The impact of using related individuals for haplotype reconstruction in population studies

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ABSTRACT

Recent studies have highlighted the dangers of using haplotypes reconstructed directly from population data for a fine-scale mapping analysis. Family data may help resolve ambiguity, yet can be costly to obtain. This study is concerned with the following question: How much family data (if any) should be used to facilitate haplotype reconstruction in a population study? We conduct a simulation study to evaluate how changes in family information can impact the accuracy of haplotype frequency estimates and phase reconstruction. To reconstruct haplotypes, we introduce an EM-based algorithm that can efficiently accommodate unrelated individuals, parent-child trios and arbitrarily large half-sib pedigrees. Simulations are conducted for a diverse set of haplotype frequency distributions, all of which have been previously published in empirical studies. A wide variety of important results regarding the effectiveness of using pedigree data in a population study are presented in a coherent, unified framework. Insight is provided into the different properties of the haplotype frequency distribution that can influence experimental design. We show that a preliminary estimate of the haplotype frequency distribution can be valuable in large population studies with fixed resources.

INTRODUCTION

There is currently a strong interest in how best to use Linkage Disequilibrium (LD) information for fine-scale mapping and association analysis of complex traits. A growing number of studies demonstrate that haplotype-based approaches may provide more power and accuracy in locating quantitative trait loci (QTL) and causative disease variants than single-locus methods (see, e.g. Zhao et al., 2003; Morris et al., 2002; Fallin et al., 2001). Since haplotypes are typically not observed *in vitro*, haplotype-based studies are likely to follow a two-step procedure: first, haplotypes are inferred from a sample of phase-unknown genotypes using a computational algorithm, and second, inferred haplotypes are fed into a multi-locus LD model, where they are treated as having been directly observed.

There are two principal approaches to inferring haplotypes from population data, both with potential drawbacks. One approach is to use family data, which may be able to deterministically resolve phase for genotypes featuring multiple heterozygous loci. However, ascertaining this information can be costly. When resources are fixed, it may actually be more efficient to use a genotype-based mapping model rather than re-allocate resources to ascertain family data for haplotype reconstruction (Grapes et al., 2004).

A second approach is to infer haplotypes directly from population data. A variety of statistical algorithms exist for random-mating populations, and good comparative surveys are available (see, e.g. Stephens and Donnelly,

2003; Zhao et al., 2003). A problem with reconstructing haplotypes using these models is that there may be considerable uncertainty associated with the inferred haplotypes. It was recently demonstrated that failing to account for this uncertainty can result in unreliable location estimates in a subsequent mapping analysis (Morris et al., 2004). These results indicate that, when possible, haplotype-based analyses should be modified to efficiently accommodate this uncertainty. When the two-stage procedure must be used, it is important to understand the factors that will limit its effectiveness. One important factor is likely to be the haplotype frequency distribution. This is because, under the standard assumption of random-mating, the data can be regarded as independent samples from this distribution.

In this paper, we illustrate the different ways in which the haplotype frequency distribution can impact the accuracy of both the phase assignments and haplotype frequency estimates. We also examine the effectiveness of using family data to improve accuracy for different frequency profiles.

To facilitate our analysis, we begin by introducing an EM-based haplotype reconstruction model that can accommodate outbred half-sib pedigrees, unrelated individuals and family-child trios. The method is efficient for arbitrarily large sibships with missing marker data, and will be of interest to studies of hierarchical population structures, including those in populations of many natural and domestic animal species.

The remainder of the paper is devoted to simulating and analyzing results using a diverse set of published haplotype frequencies. We describe sum-

mary statistics that can be calculated directly from inferred frequency data, and which can be used to predict the accuracy of phase assignment and the usefulness of family data. A wide variety of results that either extend or complement existing analysis are presented in a coherent, unified framework.

METHODS

Notation and Modelling Assumptions

We are considering a candidate region in the genome characterized by L tightly linked biallelic loci. Let $\mathbf{h} = h_1 \ldots h_M$ denote the $M = 2^L$ possible haplotypes, and let $\Theta = (\theta_1, \ldots, \theta_M)$ denote corresponding haplotype frequencies in the target population.

For a large, panmictic population, we can specify the probability of observing a given phase configuration, $z = (h_i, h_j)$ as

$$p(z = h_i, h_j | \Theta) = c \ \theta_i \theta_j \tag{1}$$

where c is 1 if the individual is a homozygote (i.e. $i = j$), or 2 if the individual is a heterozygote (i.e. $i \neq j$).

Similarly, the probability of observing a given phase unknown genotype, y, in a panmictic population is:

$$p(y | \Theta) = \sum_{z \in \mathbf{z}(y)} p(z | \Theta), \tag{2}$$

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where $\mathbf{z}(y)$ is the set of all possible phase configurations that can resolve y. Note that the size of $\mathbf{z}(y)$ increases exponentially with the number of heterozygous loci.

Haplotype Reconstruction via the EM Algorithm

Let $\mathbf{y} = y_1 \ldots y_N$ denote a sample of N phase unknown genotypes. The objective of the EM-based approach to haplotype reconstruction is to calculate the maximum likelihood estimate of Θ given $\mathbf{y}$. Haplotype frequency estimates can then be used to reconstruct phase.

An important initial assumption concerns any underlying pedigree structure of the data. It will be useful to make these assumptions explicit by characterizing the observed data using two parameters: the marker data, $\mathbf{y}$ and a representation of the underlying pedigree structure, $\mathcal{F}$. When individuals are unrelated, or no additional family information is included, we set $\mathcal{F} = \emptyset$. A central challenge of any likelihood-based pedigree model is achieving reasonable computational complexity for a given $\mathcal{F}$, and one of the principal contributions of our method is that we provide an efficient algorithm for sparse half-sib pedigrees. It will be useful to first review the EM-based approach for unrelated individuals.

EM algorithm for Unrelated Individuals: The EM algorithm for unrelated individuals has been developed and evaluated in many different contexts (see, e.g. Hill, 1974; Terwilliger and Ott, 1994; Excoffier and Slatkin, 1995; Hawley and Kidd, 1995; Fallin and Shorck, 2000; Kirk and Cardon, 2002;

Qin et al., 2002). The general approach entails augmenting the observed phase-unknown genotypes for each member in the sample by the corresponding phase configurations. We denote these latent phase configurations by $\mathbf{z} = z_1 \ldots z_N$. Each iteration of the EM algorithm requires calculating the expected log-likelihood of the augmented data:

$$E_{p(\mathbf{z}|\mathbf{y}\tilde{\Theta})} \log[p(\mathbf{y}, \mathbf{z}|\Theta)] \ = \ \sum_{i=1}^{N} \sum_{j=1}^{M} E_{p(z_i|y_i,\tilde{\Theta})} n_{ij} \log \theta_j + \text{Constant}, \quad (3)$$

where $\tilde{\Theta}$ denotes the current estimate of Θ and n_{ij} refers to the number of times haplotype j appears in the phase configuration of individual i. Once (3) has been calculated, the result is maximized with respect to Θ and the process is repeated until $\tilde{\Theta}$ converges at a maximum, $\hat{\Theta}$.

Note that without the expectation, (3) is a straightforward description of "gene counting". The added complexity incurred from using the EM algorithm is therefore attributed to calculating $p(z_i|y_i, \tilde{\Theta})$. This quantity is given by dividing equation (1) by equation (2).

The complexity is dominated by calculating the terms in (2), which increase linearly with the number of possible phase configurations. However, the number of possible phase configurations increases exponentially with the number of heterozygous genotypes, which limits the number of SNPs that can reasonably be evaluated. This constraint will apply to any EM-based approach that requires estimating the distribution of phase given marker data, including the one we are about to describe for outbred half-sib pedigrees. It is

worth mentioning that one way to accommodate a large number of markers is to use the partition ligation algorithm introduced by Niu et al. (2002). While this algorithm was developed as part of a Bayesian model, it has since been incorporated into the EM framework (Qin et al., 2002).

EM algorithm for Outbred Half-Sib Pedigrees: We now consider the case where members of $\mathbf{y}$ may be related through one of $P \leq N$ sires. If $P < N$, then the likelihood in (3) is no longer valid since the summation over N only follows from assuming each animal is unrelated.

One EM-based approach that accommodates nuclear family information is described by Rohde and Fuerst (2001). The model treats the parents as independent and augments the marker data by the latent parental phase configurations. The E-Step entails enumerating phase configurations that are consistent with parent and progeny marker data, and then evaluating each configuration. This enumerative approach is sufficient for small sibships, but it is computationally infeasible for a sib structure of moderate size. For sparse family structures, such as half-sib pedigrees with untyped parents, it is not a realistic strategy.

The starting point for our model follows a generic approach described by O'Connell (2000), and entails augmenting the data by the latent phase configurations of the founders. The E-step would then require calculating the marginal distribution of each founder given the marker data specific to that founder's pedigree.

Let the latent phase configurations of the sires and dams be denoted $\mathbf{s}$ and $\mathbf{d}$

respectively. The expectation of the augmented data (omitting the constant term) is:

$$\sum_{i=1}^{P}\sum_{j=1}^{M} E_{p(s_i|\tilde{\Theta},\mathbf{y},\mathcal{F})}\left[n_{ij}\right]\log\theta_j \quad + \quad \sum_{i=1}^{N}\sum_{j=1}^{M} E_{p(d_i|\tilde{\Theta},\mathbf{y},\mathcal{F})}\left[n_{ij}\right]\log\theta_j. \qquad (4)$$

The challenge is therefore how to calculate $p(s_i|\tilde{\Theta})$ and $p(d_i|\tilde{\Theta})$. This can be done in any number of ways, but many potentially relevant techniques have been developed in the context of human linkage analysis. In the appendix we provide a formal derivation of the approach we use for calculating the marginal distributions of the sire and dams. Briefly, inferring $p(s_i|\tilde{\Theta})$ follows a straightforward application of the peeling algorithm (Elston and Stewart, 1971). Inferring $p(d_i|\tilde{\Theta})$ can be achieved by adopting general principles used to simulate pedigree data (Ploughman and Boehnke, 1989). Using these techniques results in a computational complexity that is cubic in the number of phase configurations. This is still two orders of magnitude worse than the complexity for unrelated individuals. In the appendix, we also demonstrate how to achieve quadratic complexity for both sire and dam. In the context of haplotype reconstruction, the corresponding reduction in computational resources can be substantial.

Both the method of Rohde and Fuerst (2001) and O'Connell (2000) assume nonrecombinant haplotypes, which is realistic for small genomic regions or over regions lacking recombination hotspots. We make the same assumption and note that O'Connell (2000) outlines a strategy for dealing with recom-

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binant regions that is similar to the partition-ligation algorithm (Niu et al., 2002) for accommodating large numbers of loci.

Our algorithm also provides a unified framework to evaluate arbitrarily large half-sib pedigrees, parent-child trios (a sibship of size one) and unrelated individuals. When no additional family data is provided, results are the same as using the standard EM algorithm for unrelated individuals.

SIMULATIONS

We conduct a simulation study based on independent half-sib pedigrees using empirically derived haplotype data. Our simulation strategy is divided into the following three steps: (1) specification of a haplotype frequency distribution for the parental population; (2) simulation of genotypes for independent half-sib pedigrees; and (3) estimation of haplotype frequencies and phase configurations using alternate categories family data.

We fix the number of sampled individuals at 100 and consider sib-sizes of 1,2,5,10 and 25. The categories of family information that are used with each sample when reconstructing haplotypes are given in Table 1. Family sizes are exact, and therefore results for samples featuring a family of size 1 correspond to unrelated individuals, or, if parental genotypes are provided, to parent-child trios. One consequence of using this simulation strategy is that increasing the size of a sibship will reduce the number of independent haplotypes in a given sample. This allows us to evaluate whether the resolving

power from additional pedigree data compensates for the loss in independent haplotypes (i.e. whether the improved quality of the data compensates for the reduced quantity).

Haplotype Frequency Distributions: The most important parameter in the simulation study is the parental haplotype frequency distribution. Our analysis is based on three empirically-derived haplotype frequency distributions. The first two frequency profiles, $APOE_1$ and $APOE_2$, were provided by Fallin et al. (2001) and correspond to two sets of marker data for a control group used in an association study for Alzheimer's Disease. The third data set, $IL8_E$ was presented by Hull et al. (2001) and corresponds to haplotype frequency estimates of a European sample for six biallelic loci spanning a 7.6 kb region within the $IL8$ locus.

One of the central results from the simulation study is that the expected accuracy for any EM-based quantity will be different for each of the population frequency distributions. It will be useful to identify relevant summary statistics that capture the relative performance that can be expected for random samples from each of the populations.

Qin et al. (2002) demonstrate that the expected information in a random sample of phase unknown genotypes can be expressed as the sum of two components: the first component reflects the variance of $\hat{\Theta}$ if phase configurations are observed, while the second component reflects the loss of information because of unknown phase configurations.

The first component can be approximated by the gene diversity, $1 - \sum\limits_{i=1}^{M} \theta_i^2,$

which is a measure of the uniformity of the frequency distribution.

To describe the additional uncertainty from the unknown phase configurations, we use the expected error rate using most likely phase configuration. Consider a given phase-unknown genotype, y. The probability that the most likely phase configuration is the correct one is given by $\max p(z|y, \Theta)$. A measure of the uncertainty from not knowing phase for this genotype is $1 - \max p(z|y, \Theta)$. The expectation of incorrectly assigning phase for a random sample is therefore:

$$E(\varepsilon|\Theta) = \sum_y p(y|\Theta) \left[1 - \max p(z|y, \Theta)\right].$$
(5)

Appreciating the relevance of equation (5) in the context of accurate EM-based phase reconstruction cannot be overstated. The expression describes the number of incorrect phase assignments that is expected in a population sample when the most likely phase configuration is used and haplotype frequencies are known. It can therefore be considered a lower bound on the number of errors that are calculated from haplotype frequencies inferred by the EM algorithm.

These two statistics are presented in Table 2. If the population haplotype frequency for $APOE_1$ were known with certainty, we would expect to get no greater than 82% of the sample correct if the most likely phase criterion is used. By contrast, phase assignment using the most likely phase criterion would be virtually error free for population samples generated from the $IL8_E$

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distribution, even though the expected number of ambiguous genotypes (i.e. genotypes with two or more heterozygous loci) is similar to the $APOE_1$. Although there are multiple phase configurations that can, in theory, resolve an ambiguous genotype sampled from $IL8_E$, the vast majority of these will feature at least one haplotype that does not actually segregate in the population. This demonstrates that family data may be unnecessary for accurate phase reconstruction, even when a sample features many ambiguous genotypes.

RESULTS

We describe the results from our simulation study using two standard summary statistics based on haplotype frequency estimates and phase accuracy. For assessing haplotype frequency estimates, we use the Discrepancy metric (Excoffier and Slatkin, 1995; Kirk and Cardon, 2002), which is defined as:

$$D(\Theta; \hat{\Theta}) = \frac{1}{2} \sum_{i=1}^{2^L} \left| \theta_i - \hat{\theta}_i \right|. \tag{6}$$

For the phase configurations, we use the estimated frequencies to assign each individual their most likely phase configuration and then calculate the percentage of individuals that are incorrectly assigned. This metric is appropriate since it is the typical criterion on which haplotypes are assigned for use in a fine-scale mapping analysis.

Results for these two measures of accuracy are presented in Table 3. The table is structured to highlight a wide variety of trends, some of which are

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indexed by letters that will be referenced in the text. When referring to an entry in the table indexed by X, we will use the notation $(\mathbf{X})$. We only include indices for the $APOE_1$ results since annotating each table would have obscured trends. It will be contextually clear which distribution(s) are relevant to supporting a given statement. Similarly, we do not include standard errors. Comparative statements were verified at the 95% significance level using a paired t-test.

The table also features results from a standard analysis using the EM algorithm for unrelated individuals (shaded column). These will be useful when discussing the results from treating related individuals as unrelated (boxed section). Note that using our algorithm gives the same results as the EM algorithm for unrelated individuals when no family data is provided $(\mathbf{A})$. Hence either entry can be used to describe accuracy for 100 unrelated individuals, which is often useful as a base comparison to other scenarios that use family data.

Broadly, we will be interested in how changes in family data, sample size and frequency distribution impact each of the two measures of accuracy. We will also be interested in whether trends observed for one measure of accuracy apply to the other. Since the number of progeny is fixed at 100, we measure sample size by the number of independent haplotypes segregating in the sample.

We begin by focusing exclusively on the accuracy of haplotype frequency estimation. We then provide a similar analysis for phase reconstruction ac-

curacy, highlighting how the two metrics differ in sensitivity to family data. These two sections collectively illustrate the importance of the true haplotype frequency distribution in determining the magnitude of reconstruction error as well as the effectiveness of reallocating resources for family data. In the last section, we discuss the sensitivity of a popular case-control association test to biased frequency estimates that are inferred by treating related individuals as unrelated.

Impact of Family Data on Haplotype Frequency Estimation

We start by observing that for a given family size, increasing family information typically results in an improvement in accuracy (i.e. discrepancy decreases along a given row). However, adding family information does not always contribute to accuracy, as can been seen in the case of adding the genotype from a single parent (**C**). This is because the number of progeny is sufficient to explain the parental phase and therefore the sire genotype provides redundant information. By contrast, there is always an improvement in discrepancy if both parental genotypes are included (**D**). This is because the second parental genotype will always provide information regarding an additional independent haplotype (which follows from our assumption of one progeny per dam).

This example demonstrates how increasing the number of independent haplotypes *or* the amount of family information improves accuracy. The question of whether resources intended for population data should be reallocated for family data is concerned with whether one should be increased at the

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expense of the other. This question was addressed for the case of nuclear families v. unrelated individuals in several studies, which showed that the optimal allocation decision will be frequency-dependent (Becker and Knapp, 2002; Schaid, 2002). Our results illustrate that these frequency-dependent trade-offs between the quality and quantity of population data can be found for many pedigree configurations. Specifically, we compare the accuracy of 200 independent haplotypes from a sample of unrelated individuals to 140 independent haplotypes segregating in 20 half-sib pedigrees of size 5 with a typed sire **(F)**. For the $APOE_1$ distribution, better accuracy is achieved from using more family data and fewer independent haplotypes, while for the $APOE_2$ and $IL8_E$ distributions more independent data is preferable to family data. As discussed in the previous section, a random sample generated from the $APOE_1$ distribution will have the most uncertainty associated with phase assignments and therefore will benefit most from family data.

We also observe that when family data is ignored (i.e. related individuals are treated as unrelated) discrepancy increases with family size **(G)**. This follows since increasing family size (i.e. increasing the number of conditional dependencies in the data) implies further deviation from the assumption of unrelated (independent) individuals. We will be evaluating these results squarely in the context of association analysis at the end of this section.

Impact of Family Data on Phase Reconstruction Accuracy

The most striking result is the uniformly perfect phase reconstruction given by $IL8_E$, which provides an example of a distribution where family data is

redundant despite over 50% of a sample containing ambiguous genotypes. These results are consistent with the frequency-known error rate given in Table 2. We note that for all three distributions, the observed error rate (**A'**) is fairly close to the frequency-known error rate, which is the best-case average error rate that can be achieved when using the EM algorithm. In this context, it is reasonable to claim that EM-based phase assignments are accurate.

For the $APOE_1$ and $APOE_2$ distributions, increasing sib size and adding parental marker data always improves phase reconstruction accuracy. Specifically, as we move down a given column or across a row for either distribution, we observe a *gradual* decrease in phase reconstruction error rate. It should be noted that when resources are fixed, increasing family size decreases the number of independent haplotypes used in the subsequent study, and therefore this gain in phase reconstruction accuracy may not be justified.

While phase reconstruction error decreases with family information, it is not eliminated. Even for very large sib sizes, there is a small, but significant error when both parental genotypes are provided. Note also that for both distributions, there is also a discernable increase in the error rate when only the genotype for the common parent is provided. However, results for the $APOE_1$ distribution are consistently worse than for the $APOE_2$ distribution. These observations highlight the importance that both the frequency distribution and the pedigree structure have in determining whether resources should be allocated to ascertain family data.

Although increasing sib-size and parental marker information will both improve phase reconstruction accuracy, obtaining parental marker data is more efficient than adding more half-sibs. For both distributions, introducing genotype data for an untyped parent is more efficient than introducing as many as five additional half-sibs (**H,I**). This complements the results that show two full-sibs with untyped parents can be very inefficient in the context of optimal frequency estimation (Schaid, 2002).

It is important to recognize that reconstruction accuracy for progeny does not extend to parents. This means that parental phase may still be incorrectly reconstructed even when reconstruction is accurate for progeny. Adding half-sibs will help reconstruct phase for an untyped common parent, yet our results show that the total number of half-sibs needed to make this parental genotype redundant can be quite large. For each of the three distributions, we see that a typed sire still provides a small, but significant, improvement in discrepancy when as many as 10 progeny are available (**E**). Introducing sibs without genotyping parents can actually be worse than reconstruction from population data if this information is to be used in a subsequent LD model that relies on haplotype accuracy of both parents and progeny (Meuwissen et al., 2002; Lee and van der Werf, 2004).

Note that the frequency-dependent trade-off between independent haplotypes and family size that was observed for optimal haplotype frequency estimation (**F**) is not applicable to optimal phase reconstruction accuracy (**F'**). Although increasing family information tends to improve both haplotype reconstruc-

tion accuracy and phase reconstruction accuracy the impact of family data differs. For example, the gain achieved when introducing the genotype for one parent than for both is larger when the first parent's genotype is provided in the context of haplotype frequency estimation (**B**), yet each parent makes roughly equal contribution to accuracy in the context of phase reconstruction accuracy (**B'**).

There is an actual contradiction in trends between frequency estimation and phase assignment when related individuals are treated as unrelated. Specifically, we see that phase accuracy *improves* as the number of related individuals that are treated as unrelated increases (**G'**). This paradox can be explained by noting that for more closely related individuals, there is a higher probability that two haplotypes are IBD (i.e. more homozygosity) in the genotype data. Fallin and Shorck (2000) made a similar observation when investigating the robustness of the EM algorithm to departures from Hardy-Weinberg Equilibrium by imposing homozygosity on the haplotype data. When individuals are related, however, the appropriate comparison is not the "benefit" in phase reconstruction accuracy relative to unrelated individuals, but the loss by not properly accounting for the conditional dependencies in the data. This loss can be quite large as seen in the case of $APOE_1$ where over 10 haplotypes are incorrectly assigned by not accounting for underlying pedigree structure (**J**).

An Application to Association Analysis

We now evaluate the discrepancy that arises from treating related individ-

uals as unrelated, **(G)**, in the context of an association analysis. This is relevant for many natural and domestic populations, where, in the absence of pedigree information, a sample may be treated as unrelated even through the sampled members can be closely related. A popular test that utilizes haplotype frequencies directly is a chi-square test, which is used to detect association in a case-control study (Zhao et al., 2000). While variants of the test have been proposed (Fallin et al., 2001), the underlying principle remains that if a causative variant is linked to marker haplotype, then case haplotype frequencies should differ from those of the control frequencies. A Type I error occurs when the reconstructed frequencies for case and control data are considered significantly different, yet the markers are actually unlinked with a causative variant. Treating related individuals as unrelated might be expected to increase the probability of committing a Type I error, since variability that naturally arises between two groups sampled from the same neutral region will be accentuated when the data are dependent. To investigate the impact of using these reconstructed frequencies on a Type I error, we simulate neutral marker data for hypothetical cases and controls from two repetitions of the simulation process described above. We estimate frequencies under the assumption that individuals are unrelated and then calculated the chi-square statistic based on the reconstructed frequencies. The results are presented in the right column of Table 4. They reveal that an inflated Type I error is likely to be realized for a family size of 5 for each of the distributions. Even though the actual discrepancy statistic

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is uniformly greatest for the $APOE_1$ and least for $IL8_E$, the rate at which significance increases with family size is fairly consistent for each of the three distributions: the principal difference lies in the standard deviation. We also ran a standard single locus Hardy-Weinberg test for each of the loci and present the results on the left of Table 4. This reveals that data sets that are sufficiently related to exhibit a Type I error in case-control association analysis will not be detected using the standard single locus Hardy-Weinberg test.

DISCUSSION

The results of this study have significant implications for an experimental design using two-stage haplotype analysis. The effectiveness of a two-stage haplotype analysis will be contingent on two factors: 1) the magnitude of the estimation error, which we have shown depends on the the haplotype frequency distribution and 2) the sensitivity of the subsequent haplotype-based analysis to this estimation error, which can be determined from simulation studies.

EM-based haplotype frequency estimates are often considered accurate for sample sizes consisting of approximately 100 individuals (Fallin and Shorck, 2000; Qin et al., 2002). For smaller samples sizes, or for samples featuring large amounts of missing genotype data, a Bayesian approach may be more appropriate (Stephens et al., 2001; Niu et al., 2002). The EM algo-

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rithm is also considered to provide accurate results under departures from the random-mating assumption (Fallin and Shorck, 2000). However, our results demonstrate that failing to account for related individuals in a sample (by treating each member in the sample as independent) can lead to an appreciable increase in Type I error in a study where two populations are contrasted using EM-based haplotype frequencies. In natural populations featuring sibships, it is important to ascertain pedigree information, e.g. by sibship reconstruction methods described by Thomas and Hill (2002), before using this test.

The results of Morris et al. (2004) clearly demonstrate that phase assignments based on the most likely phase criterion should not be blindly used in a subsequent haplotype-based analysis. We show that while reconstruction errors may be unavoidable (i.e. independent of sample size), this error rate can be calculated directly from the frequency distribution. We also provide an example where the most likely phase criterion will yield perfectly accurate results, even for a sample containing a large proportion of ambiguous genotypes.

Practically, we can calculate the error rate from an estimated frequency distribution that is based on a preliminary sampling of the population. This estimated error rate can then be compared against a predetermined threshold denoting the minimum level of phase accuracy. If the predicted phase reconstruction error exceeds this threshold, then either pedigree data is required or an alternative to the two stage approach must be used.

The effectiveness of pedigree data will also depend on the haplotype frequency distribution, as well as the type of pedigree information provided. We considered two kinds of information: increasing the family size and introducing parental marker data. For half-sibs, introducing parental genotypes is more efficient than increasing family size and leaving parents untyped. In general, assessing the effectiveness of pedigree structure and frequency distribution on haplotype inference can be done through a simulation study based on the inferred frequencies.

In conclusion, we have confirmed that the biased haplotype frequency estimates that result from treating related individuals as unrelated can impact association analysis, and we have provided the appropriate statistical methods to efficiently accommodate some important population structures. We have also demonstrated how to forecast the accuracy of phase reconstruction based on the most likely phase criterion, which will determine whether pedigree data is warranted. We find that the impact of pedigree data depends upon the actual haplotype structure in the population. Since this structure will vary throughout the genome, there is unlikely to be a single optimal strategy for accurate haplotype reconstruction from markers used in genome-wide scans. Rather, the impact of various pedigree data on particular subsets of markers can be assessed through a simulation study based upon initial frequency estimates.

Table 1 - Categories of family data that can be included with a sample of phase unknown genotypes in simulation study.

Table 2 - Summary statistics for haplotype frequency estimates used in data analysis.

Table 3 - Impact of family size, family information and parental haplotype distribution on estimated haplotype frequencies, as measured by the discrepancy statistic (Left) and on phase reconstruction error (Right). Each entry for a given metric corresponds to the average value for 100 replicates. Shaded areas denote estimates that were obtained using the standard EM algorithm for unrelated individuals, boxed areas denote estimates obtained by treating related individuals as unrelated. Letters are referenced in the text.

Table 4 - Impact of treating half-sibs as unrelated on two nonparametric tests: single-locus tests for Hardy-Weinberg Disequilibrium (left) and non-homogeneity of haplotype frequency profiles for case control data in a neutral genomic region (right).

Table 1:

Category	Description
UN	No Information - All Animals Assumed Unrelated
P	Pedigree Structure Only (Parents Untyped)
PS	Pedigree Structure + Sire Genotype
PSD	Pedigree Structure + Sire and Dam Genotypes

Table 2:

	APOE$_1$	APOE$_2$	IL8$_E$
Number of Loci	4	4	6
Number of Haplotypes	13	10	9
Ambiguous Genotypes[1]	0.52	0.41	0.53
Gene Diversity	0.86	0.8	0.56
Frequency-Known Error Rate[2]	0.18	0.12	0.0003

[1] The expected proportion of a population sample that is heterozygous for at least two loci.

[2] The expected proportion of phase configurations that will be incorrectly resolved in a population sample when the most likely phase criterion is used and haplotype frequencies are known.

Table 3:

HAPLOTYPE FREQUENCY DISCREPANCIES

	Family Size	UN	P	PS	PSD
APOE$_1$	1	0.116 **A**	0.116 **A,B,F**	0.078 **B**	0.063 **B**
	2	0.123	0.117	0.095	0.070
	5	**G** 0.143	0.119	0.109 **F**	0.077
	10	0.162	0.119 **E**	0.116 **E**	0.081
	25	0.212	0.122 **C**	0.122 **C,D**	0.082 **D**
APOE$_2$	1	0.082	0.082	0.061	0.049
	2	0.091	0.088	0.077	0.060
	5	0.110	0.097	0.090	0.065
	10	0.132	0.103	0.099	0.066
	25	0.180	0.104	0.104	0.070
IL8ε	1	0.047	0.047	0.037	0.032
	2	0.054	0.054	0.051	0.040
	5	0.065	0.060	0.058	0.043
	10	0.085	0.065	0.063	0.046
	25	0.110	0.068	0.068	0.047

PHASE RECONSTRUCTION ERROR RATE

	Family Size	UN	P	PS	PSD
APOE$_1$	1	0.21 **A'**	0.21 **B',F'**	0.12 **B',H**	0.06 **B',I**
	2	0.20	0.17	0.10	0.03
	5	**G'** 0.19	0.12 **H**	0.06 **F',I**	0.02
	10	0.18 **J**	0.07 **J**	0.05	0.02
	25	0.15 **J**	0.06 **J**	0.06	0.02
APOE$_2$	1	0.13	0.13	0.08	0.04
	2	0.13	0.11	0.07	0.03
	5	0.13	0.09	0.05	0.02
	10	0.11	0.06	0.05	0.02
	25	0.11	0.05	0.04	0.02
IL8ε	1	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00

Table 4:

	Family Size	Single Locus Analysis for Hardy-Weinberg Disequilibrium[1]		Association Test using Haplotype Frequency Estimates	
		$\chi^2(1)$	sdev	$\chi^2(8)$	sdev
IL8$_E$	**1**	1.69	2.23	9.32	4.05
	5	1.71	1.87	14.53 *	6.57
	10	1.67	1.74	18.78 **	9.22
	25	2.17	2.42	26.17 ***	16.37
	50	3.55 *	4.27	--	--
		$\chi^2(1)$	sdev	$\chi^2(9)$	sdev
APOE$_2$	**1**	2.47	2.05	9.37	4.12
	5	2.29	1.99	14.76 *	7.09
	10	2.24	1.87	21.35 **	9.69
	25	2.79	2.61	35.73 ***	17.91
	50	4.50 **	4.42	--	--
		$\chi^2(1)$	sdev	$\chi^2(12)$	sdev
APOE$_1$	**1**	2.43	2.11	12.51	4.66
	5	2.43	1.99	21.41 *	7.47
	10	2.36	2.00	27.66 ***	12.24
	25	2.99	2.77	47.68 ***	19.77
	50	5.58 **	4.95	--	--

[1] For each replicate, the test statistic for the locus exhibiting the highest disequilbrum was used.

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References

Becker, T. and Knapp, M. (2002). Efficiency of haplotype frequency estimation when nuclear family information is included. *Hum. Hered.*, 54:45–53.

Elston, R. and Stewart, J. (1971). A general model for the genetic analysis of pedigree data. *Hum. Hered.*, 21:523–542.

Excoffier, L. and Slatkin, M. (1995). Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol. Biol. Evol.*, 12:921–927.

Fallin, D., Cohen, A., Essioux, L., Chumakov, I., Blumenfeld, M., Cohen, D., and Schork, N. J. (2001). Genetic analysis of case/control data using haplotype frequencies: Application to APOE locus variation and Alzheimer's Disease. *Genet. Res.*, 11:143–151.

Fallin, D. and Shorck, N. (2000). Accuracy of haplotype frequency estimation for biallelic loci, via the expectation-maximization algorithm for unphased diploid genotype data. *Am. J. Hum. Genet.*, 67:947–959.

Grapes, L., Dekkers, J., Rothschild, M., and Fernando, R. (2004). Comparing linkage disequilibrium-based methods for fine mapping of quantitative trait loci. *Genet.*, 166:1561–1570.

Hawley, M. and Kidd, K. (1995). HAPLO: a program using the EM algo-

rithm to estimate the frequencies of multi-site haplotypes. *J. Hered.*, 86:409–411.

Hill, W. G. (1974). Estimation of linkage disequilibrium in randomly mating populations. *Hered*, 33:229–239.

Hull, J., Ackerman, H., Isles, K., Usen, S., Pinder, M., Thomson, A., and Kwiatkowski, D. (2001). Unusual haplotypic structure of IL8, a susceptibility locus for a common respiratory virus. *Am. J. Hum. Genet.*, 69:413–419.

Kirk, K. M. and Cardon, L. R. (2002). The impact of genotyping error on haplotype reconstruction and frequency estimation. *Eur. J. Hum. Genet.*, 10:616–622.

Lee, S. H. and van der Werf, J. H. (2004). The efficiency of designs for fine-mapping of quantitative trait loci using combined linkage disequilibrium and linkage. *Genet. Sel. Evol.*, 36:145–160.

Meuwissen, T. H. E., Karlsen, A., Lien, S., Olsaker, I., and Goddard, M. E. (2002). Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibirum mapping. *Genet.*, 161:373–379.

Morris, A., Whittaker, J., and Balding, D. (2002). Fine-scale mapping of disease loci via shattered coalescent modeling of genealogies. *Am. J. Hum. Genet.*, 70:686–707.

Morris, A., Whittaker, J., and Balding, D. (2004). Little loss of information due to unknown phase for fine-scale linkage-disequilibirium with single-nucleotide-polymorphism genotype data. *Am. J. Hum. Genet.*, 74:945–953.

Niu, T., Qin, Z. S., Xu, X., and Liu, J. S. (2002). Bayesian haplotype inference for multiple linked single-nucleotide polymorphisms. *Am. J. Hum. Genet.*, 70:157–169.

O'Connell, J. R. (2000). Zero-recombinant haplotyping: Applications to fine mapping using SNPs. *Genet. Epidemiol.*, 19:S582–S587.

Ploughman, L. M. and Boehnke, M. (1989). Estimating the power of a proposed linkage study for a complex genetic trait. *Am. J. Hum. Genet.*, 44:543–551.

Qin, Z. S., Niu, T., and Liu, J. S. (2002). Partial-ligation-expectation-maximization for haplotype inference with single nucleotide polymorphisms. *Am. J. Hum. Genet.*, 71:1242–1247.

Rohde, K. and Fuerst, R. (2001). Haplotyping and estimation of haplotype frequencies for closely linked biallelic multilocus genetic phenotypes including nuclear family information. *Hum Mutat*, 17:289–295.

Schaid, D. J. (2002). Relative efficiency of ambiguous vs. directly measured haplotype frequencies. *Genet. Epidemiol.*, 23:426–443.

Stephens, M. and Donnelly, P. (2003). A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Genet.*, 73:1162–1169.

Stephens, M., Smith, N. J., and Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.*, 68:178–989.

Terwilliger, J. and Ott, J. (1994). *Handbook of Human Linkage Analysis.* John Hopkins University Press.

Thomas, S. C. and Hill, W. G. (2002). Sibship reconstruction in hierarchical population structures using Markov Chain Monte Carlo techniques. *Genet. Res. Camb.*, 79:227–234.

Zhao, H., Pfeiffer, R., and Gail, M. H. (2003). Haplotype analysis in population genetics and association studies. *Pharmaco*, 4:171–178.

Zhao, J. H., Curtis, D., and Sham, P. C. (2000). Model-free analysis and permutation tests for allelic associations. *Hum. Hered.*, 50:133–139.

APPENDIX

AN EFFICIENT ALGORITHM FOR EVALUATING HALF-SIB

PEDIGREES

Our objective is efficient computation of the posterior distributions for sire and dam phase configurations, which are required to calculated the complete-data log-likelihood given in (4). We begin by describing a straightforward peeling approach to obtain the posterior for the sire. We then demonstrate how the results obtained during the peeling process can be used for efficient calculation of the dam distribution. Both of these approaches achieve cubic complexity in the number of possible phase configurations. We conclude by demonstrating how complexity can be reduced to quadratic, which as described in the text, can result in substantial savings in computational resources.

To minimize notational clutter we drop explicit references to $\tilde{\Theta}$ and $\mathcal{F}$, and it should be understood that this information is given. Furthermore, since we will be talking about the distribution for a specific sire or dam, we can drop any index that refers to an order in the sample. Hence,

$$p(s_i|\tilde{\Theta}, \mathbf{y}, \mathcal{F}) \doteq p(s|\mathbf{y}) \qquad (7)$$

.

Finally, we will need to reference other members in a given sibship. For a sibship of size K, we assign an arbitrary ordering to all progeny and dam pairs, and use (y_i, d_i) $i = 1 \ldots K$ to refer to the ith pair.

Calculating the Distribution of the Sire Phase: We begin by writing the objective as

$$p(s|\mathbf{y}) = \frac{\sum_{\mathbf{d}} p(s, \mathbf{d}, \mathbf{y})}{p(\mathbf{y})}. \tag{8}$$

Using the key insight of Elston and Stewart (1971), the joint distribution is expressed as a telescopic sum:

$$p(\mathbf{y}, s) = p(s) \prod_{k=1}^{K} \left\{ \sum_{d_k} p(y_k|s, d_k) p(d_k) \right\}, \tag{9}$$

where

$$p(y_k|s, d_k) = \sum_{z \in \mathbf{z}(y_i)} p(z_i|s, d_i) p(y_i|z_i). \tag{10}$$

Peeling the jth family first entails calculating

$$p(s, \mathbf{y}_j) = \sum_{d_j} p(s, \mathbf{y}_{j-1}) p(y_j|s, d_j) p(d_j) \tag{11}$$

where $\mathbf{y}_{j-1} = y_0, y_1 \ldots y_{j-1}$ and $y_0 = \emptyset$. The likelihood is then updated to

$$p(\mathbf{y}) = p(s, \mathbf{y}_j) \prod_{k=j+1}^{K} \left\{ \sum_{d_k} p(y_k|s, d_k)p(d_k) \right\}.$$

Each family is iteratively peeled and, after the final family has been peeled, the resulting expression, $p(\mathbf{y}, s)$, can be used to calculate $p(s|\mathbf{y})$. $p(\mathbf{y})$ can then be obtained by summing out s.

Calculating the Posterior for the Dam: First, we can rewrite our objective as

$$
\begin{aligned}
p(d_j|\mathbf{y}) &= \sum_s p(s, d_j|\mathbf{y}) \\
&= \frac{1}{p(\mathbf{y})} \sum_s p(s, d_j, \mathbf{y}) \\
&= \frac{1}{p(\mathbf{y})} \sum_s p(s, \mathbf{y}_{-j})p(y_j|d_j, s)p(d_j), \quad (12)
\end{aligned}
$$

where efficient calculation of $p(\mathbf{y})$ is given above and the definition of $p(s, \mathbf{y}_{-j})$ is given by:

$$p(s, \mathbf{y}_{-j}) = p(s, y_0, y_1 \ldots y_{j-1}, y_{j+1}, \ldots y_K).$$

To calculate $p(s, \mathbf{y}_{-j})$, we must store each of the K expressions given by equation (11), i.e.

$$p(s, \mathbf{y}_j) \qquad j = 0 \ldots K. \quad (13)$$

These are sufficient to calculate $p(s, \mathbf{y}_{-j})$ since:

$$\left[\frac{p(s, \mathbf{y})}{p(s, \mathbf{y}_j)}\right] p(s, \mathbf{y}_{j-1}) = \frac{\prod_{i=1}^{K} p(y_i|s)}{\prod_{i=1}^{j} p(y_i|s)} p(s, \mathbf{y}_{j-1})$$

$$= \prod_{i=j+1}^{K} p(y_i|s) p(s, \mathbf{y}_{j-1})$$

$$= p(y_{j+1} \ldots y_K|s) p(s, \mathbf{y}_{j-1})$$

$$= p(s, \mathbf{y}_{-j}). \tag{14}$$

Further Reduction in Complexity: From equations (10) and (14), we see that the complexity of the phase distribution for both sire and dam is dominated by the expression $p(y|s, d)$, which is cubic in the number of phase configurations. To achieve quadratic complexity, we show how to calculate this expression by evaluating $p(y|s)$ and $p(y|d)$ directly.

Consider first evaluating the relevant expression for the sire where the genotype of the dam is unknown. Rather than summing over all the dam configurations, as suggested by (9), we attempt to directly evaluate

$$p(\mathbf{y}, s) = p(s) \prod_{k=1}^{K} p(y_k|s). \tag{15}$$

A given set of phase configurations, $z \in \mathbf{z}(y_k)$, can be expressed as

$$p(z = h_i, h_j | s = h_k, h_l). \tag{16}$$

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Let $t_1(s)$ denote the event that the sire transmitted h_k and let $t_2(s)$ denote the event that the sire transmitted h_l. Then (16) can be written as:

$$p(z = h_i, h_j | s = h_k, h_l) \quad = \quad p(z, t_1(s)) + p(z, t_2(s)) \tag{17}$$

$$= \quad p(z|t_1(s))p(t_1(s)) + p(z, t_2(s))p(t_2(s)) \tag{18}$$

where $p(t_1(s)) = p(t_2(s)) = 1/2$. Importantly, $p(z|t_1(s))$ and $p(z|t_2(s))$ can be calculated directly from $\tilde{\Theta}$, for example:

$$p(z|t_1(s)) = \begin{cases} \tilde{\theta}_j & k = i \\ \tilde{\theta}_i & k = j \\ 0 & \text{otherwise.} \end{cases} \tag{19}$$

When the genotype of the dam is known, the probabilities in (19) can be calculated from the distribution $p(d|\tilde{\Theta})$. Evaluating $\sum_s p(y|s, d)$ follows an analogous procedure.