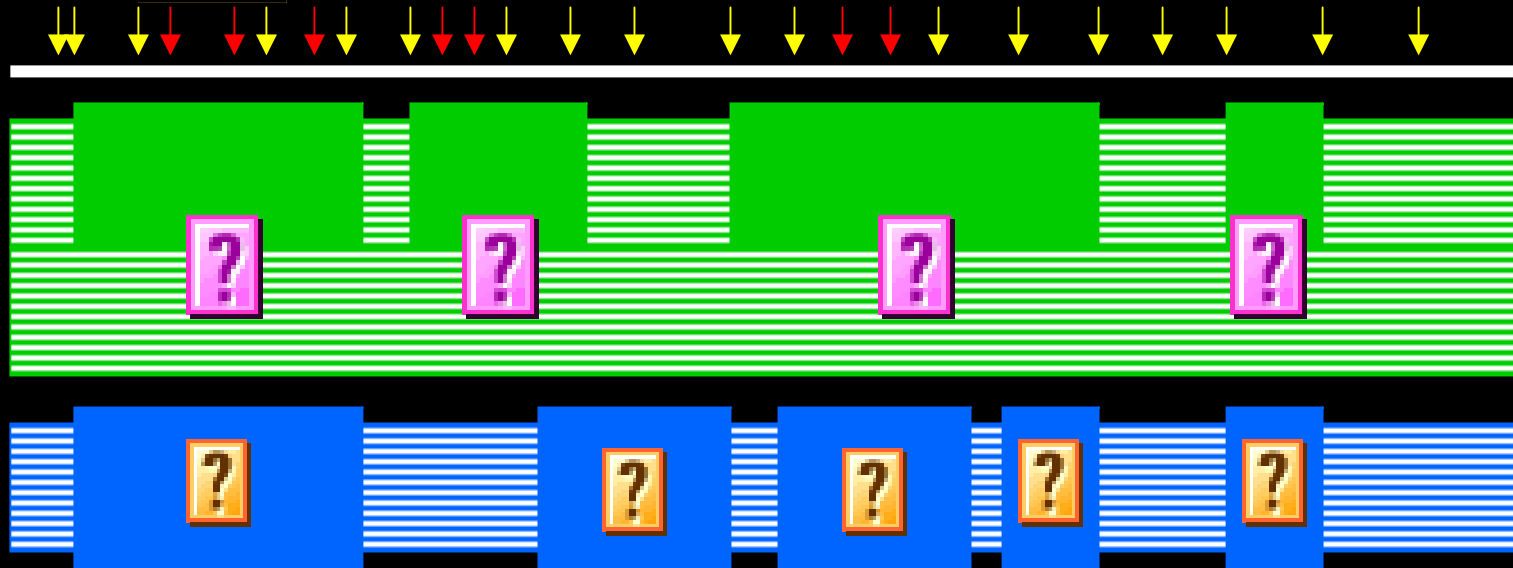
2. within each block, determine the number of SNPs that distinguishes common haplotypes (htSNPs)

3. **minimize** the total number of **htSNPs** over complete region including all blocks

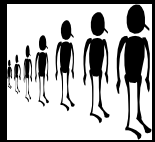
```
block1: left=1 right=9 length=9 htSnps=3 haplotypes=[000000000 (17), 111011101 (13),
111111111 (10), 000000001 (3), 111000000 (2), 000011111 (1)]
block2: left=10 right=17 length=8 htSnps=3 haplotypes=[10100001 (12), 00100000 (11),
01001000 (10), 00010110 (10), 00101000 (3)]
block3: left=18 right=21 length=4 htSnps=3 haplotypes=[0000 (15), 1111 (12), 1010 (1
2), 1110 (7)]
blocks=3 htSnps=9
extractHapBlocks.pl completed
```



Questions about the HapMap



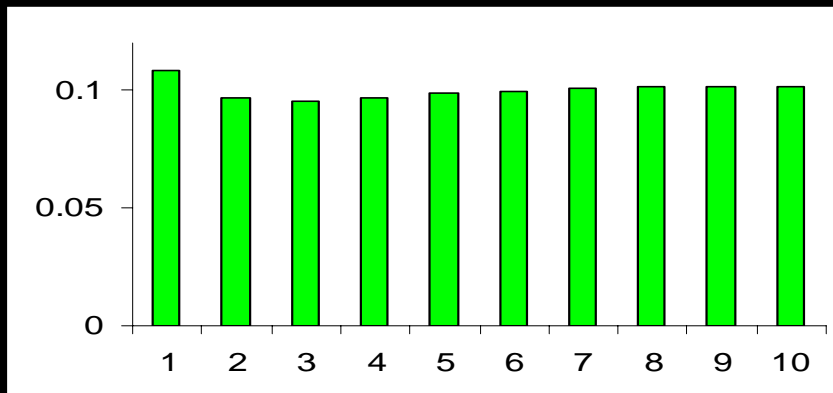
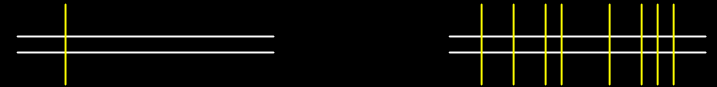
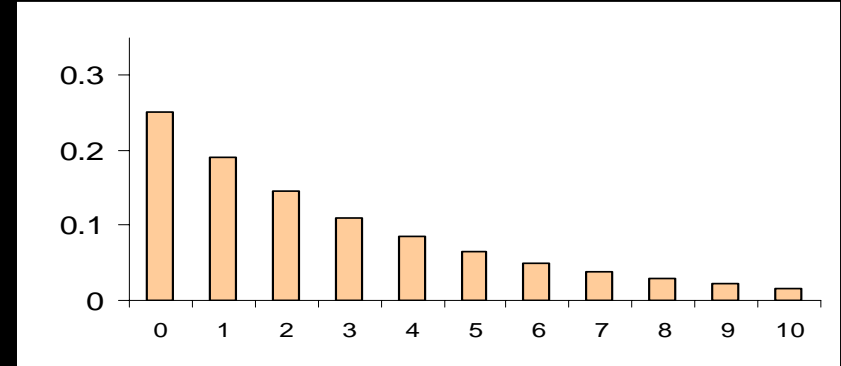
- is structure constant with sample size?
- completion, sufficient density?
- haplotype structure across populations?
- Explore human allele structure with a Population Genetic modeling and data fitting technique



Data: polymorphism distributions

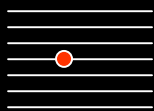
1. marker density (MD): distribution of number of SNPs in pairs of sequences

Clone 1	Clone 2	# SNPs
AL00675	AL00982	8
AS81034	AK43001	0
CB00341	AL43234	2

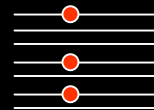


2. allele frequency spectrum (AFS): distribution of SNPs according to allele frequency in a set of samples

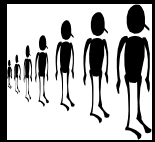
SNP	Minor allele	Allele count
A/G	A	1
C/T	T	9
A/G	G	3



“rare”

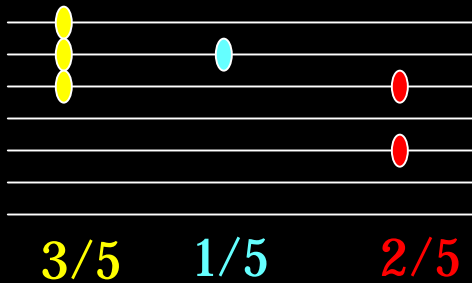
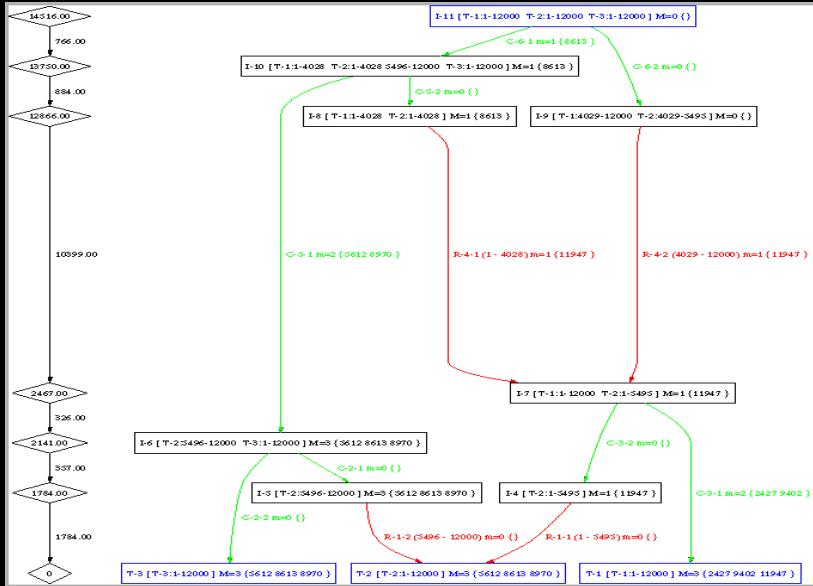


“common”



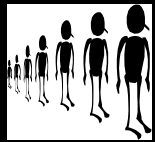
Model: processes that generate SNPs

simulation procedures



computable formulations

$$\begin{aligned}
 P(k) = & \frac{1}{1+\theta_1 L} \left(\frac{\theta_1 L}{1+\theta_1 L} \right)^k \left\{ 1 - e^{-(1+\theta_1 L)\Delta\tau_1} \left[1 + \sum_{i=1}^k \frac{[(1+\theta_1 L)\Delta\tau_1]^i}{i!} \right] \right\} \\
 & + \frac{1}{1+\theta_2 L} \left(\frac{\theta_2 L}{1+\theta_2 L} \right)^k e^{-(1+\theta_1 L)\Delta\tau_1} \left\{ 1 + \sum_{i=1}^k \frac{[(1+\theta_2 L)\frac{\theta_1}{\theta_2}\Delta\tau_1]^i}{i!} \right\} e^{-(1+\theta_2 L)\Delta\tau_2} \left\{ 1 + \sum_{i=1}^k \frac{[(1+\theta_2 L)\frac{\theta_1}{\theta_2}\Delta\tau_1 + \Delta\tau_2]^i}{i!} \right\} \\
 & + \frac{1}{1+\theta_3 L} \left(\frac{\theta_3 L}{1+\theta_3 L} \right)^k e^{-(1+\theta_1 L)\Delta\tau_1 - (1+\theta_2 L)\Delta\tau_2} \left\{ 1 + \sum_{i=1}^k \frac{[(1+\theta_3 L)\left(\frac{\theta_1}{\theta_3}\Delta\tau_1 + \frac{\theta_2}{\theta_3}\Delta\tau_2\right)]^i}{i!} \right\}
 \end{aligned}$$



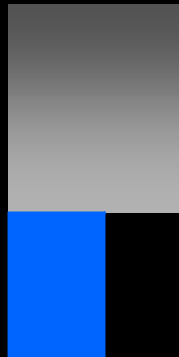
Models of demographic history

past
↓
history
↓
present

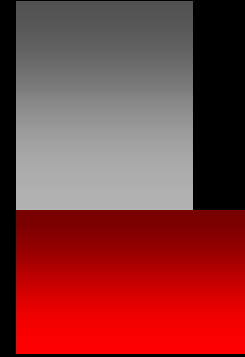
stationary



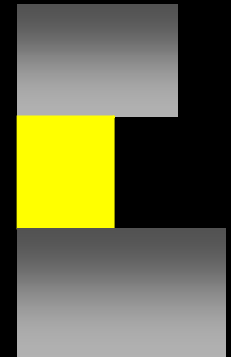
collapse



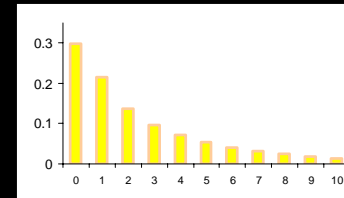
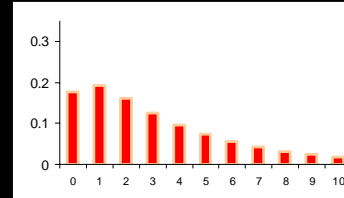
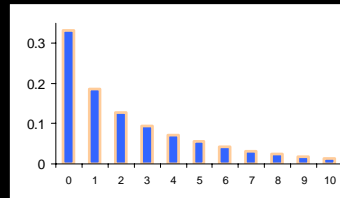
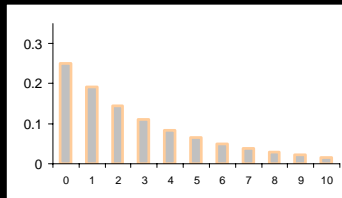
expansion



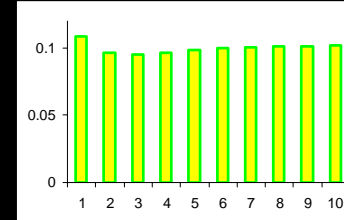
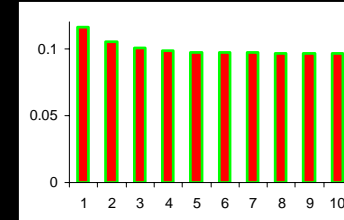
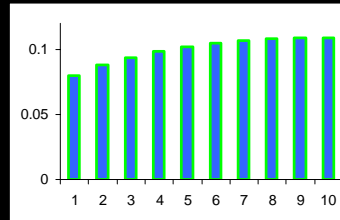
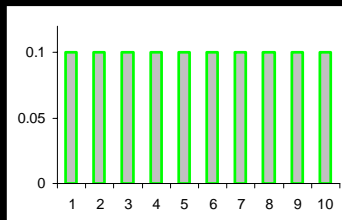
bottleneck

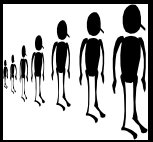


MD
(simulation)

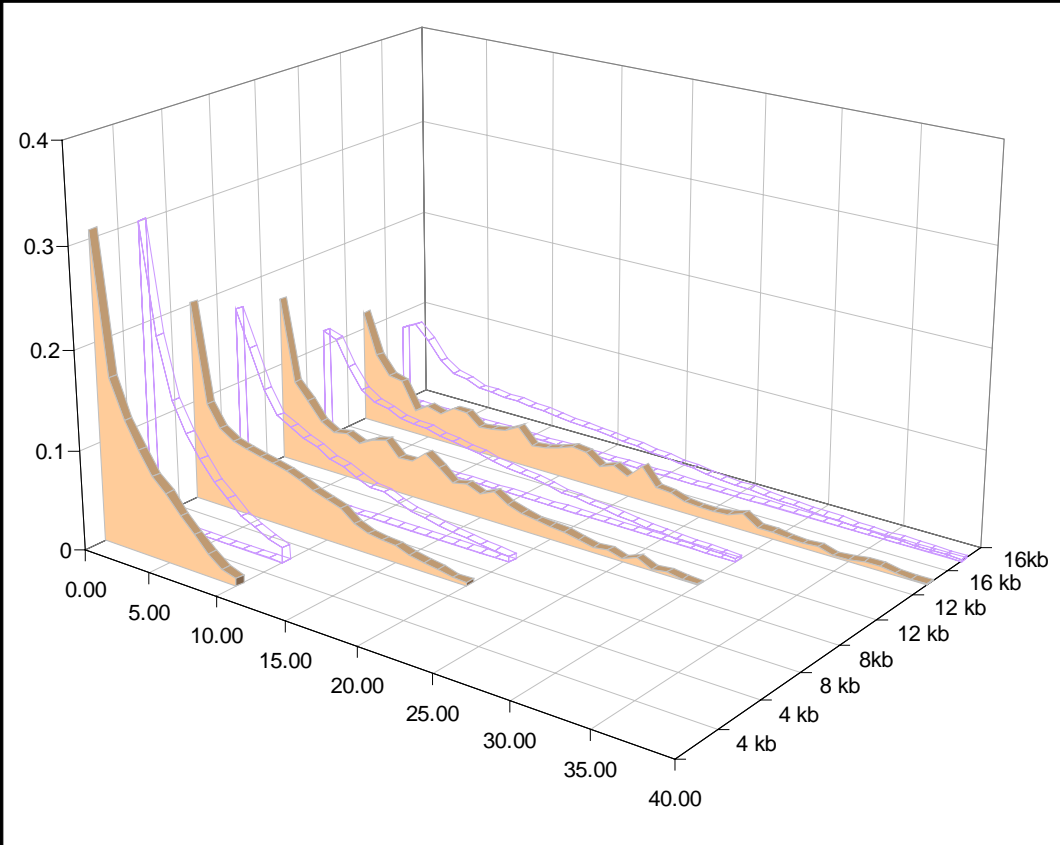


AFS
(direct form)

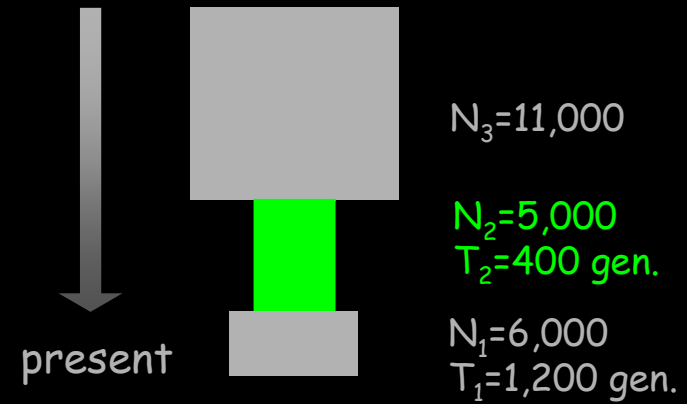




Data fitting: marker density

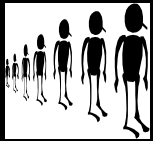


- best model is a **bottleneck** shaped population size history

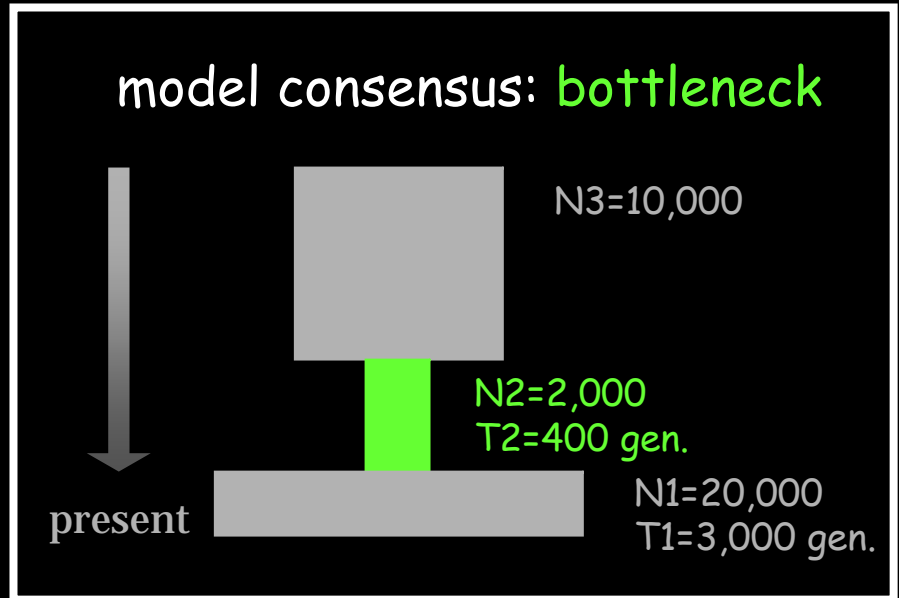
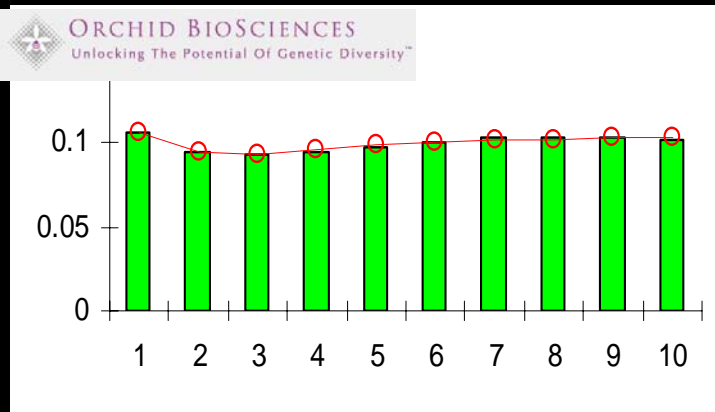
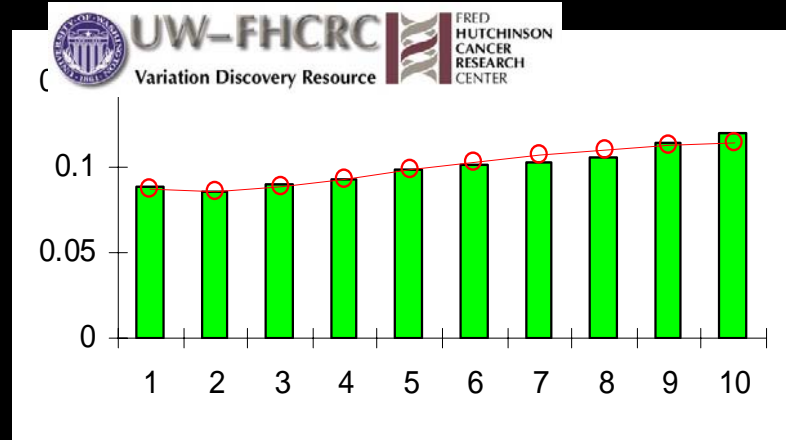
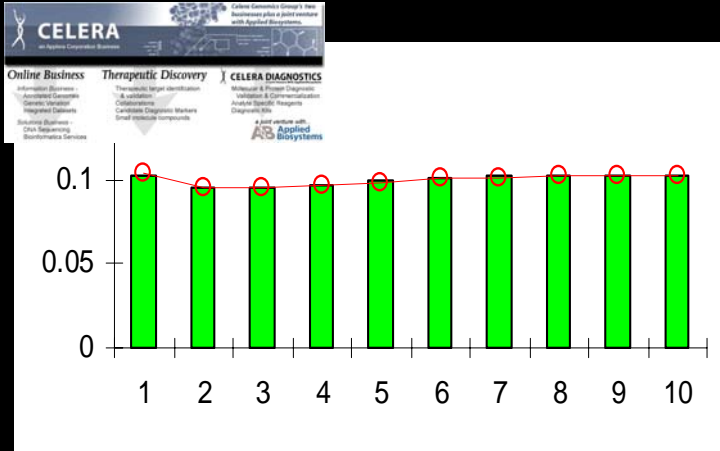


Marth *et al.*
PNAS 2003

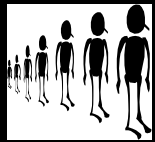
- our conclusions from the marker density data are confounded by the **unknown ethnicity** of the public genome sequence we looked at **allele frequency** data from ethnically defined samples



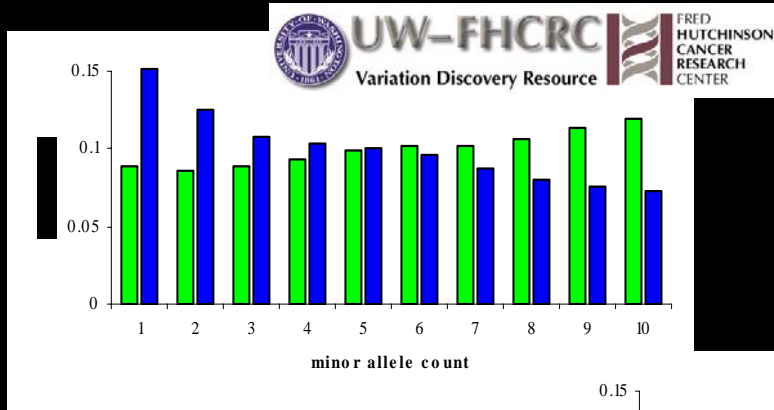
Data fitting: allele frequency



• Data from **other populations**?

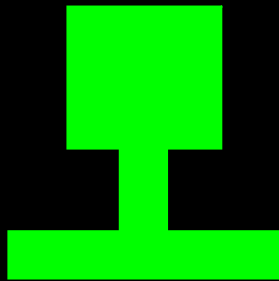
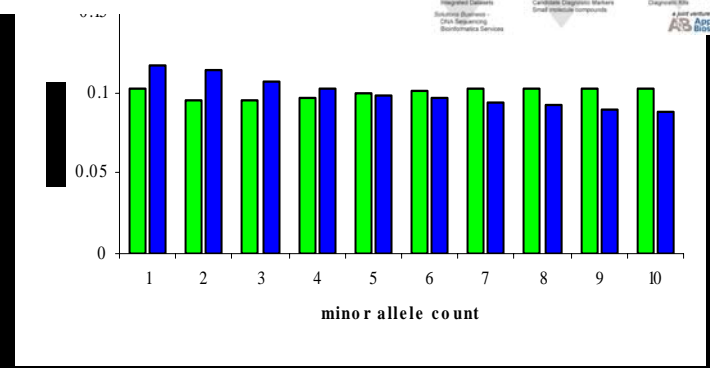
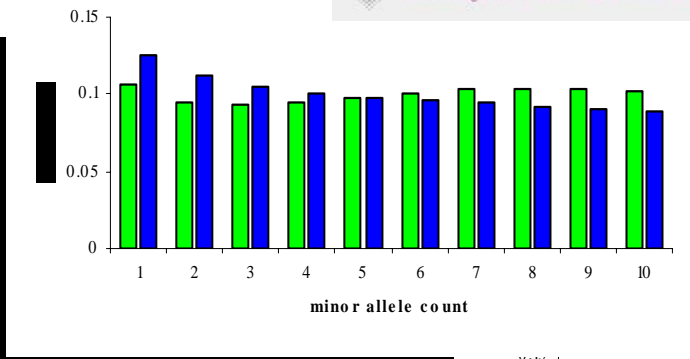


Population specific demographic history



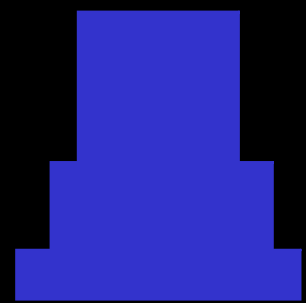
European data

African data

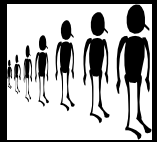


bottleneck

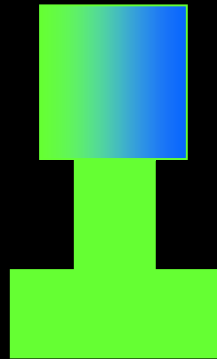
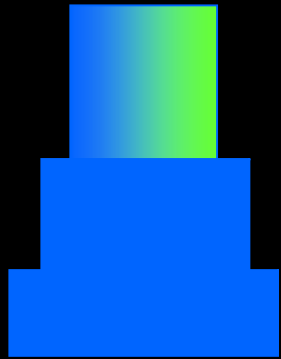
modest but uninterrupted expansion



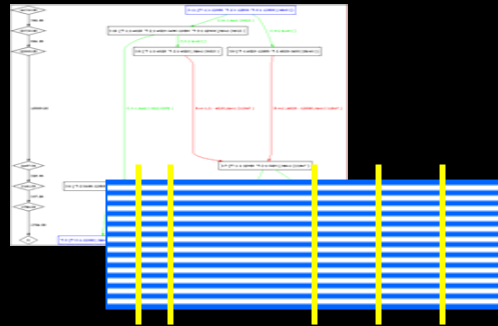
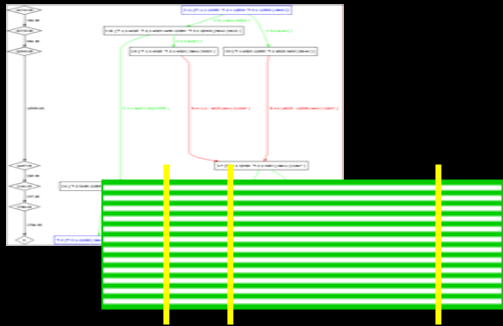
Marth *et al.*
Genetics 2004



Model-based prediction

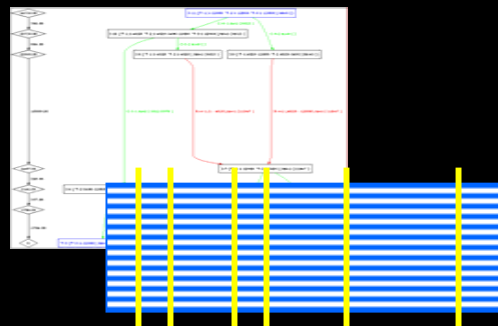
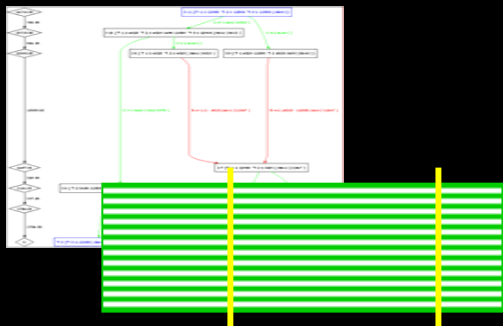


computational model
encapsulating what we
know about the process



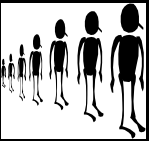
genealogy + mutations

allele structure



arbitrary number of
additional replicates

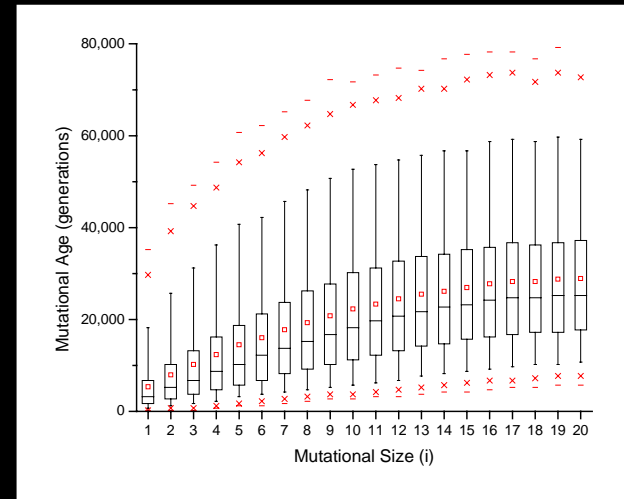
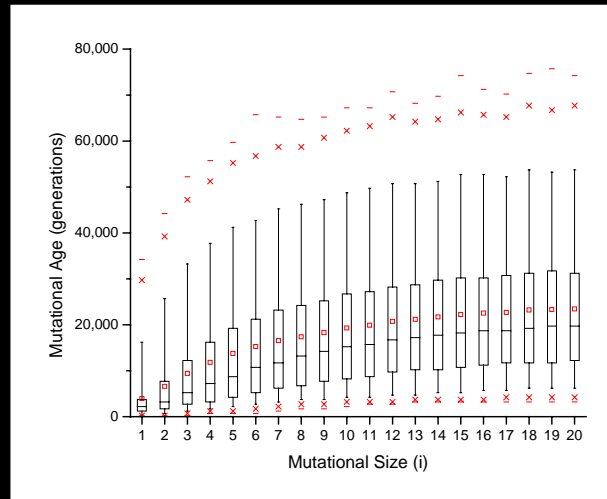
Prediction - allele frequency and age



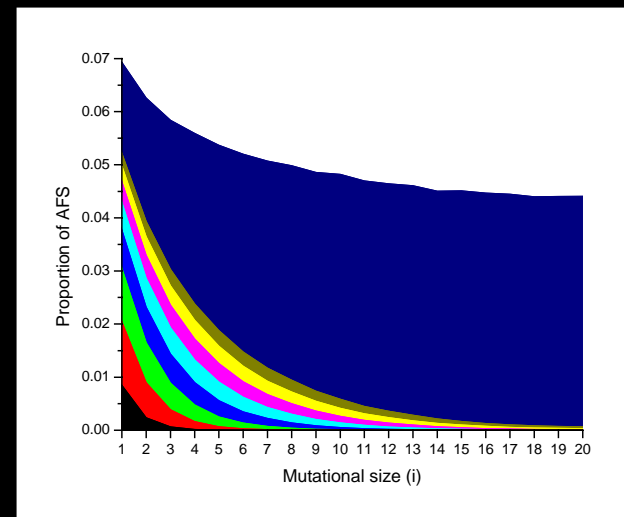
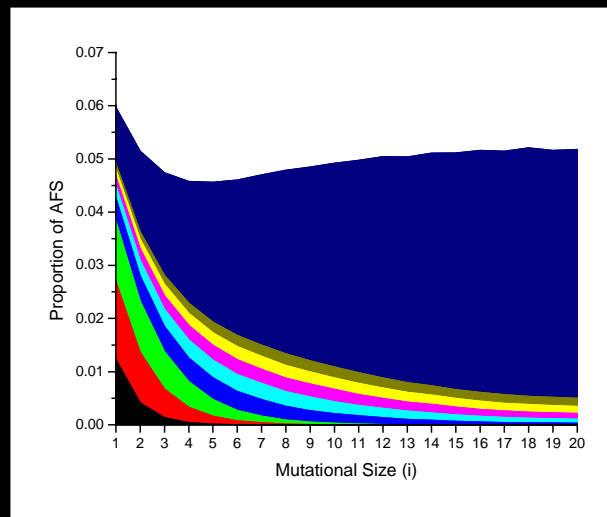
European data

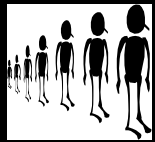
African data

average age of polymorphism

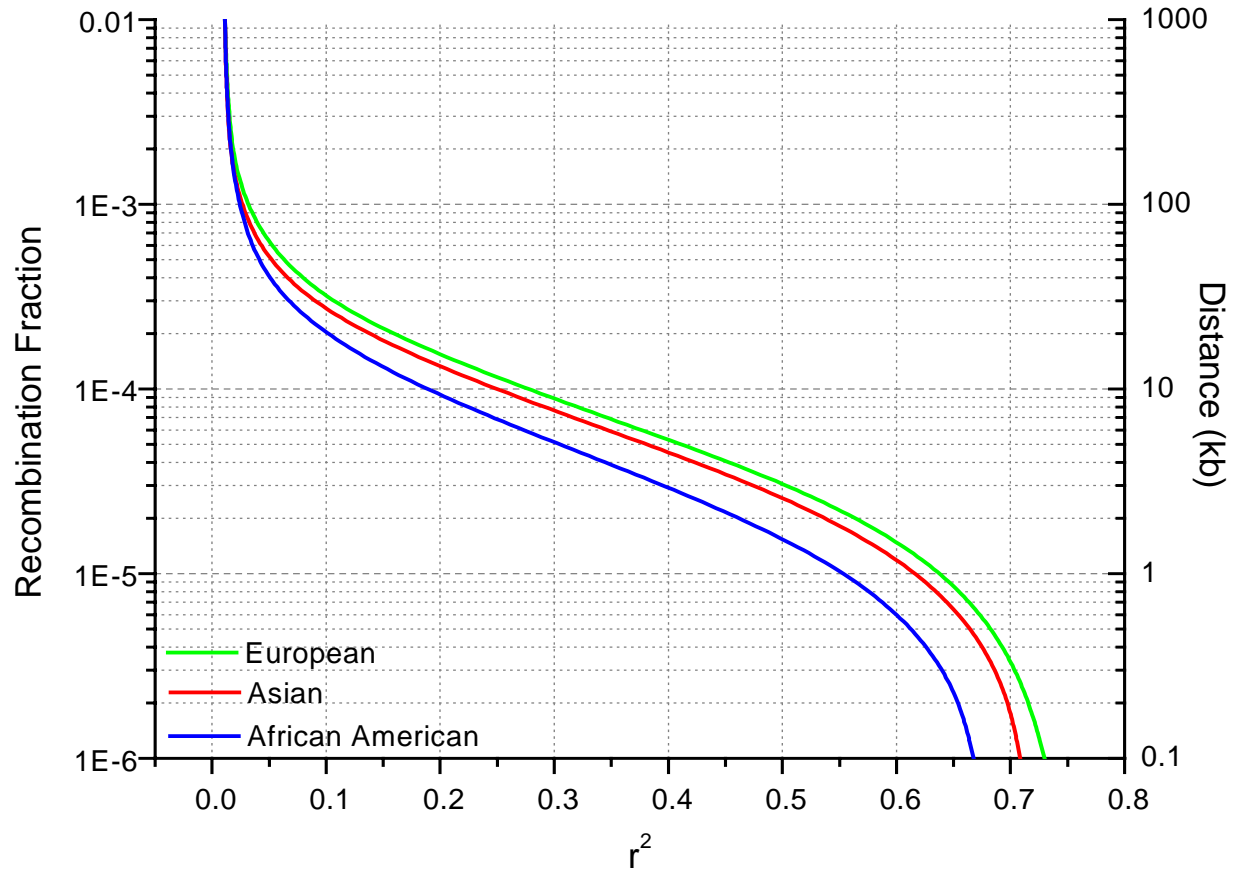


contribution of the past to alleles in various frequency classes



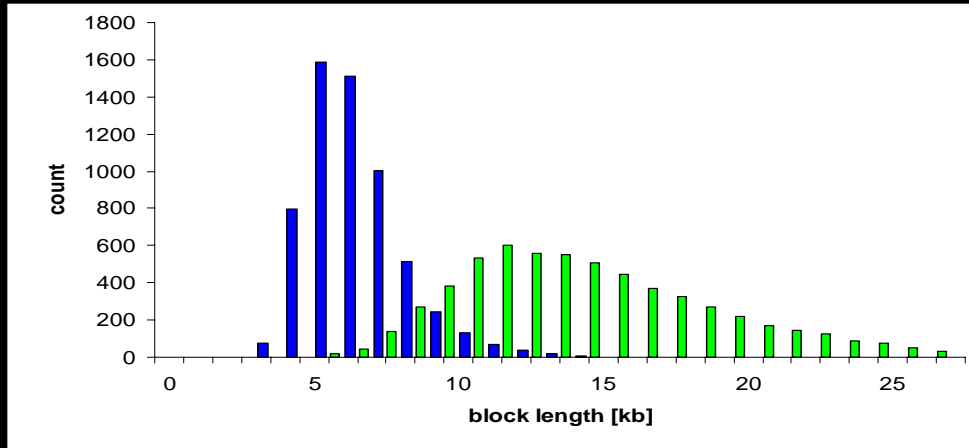


Prediction - extent of LD



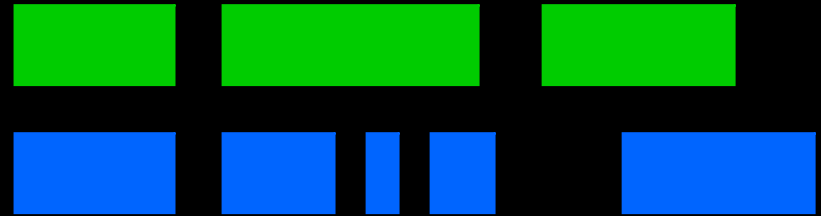


Prediction - haplotype structure

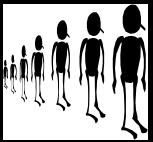


- our models predict shorter blocks in African samples than in Europeans

- what is the spatial relationship between blocks?

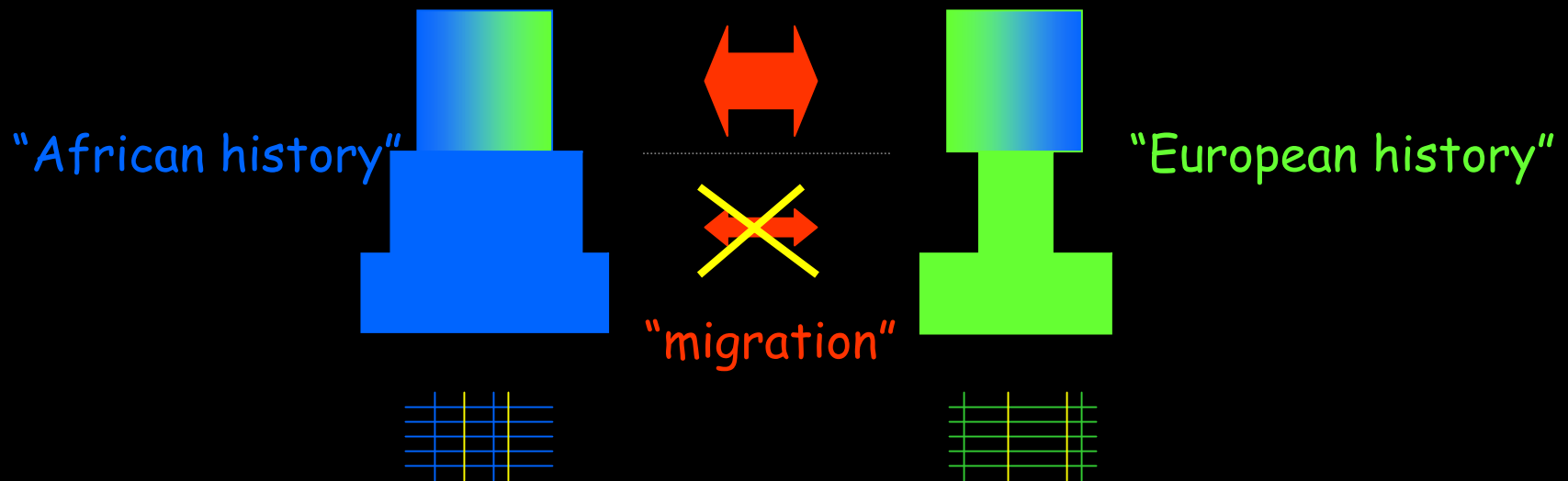


- we must connect the polymorphism structure of different human populations

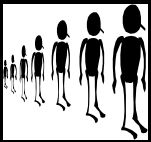


Modeling joint allele structure

- The “true” history of all human populations is interconnected
- We study these relationships with models of **population subdivision**



- The **genealogy** of samples from different populations are **connected** through the shared part of our past
- Polymorphic markers (some shared, some population-specific) and haplotypes are placed into a **common frame of reference**



Joint allele frequencies

European African	monomorphic	rare	common
monomorphic	0.0 % 0.0 %	19.9 % 13.2 %	2.3 % 1.0 %
rare	43.4 % 43.7 %	11.5 % 11.0 %	4.6 % 7.4 %
common	10.2 % 4.2 %	4.4 % 6.0 %	6.6 % 13.4 %

observation in UW PGA data

SNPs private to
European samples

shared SNPs

SNPs common in
both populations

SNPs private to
African samples

- our simple model of subdivision captures the **qualitative dynamics**
- we now have the tools to analyze joint allele structure



Generality for future samples?

- The haplotype map resource is a collection of reagents

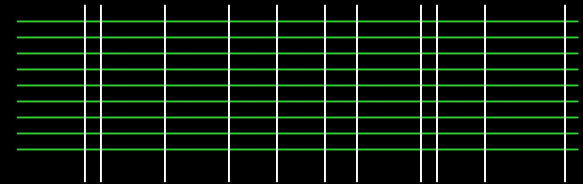
- reference samples

- common markers

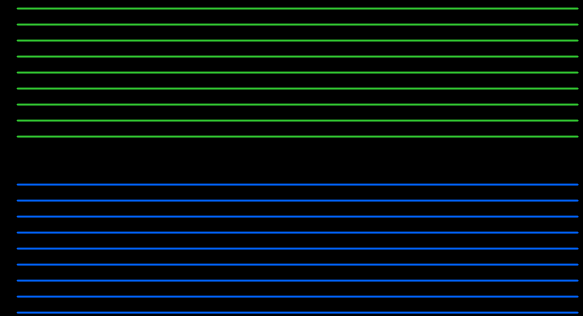
- blocks

- list of haplotypes

- frequent haplotypes

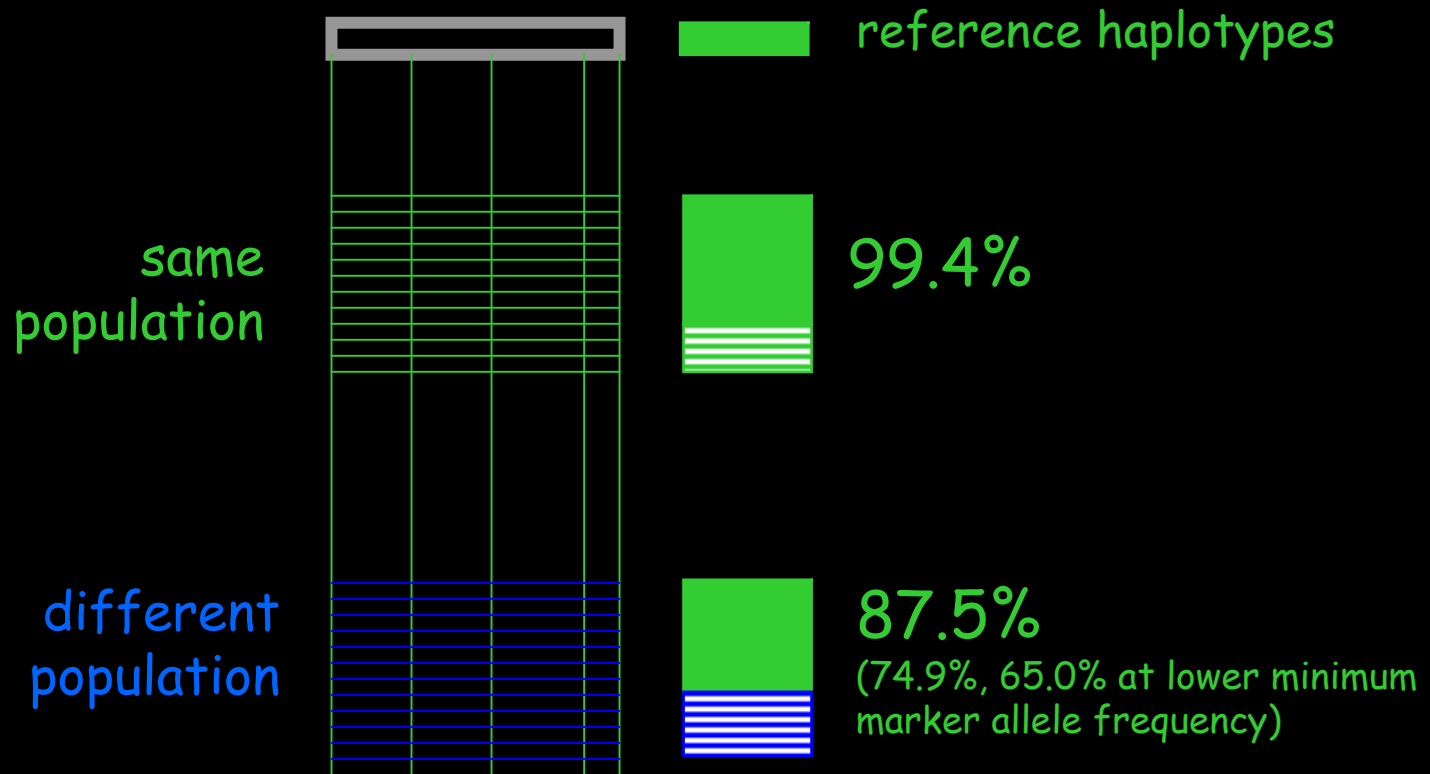


- How relevant are the reference reagents to future clinical samples (drawn from the **same** or **different** population)?





Reference haplotypes



- these computational studies inform us about the global, genome-average properties of the HapMap reagents
- what can we say about linkage in **specific local regions?**



Utility for association studies?

- No matter how good the resource is, its success to find disease causing variants greatly depend on the **allelic structure of common diseases**, a question under debate

© 2000 Nature America Inc. • http://genetics.nature.com **commentary**

How many diseases does it take to map a gene with SNPs?

Kenneth M. Weiss¹ & Joseph D. Terwilliger²

"They all talked at once, their voices a noisy impetuous babbling of uncertainty as each an inconceivable idea fell, as people will who are fascinated. They looked at..."

Linkage disequilibrium and the mapping of complex human traits

Kenneth M. Weiss and Andrew G. Clark

older variants have had more time to recombine and can have less LD than rare, younger or more geographically localized variants, so it is important to understand the pattern of LD in our genome.

LD in the human genome
Population genetic theory describes the way mutations, gene conversion, recombination, natural selection and the demographic structure of human populations affect patterns of LD. These theories vary along the genome and are generated by highly stochastic (stochastic) processes, which are...

Am. J. Hum. Genet. 69:124-137, 2001

Are Rare Variants Responsible for Susceptibility to Complex Diseases?

Jonathan K. Pritchard
Department of Statistics, University of Oxford, Oxford

On the allelic spectrum of human disease

David E. Reich and Eric S. Lander

association and linkage disequilibrium studies, depend on the existence of a relatively simple allelic spectrum in the study population". Clinical trial authorization and design of diagnostic and therapeutic interventions are also substantially more complex when the allelic spectrum is simple.

The purpose of this paper is to explore the following questions: Why is there the wide range of allelic spectra in human disease genes? To what extent is the spectrum of a disease predictable from its genetic properties? How can we learn more about the human mutation and population genetics...

- Regardless of how we describe human association structure, many questions remain about the relative merits of single-marker vs. haplotype-based strategies for medical association studies





Acknowledgements

Steve Sherry

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Andy Clark (Cornell)

Pui-Yan Kwok (UCSF)

Henry Harpending (Utah)

Jim Weber (Marshfield)

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