SNPs and Haplotype Inference

10-810, CMB lecture 10---Eric Xing

Polymorphism

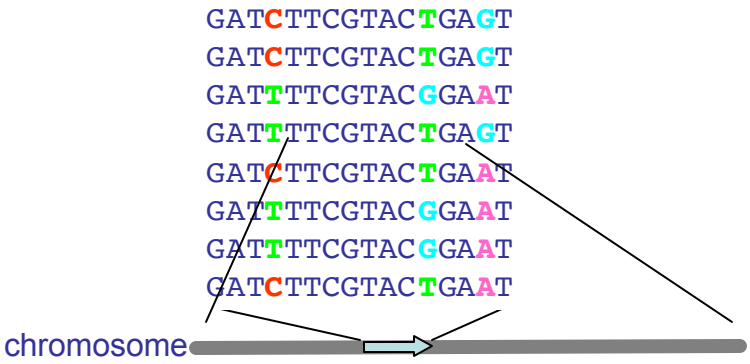
- Alleles: Alternative DNA sequences at a locus
- Technical definition: most common variant (allele) occurs with less than 99% frequency in the population
- Also used as a general term for variation
- Many types of DNA polymorphisms, including RFLPs, VNTRs, microsatellites
- 'Highly polymorphic' = many variants

Type of polymorphisms

- Single base mutation (SNP)
  - Restriction fragment length (RFLP)
  - Creating restriction sites via PCR primer
  - Direct sequencing
- Insertion/deletion of a section of DNA
  - Minisatellites: repeated base patterns (several hundred base pairs)
  - Microsatellites: 2-4 nucleotides repeated
  - Presence or absence of Alu segments

Frequency of SNPs greater than that of any other type of polymorphism

Single Nucleotide Polymorphism (SNP)



- "Binary" nt-substitutions at a single locus on a chromosome
  - each variant is called an "allele"

Single Nucleotide Polymorphism (SNP)

- More than 5 million common SNPs each with frequency 10-50% account for the bulk of human DNA sequence difference
- About 1 in every 600 base pairs
- It is estimated that ~60,000 SNPs occur within exons; 85% of exons within 5 kb of nearest SNP

Why SNPs?

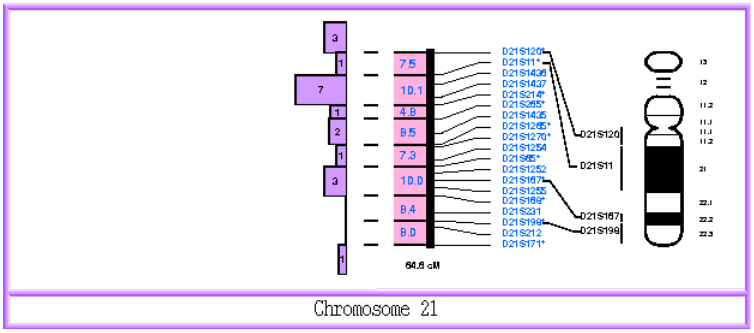
- The majority of human sequence variation is due to substitutions that have occurred once in the history of mankind at individual base pairs, SNPs (Patil et al. 2001).
- Markers for pinpointing a disease
- Association study: check for differences in SNP patterns between cases and controls
- There can be big differences between populations!
- http://snp.cshl.org/about/introduction.shtml

Linkage disequilibrium

- Relationship between alleles at different loci.
- Alleles at locus A: frequencies $p_1,\ldots, p_m$
- Alleles at locus B: frequencies $q_1,\ldots,q_n$
- Haplotype frequency for A_iB_j equilibrium value = $h_{ij} - p_i q_j$
- Linkage disequilibrium is an allelic association measure (difference between the actual haplotype frequency and the equilibrium value)
- More precisely: **gametic association**

Use of Polymorphism in Gene Mapping

- 1980s – RFLP marker maps
- 1990s – microsatellite marker maps

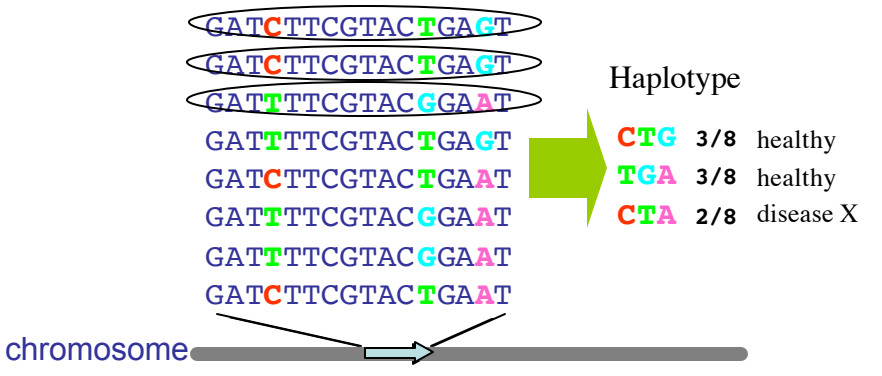


Advantages of SNPs in genetic analysis of complex traits

- Abundance: high frequency on the genome
- Position: throughout the genome (level of influence of type of SNP, e.g. coding region, promoter site, on phenotypic expression?)
- Haplotypic patterns (see later)
- Ease of genotyping
- Less mutable than other forms or polymorphisms
- Allele frequency drift (different populations)

Haplotype

-- a more discriminative state of a chromosomal region



- Consider J binary markers in a genomic region
- There are 2^J possible haplotypes
  - but in fact, far fewer are seen in human population
- Good genetic marker for population, evolution and hereditary dis eases …

Haplotype analyses

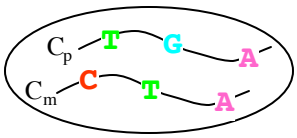
- Linkage disequilibrium assessment
- Disease-gene discovery
- Genetic demography
- Chromosomal evolution studies

Why Haplotypes

- Haplotypes are more powerful discriminators between cases and controls in disease association studies
- Use of haplotypes in disease association studies reduces the number of tests to be carried out.
- With haplotypes we can conduct evolutionary studies
- Haplotypes are necessary for linkage analysis

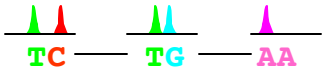
Phase ambiguity

-- haplotype reconstruction for individuals

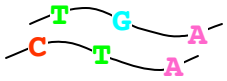


Heterozygous diploid individual

sequencing



Genotype g
pairs of alleles with association of alleles to chromosomes unknown



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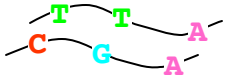


haplotype $h \equiv (h_1, h_2)$
possible associations of alleles to chromosome

This is a mixture modeling problem!

Inferring Haplotypes

- Genotype: AT//AA//CG
  - Maternal genotype: TA//AA//CC
  - Paternal genotype: TT//AA//CG
  - Then the haplotype is AAC/TAG.
- Genotype: AT//AA//CG
  - Maternal genotype: AT//AA//CG
  - Paternal genotype: AT//AA//CG
  - Cannot determine unique haplotype
- **Problem:** determine Haplotypes without parental genotypes

Identifiability

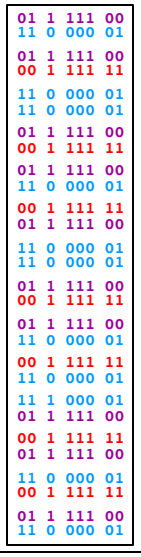
Genotype representations

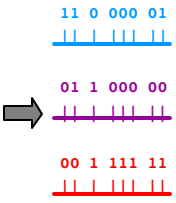
0/0 → 0
1/1 → 1
0/1 → 2

Genotypes of 14 individual

```
21 2 222 02
02 1 111 22
11 0 000 01
02 1 111 22
21 2 222 02
02 1 111 22
11 0 000 01
02 1 111 22
21 2 222 02
22 2 222 21
21 1 222 02
02 1 111 22
22 2 222 21
21 2 222 02
|| | ||| ||
```

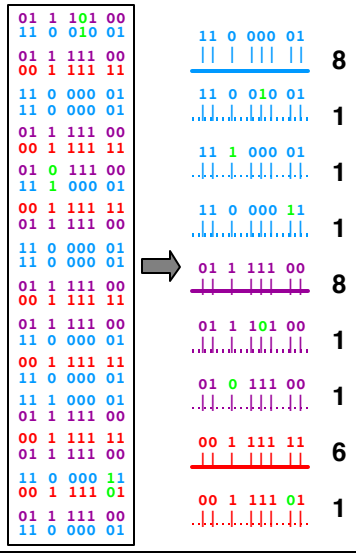
Identifiability





Parsimonious solution

?



Three Problems

1. Frequency estimation of all possible haplotypes
2. Haplotype reconstruction for individuals
3. How many out of all possible haplotypes are plausible in a population

given a random sample of multilocus genotypes at a set of SNPs

Haplotype reconstruction: Clark (1990)

- **Choose individuals that are homozygous at every locus (e.g. TT// AA//CC)**
  - **Haplotype: TAC**
- **Choose individuals that are heterozygous at just one locus (e.g. TT//AA//CG)**
  - **Haplotypes: TAC or TAG**
- **Tally the resulting known haplotypes.**
- **For each known haplotype, look at all remaining unresolved cases: is there a combination to make this haplotype?**
  - **Known haplotype: TAC**
    - **Unresolved pattern: AT//AA//CG**
    - **Inferred haplotype: TAC/AAG. Add to list.**
  - **Known haplotype: TAC and TAG**
    - **Unresolved pattern: AT//AA//CG**
    - **Inferred haplotypes: TAC and TAG. Add both to list.**
- **Continue until all haplotypes have been recovered or no new haplotypes can be found this way.**

Problems: Clark (1990)

- No homozygotes or single SNP heterozygotes in the sample
- Many unresolved haplotypes at the end
- Error in haplotype inference if a crossover of two actual haplotypes is indentical to another true haplotype
- Frequency of these problems depend on avg. heterozygosity of the SNPs, number of loci, recombination rate, sample size.
- Clark (1990): algorithm "performs well" even with small sample sizes.

Finite mixture model

The probability of a genotype *g*:

$$p(g) = \sum_{h_1, h_2 \in H} p(h_1, h_2)\, p(g \mid h_1, h_2)$$

Population haplotype pool — **Haplotype model** — **Genotyping model**

Standard settings:

- $p(g|h_1,h_2)=\mathbf{1}(h_1 \oplus h_1=g)$ noiseless genotyping
- $p(h_1,h_2)= p(h_1)p(h_2)=f_1 f_2$ Hardy-Weinberg equilibrium, multinomial
- $|H| = K$ fixed-sized population haplotype pool

$$p(g) = \sum_{\substack{h_1, h_2 \in H \\ h_1 \oplus h_2 = g}} f_1 f_2$$

EM algorithm:

Excoffier and Slatkin (1995)

Numerical method of finding maximum likelihood estimates for parameters given incomplete data.

1. Initial parameter values: Haplotype frequencies: $f_1,\ldots,f_h$
2. Expectation step: compute expected values of missing data based on initial data
3. Maximization step: compute MLE for parameters from the complete data
4. Repeat with new set of parameters until changes in the parameter estimates are negligible.

Beware: local maxima.

EM algorithm efficiency

- Heavy computational burden with large number of loci? (2^L possible haplotypes for L SNPs)
- Accuracy and departures from HWE?
- Error between EM-based frequency estimates and their true frequencies
- Sampling error vs. error from EM estimation process

Bayesian Haplotype reconstruction

- Bayesian model to approximate the posterior distribution of haplotype configurations for each phase-unknown genotype.
- $G = (G_1, \ldots, G_n)$ observed multilocus genotype frequencies
- $H = (H_1, \ldots, H_n)$ corresponding unknown haplotype pairs
- $F = (F_1, \ldots, F_M)$ M unkown population haplotype frequencies
- EM algorithm: Find F that maximizes P(G|F). Choose H that maximizes $P(H|F^{EM}, G)$.

Gibbs sampler

Initial haplotype reconstruction $H^{(0)}$.

- Choose and individual i, uniformly and at random from all ambiguous individuals.
- Sample $H_i^{(t+1)}$ from $P(H_i|G,H_{-i}^{(t)})$, where H_{-i} is the set of haplotypes excluding individual i.
- Set $H_j^{(t+1)} = H_j^{(t)}$ for j=1,…,i-1,i+1,…,n.

HAPLOTYPER:
Bayesian Haplotype Inference (Niu et al.2002)

- Bayesian model to approximate the posterior distribution of haplotype configurations for each phase-unknown genotype.
- Dirichlet priors ß=(ß$_1$,…, ß$_M$) for the haplotype frequencies F=(f$_1$,…,f$_M$).
- Multinomial model (as in EM algorithm) for individual haplotypes:
  - product over n individuals,
  - and multilocus genotype probabilities are sums of products of pairs of haplotype probabilities.

Gibbs sampler

- Haplotypes H are “missing:”

$$P(G,H \mid F) \sim \prod_{i=1,\ldots,n} f_{h_{i1}} f_{h_{i2}} \prod_{j=1,\ldots,n} f_j^{\beta_j - 1}$$

- Sample h_{i1} and h_{i2} for individual *i*:

$$P(h_{i1} = g, h_{i2} = h \mid F, G_i) = \frac{f_g f_h}{\sum_{g,h=G_i} f_{g'} f_{h'}}$$

- Sample H given H^{updated} Improving efficiency (Niu et al.)

Gibbs sampler

- **Predictive updating (Gibbs sampling)**:
  - (N(H)=vector of haplotype counts)

    $P(G,H) \sim \Gamma(|ß+N(H)|)/ \Gamma(ß+N(H))$
  - Pick an individual i, update haplotype h_i:

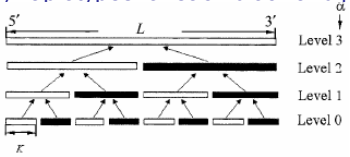
    $P(h_i =(g,h)|H_{-i},G) \sim (n_g+ ß_g)(n_h+ ß_h)$

    (n_g =count of g in H_{-i})

- **Prior Annealing**:
  - use high pseudo counts at the beginning of the iteration and progressively reduce them at a fixed rate as the sampler continues.

HAPLOTYPER Discussions

- **Missing marker data**:
  - PCR dropouts ->absence of both alleles,
  - one allele is unscored
  - Gibbs sampler adapts nicely
- **Ligation**
  - Problem: large number of loci.
  - Partition L loci into blocks of 8 and carry out block level haplotype reconstruction.
  - Record the B most probable (partial) haplotypes for each block and join them
    - **Progressive ligation**.
    - **Hierarchical ligation**.



$L=K \times 2^{\alpha}$

Phase

coalescence-based Bayesian Haplotype inference: Stephens et al (2001)

- What is $P(H_i | G, H_{-i}^{(t)})$?
- For a haplotype $H_i=(h_{i1},h_{i2})$ consistent with genotypes G_i: $P(H_i|G,H_{-i}) \sim P(H_i|H_{-i}) \sim p(h_{i1}|H_{-i})\, p(h_{i2}|h_{i1},H_{-i})$
- $p(.|H)$=conditional distribution of a future sampled haplotype given previously sampled haplotypes H.
- r=total number of haplotypes, r_a=number of haplotypes of type a, ?=mutation rate, then a choice for

$$\pi(a|H) = (r_a + ? \mu_a)/(r + ?),$$

where μ_a=prob. of type a.

PHASE, details

- This is not working when the number of possible values H_i is too large: 2^{J-1}, J=number of loci at which individual i is heterozygous. Alternatively,

$$\pi(h \mid H) = \sum_{\alpha \in E} \sum_{s=0}^{\infty} \frac{r_\alpha}{r} \left(\frac{\theta}{r+\theta}\right)^s \frac{r}{r+\theta} \left(P^s\right)_{\alpha h}$$

  where *E*=set of types for a general mutation model, *P*=reversible mutation matrix.
- I.e. future haplotype *h* is obtained by applying a random number of mutations, *s* (sampled from geometric distribution), to a randomly chosen existing haplotype, r_a (coalescent).
- Problems: estimation of *?*, dimensionality of *P* (dim P = M, the number of possible haplotypes).

PHASE Discussion

- Key: unresolved haplotypes are similar to known haplotypes
- HWE assumption, but robust to “moderate” levels of recombinations
- More accurate than EM,Clark’s and Haplotyper algorithms
- Provides estimates of the uncertainty associated with each phase call
- Problem (of both Bayesian model): dimensionality

Summary: Algorithms

Clark’s parsimony algorithm:

- simple, effective,
- depends on order of individuals in the data set,
- need sufficient number of homozygous individuals,
- Disadvantage: individuals may remain phase indeterminate, biased estimates of haplotype frequencies

EM algorithm:

- accurate in the inference of common haplotypes
- Allows for possible haplotype configurations that could contribute to a phase-unknown genotype.
- Cannot handle a large number of SNPs.

Summary: Algorithms

Haplotyper:

- Bayesian model to approximate the posterior distribution of haplotype configurations
- Prior annealing helps to escape from local maximum
- Partitions long haplotypes into small segments: block-by-block strategy
- Gibbs sampler to reconstruct haplotypes within each segment. Assembly of segments.
- **http://www.people.fas.harvard.edu/~junliu/index1.html#ComputationalBiology**

Summary: Algorithms

PHASE:

- Bayesian model to approximate the posterior distribution of haplotype configurations
- based on the coalescence theory to assign prior predictions about the distributions of haplotypes in natural populations,
- may depend on the order of the individuals,
- pseudo posterior probabilities (-> pseudo Gibbs sampler),
- lacks a measure of overall goodness.
- **http://www.hgmp.mrc.ac.uk/Registered/Option/phase.html**