

Linkage Analysis with Sequential Imputation

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Abstract

Multilocus calculations using all available information on all pedigree members are important for linkage analysis. Exact calculation methods in linkage analysis are limited in either the number of loci or the number of pedigree members they can handle. In this article, we propose a Monte Carlo method for linkage analysis based on sequential imputation. Unlike exact methods, sequential imputation can handle both a large number of loci and a large number of pedigree members. This Monte Carlo method is an application of importance sampling in which we sequentially impute ordered genotypes locus by locus and then impute inheritance vectors conditioned on these genotypes. The resulting inheritance vectors together with the importance sampling weights are used to derive a consistent estimator of any linkage statistic of interest. The linkage statistic can be parametric or nonparametric; we focused on nonparametric linkage statistics. We demonstrated that accurate estimates can be achieved within reasonable computing time. Then we performed a simulation study to illustrate the potential gain in power using our method for multilocus linkage analysis with large pedigrees. We simulated data at six markers under three models. We analyzed them using both sequential imputation and GENEHUNTER. GENEHUNTER had to drop between 38% to 54% of the pedigree members whereas our method was able to use all pedigree members. The power gains of using all of the pedigree members were substantial under two of the three models. We have implemented sequential imputation for multilocus linkage analysis in a user-friendly software package called SIMPLE.

Key Words: IBD, Monte Carlo, NPL statistics, Pedigrees, Power study

Introduction

Linkage analysis extracts inheritance information from pedigree data to evaluate the cosegregation of marker and trait alleles. Thus it is important to utilize available information on multiple markers and all pedigree members. Unfortunately, algorithms for exact analysis are computationally limited in either the number of markers or the number of pedigree members they can handle. Peeling and Hidden Markov Model (HMM) approaches are two such exact methods that are most frequently used.

Peeling [Elston and Stewart, 1971; Cannings et al., 1978] is a computational algorithm that successively aggregates inheritance information from pedigree members. The algorithm scales linearly with the number of pedigree members, but exponentially with the number of loci. Genotype elimination [Lange and Gora-dia, 1987; O'Connell and Weeks, 1999] and set-recoding [O'Connell and Weeks, 1995] have been proposed to reduce the computational requirements so that data from more loci can be processed jointly. Despite these improvements, peeling is still limited in the number of loci that it can handle.

The HMM methods model the underlying inheritance pattern as an inhomogeneous Markov chain with each entry of the transition matrix being a function of the recombination fraction between adjacent loci [Lander and Green, 1987]. The key to the algorithm is the assumption of no genetic interference. In contrast to peeling, the HMM method scales linearly with the number of loci, but exponentially with the number of pedigree members. Many improvements have been made to reduce computational requirements so that more pedigree members can be analyzed. Properties of the transition matrix [Kruglyak et al., 1995] and symmetries in founder phases [Kruglyak et al., 1996] were exploited to reduce the amount

of calculations. Fast Fourier transformations [Kruglyak and Lander, 1998] further speed up calculations. Using observed genotypes to reduce the inheritance space [Markianos et al., 2001a] and to form equivalence classes [Markianos et al., 2001b] allows for potentially more pedigree members. Idury and Elston (1997) describe a ‘divide and conquer’ algorithm which speeds up some of the calculations and allows for sex-specific recombination without any computational penalty. This ‘divide and conquer’ method was incorporated into the software package Merlin [Abecassis et al., 2002] which also uses an approximation method to expand the size of the pedigree it can handle in some cases. Other algorithmic improvements such as efficient tree traversal were made to the HMM algorithm and incorporated into Allegro [Gudbjartsson et al., 2000]. However, even with these improvements, the HMM formulation inevitably scales exponentially with the number of pedigree members.

Monte Carlo methods have been proposed to overcome these computational limitations. Two major approaches of Monte Carlo methods to linkage analysis are Markov chain Monte Carlo (MCMC) and sequential imputation. MCMC algorithms can be designed such that they scale linearly in both the number of loci and the number of pedigree members [Thompson, 2000]. Thus, MCMC is an extremely powerful estimation method that can practically deal with any number of loci and pedigree of arbitrary size and complexity [Luo et al., 2001]. However, due to strong dependencies among realizations of the Markov chain, convergence can be slow [Thompson, 2000].

Sequential imputation is another Monte Carlo method that has been successfully applied to a variety of areas [Bergman, 2001; Blake et al., 2001]. Irwin et al. (1994) illustrated how to use sequential imputation in linkage analysis to calculate

the likelihood (and hence LOD scores), utilizing the peeling algorithm for a single locus, which results in an algorithm that also scales linearly in both the number of loci and the number of pedigree members. For pedigrees that are not very complex (i.e., single-locus peelable), sequential imputation is expected to be more efficient computationally than MCMC methods in most circumstances. However, it should be noted that sequential imputation is not meant to be a replacement for MCMC, as it cannot handle very complex pedigrees, such as the 1544-member Hutterite pedigree successfully dealt with using MCMC methods [Luo et al., 2001].

This article extends the method of sequential imputation to nonparametric linkage analysis. This is an important step forward in making sequential imputation a viable alternative for linkage analysis, as nonparametric linkage analysis is frequently more suited for analyzing complex traits whose underlying genetic model is unknown or unclear.

The idea is to simulate inheritance vectors conditioned on phase-known multi-locus genotypes that were imputed sequentially. Then the inheritance vectors can be used to estimate any linkage statistic of the form [Whittemore and Halpern, 1994b; Kruglyak et al., 1996]

$$E[S(\phi, x_d) | \mathbf{x}_m] = \sum_{\phi} S(\phi, x_d) P(\phi | \mathbf{x}_m), \quad (1)$$

where \mathbf{x}_m is the observed marker data, x_d is the observed disease phenotypes and ϕ is the unobserved inheritance vector. The inheritance vector [Lander and Green, 1987] $\phi = (p_1, m_1, \dots, p_n, m_n)$ is a binary representation of the inheritance information at a location in the genome for each of the n nonfounders. The i^{th} nonfounder is assigned 2 bits, p_i and m_i , corresponding to the genetic information inherited from the father and mother. Each bit is either 1 or 0 depending

on whether it was inherited from the grandmother or grandfather, respectively. The inheritance distribution, $P(\phi|\mathbf{x}_m)$, is the distribution of the inheritance vectors conditioned on the observed marker data.

The scoring function, $S(\phi, x_d)$ for inheritance vector ϕ and observed disease phenotypes x_d , measures the amount of IBD sharing. An example of a scoring function for sib pairs is to assign a score of $\frac{1}{2}$, $\frac{1}{4}$ or 0 to a sib pair that share 2, 1 or 0 alleles IBD, respectively. Suppose two sibs have the following inheritance vector: (1,0, 1,0), which implies that they both inherited the grandmaternal allele from their father and the grandpaternal allele from their mother. Therefore they share two alleles IBD and would get a score of $\frac{1}{2}$ with this scoring function.

The class of linkage statistics represented in (1) encompasses a wide range of nonparametric IBD statistics, including S_{pairs} and S_{all} [Whittemore and Halpern, 1994a], the most popular allele sharing statistics for nonparametric analysis. We note that, if we add genetic parameters for the disease model to the score function, the statistic in the form (1) becomes a parametric statistic. In fact, the familiar LOD score is included in this class [Kruglyak et al., 1996].

The next three sections describe the algorithm and other technical issues. They are followed by a simulation study to demonstrate the potential gain in power of using larger pedigrees for multilocus linkage analysis.

Methods

The idea is to estimate the linkage statistic in formula (1) instead of calculating it exactly. We decompose the information that we have on the m markers into $\mathbf{x}_m = \{x_1, \dots, x_m\}$. We denote the unobserved ordered genotypes at the m markers, $\{y_1, \dots, y_m\}$, as \mathbf{y} . After obtaining the starting point of the sequential

imputation (step 1) we sequentially sample the ordered genotypes and calculate the appropriate importance sampling weight (steps 2 and 3). Then we sample the inheritance vector ϕ at a particular location given the sampled ordered genotypes at the m markers (step 4). Finally, we calculate the score using ϕ (step 5).

Step 1. Calculate $P(x_1)$ and sample y_1 from $P(y_1|x_1)$.

Step 2. For $t = 2, \dots, m$ we carry out the following steps:

(a) Calculate $P(x_t|x_1, y_1, \dots, x_{t-1}, y_{t-1})$.

(b) Sample y_t from $P(y_t|x_1, y_1, \dots, x_{t-1}, y_{t-1}, x_t)$.

Step 3. Form $w(\mathbf{y}) = P(x_1) \prod_{t=2}^m P(x_t|x_1, y_1, \dots, x_{t-1}, y_{t-1})$.

Step 4. Sample ϕ at a location of interest according to $P(\phi|\mathbf{y})$, where \mathbf{y} are the ordered genotypes sampled in steps 1-3. Note that $P(\phi|\mathbf{y})=P(\phi|\mathbf{y}, \mathbf{x}_m)$.

Step 5. Calculate the score $S(\phi, x_d)$.

Steps 1 to 5 are carried out N times to form $w(\mathbf{y}_1), \dots, w(\mathbf{y}_N)$ and $S(\phi_1, x_d), \dots, S(\phi_N, x_d)$. The probability calculations and the sampling in steps 1 through 3 are done by means of single locus peeling and sampling using reverse peeling (Ploughman and Boehnke 1989; Ott 1989).

The sampling of the inheritance vector in step 4 involves a series of Bernoulli trials. Since each of the bits that make up the inheritance vector are conditionally independent of each other given the in-phase genotypes, \mathbf{y} , we can sample these bits separately.

Irwin et al. (1994) show that the sampling distribution of the ordered genotypes, $P^*(\mathbf{y}|\mathbf{x}_m)$, satisfies:

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$$P^*(\mathbf{y}|\mathbf{x}_m) = \frac{P(\mathbf{y}|\mathbf{x}_m)P(\mathbf{x}_m)}{w(\mathbf{y})}.$$

From this equality it follows:

$$\begin{aligned} & E_{\phi, \mathbf{y}}[S(\phi, x_d)w(\mathbf{y})|\mathbf{x}_m] \\ &= \sum_{\phi} S(\phi, x_d) \sum_{\mathbf{y}} P(\phi|\mathbf{y})P^*(\mathbf{y}|\mathbf{x}_m)w(\mathbf{y}) \\ &= P(\mathbf{x}_m) \sum_{\phi} S(\phi, x_d) \sum_{\mathbf{y}} P(\phi|\mathbf{y})P(\mathbf{y}|\mathbf{x}_m) \\ &= P(\mathbf{x}_m)E_{\phi}[S(\phi, x_d)|\mathbf{x}_m]. \end{aligned}$$

This result and the fact that the average of the weights is an unbiased estimator of $P(\mathbf{x}_m)$ (Irwin et al. 1994) gives us a consistent estimator for the linkage statistic in (1):

$$\hat{E}[S(\phi, x_d)|\mathbf{x}_m] = \sum_{j=1}^N S(\phi_j, x_d) \frac{w(\mathbf{y}_j)}{w(+)},$$

where $w(+)=\sum_{j=1}^N w(\mathbf{y}_j)$. So the estimate is a weighted average of the scores.

In step 5 to calculate the score, $S(\phi, x_d)$, we first assign each of the founders two unique allele labels. We pass these founder allele labels down the pedigree using the sampled inheritance vector. We then measure the number of founder allele labels in common amongst the affecteds via the IBD scoring function.

The null distribution

The IBD statistic measures the amount of IBD sharing. If the amount of sharing among the affecteds is significantly more than what would be expected under the

null hypothesis of no linkage then there is evidence of linkage. Therefore it is necessary to measure the mean and variance of the scores under null hypothesis of no linkage. To estimate the null mean and variance we simply pass the founder allele labels through the pedigree with 50% probability of a particular allele label being passed on to an offspring and calculate the score. We repeat this process many times to get a sample of the scores from the null distribution. The mean and variance of this sample give unbiased estimates of the null mean and variance of the scoring function. Although one could do this exactly [Kruglyak et al., 1996] we find this to be inefficient for the size of pedigrees we are considering. Therefore, we have implemented instead the above Monte Carlo version of the GENEHUNTER procedure [Kruglyak et al., 1996]. We then standardize $\hat{E}[S(\phi, x_d) | \mathbf{x}_m]$ by the estimated null mean and null standard deviation to form the standardized statistic. Furthermore, the sampled scores under the null distribution are used to estimate the exact p-value. We note that this leads to conservative estimates of the standardized statistic and p-value as pointed out by Kruglyak et al. (1996).

To reweight or not?

In the methods described above, we sampled the inheritance vectors (step 4) at every location of interest (usually along the entire chromosome in which the markers reside) and then estimate the statistic using the sampled inheritance vectors. Alternatively, we could sample inheritance vectors at only a few locations of the chromosome and estimate the linkage statistics at neighboring locations by reweighting, another importance sampling idea exploited by Irwin et al. (1994). For instance, suppose that inheritance vectors were sampled at position d_0 . We

can estimate the statistic at a nearby location, say d_1 , by:

$$\sum_{j=1}^N S(\phi_j, x_d) \frac{P_{d_1}(\phi_j | \mathbf{y}_j) w(\mathbf{y}_j)}{P_{d_0}(\phi_j | \mathbf{y}_j) w(+)} \quad (2)$$

This reweighted statistic is a consistent estimator of the linkage statistic at d_1 .

We have found that reweighting does not perform well in estimating IBD statistics, however. This is most likely due to the fact that the distribution of the inheritance vectors under d_1 is too far away from the distribution under d_0 , resulting in large variability in (2). The computational savings in doing reweighting instead of sampling at a particular location for estimating the likelihood, as proposed by Irwin et al. (1994), can be substantial since the alternative would involve peeling. On the other hand, there was no such clear advantage in using reweighting in this application of sequential imputation as sampling inheritance vectors does not pose much computational burden at all. Hence reweighting is not adopted here.

The software package

We have implemented sequential imputation for linkage analysis in a software package called SIMPLE (Sequential Imputation for MultiPoint Linkage Estimation). The nonparametric IBD statistics currently available in SIMPLE include the score functions S_{all} and S_{pairs} [Whittemore and Halpern, 1994a; Kruglyak et al., 1996], plus others as well. Furthermore, SIMPLE can calculate LOD scores. SIMPLE takes input files with the same format as those used in GENEHUNTER, enabling the user to easily switch to SIMPLE if the pedigree is too large to be handled by GENEHUNTER in its entirety. The software is freely available from our web site.

Computational Requirements

Producing the weights and ordered genotypes (steps 1-3) takes the majority of the computing time. To complete a single iteration we need to do a single locus peel for each marker and then do reverse peeling [Ploughman and Boehnke, 1989; Ott, 1989] to sample the ordered genotypes. So the complexity and memory requirements are the same as those required to do m single locus peels. The key difference in computational cost between this algorithm and a standard peeling algorithm for linkage analysis such as that implemented in LINKAGE [Lathrop et al., 1984] is that we are only doing a single locus peel at a time so the calculations are linear in the number of markers. Efficiencies in peeling algorithms can be applied to the peeling step here to improve the overall efficiency. Currently some genotype elimination has been implemented in SIMPLE to achieve such efficiencies. As in peeling, this stage is sensitive to missing data.

In step 4 of the algorithm, we sample the inheritance vector at a location of interest, conditioned on the sample ordered marker genotypes. For one iteration this involves simulating the two inheritance bits for each of the nonfounders, resulting in the calculations being linear in the number of pedigree members. The computational time required for calculating the score (step 5 of the algorithm) depends on its complexity. In particular, the current algorithm for calculating S_{all} is computationally limited in the number of affecteds it can handle; see Markianos et al. (2001a) for a detailed discussion. Missing data has no effect on either of these last two steps since they are conditioned on complete ordered genotypes.

The memory is most influenced by the number of loci being analyzed. This is because we store the joint recombination probabilities across all loci, leading to the storage being exponential in the number of loci being analyzed. In steps 1

through 3 we store the recombination probabilities for just the markers. Whereas in steps 4 and 5 we store the recombination probabilities for the markers plus a location of interest. These probabilities are stored for all locations where the statistics are to be estimated. We could calculate these probabilities as needed to save memory, but this would lead to a large increase in computing time.

We now present a summary of results for time and memory requirements in analyzing a small, medium and large pedigree, respectively. We chose the first three pedigrees (pedigrees 1, 2 and 3) that were presented in a simulated data set from Genetics Analysis Workshop 12. The small, medium and large pedigrees have 52, 86 and 100 members, respectively. They have 15, 17 and 34 members with missing data. Eight markers, with 6-8 alleles each and an average heterozygosity of .77, were analyzed. We ran SIMPLE for 1,000 iterations and estimated S_{pairs} . GENEHUNTER was not capable of analyzing any of these pedigrees without seriously reducing the number of pedigree members. GENEHUNTER would have had to drop 24 (46%), 50 (58%) and 58 (58%) members in the small, medium and large pedigrees, respectively, to be able to analyze them. We used version 2.1.3 of GENEHUNTER here and throughout this paper.

Ped size	Steps 1-3		Steps 4 & 5	
	Time (hr:min)	Memory (MB)	Time (sec)	Memory (MB)
Small	1:37	4.3	.57	.42
Medium	1:41	4.1	1.33	.42
Large	3:47	7.5	1.61	.42

Table 1: **Time & memory requirements for 1,000 iterations.** NOTE- We report the time and memory requirements to complete 1,000 iterations of steps 1-3 and 4 & 5 of the algorithm (including the calculation of the estimate) for eight markers in each of three pedigrees of sizes small (52 members), medium (86 members) and large (100 members). Results are reported per disease location for steps 4 & 5. Note that the time units are different for steps 1-3 and steps 4 & 5.

We conducted the study on a Sun Blade 100 with an Ultrasparc IIe 500 mHz processor. This study can be used as a rough guideline to the time and memory requirements for using SIMPLE. The results are shown in table 1. In this table we show the time and memory requirements to process all 8 markers for 1,000 iterations in steps 1-3. Since the number of points where linkage statistics are estimated depends on the user, we report the time and memory requirements per point in steps 4 and 5. Because the computational time grows linearly with the number of iterations, an estimate of the time for analyzing these pedigrees with 2,000 iterations would be approximately twice the reported times, for example. On the other hand, the memory is not affected by the number of iterations. For steps 4 and 5, the computational time and memory grow linearly with the number of points to be analyzed. For example, to estimate the time and memory to analyze these 8 markers with 5 points between each pair of adjacent markers (43 points in total), multiply the reported time and memory by 43.

The time and memory requirements to produce the weights and ordered genotypes (steps 1-3) for the small and medium pedigrees were similar. Though the medium pedigree was substantially larger than the small pedigree, they both had a comparable amount of missing data. This would explain why they took similar amount of time and memory to be analyzed. On the other hand, the large pedigree had twice as much missing data and therefore took more than twice as long and almost twice as much memory as the other two pedigrees to be analyzed. The memory requirements to sample the inheritance vectors (step 4), calculate the scores (step 5) and form the weighted estimates were the same for all three pedigrees. This is expected since the number of loci (8 markers and 1 point) being analyzed was the same for all three pedigrees. On the other hand, the time

increased as the size of the pedigree increased since the number of inheritance vectors to be sampled increased accordingly.

Accuracy of estimates

We did a number of validation studies of SIMPLE using GENEHUNTER to verify that the scores were being estimated accurately within reasonable computing time. The scores were always quite close to the true scores produced by GENEHUNTER. Of course the accuracy is a function of the number of iterations. To get a rough estimate of the necessary sample size to reach a certain desired accuracy, one may run SIMPLE for a small number of iterations (say 100) to estimate the sampling variability (automatically calculated in SIMPLE), which we note is unlikely to be very accurate. From this estimate, one can calculate the necessary number of iterations. In practice we have found that 5,000 iterations is sufficient for pedigrees that we have examined.

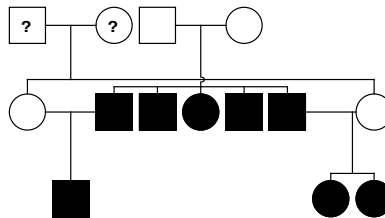


Figure 1: Pedigree used in the validation study. The individuals marked with ‘?’ have no marker data nor information on disease phenotypes.

To illustrate the accuracy of SIMPLE, we analyzed pedigree 76 of the COGA (Collaborative Studies on the Genetics of Alcoholism) data set from Genetics Analysis Workshop 11. We removed three members so GENEHUNTER could analyze it. The pedigree is shown in figure 1. Note that it has a marriage loop.

There are fourteen members in the (reduced) pedigree with four founders. Eight markers are used from chromosome one: D1S1613, D1S550, D1S532, D1S1588, D1S1631, D1S1675, D1S534, D1S1595. They have nine to twelve alleles with an average heterozygosity of .75. The markers are spaced 11.2, 8.4, 18.1, 12.5, 11.9, 9.0 and 9.8 cM apart. Two founders (14%) are missing all of their marker data. In addition, seven other members (50%) are missing data on D1S1631, two members (14%) are missing data on D1S534 and three members (21%) are missing data on other markers.

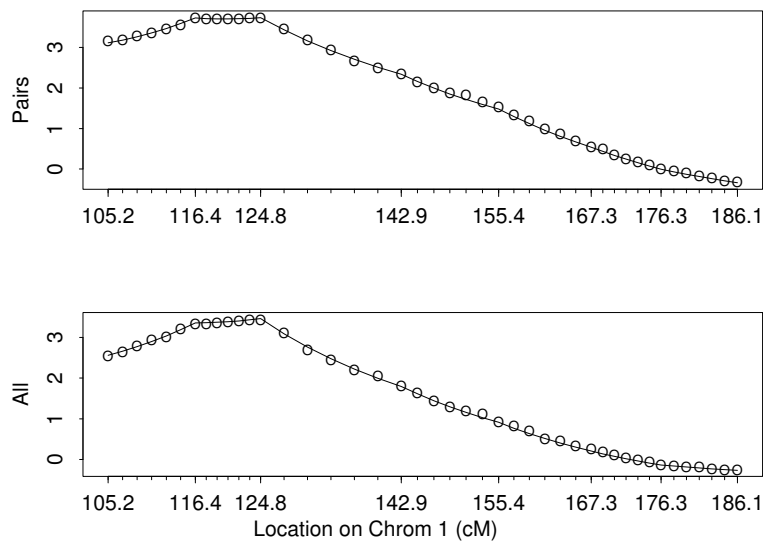


Figure 2: Standardized scores from the validation study. Scores produced by GENEHUNTER are given by the line and the scores produced by SIMPLE are plotted with circles. S_{pairs} are plotted in the top frame and S_{all} are plotted in the bottom frame. The markers are indicated by the extended tick marks and the locations in cM are indicated on the x-axis of the bottom plot.

The linkage statistics S_{pairs} and S_{all} were estimated at five locations between each adjacent pair of markers, using both GENEHUNTER and SIMPLE with 5,000 iterations. As can be seen from the plots in figure 2, the estimated standard-

ized scores produced by SIMPLE were quite close to the true scores produced by GENEHUNTER.

Power Study

To illustrate the potential benefit to multipoint linkage analysis by processing all pedigree members of a large pedigree, we performed a simulation study. We used the S_{pairs} statistic to analyze the full pedigree shown in figure 3 with SIMPLE and then with GENEHUNTER, which needed to discard some members of the pedigree. The pedigree had 37 members, 11 of whom were founders and 5 members had missing marker and disease data. The ascertainment criteria was that at least one sib in each of the seven sibships in the last generation had to be affected.

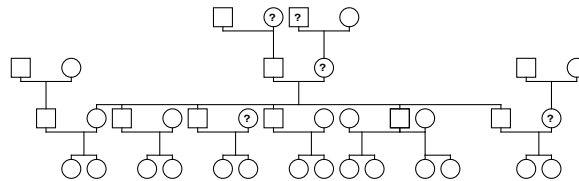


Figure 3: Pedigree structure for the power study. The individuals marked with “?” have no marker nor disease data.

We used 6 markers with equally frequent alleles for each marker. The markers were spaced 15 cM apart. We simulated the marker and disease data under three disease models. In all three cases, the disease data was simulated at a locus in the middle of the marker map at 37.5 cM. In model I, the penetrances for genotypes aa, Aa and AA were 0, .9 and .95 with a disease allele frequency $P(A) = 0.1$. In model II, the penetrances were .05, .4 and .6 with a disease allele frequency .05. In model III the penetrances were .05, .5 and .7 with a disease allele frequency of .3.

level	Model I		Model II		Model III	
	SIMPLE	GH	SIMPLE	GH	SIMPLE	GH
.01	44	40	38	26	21	19
.001	26	24	23	12	11	7
.0001	15	10	15	5	5	3
.00001	8	3	10	3	2	1

Table 2: **Power Estimates for a Single Pedigree** NOTE- Power was defined as the percentage of pedigrees that exceeded given thresholds. The thresholds used for asymptotic significance levels of .01, .001, .0001 and .00001 were 2.33, 3.09, 3.72 and 4.27, respectively.

Five hundred pedigrees were simulated under all three models. GENEHUNTER had to drop between 14 (38 %) to 20 (54%) members in order to process the pedigrees. To estimate power, we calculated the proportion of pedigrees that had maximum scores exceeding given thresholds. Four thresholds levels were entertained: 2.33; 3.09; 3.72 and 4.27, as suggested by Kruglyak et al. (1996). These thresholds correspond to asymptotic significance levels .01, .001, .0001 and .00001, respectively. The results are summarized in table 2.

From the initially simulated pedigrees, we re-sampled, with replacement, 500 data sets of size k , with k ranging from 2 to 50 pedigrees for each of the three models. We estimated powers by the proportion of data sets with standardized scores that exceeded the threshold values. The results for the three models using threshold 3.09 are shown in figure 4. We plotted the proportions for both SIMPLE and GENEHUNTER as points and included a curve that was calculated by a spline smoother [Hastie and Tibshirani, 1990]. We see that under all three models, SIMPLE yields higher powers than GENEHUNTER.

For models I and II, we calculated the minimal sample sizes needed, based on the spline smooth curve (only one of the curves is shown in figure 4; the remaining

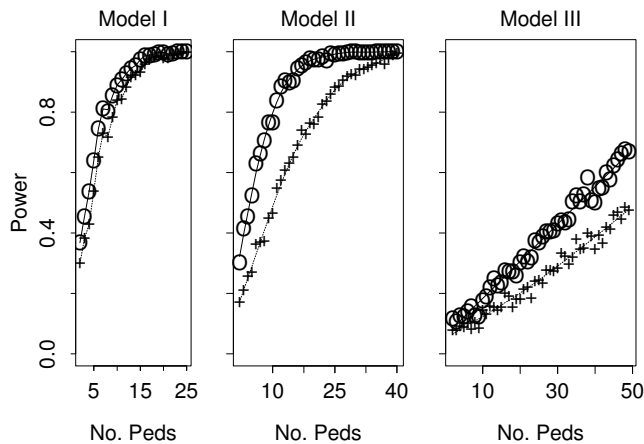


Figure 4: Power curves for SIMPLE (solid line and ‘o’) and GENEHUNTER (dashed line and ‘+’) based on a threshold of 3.09 for all three genetic models.

power	level	Model I		Model II	
		SIMPLE	GH	SIMPLE	GH
50%	.01	2	2	2	5
50%	.001	4	5	5	11
50%	.0001	6	7	8	17
50%	.00001	8	10	11	24
65%	.01	3	4	4	8
65%	.001	6	6	7	15
65%	.0001	8	10	11	23
65%	.00001	11	13	13	29
80%	.01	5	6	6	12
80%	.001	7	10	11	21
80%	.0001	11	14	14	29
80%	.00001	14	17	18	37

Table 3: **Sample Size Estimates for Models I & II** NOTE- For nominal significance levels of .01, .001, .0001 and .00001, we report the minimal sample size necessary (based on a spline fit) to achieve 50%, 65% and 80% power. The corresponding thresholds are 2.33, 3.09, 3.72 and 4.27, respectively.

plots are available from our web site), to reach 50%, 65% and 80% power for each of the threshold levels: 2.33; 3.09; 3.72 and 4.27. The results are summarized in table 3. Since the power was much weaker for model III we reported the results for powers 40%, 50% and 65% at thresholds 2.33 and 3.09 for this model. The results are summarized in table 4. For model I, SIMPLE performed slightly better than GENEHUNTER. However, for model II, SIMPLE only requires approximately half as many pedigrees as GENEHUNTER for the powers considered. In model III, GENEHUNTER needs approximately 50% more pedigrees than SIMPLE to achieve the same power. In all three models, the reduction in the number of pedigrees necessary to achieve the given powers using SIMPLE grows as the desired power increases and as the threshold becomes more stringent.

power	level	SIMPLE	GH
40%	.01	11	18
40%	.001	28	42
50%	.01	17	26
50%	.001	36	*
65%	.01	26	36
65%	.001	48	*

Table 4: **Sample Size Estimates for Model III** NOTE- For nominal significance levels of .01 and .001, we report the minimal sample size necessary (based on a spline fit) to achieve 40%, 50% and 65% power. The corresponding thresholds are 2.33 and 3.09, respectively. The cases marked by ‘*’ indicate that the required sample size is greater than 50.

Type I error

We studied the type I error rates for a data set of 15 pedigrees, which was chosen to reflect a realistic situation. To estimate type I error, we simulated marker genotypes for 10,000 pedigrees using the same pedigree structure and missing

Nominal level	Empirical	
	SIMPLE	GH
.01	.008	.005
.001	.0005	.003
.0001	0	.002
.00001	0	0

Table 5: **Type I Error Rates.** NOTE- For nominal levels of .01, .001, .0001 and .00001, we report the estimated type I error rates for a sample of 15 pedigrees. The corresponding thresholds are 2.33, 3.09, 3.72 and 4.27, respectively.

data pattern used in the power study (figure 4), fixing the last generation as all affected. From these 10,000 simulated pedigrees we re-sampled 2,000 data sets of size 15 pedigrees with replacement. We then calculated the proportion of data sets with standardized scores exceeding each of four thresholds to estimate the type I error rates. The results for both SIMPLE and GENEHUNTER are shown in table 5. GENEHUNTER dropped 17 (46%) members in each of the pedigrees simulated. The estimated type I error rates were close to the nominal significance levels.

Discussion

Linkage analysis is an important tool in localizing disease loci. When analyzing complex traits in humans, it is desirable to process many loci and use all informative members in a given pedigree. We present a Monte Carlo method to do non-parametric multipoint linkage analysis for large pedigrees: sequential imputation. This method can handle either more loci or larger pedigrees than the conventional exact calculation methods: peeling and HMM. We note that while sequential imputation and peeling can both handle large pedigrees, sequential imputation can handle more loci.

One advantage of this method over the HMM is that it can process larger pedigrees which can lead to an increase in power. We demonstrated the potential gain in power in our simulation study using S_{pairs} and three genetic models, although the magnitude of power gains varied from model to model. Substantial power gains are observed under models II and III, while the gains under model I are minimal. The different levels of power gains in the three models are due to the differences in the amount of IBD information carried by the affected individuals dropped. We note that using MCMC methods would yield comparable results as these methods can process the same data as sequential imputation. However, we would expect sequential imputation to be more efficient than MCMC for pedigrees that are not too complex, such as the pedigrees studied.

We would expect the gains in power to be even greater with S_{all} due to the nature of the statistic. Unlike S_{pairs} , S_{all} gives increasing scores to the larger number of affected pedigree members sharing an allele IBD. Since GENEHUNTER often discards affected members, we would expect this to adversely affect the power to a greater degree with S_{all} than with S_{pairs} . One drawback of using S_{all} , however, is the computational intensity of its current implementation. Markianos et al. (2001b) have addressed this issue and proposed a method to reduce the computational burden.

Another advantage of sequential imputation over the HMM method is that it can incorporate genetic interference in its calculations. Currently, in addition to Haldane's no interference model, SIMPLE can calculate linkage statistics using the chi-square model [Foss et al., 1993; Zhao et al., 1995], a recombination model that is suitable for modeling crossover interference in humans [Lin and Speed, 1996].

In the algorithm described in this paper, we decomposed the data into the information that we have at the m loci and sequentially imputed the ordered-genotypes locus by locus. We note that other decompositions are possible. For instance, one could decompose the data into sets of loci. This would involve a multilocus peel per iteration, which obviously increases the computational cost. The advantage is that it should decrease the Monte Carlo variability and hence require less iterations to reach the same accuracy. Furthermore, the order of the sequential imputation does not have to be the physical order of the loci. In fact, the simulation variability should decrease by processing the more informative loci first. SIMPLE, by default, uses the number of alleles as a measure of informativeness and sorts the loci accordingly. The user may override this default and provide his/her own process order.

Electronic-Database Information

The URL for the software and the supplementary materials:

<http://www.stat.ohio-state.edu/~statgen/SOFTWARE/SIMPLE>

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