Algorithms for relationship inference can greatly benefit association datasets containing distantly related individuals. Additionally, the terms (Blouin, 2003; Jones and Arden, 2003) and are not designed for relatives, and have applications in forensics and wildlife population and linkage studies by detecting undeclared or misspecified unrelated). PREST-plus (McPeek and Sun, 2000; Sun et al., 2002) extends this set of alternative relationships to include the third and fourth-degree relatives. RELPAIR (Epstein et al., 2000) examines eight types of relationships (full siblings, parent-child, avuncular, grandparent-grandchild, half siblings, first cousins, monozygotic twins, and unrelated). PREST-plus (McPeek and Sun, 2000; Sun et al., 2002) extends this set of alternative relationships to include the relationship types half-avuncular, half-first cousins and half-siblings first-cousins. Algorithms for pedigree reconstruction (Berger-Wolf et al., 2007; Koch et al., 2008; Riester et al., 2009, 2010) partition the individuals into sets of sibling and parent-child pairs (Bleun, 2003; Jones and Arden, 2003) and are not designed for datasets containing distantly related individuals. Additionally, the methods mentioned above cannot differentiate between rotated relationships, that is, between relationships with the same number of common ancestors and the same number of meioses separating the individuals under consideration (e.g. aunt-niece versus niece-aunt or first cousins versus great aunt-niece). Distinguishing between such relationships facilitates pedigree reconstruction from relatively distant relationships.

We developed CARROT (ClAssification of Relationships with ROTations), a new framework for relationship inference that leverages linkage information to differentiate between rotated relationships. As suggested by Skare et al. (2009), we grouped relationships between outbred individuals into three categories, based on whether the individuals under consideration have one or two common ancestors or whether one of them is the ancestor of the other. Building on the ideas of Stankovich et al. (2005) and Bercovici et al. (2010), we defined three sets of HMMs, one for each category of relationships. CARROT uses a novel heuristic to decide whether one of the two individuals under consideration is closer to the common ancestors than the other and benefits from haplotype phasing in order to distinguish between certain rotated relationships.

We demonstrated that CARROT achieves higher accuracy than maximum-likelihood approaches, such as RELPAIR and PREST-plus, using simulated data. We also applied CARROT on real data from Phase III of the HapMap Project (The International HapMap 3 Consortium, 2010). In addition to validating relationships reported by Pemberton et al. (2010), we identified previously unreported third- and fourth-degree relationships in this dataset.

1 INTRODUCTION

Algorithms for relationship inference can greatly benefit association and linkage studies by detecting undeclared or misspecified relatives, and have applications in forensics and wildlife population management. Existing methods (Epstein et al., 2000; McPeek and Sun, 2000; Sun et al., 2002) based on Hidden Markov Models (HMMs) only consider a small number of relationship types. RELPAIR (Epstein et al., 2000) examines eight types of relationships (full siblings, parent-child, avuncular, grandparent-grandchild, half siblings, first cousins, monozygotic twins, and unrelated). PREST-plus (McPeek and Sun, 2000; Sun et al., 2002) extends this set of alternative relationships to include the relationship types half-avuncular, half-first cousins and half-siblings first-cousins. Algorithms for pedigree reconstruction (Berger-Wolf et al., 2007; Koch et al., 2008; Riester et al., 2009, 2010) partition the individuals into sets of sibling and parent-child pairs (Bleun, 2003; Jones and Arden, 2003) and are not designed for datasets containing distantly related individuals. Additionally, the methods mentioned above cannot differentiate between rotated relationships, that is, between relationships with the same number of common ancestors and the same number of meioses separating the individuals under consideration (e.g. aunt-niece versus niece-aunt or first cousins versus great aunt-niece). Distinguishing between such relationships facilitates pedigree reconstruction from relatively distant relationships.

We developed CARROT (ClAssification of Relationships with ROTations), a new framework for relationship inference that leverages linkage information to differentiate between rotated relationships. As suggested by Skare et al. (2009), we grouped relationships between outbred individuals into three categories, based on whether the individuals under consideration have one or two common ancestors or whether one of them is the ancestor of the other. Building on the ideas of Stankovich et al. (2005) and Bercovici et al. (2010), we defined three sets of HMMs, one for each category of relationships. CARROT uses a novel heuristic to decide whether one of the two individuals under consideration is closer to the common ancestors than the other and benefits from haplotype phasing in order to distinguish between certain rotated relationships.

We demonstrated that CARROT achieves higher accuracy than maximum-likelihood approaches, such as RELPAIR and PREST-plus, using simulated data. We also applied CARROT on real data from Phase III of the HapMap Project (The International HapMap 3 Consortium, 2010). In addition to validating relationships reported by Pemberton et al. (2010), we identified previously unreported third- and fourth-degree relationships in this dataset.

2 METHODS

2.1 Overview of CARROT

CARROT is a framework for predicting the relationship type for a pair of individuals from their genotypes. Similarly to previous work (Epstein et al., 2000; McPeek and Sun, 2000; Sun et al., 2002), CARROT uses HMMs to compute the likelihood of the genotype data under a set of alternative relationships. Unlike existing methods, which define a different HMM for each relationship type, CARROT defines three HMM templates from which it generates HMMs for a broad range of relationships.

CARROT uses the likelihoods computed by all alternative models as features of a classifier together with additional features that quantify the overall genetic sharing between the two individuals. In this work, we use the term Identity By Descent or Identical By Descent (IBD) to refer to genomic regions inherited from the Most Recent Common Ancestors (MRCAs) of the two individuals. The additional features we consider are the percentage of IBD, the number of transitions between IBD and non-IBD regions and the haplo-frequencies of the two individuals in these regions. Roughly speaking, the haplo-frequency of an individual A in a given genomic region is the
fraction of the reference population that has the same haplotype as A in that region.

The use of phased data enables CARROT to assign different likelihoods to relationships that are indistinguishable by previous methods: in the case of an aunt-niece pair, for instance, segments inherited from the common ancestors can lie on either haplotype of the aunt, but must lie on the same haplotype of the niece. This haplotype model, combined with linkage information captured in the haplo-frequencies, gives CARROT a significant advantage in differentiating between rotated relationships compared with the existing methods.

2.2 HMMs and factorial HMMs

A HMM is a probabilistic model for capturing the dependencies of a sequence of observations $G_1, G_2, \ldots, G_n$ on a chain of unknown (or hidden) variables $S_1, S_2, \ldots, S_N$ taken from a set $S$. An HMM makes the following conditional independence assumptions: first, given $S$, $G_i$ is conditionally independent of all observations and hidden states, that is $P(G_i|S_1, \ldots, S_n, G_1, G_{n-1}) = P(G_i|S)$. Second, given $S$, $S_i$ is conditionally independent of all previous hidden states, that is, $P(S_i|S_1, \ldots, S_{i-1}) = P(S_i|S)$. An HMM is, therefore, defined by a set of transition probabilities $P(S_i|S_{i-1})$, a set of emission probabilities $P(G_i|S)$ a probability distribution over the initial states.

Often, we want to infer the value of the hidden variables from the observed data. The posterior probability $P(S|G_i)$ can be computed using the forward-backward algorithm in time $O(M(N)^2)$ (Rabiner and Huang, 1986), where $|S|$ is the number of values in $S$.

In a factorial HMM (Ghahramani and Jordan, 1997), the observation at position $i$ depends on multiple hidden variables, $S_{1i}, S_{2i}, \ldots, S_{Ni}$, which are assumed to evolve independently, that is:

$$P(S_i = s_{1i}, s_{2i}, \ldots, s_{Ni}) = \prod_{k=1}^{N} P(s_{ki} = s_{ki} | s_{-ki})$$

A factorial HMM where each hidden variable $S_i$ takes values from the set $S$ is equivalent to an HMM with hidden variables taking values from the Cartesian product $S^N$. Using the latter representation, running the forward-backward algorithm on a factorial HMM requires $O(M(|S|^N))$ time. However, by taking advantage of the independence assumptions for the hidden variables, the forward-backward algorithm can be modified to run in $O(M(|S|^T + 1))$ time, which is a significant improvement when the number of hidden variables $T$ is large.

2.3 Likelihood computation assuming linkage equilibrium

We want to infer the relationship between two individuals $A$ and $B$, genotyped at a set of $M$ unlinked SNPs. Let $H_1, H_2, H_3, H_4 \in \{C, G, T\}$ be the two haplotypes of $A$ and $B$, respectively, $G_1 = (H_1, H_2), G_2 = (H_3, H_4)$ be their ordered, or phased, genotypes, and $\theta_k$ be the probability of recombination between SNPs $k = i + 1$. Throughout this work, we assume that $\theta_k$ is the same for both sexes.

Let $R$ be a set of putative relationships for individuals $A$ and $B$. For any relationship $R \in R$, we want to compute the likelihood of $R$, or the probability of the observed genotypes under the assumption that the true relationship between $A$ and $B$ is $R_k = \text{PhG}_A(G_R)$. As noted in Skare et al. (2009), assuming that $A$ and $B$ are not inbred, their relationship must fall into exactly one of the following categories:

1. $A$ and $B$ share exactly 2 MRCAs (e.g. full siblings, first cousins);
2. $A$ and $B$ share exactly 1 MRCAs (e.g. half siblings, half cousins);
3. $A$ is the ancestor of $B$ or vice versa.

We will consider relationships $R_1$ and $R_2$, rotated, if $R_1$ and $R_2$ are in the same relationship category and the total number of meioses between the two individuals is the same in $R_1$ and $R_2$. Alternatively, we say that $R_1$ is a rotation of $R_2$.

We defined a set of HMMs for each of these three relationship categories similarly to Stankovich et al. (2005) and Bercovici et al. (2010), who defined HMMs for cousins parameterized by the number of generations between them. Unlike these methods, the state space of our models does not increase with the number of generations of the pedigree. Below, we describe our HMMs for the first type of relationships. The models for the other two categories are derived along similar lines. Given that $A$ and $B$ have two MRCAs, $C$ and $D$ (Fig. 1), the hidden state at SNP $k$ depends on the following binary variables:

1. $m_1(k)$ and $m_2(k)$ indicate whether $C$ and $D$, respectively, passed the same allele to their immediate descendants $E_1$ and $F_1$. For example, if both $E_1$ and $F_1$ inherited the maternal allele of $C$ at position $k$, then $m_1(k) = 1$. If $E_1$ received the maternal allele of $C$, and $F_1$ received the paternal allele of $C$, then $m_2(k) = 0$.

2. $m_r(k)$ and $m_r(k)$ indicate whether $E_1$ and $F_1$ passed to $E_2$ and $F_2$, respectively, the allele of $C$ and not the allele of $D$.

3. $d_k(k)$ takes the value 0 if $A$ inherited the allele that $E_2$ got from $E_1$ (which came from either $C$ or $D$) and the value 1 otherwise. That is, $d_k(k) = 0$, if for all $i > 2$, $E_i$ got from $E_{i-1}$ the allele of $E_{i-2}$ and not the allele of $G_{i-2}$. If $d_k(k) = 1$, we will say that there were off-chains donations in the lineage of $A$ at position $k$. $d_k(k)$ is defined in an analogous way for the lineage of $B$.

4. $p_k(k)$ indicates which of the alleles of $A$, $H_1(k)$ or $H_2(k)$, comes from $E_{k-1}$ and is used to capture phasing errors. $p_k(k)$ is defined in an analogous way.

Each of these variables refers to a different set of meioses in the pedigree, therefore they all evolve independently from each other.
Any recombination between loci

To account for genotyping errors, let

We refer to relationship types using the notation (mrcas

and allele frequencies to compute the emission probabilities (Epstein 

and

Now derive the transition probabilities for

Table 1. Transition probabilities for the HMMs with two MRCAs.

| Variable | \( Pr(0 \rightarrow 0) \) | \( Pr(1 \rightarrow 0) \) |
|----------|----------------|----------------|
| \( m_e \) | \( \frac{\theta_e}{2} \left( 1 - \theta_e \right)^2 \) | \( \frac{2\theta_e(1 - \theta_e)}{n} \) |
| \( m_c \) | \( 1 - \theta_c \) | \( \theta_c \) |
| \( d_A \) | \( (1 - \theta_d)^{p_{mEC}} \) | \( \left( \sum_{m=0}^{p_{mEC}} \left( \frac{\theta_d(1 - \theta_d)}{n} \right)^m \right) \left( \frac{(1 - \theta_d)^{p_{mEC} - 1}}{n} \right) \) |
| \( p_A \) | \( 1 - \omega \) | \( \omega \) |

\( Pr(i \rightarrow j) \) is the probability that a variable transitions from state \( i \) at SNP \( k \) to state \( j \) at SNP \( k+1 \). \( gen_A \) and \( gen_B \) are the generations between the MRCAs and each of \( A \) and \( B \) (Fig. 1). \( \theta \) is the recombination probability between SNPs \( k \) and \( k+1 \), and \( \omega \) is the probability of a phasing error. The transition probabilities for the variables \( m_e, m_c, p_A \) and \( d_A \) are derived similarly.

The transition probabilities for all the variables are shown in Table 1. We now derive the transition probabilities for \( d_A \). Let \( gen_A \) be the number of generations between the MRCAs and \( A \) (Fig. 1). The number of meioses between \( E \) and \( A \) is \( \theta_e - 1 \). Assume that \( d_A(k) = 0 \), that is, the allele at \( E \) inherited from the MRCAs at locus \( k \) was passed down to \( A \). Any recombination between loci \( k \) and \( k+1 \) would result in \( d_A(k+1) = 1 \), therefore \( Pr(d_A(k+1) = 0 | d_A(k) = 0) = 1 - (1 - \theta_d)^{p_{mEC}} \). If \( d_A(k) = 1 \), then there exists at least one off-chain donation in the \( gen_A - 1 \) meioses between \( E \) and \( A \). The probability that there are exactly \( n \) off-chain donations between \( E \) and \( A \) is \( \left( \frac{\theta_d}{n} \right) \left( 1 - \theta_d \right)^{p_{mEC} - n} \). Given that there are exactly \( n \) off-chain donations at SNP \( k \), the probability that there are no off-chain donations at SNP \( k+1 \) is \( (1 - \theta_d)^{p_{mEC} - 1} \). Therefore:

\[
\frac{Pr(d_A(k) = 0 | d_A(k+1) = 1)}{Pr(d_A(k) = 1)} = \left( \frac{\theta_e}{n} \right) \left( 1 - \theta_e \right)^{p_{mEC} - 1} \left( \frac{\theta_d}{n} \right) \left( 1 - \theta_d \right)^{p_{mEC} - n} \left( 1 - \theta_d \right)^{-n} = \frac{1}{\theta_d} \left( \frac{\theta_e}{n} \right) \left( 1 - \theta_e \right)^{p_{mEC} - 1} \left( 1 - \theta_d \right)^{p_{mEC} - n} \left( 1 - \theta_d \right)^{-n}
\]

Given \( k \), we can determine the IBD status at SNP \( k \) and use population allele frequencies to compute the emission probabilities (Epstein et al., 2000). To account for genotyping errors, let \( x \) be the probability of a genotyping error, and \( f(x,y) \) be the probability that allele \( x \) is genotyped as \( y \):

\[
f(x,y) = \begin{cases} 1 - x & \text{if } x = y \\ x & \text{if } x \neq y \\ \end{cases}
\]

Then:

\[
Pr(H_k|a,x,y) = \sum_{c,C} q_c f(x,c) f(y,C) \text{ and } H_{k+1} \text{ are IBD} = \sum_{c,C} q_c f(x,c) f(y,C)
\]

where \( q_c \) is the frequency of allele \( c \) in the reference population. The rest of the emission probabilities are adjusted in a similar way.

2.4 Relationship notation

We refer to relationship types using the notation (mrcas, \( gen_A \), \( gen_B \)). The variable mrcas is 2 when individuals \( A \) and \( B \) share two MRCAs and 1 otherwise. Unless \( A \) is the ancestor of \( B \) or vice versa, \( gen_A \) and \( gen_B \) are the number of generations between the MRCAs(A) and \( A \) and \( B \), respectively. If \( A \) is the ancestor of \( B \), then \( gen_B \) is set to \(-1 \), and \( gen_A \) is the number of generations between \( A \) and \( B \). For close relationships, we prefer to use the usual verbal description, unless space is limited. Table 2 shows the numerical notation for some common relationships.

2.5 Incorporating linkage information

HMMs that use unlinked markers have limited power to distinguish between relationships of the same degree (Sun et al., 2002). Linkage information can help disambiguate such relationships. Assume, for instance, that we want to determine whether the relationship between individuals \( A \) and \( B \) is first cousins or great aunt-niece. An IBD block between \( A \) and \( B \) implies that they inherited overlapping genomic segments from their MRCAs (Fig. 2). If \( A \) and \( B \) are first cousins, the two scenarios of Figure 2 are equally likely. However, if \( A \) is closer to the MRCAs than \( B \), then it is more likely that \( A \) inherited a larger segment from the common ancestor than \( B \) (scenario (a)), because we expect fewer recombination between the MRCAs and \( A \) than between the MRCAs and \( B \). Therefore, if we compare the haplotypes of \( A \) and \( B \) in a small window around the IBD transitions to the haplotypes of a reference population, we are more likely to find a match for the haplotype of \( A \), than for the haplotype of \( B \).

To quantify this intuition, assume that there is a transition in IBD status between SNPs \( k \) and \( k+1 \), and let \( H_k(k+w,1,k+w) \) be the haplotype of individual \( i \) at positions \( k-w+1, k-w+2, ..., k+w \), that is in a window of size \( 2w \) around \( k \). The haplotype frequency of \( A \) is defined as:

\[
C_A = \frac{\sum_i f(H_k(k+w,1,k+w), H_k(k-w+1, k+w))}{N}
\]

where the sum is over all haplotypes in the reference population, \( N \) is the number of such haplotypes, and \( f(H_k(k+w,1,k+w), H_k(k-w+1, k+w)) \) is the probability that \( H_k(k-w+1, k+w) \) is genotyped as.
The positions of transitions in IBD status are estimated from the posterior probabilities of IBD of the HMMs using two cutoffs, \( c_{\text{northern}} \) and \( c_{\text{western}} \). Positions with posterior probability for IBD larger than \( c_{\text{northern}} \) and \( c_{\text{western}} \) are called IBD. To distinguish between relationships of the same degree, we consider \( k = w + 1, k + w \) as the transition window.

### 2.6 Classifying relationships

Existing methods for relationship inference (Epstein et al., 2000; McPeek and Sun, 2000; Sun et al., 2002) use HMMs to compute the likelihood of the observed genotypes under a set of alternative relationships and then select the relationship that maximizes that likelihood. As explained in Section 2.5, when \( A \) and \( B \) are equally distant from their common ancestors, \( r \) is expected to be 0.5. A value of \( r \) much larger than 0.5 indicates that \( C \) is more distant from the common ancestors than \( A \). Similarly, a value of \( r \) much smaller than 0.5 indicates that \( B \) is more distant from the common ancestors than \( A \). It is possible that some of the fourth-degree relatives were also excluded during this process. We note, however, that the goal of this analysis was not to exhaustively identify all the relationships in the populations studied, but rather to demonstrate the applicability of our method to a real dataset.

For the pairs that passed the above cutoff, we ran our HMMs for all relationships of degree up to 5, using previously reported unrelated individuals of the corresponding population to compute allele and haplotype frequencies. The phasing error parameter \( w \) was set to 0.1%. To infer the relationships of these pairs, we trained our classifiers on 100 pairs of transitions on a set of simulated individuals distinct from the training and testing sets.

### 2.8 Comparison with RELPAIR and PREST-plus

We compared our method to RELPAIR, version 2.0.1 (Epstein et al., 2000) and PREST-plus, version 4.09 (McPeek and Sun, 2000; Sun et al., 2002), on simulated individuals created as described in Section 2.7. PREST-plus was run on the same 79,681 unlinked SNPs that was used to run CARROT’s HMMs. RELPAIR cannot handle more than 9999 markers, so, similarly to Pemberton et al. (2010), we ran it on 5 random subsets of the unlinked SNPs of size 9961 SNPs each. Each subset was created by dividing the 79,681 SNPs into non-overlapping windows of size 8, and then randomly selecting one SNP from each window. The prediction of RELPAIR for a given pair of individuals was set to the relationship predicted in the majority of the five runs.
of individuals for each relationship of degree up to 5 and 100 unrelated individuals, simulated as described in Section 2.7.

We verified our predictions for the first- and second-degree relationships by comparing them with the relationships reported in HapMap and the predictions of Pemberton et al. (2010). To verify our predictions for relationships of higher degree, we detected sets of three or more individuals that were all predicted to be related to each other and examined whether our predictions for these individuals were mutually consistent. We were very conservative in our verification process, reporting newly discovered third- and fourth-degree relationships as verified only if they had strong support from relationships of smaller degrees.

Pedigrees were drawn using HaploPainter, version 1.043 (Thiele and Nürnberg, 2005).

3 RESULTS

3.1 Results on simulated data

Comparison with maximum-likelihood methods: We compared CARROT with two existing methods for relationship inference, RELPAIR (Epstein et al., 2000) and PREST-plus (McPeek and Sun, 2000; Sun et al., 2002). Both these methods use HMMs similar to those defined in Section 2.3 to compute the likelihoods of a set of alternative relationships and select the relationship within the set with the maximum likelihood. RELPAIR only considers the relationships such as full siblings, parent–child, avuncular, grandparent–grandchild, half siblings, first cousins, monoyzocytic twins and unrelated. PREST-plus considers the relationships examined by RELPAIR as well as the relationships half avuncular, half first cousins and half-siblings-first-cousins [see Sun et al. (2002) for the definition of that relationship]. Neither RELPAIR nor PREST-plus differentiate between rotated relationships, so for example they do not distinguish between a grandparent–grandchild pair and a grandchild–grandparent pair.

We first compared CARROT, RELPAIR and PREST-plus on the set of relationships that can be handled by RELPAIR, except twins, which are not considered by CARROT. We simulated 100 pairs of phased individuals for each of the relationships full siblings, parent–child, aunt/uncle–niece/nephew, grandparent–grandchild, half siblings, first cousins and unrelated. We ran RELPAIR and PREST-plus on all pairs using the seven aforementioned alternative relationships. The performance of CARROT was assessed using 10-fold cross-validation: The simulated pairs were divided into 10 subsets each containing 10 pairs from each relationship. CARROT was trained on 9 of the subsets, that is, on 90 pairs from each relationship, and tested on the remaining subset. This process was repeated 10 times and the accuracy was averaged over the 10 runs.

Figure 3 shows the number of pairs that were predicted correctly by each method. Although all three methods achieved excellent accuracy for the first-degree relationships, CARROT clearly outperformed both RELPAIR and PREST-plus on second degree pairs, particularly on half siblings. RELPAIR and PREST-plus classified more than half (56 and 68%, respectively) of the half sibling pairs as avuncular. We note that the difference in performance between RELPAIR and PREST-plus can probably be attributed to the fact that RELPAIR was run on smaller sets of SNPs (see Section 2.8).

We also compared CARROT with PREST-plus on the set of nine relationships which includes the seven relationships above as well as half avuncular and half first cousins. As shown in Figure 4, CARROT outperformed PREST-plus for most relationships as well as on average. The only relationship for which CARROT performed worse than PREST-plus was first cousins: 13% of the first cousins pairs were classified as half avuncular by CARROT, probably because the haplo-frequencies of these two relationships are similar.

As mentioned above, RELPAIR and PREST-plus use HMMs to compute the likelihoods of a set of alternative relationships and select the relationship with the maximum likelihood. However,
Comparison between CARROT and two other approaches for relationship inference

Table 3.

| Method/Degree | 1  | 2  | 3  | 4  | 5  |
|---------------|----|----|----|----|----|
| Max likelihood| 100| 98.6| 62.29| 39.33| 26.73|
| CARROT likelihoods| 100| 100| 74| 43.78| 29|
| CARROT likelihoods| | 100| 84| 57.56| 34.45|

Differentiating between rotated relationships: to evaluate the accuracy of CARROT in distinguishing between rotations of relationships, we simulated 100 pairs of individuals for each of the possible relationships of degree up to five, including all rotated relationships. We first assumed that the degree of each relationship was known, and ran CARROT separately for each degree. We started by examining the ideal case of perfect phasing, so we set the probability of phasing errors, $\omega$, to zero.

We assessed CARROT’s accuracy using 10-fold cross-validation, as described in the previous section: the simulated pairs were divided into 10 subsets each containing 10 pairs from each relationship. CARROT was trained on nine of the subsets and tested on the remaining subset. This process was repeated 10 times and the accuracy was averaged over all 10 runs. We defined the prediction accuracy as the number of pairs that were classified in the correct relationship.

### Table 4. Classification accuracy of CARROT on third-degree relatives

| Rel. | 2,0,2 | 2,1,1 | 2,2,0 | 1,1,2 | 1,0,1 | 1,1,0 | 1,2,−1 |
|------|-------|-------|-------|-------|-------|-------|-------|
|      | 2,0,2 | 2,1,1 | 2,2,0 | 1,1,2 | 1,0,1 | 1,1,0 | 1,2,−1 |
| Max likelihood | 88 | 84 | 1 | 96 | 11 | 11 | 1 |
| CARROT likelihoods | 80 | 88 | 24 | 90 | 24 | 24 | 1 |
| CARROT | 80 | 68 | 80 | 80 | 80 | 80 | 80 |

The effect of phasing errors: the phasing error rate is defined as the proportion of successive pairs of heterozygote SNPs that are phased incorrectly with respect to each other. To examine the effect of phasing errors on the classification accuracy of CARROT, we simulated 100 pairs of individuals for each of the third-degree relationships, as shown. As expected, the accuracy of our classifiers drops as the degree of the relationship increases. However, even within the same degree, some relationships are much harder to predict correctly than others. For example, the two half-avuncular relationships, (1, 0, 1) and (1, 1, 0), are hard to differentiate from each other, since in practice the degree of the individuals from their MRCA is not enough for the haplotype frequencies to distinguish them from a balanced relationship where both individuals are equally distant from the MRCA. Similarly, although the average accuracy for the fifth-degree relationships was 34.45%, the (2, 4, 0) and (2, 3, 4) pairs were predicted correctly in 84% of the cases.

When run on first- and second-degree relatives, CARROT achieved perfect performance. The results for the third- and fourth-degree relationships are summarized in Tables 4 and 5, respectively.

The average accuracy over all the pairs of the corresponding degree was 83.86 and 56.89%, respectively. For the fifth-degree relationships, the average accuracy was 34.45% (full results not shown). As expected, the accuracy of our classifiers drops as the degree of the relationship increases. However, even within the same degree, some relationships are much harder to predict correctly than others. For example, the two half-avuncular relationships, (1, 0, 1) and (1, 1, 0), are hard to differentiate from each other and from first cousins, since the difference in the distance of each of the individuals from their MRCA is not enough for the haplotype frequencies to distinguish them from a balanced relationship where both individuals are equally distant from the MRCA. Similarly, although the average accuracy for the fifth-degree relationships was 34.45%, (the (2, 4, 0) and (2, 3, 4) pairs were predicted correctly in 84% of the cases.)

### Predictions across degrees:

Since in practice the degree of the relationship is not necessarily known, we also performed cross validation on a set of 200 pairs of individuals from each of the relationships of degree up to 5, including rotated relationships, as well as 200 pairs of unrelated individuals. The average classification accuracy was 57.5%, varying widely for different degrees: all the first-degree pairs were classified correctly; the average accuracy for the second-degree pairs was 99.5%, for the third-degree pairs 76.57%, for the fourth-degree pairs 46% and for the fifth-degree pairs 23.36%. Finally, 90% of the unrelated individuals were classified correctly. We note that there was a small decrease in accuracy compared with the results of the previous section, because some of the pairs were classified in relationships of the incorrect degree, while this was never the case when only within-degree predictions were made. Table 6 shows the percentage of pairs that were classified in a relationship of the correct degree for each of the degrees examined. On average, the correct degree was predicted for 89.83% of the pairs.

### The effect of phasing errors:

The phasing error rate is defined as the proportion of successive pairs of heterozygote SNPs that are phased incorrectly with respect to each other. To examine the effect of phasing errors on the classification accuracy of CARROT, we simulated 100 pairs of individuals for each of the third-degree relationships.
Yoruba individuals from Ibadan, Nigeria (YRI). These populations were selected because they contained both previously reported and column $j$ is the percentage of pairs of relationship $i$ that were predicted to be of relationship $j$.

The value at row $i$ and column $j$ is the percentage of pairs of relationship $i$ that were classified in a relationship of degree $j$.

| Relationship reconstruction |

Table 5. Classification accuracy of CARROT on fourth-degree relatives

| Rel | 2,0,3 | 2,1,2 | 2,2,1 | 2,3,0 | 1,–1,3 | 1,0,2 | 1,1,1 | 1,2,0 | 1,3,–1 |
|-----|-------|-------|-------|-------|--------|-------|-------|-------|--------|
| 2,0,3 | 76 | 4 | 1 | – | 8 | 8 | 3 | – | – |
| 2,1,2 | 1 | 42 | 24 | – | – | 15 | 11 | 7 | – |
| 2,2,1 | – | 24 | 43 | – | – | 5 | 13 | 15 | – |
| 2,3,0 | – | – | 4 | 73 | – | 1 | 3 | 10 | 9 |
| 1,–1,3 | 4 | 1 | – | – | 80 | 13 | – | 2 | – |
| 1,0,2 | – | 13 | 9 | – | – | 52 | 14 | 12 | – |
| 1,1,1 | – | 8 | 19 | – | – | 28 | 14 | 31 | – |
| 1,2,0 | – | 5 | 13 | – | – | 13 | 17 | 52 | – |
| 1,3,–1 | – | 14 | 1 | 4 | – | 2 | 1 | 12 | 80 |

The value at row $i$ and column $j$ is the percentage of pairs of relationship $i$ that were predicted to be of relationship $j$.

Table 6. Ability of CARROT to predict the degree of a relationship

| Degree | 1 | 2 | 3 | 4 | 5 | Unrel |
|--------|---|---|---|---|---|-------|
| 1      | 100 | – | – | – | – | – |
| 2      | – | 99.5 | 0.5 | – | – | – |
| 3      | – | 0.57 | 93.57 | 5.71 | 0.14 | – |
| 4      | – | – | 3.94 | 82.06 | 14 | – |
| 5      | – | – | – | 12.41 | 86.64 | 0.95 |
| Unrel  | – | – | – | – | 10 | 90 |

The value in row $i$ and column $j$ is the percentage of pairs of degree $i$ that were classified in a relationship of degree $j$.

relationships, introduced phasing errors at various rates in the range 0–1% and performed 10-fold cross-validation, as described above. We observed that, as the phasing error increased from 0% to 1%, there was an almost linear decrease in classification accuracy from 84% to 67.57%, although for phasing errors in the range 0.0–0.1%, the accuracy was almost unaffected. Current algorithms for statistical phasing of genotype data, such as BEAGLE (Browning and Browning, 2007), have phasing error rates ranging between 0.05% and 6%, depending on the number of genotyped individuals and markers. Recently, however, Fan et al. (2011) and Yang et al. (2011) proposed new experimental techniques for whole-chromosome haplotyping, which promise to achieve much smaller error rates than current methods. Given these recent developments in phasing techniques, we believe that the phasing error requirements of our algorithm are realistic.

3.2 Inference of relationships in Phase III of HapMap

Phase III of the HapMap dataset contains genotypes of 1184 individuals from 11 populations in more than a million SNPs. To facilitate phasing, many of these individuals are connected in trios (two parents and an offspring) or duos (a parent and an offspring). Recently, however, Pemberton et al. (2010) discovered the first cousin pairs NA19664–NA19685 and NA19664–NA19662, therefore CARROT reconstructs this pedigree even in the absence of genotype data for the mother of NA19685 and NA19662. A list of all the previously unreported relative pairs detected by CARROT is given in the supplement.
Table 7. Predictions of CARROT on four HapMap populations

| Pop | Degree | Reported relatives | New relatives | Total |
|-----|--------|--------------------|---------------|-------|
|     | Correct | Degree only | Incorrect | Correct | Unverified | Degree only | Incorrect |
| CEU | 1       | 97               | –            | –       | –         | –           | –         |
|     | 2       | 2                | –            | –       | –         | –           | –         |
|     | 3       | –                | –            | –       | –         | –           | –         |
|     | 4       | –                | –            | –       | 13        | 1           | –         |
| Total|         | 99               | –            | –       | 13        | 1           | –         |
| MXL | 1       | 56               | –            | –       | –         | –           | –         |
|     | 2       | 7                | –            | –       | –         | –           | –         |
|     | 3       | –                | –            | –       | 2         | 1           | –         |
|     | 4       | –                | –            | –       | 2         | –           | 1         |
| Total|         | 63               | –            | –       | 3         | 1           | 1         |
| YRI | 1       | 105              | –            | –       | –         | –           | –         |
|     | 2       | 2                | 1            | –       | –         | –           | –         |
|     | 3       | –                | –            | –       | 1         | 3           | 1         |
|     | 4       | –                | –            | –       | 1         | 3           | –         |
| Total|         | 107              | 1            | –       | 2         | 6           | 1         |
| ASW | 1       | 46               | –            | –       | –         | –           | –         |
|     | 2       | 7                | 3            | –       | –         | 1           | –         |
|     | 3       | –                | –            | –       | 7         | 4           | 1         |
|     | 4       | –                | –            | –       | 1         | 21          | –         |
| Total|         | 53               | 3            | 3       | 8         | 25          | 2         |

The results are grouped per population and relationship degree. New Relatives are relative pairs identified by CARROT that have not been previously reported. Correct predictions are predictions that agree with previously reported relationships. The column ‘Degree Only’ refers to cases where CARROT predicted correctly only the degree of the relationship. ‘Unverified’ relationships are those for which we could not determine whether CARROT’s prediction was correct or not.

Fig. 5. Example of a pedigree identified by CARROT: the pedigree involves three families from the MXL population, M008, M010 and M012.

We note that, although for the CEU and MXL populations, the accuracy of our algorithm is consistent with the simulation results of Section 2.7, in the other two populations there was a drop in accuracy. This difference in performance might be a result of an increased phasing error in the YRI and ASW populations. Additionally, in the ASW population, the degree of relatedness is unexpectedly high, with 94 identified relationships in a sample of 83 individuals. We observed, for example, that 14 individuals from eight HapMap families (2483, 2487, 2488, 2489, 2490, 2492, 2495, and 2496) are connected to each other. With less than 50 presumably unrelated individuals ([there are 53 individuals reported as unrelated in the HapMap ASW dataset, even if we ignore the relationships reported here and in Pemberton et al. (2010)], the estimation of allele and haplotype frequencies becomes problematic, especially in an admixed population like ASW.

To help determine whether the previously unreported pairs detected by CARROT are predicted correctly, we ran KING (Manichaikul et al., 2010) on all the identified third-degree relatives. KING uses identity by state to distinguish relatives of degree up to three from unrelated individuals. Six out of the seven pairs of individuals that were predicted to be third-degree relatives by CARROT and could not be otherwise verified, were also predicted to be third-degree relatives by KING. We thus concluded that these six pairs are most likely true third-degree relatives. We notice that KING correctly predicted only 13 out of the 16 verified third-degree relatives. It is therefore likely that the last unverified third-degree prediction for which there was a disagreement between CARROT and KING is also a true third-degree pair.

4 DISCUSSION
We presented CARROT, a novel framework for relationship inference that uses linkage information to infer more distant relationships than existing methods and to distinguish between rotated relationships, that is, relationships with the same number of common ancestors and the same number of meioses separating the individuals under consideration (e.g. aunt–niece versus niece–aunt or first cousins versus great aunt–niece). We demonstrated that CARROT achieved superior accuracy on relationships of degree up to four, clearly outperforming previous methods, such as RELPAIR.
and PREST-plus on simulated data. We also applied CARROT on data from four HapMap populations, ASW, CEU, MXL and YRI and correctly identified the vast majority of the first- and second-degree relatives recently detected by Pemberton et al. (2010). Additionally, CARROT detected 67 previously unreported third- and fourth-degree relative pairs.

A possible shortcoming of CARROT stems from its current reliance on phased data. However, state of the art statistical methods for phasing achieve phasing errors smaller than 1% (Browning and Browning, 2007; Howie et al., 2009) when large cohorts and dense SNP panels are used. Additionally, novel experimental phasing techniques have achieved haplotyping of whole chromosomes with accuracy of 99.8% (Fan et al., 2011; Yang et al., 2011).

Methods for detecting genealogical relationships and for disambiguating between rotated relationships can facilitate pedigree reconstruction from cohorts such as the HapMap and WTCCC (Wellcome Trust Case Control Consortium, 2007) datasets, where most individuals are not expected to be closely related. Existing algorithms for pedigree reconstruction that are based on the detection of parent-offspring and sibling pairs cannot be applied in such datasets. In studies such as WTCCC, that are based on the premise that the individuals in the dataset are unrelated, putative relatives are usually removed or reweighted. Knowledge of the exact pedigrees, however, allows a more informed selection of the individuals to be removed. More importantly, instead of discarding the information captured in related individuals, one can leverage the reconstructed relationships in a meta-analysis step. Combining the linkage analysis from multiple reconstructed partial pedigrees with the association analysis over the remaining unrelated individuals can potentially increase the power of such studies. CARROT was designed with such applications in mind and aims at bridging the gap between algorithms for pedigree reconstruction from close relatives and traditional relationship inference methods.

ACKNOWLEDGEMENTS

We would like to thank M. Schaub for helpful discussions, as well as the two anonymous reviewers for their comments.

Funding: National Institutes of Health (HG005570-01 to S.K.-P., HG005596-01 to D.K.H., HG005570-01 to S.B.); the National Science Foundation (DBI-0640211-002 to S.B.); a William H. Hewlett Stanford Graduate Fellowship (to S.J.A.); National Science Foundation Fellowship (to S.J.A.).

Conflict of Interest: none declared.

REFERENCES

Bucuicic, S. et al. (2010) Estimating genome-wide IBD sharing from SNP data via an efficient hidden Markov model of LD with application to gene mapping. Bioinformatics 26, 1175–1182.

Bergs-Wolf, T. et al. (2007) Reconstructing sibling relationships in wild populations. Bioinformatics 23, 49–56.

Bloom, M.S. (2005) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends Ecol. Evol. 20, 503–511.

Epstein, M.P. et al. (2009) Improved inference of relationship for pairs of individuals. Am. J. Hum. Genet. 87, 1210–1231.

Fan, H.C. et al. (2011) Whole-genome molecular haplotyping of single cells. Nat. Biotechnol. 29, 51–57.

Ghahremani, E. and Jordan, M. (1997) Factorial hidden Markov models. Mach. Learn., 29, 245–273.

Hallane, J.B.S. (1919) The combination of linkage values, and the calculation of distances between the loci of linked factors. J. Genet., 26, 299–309.

Howie, B.N. et al. (2009) A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet., 5, e1000529.

The International HapMap 3 Consortium (2010) Integrating common and rare genetic variation in diverse human populations. Nature, 467, 52–58.

Jones, A.G and Anden, W.R. (2003) Methods of parentage analysis in natural populations. Mol. Ecol., 12, 2511–2523.

Koch, M. et al. (2008) Pedigree reconstruction in wild cichlid fish populations. Mol. Ecol., 17, 4500–4511.

Mancias, N. et al. (2010) Robust relationship inference in genome-wide association studies. Bioinformatics, 26, 2867–2873.

McPeek, M.S. and Sun, L. (2000) Statistical tests for detection of misspecified relationships by use of genome-screen data. Am. J. Hum. Genet., 66, 1076–1094.

Pemberton, T.J. et al. (2010) Inference of Unexpected Genetic Relatedness among Individuals in HapMap Phase III. Am. J. Hum. Genet., 87, 457–464.

Purcell, S. et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet., 81, 559–575.

Rahm, L.R. and Huang, B.R. (1986) An introduction to hidden Markov models. IEEE Acoust. Speech. Sign. Process. Mag., 3, 4–16.

Rieck, T. et al. (2009) FRANz: reconstruction of wild multi-generation pedigrees. Bioinformatics, 25, 2134–2139.

Rieck, T. et al. (2010) Reconstruction of pedigrees in clonal plant populations. Theor. Popul. Biol., 78, 109–117.

Fan, H.C. et al. (2009) Identification of distant family relationships. Bioinformatics, 25, 2376–2382.

Stankevicius, T. et al. (2005) Identifying nineteenth century genealogical links from genotypes. Hum. Genet., 117, 188–199.

Sun, L. et al. (2002) Enhanced pedigree error detection. Hum. Hered., 54, 99–110.

Theile, H. and Nürnberg, P. (2005) HaploPainter: a tool for drawing pedigrees with complex haplotypes. Bioinformatics, 21, 1730–1732.

Wellcome Trust Case Control Consortium (2007) Genome-wide association study of 144000 cases of seven common diseases and 3000 shared controls. Nature, 447, 661–678.

Yang, R. et al. (2011) Completely phased genome sequencing through chromosome sorting. Proc. Natl Acad. Sci. USA, 108, 12–17.