Computing the Minimum Recombinant Haplotype Configuration from Incomplete Genotype Data on a Pedigree by Integer Linear Programming

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Abstract

We study the problem of reconstructing haplotype configurations from genotypes on pedigree data with missing alleles under the Mendelian law of inheritance and the minimum recombination principle, which is important for the construction of haplotype maps and genetic linkage/association analyses. Our previous results show that the problem of finding a *minimum-recombinant haplotype configuration (MRHC)* is in general NP-hard. The existing algorithms for MRHC either are heuristic in nature and cannot guarantee optimality, or only work under some restrictions (on *e.g.* the size and structure of the input pedigree, the number of marker loci, the number of recombinants in the pedigree, *etc.*). In addition, most of them cannot handle data with missing alleles and, for those that do consider missing data, they usually do not perform well in terms of minimizing the number of recombinants when a significant fraction of alleles are missing. This paper presents an effective integer linear programming (ILP) formulation of the MRHC problem with missing data and a branch-and-bound strategy that utilizes a partial order relationship and some other special relationships among variables to decide the branching order. The partial order relationship is discovered in the preprocessing of constraints by considering unique properties in our ILP formulation. A directed graph is built based on the variables and their partial order relationship. By identifying and collapsing the strongly connected components in the graph, we may greatly reduce the size of an ILP instance. Non-trivial lower and upper bounds on the optimal number of recombinants are introduced at each branching node to effectively prune the search tree. When multiple solutions exist, a best haplotype configuration is selected based on a maximum likelihood approach. The paper also shows for the first time how to incorporate marker interval distance into a rule-based haplotyping algorithm. Our results on simulated data show that the algorithm could recover haplotypes with 50 loci from a

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pedigree of size 29 in seconds on a standard PC. Its accuracy is more than 99.8% for data with no missing alleles and 98.3% for data with 20% missing alleles in terms of correctly recovered phase information at each marker locus. A comparison with a statistical approach SimWalk2 on simulated data shows that the ILP algorithm runs much faster than SimWalk2 and reports better or comparable haplotypes on average than the first and second runs of SimWalk2. As an application of the algorithm to real data, we present some test results on reconstructing haplotypes from a genome-scale SNP data set consisting of 12 pedigrees that have 0.8% to 14.5% missing alleles.

Keywords: Haplotyping, pedigree analysis, recombination, missing data imputation, integer linear programming, branch-and-bound algorithm

1 Introduction

With the completion of the Human Genome Project [16, 33], an almost complete human genomic DNA sequence has become available, which is essential to the understanding of the functions and characteristics of human genetic material. An important next step in human genomics is to determine genetic variations among humans and the correlation between genetic variations and phenotypic variations such as disease status, quantitative traits, *etc.* To achieve this goal, an international collaboration, namely, the international HapMap project, was launched in October, 2002. The main objective of the HapMap project is to identify the *haplotype* structure of humans and common haplotypes among populations. However, the human genome is a diploid and, in practice, haplotype data are not collected directly, especially in large scale sequencing projects mainly due to cost considerations. Instead, genotype data are collected routinely in large sequencing projects. Hence, efficient and accurate computational methods and computer programs for the inference of haplotypes from genotypes are highly needed.

The input data for haplotype reconstruction can be divided into three categories: SNP segments from an individual [20, 22] or pooled samples [26], genotype data with *pedigree* information, and genotype data without pedigree information (also called *population* data sometimes) [8, 9, 12, 24, 31]. A recent comprehensive review of computational methods for haplotype inference can be found in [3]. Some other overviews of combinatorial and statistical methods for haplotype inference on population data can be found in [13, 14]. This paper focuses on genotype data with pedigrees. It is generally believed that haplotypes inferred from pedigrees are more accurate than those from population data. Moreover, some family based statistical association tests such as TDT (*i.e. Transmission Disequilibrium Test*) and its variants (*e.g.* [28, 36] among others) require access to haplotype information of each member in a pedigree. The existing computational methods for haplotyping pedigree data can be divided into two categories: statistical methods and rule-based (*i.e.* combinatorial) methods. The goal of statistical approaches [1, 11, 19, 21, 29, 30] is to find a haplotype solution with the maximum likelihood under certain assumptions. But statistical methods are usually time consuming and thus cannot handle large or moderately large data sets, especially for data sets with dense markers. On the other hand, rule-based approaches are usually fast, although they do not normally provide numerical assessments of the reliability of their results. Nonetheless, by utilizing some reasonable biological assumptions, such as the *minimum recombination principle*, rule-based methods have proven to be powerful and practical [25, 27, 32, 34]. The minimum recombination principle basically says that genetic recombination is rare for closely linked markers and thus haplotypes with fewer recombinants should be preferred in a haplotype reconstruction [25, 27]. The principle is well supported by practical data. For example, recently published experimental results [6, 10, 15] demonstrate that, in the case of human, the number of distinct haplotypes is limited. Moreover, the genomic DNAs can probably be partitioned into long blocks such that recombination within each block is rare or even nonexistent.

Since the maximum likelihood (ML) and MRHC formulations have different objectives, they might be the most applicable in different situations. In general, the ML formulation is more appropriate for sparse markers where the assumption of linkage equilibrium is likely to hold, while the MRHC formulation is more effective for dense markers where the expected number of recombination events is small. On the other hand, ML methods can also be used to generate haplotype solutions with few recombinants for dense markers and rule-based formulation could also be extended to take into consideration the marker interval distance for sparse markers, as demonstrated later in this paper. In fact, one may combine both approaches nicely in order to get a better performance.

1.1 Previous Work on Rule-Based Haplotype Reconstruction on Pedigrees

Qian and Beckmann [27] proposed a rule-based algorithm to reconstruct haplotype configurations for pedigree data, based on the minimum recombination principle. From now on, we refer to their algorithm as MRH. Given a pedigree and the genotype information for each member with possibly missing alleles, MRH attempts to find a haplotype configuration such that the total number of recombinants (or recombination events) in the whole pedigree is minimized. We call the above problem the *minimum-recombinant haplotype configuration (MRHC)* problem. In recent papers [7, 17, 18], we showed that MRHC is in general NP-hard, even for pedigrees without mating loops, and developed an iterative heuristic algorithm, called *block-extension*, for MRHC that is much more efficient than MRH. Our preliminary experiments showed that the algorithm block-extension is often able to compute an optimal solution or nearly optimal solution when the minimum number of recombinants required is small [17, 18]. However, its performance deteriorates significantly when the input data requires more (*e.g.* 4 or more) recombinants. We have also devised an efficient exact algorithm based on Gaussian elimination for solving MRHC on pedigree data that requires no recombinants. In fact, the algorithm can find all haplotype configurations incurring no recombinants [17, 18]. More recently, two dynamic programming algorithms [7] are developed for general pedigrees of small sizes and loopless pedigrees with a small number of marker loci.

However, these existing algorithms for MRHC either are heuristics in nature (*e.g.* MRH and block-extension) and cannot guarantee optimality, or only work under some restrictions on the size and structure of the input pedigree, the number of marker loci, the number of recombinants in a pedigree, *etc.* Furthermore, most of them cannot handle missing data and, for those that consider missing data, their performance in terms of minimizing the number recombinants drops significantly in the presence of a moderately large amount of missing alleles. In practice, pedigree data often contains a significant amount of missing alleles. For example, as much as 14.5% of the alleles belonging to a block could be missing in the pedigree data studied in [10]. Some of the livestock pedigree data that we have examined recently contained an even larger fraction of missing alleles. Unfortunately, consistent imputation of missing alleles is NP-hard even if we do not care about haplotypes and recombination [2].

1.2 Our Results

This paper presents an effective integer linear programming formulation of MRHC with missing alleles that integrates missing data imputation and haplotype inference, and a branch-and-bound strategy that utilizes a partial order relationship and some other special relationships among variables to decide the branching order. The partial order relationship is discovered in the preprocessing of constraints by taking advantage of some special properties in the ILP formulation. A directed graph is built based on the variables and their partial order relationship. By identifying and collapsing strongly connected components in the graph, we may greatly reduce the size of an ILP instance. Non-trivial lower and upper bounds on the optimal number of recombinants are estimated at each branching node to prune the branch-and-bound search tree. When multiple solutions exist, a best haplotype configuration is selected based on a maximum likelihood approach. The algorithm also incorporates the marker interval distance into the formulation whenever it is known, which overcomes the inadequacy of many rule-based algorithms that ignore the important information.

The test results on simulated data using three pedigree structures demonstrate that the above algorithm, which will be referred to as simply as algorithm ILP from now on, is very efficient. For example, it could recover haplotypes with 50 loci on a pedigree of size 29 in seconds on a regular PC. It outruns MRH v0.2 on biallelic data while guaranteeing the minimum number of recombinants. With respect to performance in terms of correctly recovered phase information at each marker locus, ILP was able to recover correct phase information at more than 99.8% of the marker loci for data with no missing alleles and 98.3% of the marker loci for data with as many as 20% missing alleles.

A comparison of the algorithm with a well known statistical approach SimWalk2 [30] on simulated data with evenly and unevenly spaced markers also demonstrates the effectiveness and soundness of the ILP algorithm. The test results show that ILP is much faster than SimWalk2 and the difference is in the order of 6 to 35-fold. The difference increases drastically with the increase of the size of the input pedigree and the number of markers. In terms of accuracy, the ILP algorithm outperforms SimWalk2 on its first run and reports comparable results as the second run of SimWalk2.

As an application to real data, we have applied the algorithm ILP to a genome-scale data set that consists of 12 multi-generation human pedigrees studied in a recent paper [10]. We focus on each of the blocks inferred in [10] and compare the haplotyping results of ILP with those of the EM algorithm used in [10]. The comparison shows that ILP outputs haplotype configurations that require a few recombinants and result in roughly the same set of *common haplotypes* (*i.e.* haplotypes that occur with a frequency at least 5%) as the EM algorithm. We also compare ILP with the algorithm block-extension on chromosome 3 consisting of 10 blocks. The results show that ILP often finds solutions that require fewer recombinants than those returned by block-extension or MRH, which has a similar performance. Out of the solutions for 120 ($= 12 \cdot 10$) data sets found by ILP, only 2 requires recombinants while 18 solutions output by block-extension require recombinants. This difference is mainly due to different methods were used to impute missing alleles.

1.3 Organization of the Paper

The rest of this paper is organized as follows. We introduce briefly the biological background of the MRHC problem and some relevant terms in Section 2 and the integer linear program formulation in Section 3. In Section 4, we explore some special properties in the constraints of the ILP formulation and define several useful relationships among the variables. The statistical assessments on multiple optimal solutions are presented in Sections 5 and the extension to incorporate marker interval distances into the formulation is shown in Section 6. Section 7 shows the experimental results on simulated data sets and on a real data set. We conclude the paper with a few remarks about possible future work in Section 8.

2 Preliminaries

The genome of an organism consists of *chromosomes* that are double strand DNAs. Locations on a chromosome can be labelled using *markers*, which are small segments of DNA with some specific features. A physical position of a marker on a chromosome is called a *marker locus* and a marker state is called an *allele*. A set of markers and their positions define a *genetic map* of chromosomes. There are many types of markers. The two most commonly used markers are *microsatellite* markers and *SNP (single nucleotide polymorphism)* markers. Different sets of markers have different properties, such as the total number of distinct allelic states at a locus, frequency of each allele, distance between two adjacent loci, *etc.* A microsatellite marker usually has several different alleles at a locus (called *multi-allelic*) while an SNP marker can be treated as a *biallelic*, which has two alternative states. The average distance between two SNP marker loci is much smaller than the average distance between two microsatellite marker loci, thus making SNP markers superior to other markers in gene fine-mapping. In *diploid* organisms, chromosomes come in pairs. The status of two alleles at a particular marker locus of a pair of chromosomes is called a *marker genotype*. The genotype information at a locus will be denoted using a set, *e.g.* $\{a, b\}$. If the two alleles a and b are the same, the genotype is *homozygous*. Otherwise, it is *heterozygous*. A *haplotype* consists of all alleles, one from each locus, that are on the same chromosome. Figure 1(A) illustrates the above concepts, where alleles are represented by their numerical IDs.

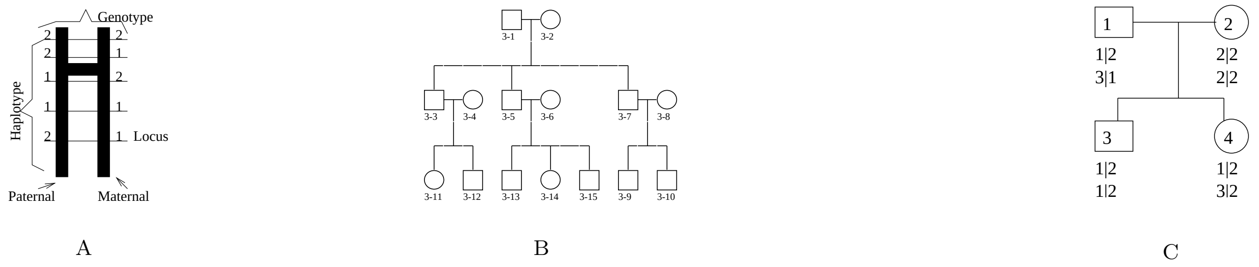


Figure 1: A. The structure of a pair of chromosomes from a mathematical point of view. B. An illustration of a pedigree with 15 members. C. An example of recombination event.

A pedigree can be defined formally as follows.

Definition 2.1 *A pedigree graph is a weakly connected directed acyclic graph (DAG)* $G = \{V, E\}$, *where* $V = M \cup F$, M *stands for the male nodes,* F *stands for the female nodes. The in-degree of each node is* 0 *(founders) or* 2 *(non-founders). If the in-degree of a node is* 2*, one edge must start from a male node (called father) and the other edge from a female node (called mother) and the node itself is a child of its parents (father and mother).*

A subgraph containing the father, mother, and child nodes is called a *nuclear family*. A *mating loop* consists of two distinct paths from a node x to a node y. For convenience, we will use conventional drawings of pedigrees throughout the paper. Figure 1(B) illustrates an example pedigree. [1] Figure 3(B) shows a pedigree with a mating loop. The Mendelian law of inheritance states that the alleles of a child must come from the alleles of its parents at each marker locus (*i.e.* assuming no mutations within a pedigree). In other words, the two alleles at each locus of the child have different origins: one is from its father (which is called the *paternal* allele) and the other from its mother (which is called the *maternal* allele). Usually, a child inherits a complete haplotype from each parent. However, *recombination* may occur, where the two haplotypes of a parent get shuffled due to a crossover of chromosomes and one of the shuffled copies is passed on to the child. Such an event is called a recombination event and its result is called a *recombinant*. Since markers

[1] The pedigree diagrams in this paper were generated using WPEDRAW [5].

are usually short DNA sequences, we assume that recombination only occurs between markers. Figure 1(C) illustrates an example where the paternal haplotype of member 3 is the result of a recombinant. Paternal allele and maternal allele at each locus is separated by a "|" in this figure.

We use the term *haplotype configuration* to describes not only the paternal and maternal haplotypes of an individual, but also the grandpaternal or grandmaternal origin of each allele on the haplotypes. Observe that the number of recombinants required in a pedigree can be easily computed once the haplotype configuration of each member of the pedigree is given. The following problem, called MRHC in the above, is known to be NP-hard [17, 18], which trivially implies MRHC with missing alleles is also NP-hard.

Definition 2.2 *(MRHC) Given a pedigree and genotype information for each member of the pedigree, find a haplotype configuration for the pedigree that requires the minimum number of recombinants.*

3 An ILP Formulation of MRHC with Missing Alleles

We first introduce variables needed in the formulation. Consider an input pedigree with genotype information. Let n denote the size of the pedigree, and m the total number of marker loci. For a marker locus j, let t_j denote the total number of distinct alleles that occur at locus j, and $M_j = \{m_1^j, m_2^j, ..., m_{t_j}^j\}$ the set of all possible alleles at locus j, where $m_k^j \geq 1$. For each member i and locus j, we introduce $2t_j$ binary indicator variables $f_{i,k}^j$ and $m_{i,k}^j$ $1 \leq k \leq t_j$, denote the paternal allele and maternal allele of member i at locus j, respectively. Namely, $f_{i,k}^j = 1$ ($m_{i,k}^j = 1$) if and only if the paternal (maternal) allele of member i at locus j is m_k^j. For each non-founder member i and locus j, we introduce two indicator variables $g_{i,1}^j$ and $g_{i,2}^j$. Such information is unnecessary for the founders. The variable $g_{i,1}^j$ indicates the grandparental origin of i's paternal allele at locus j, *i.e.* $g_{i,1}^j = 0$ (or 1) if i's paternal allele is copied from its father's paternal (or maternal, respectively) allele. The variable $g_{i,2}^j$ is defined for i's maternal allele at locus j in a similar way.

The haplotype (*i.e.* phase) information and the grandparental origin of each allele at non-founders are completely defined by the above f, m and g variables. Hence, we can easily formulate an integer program for MRHC with missing alleles using these variables, the genotype information, and the Mendelian law of inheritance. However, it is not obvious how we can represent the total number of recombinants in the pedigree as a linear function of these variables. In order to make the objective function linear, we introduce a variable $r_{i,l}^j$ for each pair of "adjacent" variables $g_{i,l}^j$ and $g_{i,l}^{j+1}$ ($1 \leq j \leq m-1$ and $l = 1, 2$) to count the number of recombinants. Here, $r_{i,l}^j = 1$ if and only if $g_{i,l}^j \neq g_{i,l}^{j+1}$. The total number of recombinants can thus be described as:

$$\sum_{\text{non-founder } i} \sum_{j=1}^{m-1} (r_{i,1}^j + r_{i,2}^j) \tag{3.1}$$

3.1 The Constraints

For each member i and locus j, the f and m variables have to satisfy the following constraints:

$$\sum_{k=1}^{t_j} f_{i,k}^j = 1, \sum_{k=1}^{t_j} m_{i,k}^j = 1 \tag{3.2}$$

Given the genotype information (denoted as $\{a, b\}$) of member i at locus j, we have the following constraints:

$$\begin{aligned}
\{m_r^j, m_s^j\} &\Rightarrow \{f_{i,r}^j + f_{i,s}^j = m_{i,r}^j + m_{i,s}^j = 1, \\
&\qquad f_{i,r}^j + m_{i,r}^j = f_{i,s}^j + m_{i,s}^j = 1\} && (3.3)\\
\{m_r^j, m_r^j\} &\Rightarrow \{f_{i,r}^j = 1, m_{i,r}^j = 1\} && (3.4)\\
\{m_r^j, 0\} &\Rightarrow \{f_{i,r}^j + m_{i,r}^j \geq 1\} && (3.5)
\end{aligned}$$

where $m_r^j, m_s^j \in M_j, m_r^j \neq m_s^j$, and 0 stands for a missing allele. If both alleles are missing at the locus, no further constraints (other than constraint 3.2) are provided.

Following the Mendelian law of inheritance, the f, m and g variables must satisfy constraints:

$$f_{i,k}^j - f_{f,k}^j - g_{i,1}^j \leq 0 \tag{3.6}$$

$$f_{i,k}^j - m_{f,k}^j + g_{i,1}^j \leq 1 \tag{3.7}$$

where $1 \leq k \leq t_j$ and f in the subscript denotes i's father. Constraint 3.6 ensures that if i's paternal allele is supposed to originate from its father's paternal allele (*i.e.* when $g_{i,1}^j = 0$), then the two alleles must be the same. In other words, constraint 3.6 implies that if $f_{i,k}^j = 1$, $f_{f,k}^j$ must be 1 when $g_{i,1}^j = 0$. Constraint 3.7 deals with the case $g_{i,1}^j = 1$ in a similar way. The constraints relating i to its mother can be defined in the same way. Recall that variable $r_{i,k}^j$ is the exclusive-or of variables $g_{i,k}^j$ and $g_{i,k}^{j+1}$. The following four constraints will ensure this relationship.

$$r_{i,k}^j - g_{i,k}^j - g_{i,k}^{j+1} \leq 0 \tag{3.8}$$

$$r_{i,k}^j + g_{i,k}^j + g_{i,k}^{j+1} \leq 2 \tag{3.9}$$

$$-r_{i,k}^j + g_{i,k}^j - g_{i,k}^{j+1} \leq 0 \tag{3.10}$$

$$-r_{i,k}^j - g_{i,k}^j + g_{i,k}^{j+1} \leq 0 \tag{3.11}$$

Finally, since all the variables are binary integers, we have constraint

$$\begin{gathered}
f_{i,k}^j, m_{i,k}^j, g_{i',l}^j, r_{i',l}^{j'} \in \{0, 1\}, \\
1 \leq j \leq m, 1 \leq i \leq n, 1 \leq k \leq t_j, \\
1 \leq j' \leq m - 1, \text{non} - \text{founder } i', 1 \leq l \leq 2
\end{gathered} \tag{3.12}$$

The above binary integer linear program defines exactly the MRHC problem with missing alleles. Observe that it implicitly contains the problem of checking Mendelian consistency of genotype data with missing alleles on a pedigree, which is known an NP-hard problem [2]. The total numbers of variables and constraints are linear in the input size. Since it uses binary representation, the actual number of variables could be quite large for multiallelic data. In the next subsection, we will try to simplify the formulation a bit.

3.2 A Simplified Formulation

The main idea of our simplification is to explicitly explore the dependency relationship between variables in the system and try to remove as much redundancy as possible without complicating the constraints too much. Observe that constraints 3.3 and 3.4 supersede constraint 3.2. So, we only need to keep constraint 3.2 when some alleles are missing. We can thus replace constraints 3.2 and 3.5 by:

$$\begin{aligned} \{0,0\} &\Rightarrow \{\sum_{k=1}^{t_j} f_{i,k}^j = 1, \sum_{k=1}^{t_j} m_{i,k}^j = 1\} && (3.13) \\ \{m_r^j, 0\} &\Rightarrow \{-f_{i,r}^j - m_{i,r}^j \le -1, \\ &\qquad \sum_{k=1}^{t_j} f_{i,k}^j = 1, \sum_{k=1}^{t_j} m_{i,k}^j = 1\} && (3.14) \end{aligned}$$

Furthermore, since there are only two variables in each equality of constraint 3.3, fixing the value of any variable would determine the other three variables. We arbitrarily select one of the four variables as the *representative*, and substitute appropriately the representative for the other three variables in the system. Constraint 3.3 can then be removed. Constraint 3.4 can be removed if we replace its variables by constant values in the system. Constraints 3.6 and 3.7 will be kept only if they remain non-trivial after constant variables are replaced. Constraints 3.13 and 3.14 may also contain equality constraints with only two variables after constant variables are replaced. Those equality constraints with two variables can be treated similarly as constraint 3.3.

4 Exploring the Constraints

Because of the NP-hardness of MRHC, it is unlikely to find an efficient polynomial-time algorithm to solve the above ILP formulation. We adopt a widely used strategy, *branch-and-bound*, to search for an optimal solution. A comprehensive treatment of integer linear programming techniques based on branch-and-bound can be found in [35]. Basically, the branch-and-bound method solves an ILP instance by dropping the integer constraint (*i.e.* linear relaxation) to obtain a lower bound of the instance. The procedure terminates when the optimal solution of the relaxed instance is integral or larger than some estimated upper bound, or no feasible solutions exist. Otherwise, it branches on some selected variables and creates some sub-instances. The process iterates until all sub-instances have been considered or pruned. Clearly, different branching orders may have a large impact on the size of the search tree. In this section, we consider some special properties of the above ILP formulation and use them to guide the branching process. We will also use additional lower bounds derived from nuclear families and upper bounds derived by the block-extension heuristic to prune the search tree.

4.1 A Partial Order Relationship

The replacement of constant variables in inequality constraints may result in many inequality constraints with two variables, which define a partial order relationship among the involved variables as given below. For convenience, let us drop the subscript of a variable in the following and denote

$$y^\gamma = \begin{cases} y & \text{if } \gamma = 1 \\ 1 - y & \text{if } \gamma = 0 \end{cases}$$

Each inequality constraint involving two variables can be expressed in the form

$$y_i^\alpha \leq y_j^\beta \tag{4.1}$$

We define a directed graph G on variables involved in the above inequality constraints as follows. For each variable y^γ, G contains a vertex $v(y^\gamma)$. There is an edge from $v(y_i^\alpha)$ to $v(y_j^\beta)$ if inequality 4.1 holds. It is easy to see that for all the vertices in a *strongly connected component* (SCC) of G, their corresponding variables must have the same value in a feasible solution. By identifying and collapsing the SCCs of G, we can remove many variables and simplify their associated inequality constraints as we did for the two-variable equality constraints before. The SCCs can be constructed by using a standard depth-first search (DFS) [4].

Furthermore, the following rules can be used to detect inconsistency and variables with "forced" constant values. Rule 1 states that a variable and its complement cannot occur in the same SCC S. Rule 2 states that if a variable is smaller (or larger) than another variable and the complement of this variable, it must be 0 (or 1, respectively). Rule 3 states if a variable is smaller than its complement, it must be 0.

Rule 1:

$$v(y^0), v(y^1) \in S \Rightarrow \text{Inconsistency}$$

Rule 2:

$$y_i^\alpha \leq y_j^\beta \bigwedge y_i^\alpha \leq y_j^{1-\beta} \Rightarrow y_i^\alpha = 0$$
$$y_i^\alpha \leq y_j^\beta \bigwedge y_i^{1-\alpha} \leq y_j^\beta \Rightarrow y_j^\beta = 1$$

Rule 3:

$$y_i^\alpha \leq y_i^{1-\alpha} \Rightarrow y_i^\alpha = 0$$

The above simplifications may result in new equality constraints with two variables and constant variables. We can repeat the steps in Section 3.2 to further reduce these variables and constraints.

Not only does the directed graph provide a way to reduce variables and constraints, the partial order obtained on the variables after shrinking each SCC can also guide the selection of branching variables. Observe that, if $y_i^\alpha \leq y_j^\beta$, then $y_i^\alpha = 1$ implies $y_j^\beta = 1$. So, we perform a topological sort on the shrunk graph (which is a directed acyclic graph, or DAG). For each vertex v, we define the weight of v as the number of successors of v in the topological sort. When branching, we consider the variables in the topological sorted order and always take the 1-branch first for each variable.

4.2 Equality Constraints with Three or More Variables

The procedure in Section 3.2 removes all equality constraints from constraints 3.3, 3.13 and 3.14 with two variables. This leaves some equality constraints with more than two variables as given in constraints 3.13 and 3.14. These equality constraints could be modified in the above simplification process (*i.e.* some variables can be replaced and constants substituted in), but we may assume without loss of generality that each of them still has the form of $\sum_{k=q}^{p} f_{i,k}^{j} = 1$ (or $\sum_{k=q}^{p} m_{i,k}^{j} = 1$) because the variables are binary integers. [2] Observe that, in such an equality constraint, fixing a variable to one would make all other variables zero. So, we say that the variables in each equality constraint form an *exclusion* set (for lack of better terms). We can take advantage of this information when selecting branching variables by considering the variables from an exclusion set consecutively and taking the 1-branch first for each variable. We define the weight of a variable in such an exclusion set as the size of the set minus one.

4.3 Lower Bounds from Nuclear Families

When dealing with data sets that require a large number of recombinants (*e.g.* data in linkage analysis involving markers separated by large genetic distances), the linear relaxation usually does not give a tight lower bound on the number of minimum recombinants. Observe that an effective lower bound must involve variables from more than 1 locus and MRHC is already NP-hard for data with 2 loci. Hence, we have to work with substructures of the input pedigree. A natural substructure in a pedigree is a nuclear family. A nuclear family constitutes a small instance of MRHC and can usually be solved in a much shorter time. The solution for a nuclear family gives a valid lower bound concerning the r variables from the family and the sum of these lower bounds forms a lower bound for the whole pedigree. The lower bound can be computed in advance for the root node of the branch-and-bound search tree and updated at each branching node, but the latter might incur big time overhead. A sensible strategy is to keep track of the difference between the

[2]If it is necessary, we could introduce some complementary variables to replace expressions of the form $1 - x$ in an equality constraint. If a variable and its complement both appear in (different) equality constraints, we could derive new equalities without such a variable or its complement by summing up appropriate equalities.

current upper bound and lower bound, and update the lower bound only when the difference is larger than a predefined threshold.

4.4 An Upper Bound

When the input data require a small number of recombinants (*e.g.* in the case of SNP data), a tight upper bound could be more effective than the above lower bound because many nuclear families could be realized with 0 recombinants. In this case, the block-extension algorithm [17, 18] can be used to estimate an upper bound because it is efficient and accurate when the number of recombinants is small.

In our implementation of the above ILP algorithm, we solve the linear relaxations of the ILP instances by using the IBM Optimization and Solution Library (OSL). A complete pseudo-code of the branch-and-bound algorithm that summarizes the discussions in sections 3 and 4 is given in Figure 2.

5 Statistical Assessment of Multiple Solutions

The above branch-and-bound algorithm in fact finds all solutions with the minimum number of recombinants. We can further choose the "best" one from these optimal solutions using a maximum likelihood approach if the genetic distances between markers are given. An alternative treatment is to output all solutions together with their associated probabilities. Both approaches require the calculation of the likelihood of a haplotype solution given the genotype information. Because the number of optimal haplotype solutions is usually small, this calculation is much easier than calculating the maximum likelihoods for all feasible solutions [21]. Let H denote a haplotype solution and G the input genotypes. Let $f(i)$ and $m(i)$ denote the father and mother of an individual i, and h_i and g_i the haplotypes and genotype of i. The likelihood of the haplotype configuration H given the genotypes G is

$$P(H|G) = \prod_{\text{founder } i} P(h_i|g_i) \prod_{\text{non}-\text{founder } i} P(h_i|h_{f(i)}, h_{m(i)}), \tag{5.1}$$

where the term $P(h_i|g_i)$ can be obtained under Hardy-Weinberg equilibrium assumption, if prior knowledge about haplotype frequencies is known. The transmission probability $P(h_i|h_{f(i)}, h_{m(i)})$ can be calculated under the assumption that recombination events are independent (no interference) and uniformly distributed.

If our input is actually a population of pedigrees (as in the case of [10]), we can further estimate the population haplotype frequencies and the probability of observing the genotypes in each pedigree given the estimated haplotype frequencies by an expectation-maximization (EM) algorithm, under the assumption that all the founders are independent. The EM algorithm works by summing over all possible optimal solutions for each pedigree, weighted according to their relative likelihoods.

```
Algorithm ILP
Input: Genotype data of a pedigree with possible missing alleles
Output: Haplotypes for all the members in the pedigree
Data structure: Constant variable set $C$, the set of representatives ($R$) of some variables, constraints $S$, global
upper bound $gub$ and lower bound $glb$, exclusion sets $I$, partial order relationship $O$, partial order graph $G$ and
topological sorted order $L$, instance list $P$, current instance $p$, and local lower bound $lb$ by linear relaxation.

1. //Init:
2. Collect $C$, $R$ and $S$, and calculate $gub$ by BE and $glb$ from nuclear families.

3. //Preprocessing
4. Iterate until no new updates exist:
       - Update $R$ and $S$ by removing constant variables;
       - Update $O$ and build $G$;
       - Find the SCCs of $G$ and update $R$;
       - Detect variables forced into constants and put them in $C$;
5. Prepare $I$ and $L$, set the current instance as $p$, run steps 6–10 until $P$ is empty.

6. //Branch-and-bound
7. Solve $p$ by linear relaxation to obtain a local lower bound $lb$.
       - If $p$ is infeasible or $lb \geq gub$, mark it as processed;
       - If the solution is integral, update $gub$;
       - Otherwise, continue to branch.
8. //Branching and selection
9. If $I \neq \phi$ or $L \neq \phi$, select a variable with the largest weight to branch, select the instance that results from
   the 1-branch as $p$, and put the outcome of the 0-branch into $P$;
10. Otherwise select a variable from the objective function, select the outcome of the 0-branch as $p$, and put
   the outcome of the 1-branch into $P$.

11. Output the optimal solutions or report that the instance is infeasible.
```

Figure 2: A pseudo-code of the ILP algorithm.

More specifically, an arbitrary optimal solution is selected from each pedigree and the haplotype frequencies are estimated according to Equation 5.2. Based on the estimated frequencies, a haplotype solution with the maximum probability (Equation 5.3) is then chosen for each pedigree, which will serve the input of the next iteration. Let h_i^1 and h_i^2 denote the two haplotypes of a founder i for any given optimal solution, and N the total number of founders. Let $\pi(h_i^1)$ denote the number of haplotypes h_i^1 occurring in all founders. Then the expected frequency of haplotype h_i^1 is simply

$$\hat{f}(h_i^1) = \frac{\pi(h_i^1)}{2N}. \tag{5.2}$$

The probability of observing genotypes G and a haplotype solution H in a pedigree is

$$P(G, H|\hat{f}) = \prod_{\text{founder } i} \hat{f}(h_i^1)\hat{f}(h_i^2) \prod_{\text{non-founder } i} P(h_i|h_{f(i)}, h_{m(i)}). \tag{5.3}$$

6 An Extension to MRHC and the ILP Algorithm

Most rule-based algorithms do not take into consideration the interval distances between adjacent markers, which is undesirable, especially for markers that are unevenly spaced and are separated by relatively large interval distances. In this case, the optimal solutions based on MRHC may be quite different from the true solutions. With the ILP formulation, one can easily incorporate into the objective function a coefficient for each marker interval that represents the likelihood of recombination. In this section, we propose several simple ways to achieve this. In general, we could define a coefficient w^j for each marker interval $j, 1 \le j \le m-1$, which is a function of the genetic/physical distance (denoted as d^j) between marker j and marker $j+1$. Revise the objective function as

$$\sum_{\text{non-founder } i} \sum_{j=1}^{m-1} w^j (r_{i,1}^j + r_{i,2}^j), \tag{6.1}$$

where $w^j = f(d^j)$ for some decreasing function $f(\cdot)$ that can be specified by the user. For example, if we are using genetic distances between markers, we may choose $f(d) = 1/d$. An alternative would be to use some mapping function [3] $m(\cdot)$ to transform the genetic distance to recombination rate and let $f(d) = 1 - m(d)$. If we are given physical distances instead, we may first find the correspondence between physical distances and genetics distances according to some prior knowledge and then apply the functions mentioned above.

7 Experimental Results

We have implemented the above algorithm ILP as a module of our *PedPhase* program, which is available at website `http://www.cs.ucr.edu/~jili/haplotyping.html`. To evaluate the efficiency of ILP, we first compared ILP, block-extension (BE, also in PedPhase) and MRH v0.2 [27]

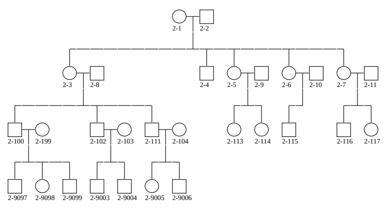
[3]Like Haldane's mapping function or Kosambi's mapping function among others [23].

on simulated genotype data in terms of efficiency on three different pedigree structures. The results show that, as an exact solution, our ILP is in fact faster than MRH v0.2 on SNP data. We also evaluated how the number of marker loci, the size of a pedigree, the number of recombinants, and the amount of missing alleles affect the efficiency. An advantage of using simulated data here is that we know the true haplotype configurations and the number of recombinants. Hence, we can evaluate the accuracy of ILP in terms of the percentage of markers with haplotypes correctly inferred. We further compared ILP and SimWalk2 on simulated data sets and the results show that ILP is much faster than SimWalk2. We defined three measures of accuracy, namely, the percentage of alleles correctly recovered, the number of recombinants inferred and the likelihoods of haplotype solutions. ILP outperformed SimWalk2 on its first run and reported comparable results as the second run of SimWalk2. As an application to real data, we tested ILP on a genome-scale data set that consists of 12 multi-generation human pedigrees studied in a recent paper [10]. We focused on each of the inferred blocks and compare the results of ILP with those of the EM algorithm used in [10]. The comparison shows that ILP outputs haplotype configurations that require a few recombinants and result in roughly the same set of common haplotypes as the EM algorithm. We also compared ILP with block-extension on chromosome 3 consisting of 10 blocks. The results show that ILP often finds solutions that require fewer recombinants than those returned by block-extension. The details of the test results are given in the following subsections.

7.1 Efficiency and Accuracy of ILP on Simulated Data

We used a method similar to those in [7, 17, 18] to generate simulated data sets. Three different pedigree structures were considered. One is a small pedigree with 15 members as shown in Figure 1. The second is a medium-sized pedigree with 29 members as shown in Figure 3 (left) and the third is a pedigree of 17 members with a mating loop as shown in Figure 3 (right). Both multi-allelic (with 6 alleles per locus) and biallelic data were considered. In order to test the worst-case performance of the algorithms, the alleles were generated following a uniform frequency distribution to maximize the chance of heterozygosity. Three different numbers of loci, namely 10, 25 and 50 were considered. The number of recombinants used in generating each pedigree ranged from 0 to 4. In addition, we considered the rate of missing alleles as 5%, 10%, 15%, and 20%. For each data set, 100 copies of random genotype data were generated. The total number of data sets used is 45000 $(= 3 \cdot 2 \cdot 3 \cdot 5 \cdot 5 \cdot 100)$.

The test results demonstrate that ILP is slower than block-extension, but faster than MRH v0.2 on biallelic data (although it is a little bit slower than MRH v0.2 on multi-allelic data), as described in Table 1. In the table, the first column indicates the combination of parameters: the size of the pedigree, the number of loci in each member, the number of distinct alleles allowed at each locus, and the number of recombinants used to generate the genotype data, respectively. The time used by each program in this section is the total time for 100 random runs for each parameter



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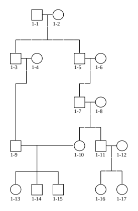

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Figure 3: A. A pedigree with 29 members. B. A pedigree with 17 members and a mating loop.

Table 1: Speeds of BE, MRH and ILP on multi-allelic and biallelic markers.

Parameters	BE	MRH	ILP
(17,10,6,0)	2.1s	7s	34s
(17,10,6,4)	2.1s	11s	37s
(15,25,6,0)	2.7s	18s	2m34s
(15,25,6,4)	2.9s	33s	3m9s
(29,10,6,0)	3.2s	10s	1m49s
(29,10,6,4)	3.1s	15s	1m57s
(29,25,6,0)	15s	4m	15m2s
(29,25,6,4)	10s	2m6s	15m10s
(17,10,2,0)	1.9s	15s	20s
(17,10,2,4)	2.3s	1m11s	23s
(15,25,2,0)	4.7s	10m50s	1m6s
(15,25,2,4)	4.8s	13m49s	1m18s
(29,10,2,0)	2.8s	6m26s	44s
(29,10,2,4)	2.7s	3m46s	50s
(29,25,2,0)	2.3s	2h7m	3m41s
(29,50,2,0)	16s	45h	15m21s

combination on a Pentium IV with 1.7GHz CPU and 512MB RAM.

Figure 4(A) uses a bar diagram to show how the speed of ILP is affected by the input size (*i.e.* number of marker loci and size of a pedigree) on biallelic data, when the number of recombinant is fixed as 1. For example, ILP requires a total of 20 minutes for 100 runs on a pedigree with 29 members and 50 loci. This suggests that ILP is efficient for MRHC instances of practical sizes. To consider the effect of the number of recombinants and the rate of missing data, Figure 4(B) indicates that the running time will increase with these two parameters, mainly because the number of free variables in the system will increase. But unlike parameters such as the pedigree size and the number of loci, for which the time increases exponentially as expected, the growth rates in Figure 4(B) are more like linear functions. In this figure, the number of marker loci is 50 and the size of the pedigree is 29.

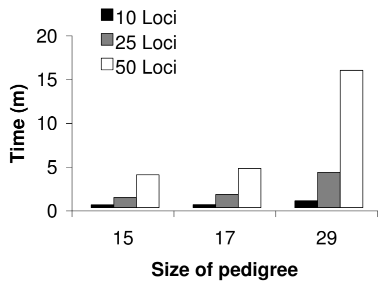
Not only does ILP solve MRHC with missing data optimally, the results in Figure 4(C) demonstrate that the algorithm is good at recovering true haplotypes. Its overall accuracy is better than 98% in terms of the number of missing alleles correctly recovered and the number of loci with haplotypes correctly inferred, for missing rate as many as 20%. Its accuracy on data with no missing alleles is even better; More than 99.8% of the loci were correctly phased. The few errors were mainly due to existence of multiple optimal solutions. These results also show that, when the number of recombinants are few and the recombination events are randomly distributed, the true haplotype configuration is often a minimum recombinant haplotype configuration. In contrast, similar simulations in [17, 18] show that the performance of block-extension could fall below 80% on pedigrees with mating loops or data that require a moderately large number (*i.e.* ≥ 4) of recombinants.

7.2 Comparison with SimWalk2

Standard linkage programs such as GeneHunter [19] and SimWalk2 [30] can also infer haplotypes based on genotype data of a pedigree. The aim of these statistics-based approaches is to find a haplotype configuration with the maximum likelihood under the assumed model. Exact algorithms for finding the most probable haplotype configuration can only work for small data sets, where the number of consistent haplotype configurations for a given pedigree is in a manageable range. Important sampling techniques such as MCMC (Markov chain Monte Carlo) are commonly used, such as in SimWalk2, to find an approximate solution for more complicated data sets within a reasonable time frame.

As mentioned in the introduction, the objectives and application ranges of the ML and MRHC formulations are not exactly the same, but overlap exists in both aspects. So it is useful to know how the ILP algorithm performs under different assumptions when compared to ML methods and how the maximum parsimony principle of minimizing the total number of recombinants reflects the truth.

We took a widely-used, state of the art ML-based program SimWalk2 version 2.89 and compared



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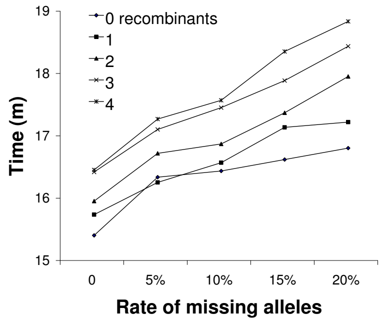

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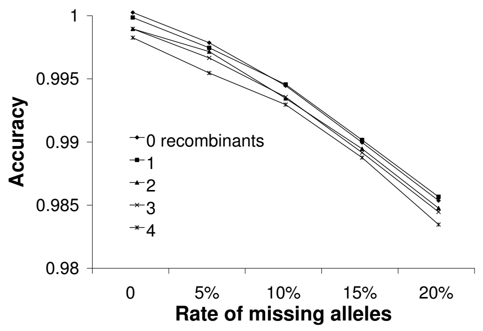


C

Figure 4: Some simulation results on ILP. A. Effect of problem size on speed. B. Effect of number of recombinants and rate of missing alleles on speed. C. Effect of number of recombinants and rate of missing alleles on accuracy.

its results on two data sets with the results of the ILP algorithm. The first data set contains the three different pedigree structures mentioned above. The total genetic length of the segment is fixed as 20cM while the total numbers of markers analyzed are 11, 26 and 51 respectively for each pedigree. The markers are evenly distributed for the first data set thus the marker interval distances are 0.2, 0.08 and 0.04 cM. The numbers of non-founders in each pedigree is 10, 11 and 20 respectively, and thus the expected numbers of recombinants in each pedigree is 4, 4.4 and 8. For the second data set, we selected the pedigree with 29 members (Figure 3(A)) and fixed the number of markers as 26. The recombination fractions between markers were chosen uniformly at random from the range [0.001, 0.061]. The total number of expected recombinants in a pedigree is 26.9. For both data sets, we considered biallelic markers with equal frequencies. The data were generated according to Haldane's model of recombination assuming no interference. In generating the simulated data, the founders' alleles were sampled first according to the allele frequencies. The inheritance of alleles in children followed a widely used scheme described below. The first allele of each child was randomly chosen from its parent's paternal or maternal allele. The child inherited that haplotype until a recombination occurred, whereafter it started to inherit the other haplotype of its parent. The probability of the recombination event in each interval was determined by the recombination fraction associated with it. For each parameter combination, 100 copies of random data were generated. The marker interval distances and allele frequencies were assumed known to both programs.

For the first data set, we compared the efficiency and the accuracy of the two programs. Our current implementation of ILP outputs one of the optimal solutions. We compared it with the haplotype result of the first run of SimWalk2. ILP is much faster than SimWalk2 as shown in Table 2, where the time is the total time used for 100 data sets on a Pentium IV computer with 256MB RAM and Window 2000 run on Linux through VMware. The difference is in the order of 6 to 35-fold. The difference increases drastically with the increase of the size of the input pedigree and the number of markers. We measured the accuracy using the percentage of correctly recovered alleles, which is called point accuracy, and the number of recombinants in a pedigree. The results in Table 2 and 3 showed ILP outperformed SimWalk2 on both measures. The average point accuracy of ILP is around 99% compared to 91% by SimWalk2. As shown in Table 3, the number of recombinants per pedigree found by ILP was very close to the expected and real numbers of recombinants across all the data sets tested. ILP might underestimate the total number of recombinants by its definition, but the difference was around 1 in this simulation. For data set with 11 markers, the difference of reported recombinants by SimWalk2 and the real number of recombinants was also around 1. But with the increase of markers, SimWalk2 reported much more recombinants than those in the real solutions.

Notice that SimWalk2 may not find a satisfactory solution on its first run. For the second data set, we compared the results of ILP with the results of SimWalk2 on its first and second runs. A

Table 2: Comparison of ILP and SimWalk2 in terms of speed and point accuracy.

	Efficiency		Accuracy	
Parameters	ILP	SimWalk2	ILP	SimWalk2
(15,11)	24m	2h12m	99.4	91.5
(15,26)	1h3m	10h18m	99.7	90.4
(15,51)	2h6m	44h45m	99.7	91.0
(17,11)	25m	2h46m	98.9	91.1
(17,26)	1h4m	13h42m	99.2	91.2
(17,51)	2h9m	48h8m	99.3	91.6
(29,11)	48m	7h2m	99.4	91.4
(29,26)	2h6m	35h40m	99.6	91.7
(29,51)	4h35m	155h46m	99.5	91.3

Table 3: Comparison of ILP and SimWalk2 in terms of the number of recombinants.

Parameters	Expected	Real	ILP	SimWalk2
(15,11)	4	4.02	3.08	3.63
(15,26)	4	3.99	3.46	9.25
(15,51)	4	3.87	3.64	19.31
(17,11)	4.4	4.31	3.12	3.57
(17,26)	4.4	4.3	3.49	6.70
(17,51)	4.4	4.46	3.92	11.62
(29,11)	8	7.54	5.66	9.18
(29,26)	8	8.18	7.13	22.98
(29,51)	8	8.70	8.04	46.18

pedigree needs to be rerun by SimWalk2 if the number of recombinants in its current solution is larger than the expected number of recombinants, or its current solution is found in the late stage of the search procedure. So, by running multiple times, SimWalk2 will likely find a solution with a smaller number of recombinants than that of the first run, as well as a better likelihood. Since for model-based methods such as SimWalk2, the measure of a haplotype solution is its likelihood, it is important to know how rule-based methods like ILP perform in terms of the likelihood measure. In a more realistic setting, as found in the second data set where the marker interval distance varies randomly, ILP was also made to report the likelihood of a haplotype solution. In order to compare the results with SimWalk2, the likelihood of a haplotype found by ILP was calculated according to the definition in [29] while omitting the phenotype and the penetrance probability terms, which is slightly different from the formula 5.1. The test results as shown in Figure 5 demonstrated that haplotypes inferred by ILP were better than those generated by the first run of SimWalk2, although ILP used much less time than SimWalk2. The results of ILP were obtained by incorporating the maker interval distance into the objective function as mentioned in Section 6. The function we used was simply one minus the recombination fraction. Figure 5(A) shows the log10 likelihoods of two methods on 100 data sets. The vertical lines and the open circles represent the results of ILP and the solid circles represent the results of SimWalk2. Figure 5(B) shows the differences of log10 likelihoods of ILP and SimWalk2. The results show that ILP was better than SimWalk2 in

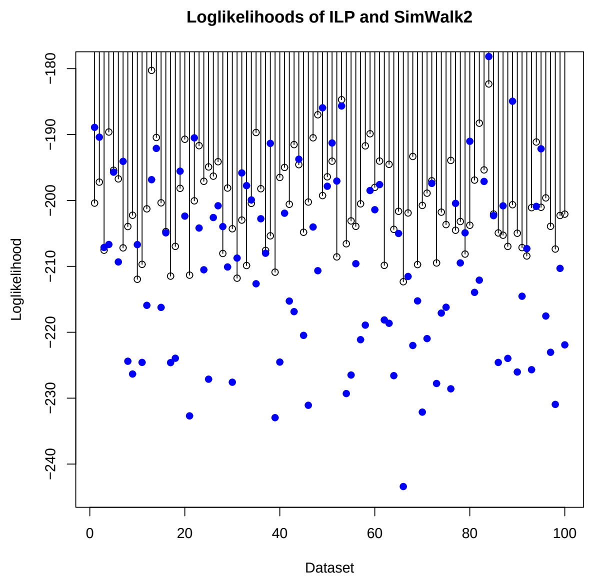
75 out of the 100 data sets. The mean of the differences was around 10. With much more time consumed, the results of the second run of SimWalk2 were much better than its first run, while the results from ILP were still comparable as shown in Figure 6. The time used for ILP, the first run of SimWalk2 and the second run of SimWalk2 was 2, 27 and 94 hours, respectively. It is still possible that SimWalk2 could identify more probable haplotypes if time allowed. We plan to compare the two programs more thoroughly in our future study on data with missing alleles.

7.3 A Genome-Scale Haplotype Reconstruction

We have also tested ILP on a real data set from Whitehead/MIT Center for Genome Research. Gabriel *et al.* [10] recently reported results on a genome-scale SNP haplotype block partition and haplotype frequency estimation project. Their original data set consists of 4 populations and 54 autosomal regions, each with an average size of 250K bps, spanning 13.4M bps (about 0.4%) of the human genome. Haplotype blocks were defined using the normalized linkage disequilibrium parameter D'. Blocks with fewer than four markers are omitted from further consideration. Within each block, haplotypes and their frequencies were calculated via an EM algorithm from [9]. One of the populations (European) has pedigree information and was used in our study. There are totally 93 members in the European population, separated into 12 multi-generation pedigrees (each with 7-8 members). The genotyped regions are distributed among all the 22 autosomes and each autosome contains 1 to 10 regions. The overall allele missing rate in a block is between 0.8% and 14.5%. We downloaded the SNP genotype data and pedigree structures from Whitehead/MIT Center for Genome Research website (`http://www-genome.wi.mit.edu/mpg/hapmap/hapstruc.html`), and obtained the results of the EM algorithm concerning common haplotypes and their frequencies in the population from the authors of [10]. [4] ILP was able to reconstruct the haplotypes for all regions and pedigrees accurately, by taking advantage of the available haplotype block structure. A comparison of the EM algorithm and ILP in terms of the common haplotypes that they output is given in Table 4. The first column of the table is the chromosome number and the second column is the number of blocks with more than four markers (no blocks with length larger than four in chromosome 19). Columns 3 and 4 are the average numbers of common haplotypes per block found by EM and ILP, respectively. Column 5 is the average number of different common haplotypes output by the two methods, which is usually about 10% of the common haplotypes output by each method. Column 6 is the average number of recombinants in each pedigree and each block as found by ILP, which is close to zero. A detailed description of the haplotypes found by ILP for the data set will be available at website `http://www.cs.ucr.edu/~jili/haplotyping.html`.

We further compared the results of ILP with our previous experiment on the block-extension and EM algorithms on chromosome 3 reported in [17]. (The results of the EM algorithm was

[4]Here, the pedigree information was used first to resolve the phases of some heterozygous loci using the Mendelian law of inheritance before the EM algorithm was run on founders.



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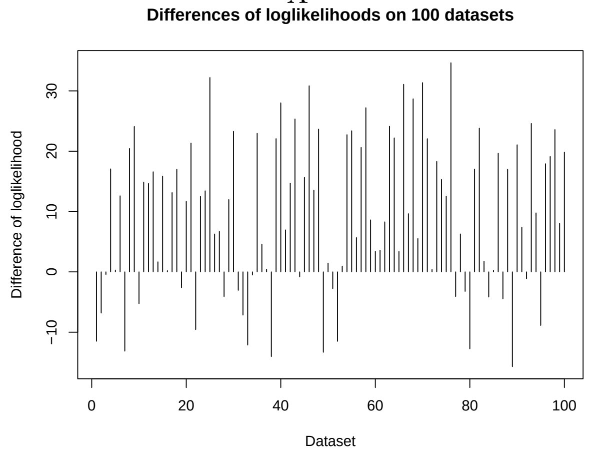

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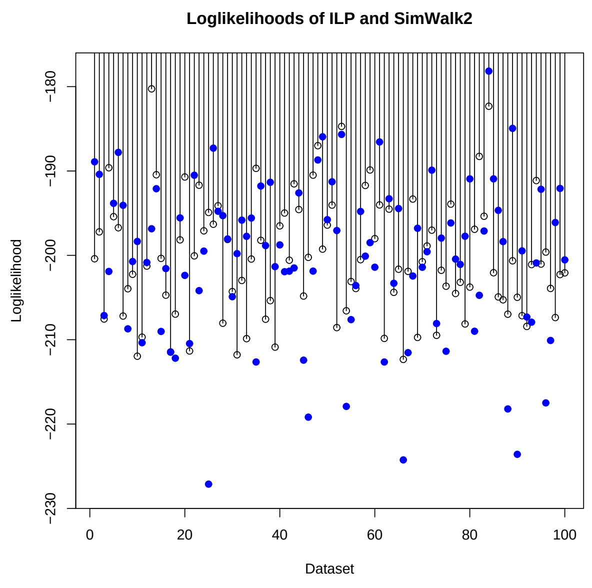
Figure 5: Comparison of the likelihoods of ILP with the first run of SimWalk2 on 100 data sets. A. The log10 likelihoods by the two programs. The vertical lines and the open circles represent the results of ILP and the solid circles represent the results of SimWalk2. B. The differences of log10 likelihoods of ILP and SimWalk2.



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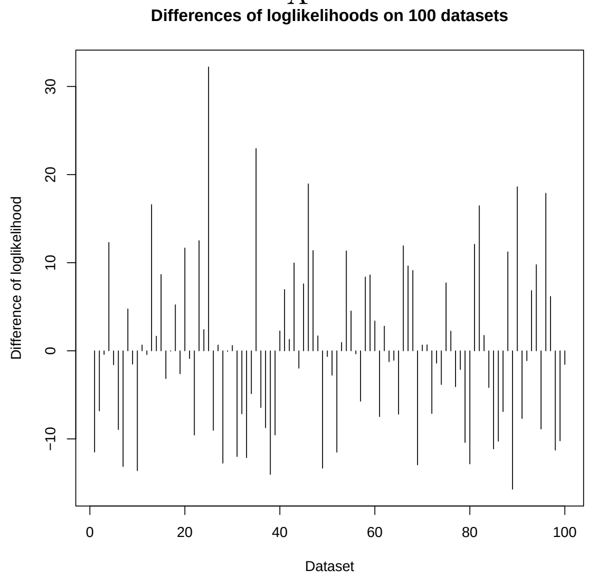

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Figure 6: Comparison of the likelihoods of ILP with the second run of SimWalk2 on 100 data sets. A. The log10 likelihoods by the two programs. The vertical lines and the open circles represent the results of ILP and the solid circles represent the results of SimWalk2. B. The differences of log10 likelihoods of ILP and SimWalk2.

Table 4: Comparison of the EM and ILP algorithms on a human genome SNP data.

Chr	# of blocks	EM	ILP	Mismatch	Recombs(ILP)
1	22	3.82	4.00	0.45	0.034
2	6	3.33	4.00	0.67	0.000
3	10	3.9	4.00	0.50	0.033
4	7	3.57	3.29	0.14	0.048
5	7	3.86	4.12	0.43	0.024
6	11	3.55	3.54	0.67	0.008
7	9	2.67	3.33	0.22	0.037
8	8	3.63	3.38	0.25	0.000
9	3	3.67	4.33	1.33	0.333
10	7	4.14	3.57	0.71	0.095
11	5	3.40	3.60	0.40	0.083
12	6	3.00	2.83	0.17	0.00
13	6	3.67	3.83	0.50	0.042
14	4	3.50	3.50	0.00	0.000
15	3	3.33	4.33	1.00	0.028
16	4	3.50	3.75	0.25	0.125
17	2	2.5	2.00	0.50	0.000
18	4	3.25	3.25	0.25	0.125
20	2	4.00	4.00	0.00	0.000
21	1	2.00	3.00	1.00	0.167
22	8	4.12	3.88	0.50	0.021

Table 5: The regions and blocks on chromosome 3.

Name	Length	SNPs	Blocks	SNPs/block	Missing rate
16a	40	14	1	5	7.96%
16b	106	53	1	6	3.76%
			2	4	2.69%
17a	186	70	1	6	4.70%
			2	5	1.50%
			3	4	7.80%
			4	6	6.27%
18a	286	74	1	16	3.70%
			2	6	5.73%
			3	4	2.15%

Table 6: Common haplotypes and their frequencies obtained by block-extension, ILP and the EM method. In haplotypes, the alleles are encoded as 1=A, 2=C, 3=G, and 4=T.

Block	Common haplotypes	EM	BE	ILP
16a-1	4 2 2 2 2	0.4232	0.3817	0.3750
	3 4 3 4 4	0.2187	0.1720	0.2187
	4 2 2 2 4	0.2018	0.1935	0.1979
	3 4 2 2 4	0.1432	0.1613	0.1458
sum		0.9869	0.9085	0.9374
16b-1	3 2 4 1 1 2	0.8014	0.7634	0.7813
	1 3 2 3 3 4	0.0833	0.0753	0.0833
sum		0.8847	0.8387	0.8646
16b-2	4 1 2 2	0.5410	0.4892	0.5104
	2 3 3 4	0.2812	0.2581	0.2500
	2 3 3 2	0.1562	0.1344	0.1562
sum		0.9784	0.8788	0.9166
17a-1	3 1 3 4 4 4	0.3403	0.3172	0.2917
	1 3 3 2 4 2	0.3021	0.2419	0.2500
	3 3 2 4 2 4	0.1354	0.0914	0.0938
	3 3 3 4 4 4	0.1021	0.1183	0.1354
	3 3 2 4 4 4	0.0681	0.0806	0.0729
	1 3 3 2 4 4	0.0521		
sum		1.0000	0.8494	0.8438
17a-2	2 3 2 4 2	0.3542	0.2903	0.3229
	3 3 4 2 4	0.3333	0.2957	0.3125
	3 3 4 4 2	0.1458	0.1344	0.1563
	3 4 4 4 4	0.1250	0.1452	0.1250
sum		0.9583	0.8656	0.9167
17a-3	4 4 3 1	0.4129	0.4355	0.4167
	3 1 1 2	0.2813	0.2258	0.2292
	4 1 3 1	0.2363	0.1935	0.2188
	4 1 3 2	0.0696	0.0753	0.0729
sum		1.0000	0.9301	0.9376
17a-4	3 4 4 1 2 4	0.3854	0.3710	0.3436
	2 3 2 4 3 2	0.3333	0.2903	0.3021
	3 4 2 4 2 4	0.2500	0.1881	0.2188
sum		0.9687	0.8494	0.8645
18a-1	1444231214144132	0.2697	0.2473	0.2396
	1444111214144132	0.2396	0.2151	0.2083
	1444131214144132	0.1887	0.2204	0.1979
	4222133313412211	0.1250		
	1444231234144132	0.0833	0.0699	0.0729
	4444133214144132			0.0521
sum		0.9063	0.7527	0.7708
18a-2	3 1 2 4 4 2	0.4967	0.4892	0.4271
	1 3 2 4 3 4	0.2604	0.1935	0.1667
	3 1 2 2 4 2	0.1271	0.0753	0.0938
	1 3 4 4 4 4	0.0938	0.0806	0.0729
	1 3 2 4 3 2		0.0538	0.0625
	3 1 2 4 4 4			0.0521
sum		0.9780	0.8924	0.8751
18a-3	2 2 1 1	0.4186	0.4032	0.3854
	4 3 3 3	0.2188	0.1935	0.2188
	2 3 1 1	0.2064	0.2204	0.2396
	4 3 1 3	0.1250	0.1559	0.1146
sum		0.9688	0.9730	0.9584

obtained from the authors of [10].) There are 4 regions in the chromosome 3 data and each region is partitioned into 1 to 4 blocks. The region name, physical length (kbps), number of blocks, SNPs in each block and percentage of missing alleles of each block are summarized in Table 5. In the experiment, the algorithm block-extension imputed missing alleles by sampling the alleles according to their estimated frequencies. On the other hand, ILP inferred missing alleles so that the number of required recombinants is minimized in the final haplotype solution. In other words, both missing data imputation and the reconstruction of haplotype configurations were combined in one framework. After haplotypes were inferred for the members of all pedigrees, population haplotype frequencies were estimated by counting the founders' haplotypes. Such frequency information can be used to estimate the likelihood of the haplotypes in a pedigree as described in Section 5. The common haplotypes, their frequencies and their total frequency in each block of chromosome 3 estimated by block-extension, ILP and the EM algorithm are summarized in Table 6. The majority of the common haplotypes identified by the three algorithms are the same. The small number of differences mainly concern haplotypes with frequencies close to 5%. Similar patterns were also observed in the tests on other chromosomes. Furthermore, for common haplotypes shared by the three algorithms, all three algorithms gave frequencies close to each other, although the frequencies given by ILP and block-extension are in general smaller than those found by the EM algorithm. This is perhaps mainly due to different strategies used for imputing missing alleles and the fact that the EM algorithm only used the founders of the pedigrees in its computation.

We have also looked at the number of recombinations required in the solutions found by ILP. Out of the 120 data sets derived from the 10 blocks in chromosome 3 and the 12 pedigrees, only 2 data sets had solutions that require recombinants. In contrast, 18 data sets had recombinants in the solutions found by block-extension [17]. The difference could be due to different methods for missing data imputation and the fact that block-extension is a heuristic algorithm.

8 Concluding Remarks

Our simulation results have demonstrated the soundness of the minimum recombination principle. It works not only for tightly linked dense markers like SNPs, where the expected number of recombinants in a pedigree is small, but also for data with moderate size of recombinants. The haplotype solutions identified by ILP could recover the correct phase information in most cases and achieve large likelihoods as well. The ILP algorithm is an effective tool for solving the MRHC problem on human pedigrees with practical sizes and missing data. It also incorporates the marker interval distance into the formulation, thus may handle data with loose markers. Our experimental results also show that ILP could identify a haplotype solution using much shorter time than SimWalk2 and its solution usually has a large likelihood. It is a natural thought to combine ILP with SimWalk2, or any other maximum likelihood approaches relying on random search, to save time in the search for the most probable solution. For example, one may use the ILP solution as the initial starting point for SimWalk2.

The results on the simulated data show that the minimum number of recombinants in a pedigree is very close to the expected number of recombinants or the real number in that pedigree. One natural extension to the MRHC problem, denoted as MRHC-k, is how to find all haplotype solutions of which the number of recombinants is smaller than the minimum number of recombinants plus k. We may expect with more confidence that the true haplotype solution is among solutions of MRHC-k.

Like many other haplotyping algorithms, the MRHC formulation and the ILP approach are designed for data from a pedigree and a segment of some chromosome. It is important to combine whole genome data on multiple pedigrees and solve them directly, especially for the purpose of whole genome association studies and the understanding of the population haplotype structure. There should be no doubt about the superiority in accuracy of this approach compared to methods using unrelated individuals. Further investments are needed.

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