The Clark Method of Phasing

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- The phasing problem now is related to PCR ambiguity in diploid individuals
- For example, suppose that the two chromosomes are:

$$\begin{array}{ccccccc} - - - - - & & * & - - - -- & & * & - - - -- \\ - - - - - & & * & - - - -- & & * & - - - -- \\ & A_0/A_1 & & & B_0/B_1 & & \end{array}$$

mother

father

- How can we resolve this ambiguity?

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- If there are k ambiguous sites, there are an exponential (in k) number of possible explanations of the ambiguity

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$$\textit{Haplotypes}: \quad \begin{matrix} 1) & 001010 \\ 2) & 011000 \end{matrix} \longrightarrow \textit{Genotype}: 0\left(\tfrac{0}{1}\right)10\left(\tfrac{0}{1}\right)0$$

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- If we were given just the genotype, then there are two possible explanations ($k = 2$)

$$\begin{array}{ll} 0\underline{0}10\underline{0}0 & 0\underline{0}10\underline{1}0 \\ 0\underline{1}10\underline{1}0 & 0\underline{1}10\underline{0}0 \end{array}$$

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- '2' denotes ambiguous site
- haplotype will be a sequences of 0 and 1's [ex. 0101010]
- An explanation of a genotype will be a *pair* of haplotypes

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- Two easy cases:
  - Homozygous: genotype with only 0's and 1's. The mother and the father chromosome have the same composition.
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- Multiple heterozygote case: More than single '2's in the genotype [ex. 01212]
- Number of explanations for a genotype with k ambiguous sites is 2^{k-1} [in this case 01010, 01011, 01110, 01111]

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- Starting with the 'easy' cases, we are able to resolve 3 of the 5 individuals:

Individual	*Inferred Mate – Pair*
20000 :	10000 – 00000
00100 :	00100 – 00100
01000 :	01000 – 01000

The Clark Method of Phasing

- For the unresolved chromosomes (01202, 12121), we have two possible explanations for each:

Individual	*Explanation* 1	*Explanation* 2
01202 :	01101	01001
	01000	01100
12121 :	10101	11101
	11111	10111

The Clark Method of Phasing

Now, if we look at the possible explanations together, we see that there is overlap between the inferred mate-pairs from the simple cases and the possible explanations for the ambiguous cases.

Individual	*Inferred Mate − Pair*
20000 :	10000 – 00000
00100 :	00100 – 00100
01000 :	**01000 – 01000**

Individual	*Explanation* 1	*Explanation* 2
01202 :	01101	01001
	01000	01100
12121 :	10101	11101
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The Clark Method of Phasing

- Notation: G = H $\oplus$ H' denotes that haplotype pair (H,H') is an explanation for G
- Notation: 'H $\longrightarrow^{C}$ G' denotes G can be resolved using $H \in C$, i.e., there exists H' s.t. $G = H \oplus H'$, and we call H' the *inferred haplotype*
- Example: For G=02112:

$$\begin{array}{rc} & \textit{Possible Mate} - \textit{Pairs} \\ G: & 00110 \oplus 01111 \\ & 01110 \oplus 00111 \end{array}$$

The Clark Method of Phasing

Clark Algorithm

1. Find all homozygotes and single heterozygotes and make a list of all of the haplotypes involved in the unique explanations.
2. While there are remaining genotypes that are unresolved, attempt to find a haplotype from the list that helps resolve some unresolved genotype. If such a haplotype exists, add the corresponding mate-pair to the list of haplotypes and label the genotype as resolved.

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Example: If the genotype is 12121, then 10101 is the *mate-pair* of 11111 in the explanation of genotype 12121

- If we are able to find a haplotype in the list and thus explain the ambiguous haplotype, we have inferred a uniquely inferred additional haplotype, that of the mate-pair.

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  - If there are no homozygotes or single heterozygotes, then the algorithm cannot start.
  - The order in which you resolve the haplotypes matters. A different ordering may produce a different haplotype list.
- What is the probability of the algorithm stopping prematurely or not being able to start?

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- How can we estimate the number of 2's?
- Population model: Infinite Sites Model (at most *one* mutation can happen at any site on the chromosome)

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- expected number of mismatches of a DNA sequence:

$$\Theta = L\Theta_{nt}$$

where

- L = length of the sequence
- $\Theta_{nt} = 4N\mu$
- N = the effective population size
- μ is the mutation rate per nucleotide per generation

The Clark Method of Phasing

- For example, in Drosophila, $\Theta = .005L$.

$$Pr(\text{2 sequences have m mismatches}) = \frac{1}{\theta+1}\left(\frac{\theta}{\theta+1}\right)^m$$

- In the infinite sites model,

$$Prob(\text{2 genes identical}) = \frac{1}{1+\theta}(m=0)$$

- Probability of two different genes is

$$1 - Prob(\text{2 genes identical}) = \frac{\theta}{1+\theta}$$

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Thus, if $\Theta > 0.5$, Clark's algorithm will work well.

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- How can we deal with the 'orphan' genotypes? What is the probability of not finishing?
- The answer depends on the algorithm implementation and the order in which you resolve the haplotypes.

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- **Maximum Resolution Problem:**

  Input: A set of vectors, ambiguous (0,1,2) and resolved (0,1) [genotypes]
  Output: Maximum number of ambiguous vectors that can be resolved by successive applications of Clark's Rule.
- Equivalent to minimize the number of orphan genotypes

The Clark Method of Phasing

Theorem

The Maximum Resolution Problem is NP-complete.

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The Maximum Resolution Problem is NP-complete.

The proof for this theorem is based on a reduction to the satisfiability problem (SAT).

Proof.

- Let X_1, X_2, X_3, and X_4 be variables, and let $(x_1, \bar{x_1}, x_2, \bar{x_2}, x_3, \bar{x_3}, x_4, \bar{x_4})$ be literals. The satisfiability problem is
  - **Input**: Boolean function F
  - **Output**: Is there a truth assignment that makes F true? If so, find such an assignment.

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  - **Input**: Boolean function F
  - **Output**: Is there a truth assignment that makes F true? If so, find such an assignment.
- We are going to attempt to create a 1-1 correspondence between the boolean logic and haplotypes using the above function F.

The Clark Method of Phasing

- To the function F, we associate a set of genotypes (corresponding to columns in the matrix). The number of rows in the matrix will be # variables + 2 $\times$ #variables +#clauses +1 = 3V +C +1.

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- We fill in rows C_1, C_2, C_3 in the following manner: if X_i is absent from a clause, put '1' in both T_i and F_i. If X_i is present in a clause, place '1' in T_i and '0' in F_i. If $\overline{X_i}$ is present in a clause, then place a '0' in T_i and a '1' in F_i.

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- Fill in columns $S_1 \rightarrow S_4$ by:

$$\begin{cases} \text{'0'} & \text{if 00} \\ \text{'1'} & \text{if 11} \\ \text{'2'} & \text{if 01 or 10} \end{cases}$$

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- Place a '2' in C_2. $\bar{x_1}$ appears in C_3, but no other clause. Place a '2' in C_3. etc. (Note: the rows x_1 and $\bar{x_1}$ should sum to the X_1 row in the C columns.)

The Clark Method of Phasing

How do we fill in the bottom right corner of the table?

- We have a clause set $C_1 \ldots C_c$, one for each clause. All will be ambiguous vectors
- For each K=1,2,...c the first v positions of the vector C_k are zero except for position i such that either x_i or $\bar{x}_i$ appears in C_k. We are blind to the actual truth value
- For the next 2v positions [the literals], place a zero except for any position v+2i-1 where x_i appears in clause C_k or position v+2i where $\bar{x}_i$ appears in clause C_k. These positions are set to 2
- For each r from 1 to c, position 3v+r of C_k =0 if and only if r=k [the diagonal].
- For r$\neq$k position 3v+r = 2 if and only if clause k and r contain a variable in common [not necessarily a literal in common].
- Otherwise, position 3v+r =1. [This assignment captures the ambiguity related to the literals that are contained in multiple clauses. You may have both x_i and $\bar{x}_i$ present in F]

The Clark Method of Phasing

We reduce SAT to MR. Start with a generic boolean formula F with C clauses and V variables X_1 ... X_v. F takes a set of vectors V(F) that are ambiguous and resolved as input to the MR problem [the columns of out table drawn above]. We want to show that F has a satisfying truth assignment *iff* V(F) has the maximum number of ambiguous vectors explained by a series of Clark Rules. Recall that $F = (x_2 \vee x_3 \vee \bar{x_4}) \wedge (x_1 \vee \bar{x_2} \vee x_4) \wedge (\bar{x_1} \vee \bar{x_3})$.

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How can we interpret resolution by the Clark Method?

- Suppose that you pick colum T_1 to resolve one of the other columns; this is interpreted as setting the literal X_1 to true.

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- Suppose that you pick colum T_1 to resolve one of the other columns; this is interpreted as setting the literal X_1 to true.
- Suppose that we try to resolve column [haplotype] S_1. S_1 can be resolved by using either the T_1 or F_1 columns. By picking one of the columns, we are fixing X_1 as true or false.
- Using the haplotypes obtained in the resolution of the S columns along with the T and F columns, can we resolve the columns $C_1 \rightarrow C_3$? (NO)

The Clark Method of Phasing

	T_1	F_1	T_2	F_2	T_3	F_3	T_4	F_4	S_1	S_2	S_3	S_4	C_1	C_2	C_3
X_1	1	1	0	0	0	0	0	0	1	0	0	0	0	2	2
X_2	0	0	1	1	0	0	0	0	0	1	0	0	2	2	0
X_3	0	0	0	0	1	1	0	0	0	0	1	0	2	0	2
X_4	0	0	0	0	0	0	1	1	0	0	0	1	2	2	0
x_1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
$\bar{x_1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
x_2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
$\bar{x_2}$	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
x_3	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
$\bar{x_3}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
x_4	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
$\bar{x_4}$	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
C_1	1	1	1	0	1	0	0	1	1	2	2	2	0	2	2
C_2	1	0	0	1	1	1	1	0	2	2	1	2	2	0	2
C_3	0	1	1	1	0	1	1	1	2	1	2	1	2	2	0
C_B	0	0	0	0	0	0	0	0	2	2	2	2	1	1	1

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- Recall the notation $R \rightarrow^{C} A$ where R = resolved haplotype and A = ambiguous haplotype.
- If we call A[i] the i-th site of A and R[j] the j-th site in R then the notation means that if A[i] = 0 or 1 then R[i] = 0 or 1 and R[i] = A[i].

Maximum Resolution Problem

Further explanation of the NP-completeness proof: For a more full presentation, see Dan Gusfield's paper

Inference of Haplotypes from Samples of Diploid Populations: Complexity and Algorithms, J. Computational Biology August 2001.

Remarks (Table Setup)

- For every variable, there are two columns T and F
- A column of selectors exists for each random variable
- A column exists for each clause
- The first V rows are associated with the random variables
- The next set of rows are associated with the literals
- The final set of rows corresponds to clauses, including the mysterious (for a little while longer anyways) C_b
- $T_1, F_1, ..., T_4, F_4$ are all *resolved* columns while the rest are *unresolved*

The Clark Method of Phasing

Remarks (General Properties)

- $T_i \rightarrow^C S_i$ or $F_i \rightarrow^C S_i$ but $T_i \not\rightarrow^C S_j$ and $F_i \not\rightarrow^C S_j$. i.e. Column T_i can be applied by Clark Rule to column S_i but to no other selector column.
- At most one T or F can be applied to any S. i.e. set X_1 to either T or F, but not both!
- We interpret as follows: if $T_i \rightarrow^C S_i$ as '$X_i = \text{true}$'; if $F_i \rightarrow^C S_i$ as '$X_i = \text{false}$'. Suppose $T_1 \rightarrow^C S_1$ $F_2 \rightarrow^C S_2$ $F_3 \rightarrow^C S_3$ $T_4 \rightarrow^C S_4$, so the *inferred* vectors are $R_1 = S_1 \ominus T_1,\ R_2 = S_2 \ominus F_2,\ R_3 = S_3 \ominus F_3,\ R_4 = S_4 \ominus T_4$
- R_1, R_2, R_3, R_4 can be applied only to C_1, C_2 and C_3. [Consider the Blocking Clause C_b] The last entry of R_1 to R_4 will be a 1
- No T or F can be applied to the C vectors [Because of the Blocking Clause, C_b]
- $T_i \rightarrow^C S_i$, then $R_k \rightarrow^C C_k$ *iff* the literal x_i appears in C_k. Similarly, $F_i \rightarrow^C S_i$ then $R_k \rightarrow^C C_k$ *iff* the literal $\bar{x}_i$ appears in C_k.