Haplotype Inference on Pedigrees with Recombinations, Errors, and Missing Genotypes via SAT solvers

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Abstract. The Minimum-Recombinant Haplotype Configuration problem (MRHC) has been highly successful in providing a sound combinatorial formulation for the important problem of genotype phasing on pedigrees. Despite several algorithmic advances and refinements that led to some efficient algorithms, its applicability to real datasets has been limited by the absence of some important characteristics of these data in its formulation, such as mutations, genotyping errors, and missing data. In this work, we propose the Haplotype Configuration with Recombinations and Errors problem (HCRE), which generalizes the original MRHC formulation by incorporating the two most common characteristics of real data: errors and missing genotypes (including untyped individuals). Although HCRE is computationally hard, we propose an exact algorithm for the problem based on a reduction to the well-known Satisfiability problem. Our reduction exploits recent progresses in the constraint programming literature and, combined with the use of state-of-the-art SAT solvers, provides a practical solution for the HCRE problem. Biological soundness of the phasing model and effectiveness (on both accuracy and performance) of the algorithm are experimentally demonstrated under several simulated scenarios and on a real dairy cattle population.

1 Introduction

After the first draft of the human genome was published in 2000, a huge research effort has been devoted to the discovery of genetic differences among same-species individuals and to the characterization of their impact to the expression of different phenotypic traits such as disease susceptibility or drug resistance. Moreover, the completion of several livestock genomes and the recent introduction of genomic data into breeding programs widened the interest of
these studies to other species. Most of these efforts are driven by the *International HapMap Project* [11], which discovered, investigated and characterized millions of genomic positions (called *loci* or *sites*) where different individuals carry different genetic subsequences (called *alleles*). In practice, unordered pairs of alleles coming from both parents of each individual studied are routinely collected, since determining the parental source of each allele is too time-consuming and expensive to be performed on large studies [3]. The pairs of alleles located at a given set of loci of an individual are called the (multi-locus) *genotype* of the individual, while the sequence of alleles that were inherited from a single parent is called a *haplotype*. The advance of high-throughput and high-density genotyping technologies, combined with a consistent reduction of genotyping costs, has led to a great abundance of genotypic data. Such genotypes (also called SNP genotypes) are generally biallelic (i.e., at each locus only two distinct alleles are observed in the population) and they will be the focus of this work. A number of association studies based on SNP genotypes have been carried out but, since haplotypes substantially increase the power of genetic variation studies [13], accurate and efficient computational prediction of haplotypes from genotypes is highly desirable. Mendelian inheritance laws, which govern the transmission of genetic material from parents to children, have been effectively used to improve the accuracy of haplotyping methods. The general problem that we have just described is called in the literature Haplotype Inference (HI) on pedigrees, and it asks for a haplotype configuration consistent with a given genotyped pedigree. While the problem has been successfully tackled by classic statistical methods (such as Lander-Green [5] and Elson-Stewart [4]), the increasing density and length of SNP genotypes pose new hurdles. In fact those methods are not designed to scale well on large datasets and they do not take directly into account the presence of Linkage Disequilibrium among loci in the founder population.

Combinatorial formulations have been proposed to overcome such limitations. Since there can exist an exponential number of consistent haplotype configurations, we need an additional criterion for choosing the “right” haplotype configuration among those compatible with the input data. The genetic linkage between neighbouring loci has inspired a parsimonious formulation of the HI problem that looks for a configuration minimizing the number of recombination events in the resulting haplotyped pedigree. This formulation, called **Minimum-Recombinant Haplotype Configuration (MRHC)** [6, 9], has been shown to be a successful approach. The aim of this formulation is the computation of a haplotype configuration which is consistent with an input genotyped pedigree and induces the minimum number of recombinations. The formulation naturally arises since recombinations are the most common source of genetic variation. However, the HI problem where mutations were allowed instead of recombinations has been solved by an ILP-based algorithm [14]. An efficient heuristic when both recombinations and mutations are allowed has been presented in [8].

Despite several remarkable advances in genotyping technologies, genotypes are usually affected by a small percentage of errors and missing data which, even at very small rates (< 0.5%), could heavily affect the outcome of subsequent anal-
yses. Moreover, missing genotypes could represent a significant portion (5% or more) of the dataset due to uncertainty in genotype call procedures or unavailability of the DNA sample of some individuals of the pedigree. These aspects shared by almost all actual datasets were not considered in the original MRHC formulation, limiting its applicability and practical relevance.

In this paper, we propose the Haplotype Configuration with Recombinations and Errors (HCRE) problem, which generalizes the MRHC formulation by allowing genotyping errors and missing data in addition to recombination events. Polynomial-time exact algorithms for HCRE are unlikely to exist since the problem is APX-hard even on simple instances. The main contribution of this paper is a practical exact algorithm for HCRE, based on a reduction from HCRE to the well-known Satisfiability problem (SAT), for which extremely efficient solvers are known. Our reduction exploits some characteristics of one of the best performing SAT solvers, CryptoMiniSat [10], in order to compute a solution for most of the practical instances with modest computing resources. An extensive experimental evaluation of our algorithm under several simulated scenarios and on a real dairy cattle population demonstrates its accuracy and performance.

2 The Computational Problem

In this section we define the basic notions that will be studied in the rest of the work. A pedigree graph is an oriented acyclic graph \( P = (V, E) \) such that (i) vertices correspond to individuals and are partitioned into male and female vertices (i.e., \( V = M \cup F, M \cap F = \emptyset \)), (ii) each vertex has indegree 0 or 2, and (iii) if a vertex has indegree 2, then one edge must come from a male node and the other from a female node. If a vertex \( v \) has indegree 0, then \( v \) is called founder. For each edge \((p, c) \in E\), we say that \( p \) is a parent of \( c \) and \( c \) is a child of \( p \). More precisely, \( p \) is the father (mother, resp.) of \( c \) if \( p \) is male (female, resp.).

The (possibly incomplete) genotype of an individual \( i \) is a \( n \)-long vector \( g_i \) over the set \( \{0, 1, 2, *\} \) where we follow the convention of encoding the unordered pair of alleles \( \{0, 0\} \) as 0, \( \{1, 1\} \) as 1, and \( \{0, 1\} \) as 2, while * represents a missing (or “not called”) genotype. A genotype is complete if it does not contain the * element, otherwise is incomplete. An individual \( c \) is said to be heterozygous in a given locus \( l \) if \( g_{c}[l] = 2 \), and homozygous if \( g_{c}[l] \in \{0, 1\} \). A haplotype configuration \( H \) is an assignment of a pair of haplotypes \( (h_0^l, h_1^l) \) to each individual \( i \) and a pair of source vectors \( (s_{f,i}, s_{m,i}) \) to each non-founder individual \( i \) of the pedigree (where \( f \) and \( m \) are the parents of \( i \)). Both a haplotype and an source vector are binary \( n \)-long vectors. In a haplotype 0 and 1 are the two (major and minor) alleles. Informally, the source vector \( s_{p,i} \) associated with a haplotype \( h^l_i \), where \( p \) is a parent of \( i \), indicates if the allele \( h^l_i[l] \) has been inherited from the paternal \( (s_{p,i}[l] = 0) \) or maternal \( (s_{p,i}[l] = 1) \) haplotype of \( p \). More formally, let \( h_i = (h_0^l, h_1^l) \) be the haplotypes of a non-founder individual \( i \). Then \( h_i \) is consistent with the Mendelian laws of inheritance for a locus \( l \) if (a) \( h_0^l[l] = h_{f[l]}^{s_{f,i}[l]} \) and (b) \( h_1^l[l] = h_{m[l]}^{s_{m,i}[l]} \) where \( f \) is the father of \( i \), and \( m \) is the mother of \( i \).
Let \( h_i = (h_0^i, h_1^i) \) be the haplotypes of an individual \( i \) with genotype \( g_i \). Then \( h_i \) is consistent with \( g_i \) in a locus \( l \) if \( h_0^i[l] = h_1^i[l] = g_i[l] \) if \( l \) is homozygous, and \( \{h_0^i[l], h_1^i[l]\} = \{0, 1\} \) if \( l \) is heterozygous. A haplotype \( h_p^i \) of individual \( i \) inherited from its parent \( p \) contains a recombination at locus \( l > 0 \) if \( s_{p,i}[l-1] \neq s_{p,i}[l] \).

In this work, we study the \((r, e)\)-HC problem, which formalizes the HI problem on pedigrees allowing the presence of recombinations and genotype inconsistencies. If \( r \) or \( e \) are *, then the corresponding values are intended as unbounded.

The \((r, e)\)-HC problem generalizes the MINIMUM-RECOMBINANT HAPLOTYPE CONFIGURATION (MRHC) problem [6], where only recombinations are allowed and all the genotypes are assumed to be complete and correctly called.

**Problem 1.** \((r, e)\)-Haplotype Configuration problem \((r, e)\)-HC.

**Input:** A genotyped pedigree \( P \) with possibly incomplete genotypes (i.e., \( g_i[l] = * \) for some individual \( i \) and locus \( l \)).

**Output:** A haplotype configuration \( H \) for the genotyped pedigree \( P \) such that:

1. \( H \) is consistent with the Mendelian laws of inheritance for each individual \( i \) and locus \( l \);
2. \( H \) is consistent with the observed genotypes in all but at most \( e \) cases;
3. \( H \) contains at most \( r \) recombinations.

Notice that, while we motivated our problem with a parsimonious principle, we preferred not to formulate \((r, e)\)-HC as an optimization problem. In fact, any cost function that melds the number of recombinations and errors (i.e., by applying some weights to them) would potentially introduce some bias in the computed solution. Instead, the two parameters \( r \) and \( e \) directly map to two important characteristics of the dataset, the genetic distance among the markers and the quality of the collected genotypes, for which the researcher could provide good estimates. As an example, the recombination rate between adjacent markers in high-density panels is about \( 10^{-6} \) while the genotyping error rate (after quality check filters) is generally less than 0.5%.

### 3 Reducing \((r, e)\)-HC to SAT

A main technical device of this work consists of a reduction from \((r, e)\)-HC to SAT. In this work, an instance of SAT is a set of “extended clauses”, where each extended clause is either the disjunction or the exclusive-OR of literals (i.e., variables or their negation). The instance is satisfiable if and only if all the extended clauses are satisfiable. The main reason for choosing such a generalization is that our reduction is slightly simplified by its use since XORs of literals are additions over the \( \mathbb{Z}_2 \) field. Moreover the SAT solver that we selected, CryptoMiniSat [10], is designed to solve those instances. For simplicity, we slightly abuse the language by indicating also the exclusive-OR of literals with the term “clause”. In the following we denote with \( \oplus \) the exclusive-OR, with \( = \) the equivalence, with \( \land \) the conjunction, with \( \lor \) the disjunction, and with \( \neg \) the negation.

In order to gently guide the reader, first we present the reduction for the case of unbounded number of recombinations and errors. Then, we will deal with the general problem \((r, e)\)-HC. In our reduction we use some Boolean variables:
- \( p_i[l], m_i[l] \), alleles of the paternal and maternal haplotypes of individual \( i \) at locus \( l \) (i.e., \( h^0_i[l] \) and \( h^1_i[l] \)) where \( false \) is equal to 0 and \( true \) is equal to 1;
- \( s_{p,i}[l] \), which is \( true \) if the source vector of individual \( i \) (w.r.t. parent \( p \)) at locus \( l \) is equal to 1, and \( false \) if it is equal to 0;
- \( r_{p,i}[l] \), which is \( true \) if a recombination has occurred between individual \( i \) and its parent \( p \) at locus \( l \) (i.e., if \( s_{p,i}[l-1] \neq s_{p,i}[l] \));
- \( e_i[l] \), which is \( true \) iff the haplotype configuration is not consistent with the observed genotypes for individual \( i \) at locus \( l \).

To better describe our model and the associated SAT instance, we will put on the left the constraint we are imposing and on the right the corresponding clauses, preceded by the condition under which the constraint must hold. A \((*,*)\)-HC instance can be encoded by the logic formula consisting of the conjunction of the following clauses which encodes three kinds of constraints: constraints for Mendelian laws consistency, constraints for genotype consistency, and constraints for recombination representation. The first class of constraints ensures the consistency with the Mendelian laws of inheritance between a non-founder individual \( i \) and its parent \( p \). In other words, each allele of the haplotype of \( i \) inherited from \( p \) must be equal to one allele of one of the haplotypes of \( p \) depending on the variable \( s_{p,i} \). We abstract from the gender of \( p \) and use \( c_{p,i}[l] \) to indicate the variable \( p_i[l] \) if \( p \) is father of \( i \) and \( m_i[l] \) if \( p \) is mother of \( i \).

For each individual \( i \), each parent \( p \) of \( i \), and each locus \( l \):

\[
\begin{align*}
\left( (p_i[l] \land \neg s_{p,i}[l]) \oplus (m_i[l] \land s_{p,i}[l]) \right) & = c_{p,i}[l] \\
\text{s}_{p,i}[l] \lor p_i[l] \lor \neg c_{p,i}[l] & \\
\text{s}_{p,i}[l] \lor \neg p_i[l] \lor c_{p,i}[l] & \\
\neg s_{p,i}[l] \lor m_i[l] \lor \neg c_{p,i}[l] & \\
\neg s_{p,i}[l] \lor \neg m_i[l] \lor c_{p,i}[l] & \\
\neg p_i[l] \lor \neg m_i[l] \lor c_{p,i}[l] & \\
p_i[l] \lor m_i[l] \lor \neg c_{p,i}[l] & \\
\end{align*}
\tag{3.1}
\]

Notice that the last two clauses of \( (3.1) \) are implied by the other clauses. However, their explicit inclusion in our formulation improves the propagating behaviour and the overall efficiency of the SAT solver \[2\].

The second class of constraints ensures that the computed haplotypes are either consistent with the observed genotypes or that variable \( e_i[l] \) is \( true \). This leads to three different equations depending on the observed genotype (clearly no constraint is needed for an individual \( i \) and locus \( l \) where the genotype \( g_i[l] \) is missing).

For each individual \( i \) and locus \( l \) such that \( g_i[l] = 0 \):

\[
\begin{align*}
e_i[l] \neq (p_i[l] = m_i[l] = 0) & \\
e_i[l] \lor p_i[l] \lor m_i[l] & \\
e_i[l] \lor \neg p_i[l] & \\
e_i[l] \lor \neg m_i[l] & \\
\end{align*}
\tag{3.2}
\]
For each individual $i$ and locus $l$ such that $g_i[l] = 1$:

$$e_i[l] \neq (p_i[l] = m_i[l] = 1)$$

$$\begin{cases}
\neg e_i[l] \lor \neg p_i[l] \lor \neg m_i[l] \\
e_i[l] \lor p_i[l] \\
e_i[l] \lor m_i[l]
\end{cases}$$  

(3.3)

For each individual $i$ and locus $l$ such that $g_i[l] = 2$:

$$e_i[l] = (p_i[l] = m_i[l])$$

$$e_i[l] \oplus p_i[l] \oplus m_i[l]$$  

(3.4)

The last class of constraints ensures that $r_{p,i}[l]$ is true when there is a recombination between individual $i$ and its parent $p$ at locus $l$ (i.e., $s_{p,i}[l-1] \neq s_{p,i}[l]$).

For each individual $i$, each parent $p$ of $i$, and each locus $l > 1$:

$$r_{p,i}[l] = (s_{p,i}[l-1] \neq s_{p,i}[l])$$

$$\neg r_{p,i}[l] \oplus s_{p,i}[l-1] \oplus s_{p,i}[l]$$  

(3.5)

The conjunction of all previous clauses is an instance of $(\ast, \ast)$-HC. Notice that the total number of variables and clauses is $O(nm)$, where $n$ is the pedigree size and $m$ the length of the genotype.

To bound the total number of errors and recombination, we simply have to add two cardinality constraints:

$$\sum_{\text{individual } i \text{ locus } l} e_i[l] \leq e$$

$$\sum_{\text{individual } i \text{ parent } p \text{ of } i \text{ locus } l} r_{p,i}[l] \leq r$$  

(3.6)

Converting a cardinality constraint $\sum_{1 \leq i \leq n} v_i \leq k$ in a compact Boolean formula is not straightforward since a naïve approach would consider all the subsets of $k$ elements and, thus, would produce an exponential number of clauses, ruling out even moderate instances. This problem of devising an “efficient” encoding of a cardinality constraint into a CNF formula has been investigated in the constraint programming literature and a promising approach has been recently proposed. This technique is based on the construction of cardinality networks [1] and essentially sorts the set $\{x_1, \ldots, x_n\}$ of variables composing the constraint obtaining a permutation $(y_1, \ldots, y_n)$ such that whenever $y_i$ is false, then all variables $y_j$ for $j > i$ are also false. Consequently, bounding the number of recombinations and errors consists of forcing the variables $y_{i+1}$ or $y_{e+1}$ to be false. Two advantages of the cardinality network approach are that only $O(n \log^2 k)$ additional clauses are required and that arc-consistency under unit propagation is preserved, which improves the performances of modern SAT solvers. We refer the reader to [1] for the detailed description of the required clauses.

4 Experimental Results

An implementation of our algorithm, called reHCstar, is available at [http://www.algolab.eu/reHCstar]. We used our implementation to investigate feasibility, accuracy and performance of our approach under several scenarios. First,
we analyzed the effect of changing the main parameters (such as pedigree size, missing rate, etc.) on the accuracy and the performance of our algorithm. Second, we compared reHCstar with MePhase \[14\], which is a combinatorial approach to a slightly different formulation of the Haplotype Inference problem on pedigrees. Third, we present the evaluation of reHCstar on a complex pedigree of a real bovine population. We tested on such pedigree the soundness of the \((r, e)\)-HC formulation and the ability of reHCstar to handle these instances.

Evaluation on Random Instances. We evaluated how the main problem parameters – pedigree size \((n)\), genotype length \((m)\), recombination probability \((\theta)\), error probability \((\varepsilon)\), and missing probability \((\mu)\) – affect the accuracy and the performance of reHCstar. For each choice of the parameters, we generated 10 different random pedigree graphs. Then we generated 10 random haplotype configurations for each pedigree as follows. Two haplotypes have been randomly generated for each founder of the pedigree. Haplotypes of non-founder individuals have been uniformly sampled from those of their parents and a recombination has been applied at each locus with probability \(\theta\). Genotypes of the individuals are then computed from their haplotypes. Finally, each locus of each individual genotype has been replaced with a different pair of alleles with probability \(\varepsilon\) and “masked” with probability \(\mu\) (to simulate missing genotypes). Accuracy of the results computed by reHCstar on each instance has been evaluated with respect to the original haplotype configuration according to 

| genotype imputation error rate (the fraction of the missing genotypes that have been incorrectly imputed) and haplotyping error rate (the fraction of alleles that have been incorrectly predicted). The latter ratio has been computed for the set of all loci and for the set of non-missing genotypes. Performance has been evaluated considering the average and the maximum running time of reHCstar on a standard workstation with a 2.8GHz CPU. We have chosen a base set of reasonable values for the parameters: \(n = 50, m = 50, \theta = 0.005, \varepsilon = 0.005, \mu = 0.05\). We performed five series of tests; in each of these series only one parameter is allowed to assume different values, while the other four parameters are fixed to the base value. Moreover we fixed the values of \(r\) and \(e\) on each instance as constant proportions \(r_r\) and \(e_r\) of the total number of recombination and error variables, respectively. The base values of the maximum recombination rate \(r_r\) and the maximum error rate \(e_r\) is 0.012. Since a random generator can produce instances where the actual error or recombination rate is larger than \(\theta\) and \(\varepsilon\), the actual values of \(r\) and \(e\) given to reHCstar must be chosen appropriately.

Table 1 summarizes the accuracy and the performance of reHCstar on two series that are representative of this experimental part. Due to space constraints, we presented the complete table in the supplementary material (Tab. A.1) and we only sketch the main conclusions. We notice that the average genotype imputation error rate is always below 21\%, the average haplotyping error rate is always below 8\% and the average running time is always below 9 minutes, while the instance that took the longest time terminated in 52 minutes. The best case for each row is boldfaced in order to show the effects that parameter variations
Table 1. Summary of accuracy and performance obtained by reHCstar on randomly generated instances. Each table refers to a series of tests where only one parameter has been varied. The base values of the parameters are: \( n = 50, m = 50, \theta = 0.005, \varepsilon = 0.005, \mu = 0.05 \). The best result for each row is boldfaced.

(a) Increasing pedigree size \((n)\)

| Pedigree size \( n \) = | 50 | 100 | 200 |
|------------------------|----|-----|-----|
| No. of completed instances | 100/100 | 100/100 | 100/100 |
| Avg. genotype imputation error rate | 0.161 | 0.151 | 0.149 |
| Avg. haplotyping error rate | 0.043 | 0.039 | \textbf{0.033} |
| Avg. haplotyping error rate (wo/missing) | 0.037 | 0.034 | \textbf{0.027} |
| Avg. running time (in seconds) | 15.1 | 82.3 | 359.4 |
| Max. running time (in seconds) | \textbf{72.3} | 479.9 | 1129.3 |

(b) Increasing genotype length \((m)\)

| Genotype length \( m \) = | 50 | 100 | 200 |
|------------------------|----|-----|-----|
| No. of completed instances | 100/100 | 100/100 | 100/100 |
| Avg. genotype imputation error rate | \textbf{0.170} | 0.174 | 0.174 |
| Avg. haplotyping error rate | 0.043 | 0.049 | 0.063 |
| Avg. haplotyping error rate (wo/missing) | 0.038 | 0.045 | 0.059 |
| Avg. running time (in seconds) | \textbf{17.0} | 93.6 | 505.0 |
| Max. running time (in seconds) | 85.7 | 770.4 | 3127.5 |

has on the outcome. The main noteworthy, albeit predictable, observation is that larger pedigrees require more computational resources, but result in a more accurate prediction.

**Comparison with a State-of-the-Art Method.** In this second part, we compared accuracy and performance of reHCstar with another state-of-the-art approach for the haplotype inference problem on pedigrees: MePhase \[14\]. A second approach, PedPhase 3.0 \[7\], was initially considered. A preliminary test revealed that PedPhase terminated without giving a solution or giving an error message on a significant subset of instances and we preferred not to include it in this comparison. MePhase is a heuristic algorithm for the haplotype configuration with mutation and errors problem on tree pedigrees (i.e., pedigrees such that each pair of individuals are connected by at most one directed path) and it is based on an ILP formulation derived from a system of linear equations over \( \mathbb{Z}_2 \) \[15\]. This formulation of the HI problem asks for a haplotype configuration of a given genotyped pedigree allowing mutations, genotyping errors, and missing genotypes, while in this work we allow recombinations, genotyping errors, and missing genotypes. As a consequence, the comparison with MePhase involved randomly generated haplotype configurations on tree pedigrees (opposed to the general pedigrees used in the first part) without mutations and recombinations. To ensure a proper comparison even if our approach and MePhase were designed for slightly different problems, the experimental evaluation is similar to the one described by the authors of MePhase in \[14\]. Tree pedigrees have been generated using the random generator proposed by Thomas and Cannings \[12\]. Random
haplotype configurations have been assigned to the tree pedigrees just as in the previous experimental part. In this case, we analyzed the effects of the following problem parameters on the accuracy and performance of MePhase and reHC-star: average nuclear family size (denoted with \(f\), and represents the average number of individuals that compose a nuclear family), genotype length (\(m\)), error probability (\(\varepsilon\)), and missing probability (\(\mu\)). We generated five different tree pedigrees for each value of \(f\) ranging from 3 to 6. For each pedigree and for each combination of the remaining parameters \((m, \varepsilon, \mu)\), six random haplotype configurations have been computed. We considered the following set of parameter values: \(m \in \{50, 100\}\), \(\varepsilon \in \{0, 0.005, 0.01\}\), and \(\mu \in \{0, 0.05, 0.1, 0.2\}\) for a total of 2880 instances. Table 2 summarizes the results of the comparison. Accuracy of the two approaches in imputing the missing genotypes and reconstructing the haplotype configuration is similar: reHCstar is definitely more accurate than MePhase on smaller families (\(f \geq 4\)), while MePhase is slightly more accurate on larger families (\(f \geq 5\)). A likely reason is that MePhase computes haplotype configurations with fewer genotyping errors than reHCstar on larger families, as witnessed by the fact that, on those families, the average number of errors is smaller for MePhase than for reHCstar. Also, on those families, precision and recall (i.e., the fraction of original and computed errors that have been correctly identified) are better for MePhase than for reHCstar.

However, reHCstar is considerably more efficient than MePhase: the difference is most remarkable on large families (\(f = 6\)) where MePhase took 1092 seconds on average compared to the 23 seconds required by reHCstar. Moreover, MePhase was not able to solve 266 of the original 2880 instances (9.2%) within a time limit of an hour for each instance, while reHCstar completed the whole dataset within the same time limit. MePhase could not solve within the time limit 31.2% of the instances with \(f = 6\). Overall, reHCstar is much faster and more scalable than MePhase while maintaining comparable accuracy.

**Evaluation on a Real Genotyped Pedigree.** In the last part, we evaluated our approach on a real genotyped pedigree. The pedigree describes part of a dairy cattle population that has been obtained from the Italian Brown Swiss Breeders Association (ANARB). Genotypes have been obtained from a BovineSNP50 BeadChip and were restricted to 2570 loci on Chromosome 6. The pedigree (represented in the Supplementary Figure S.1) contains 207 individuals – 130 males and 77 females – with 93 founders. Unlike humans pedigrees, livestock pedigrees are composed by large families of half-siblings. In fact, the pedigree contains two bulls that generated 17 and 15 offspring, respectively, while other 12 bulls generated 50 offspring. A second typical characteristic of livestock pedigrees is the presence of several individuals that are not genotyped. The reason is twofold: the introduction of genotyping technologies for livestock species is recent. Therefore, most of the animals of the pedigree are not alive and cannot be genotyped. Moreover, the extraction of genotypes is quite expensive, hence it is performed only on animals of higher commercial value, such as
Table 2. Results of the experimental comparison between MePhase and reHC-star. The first column \((f)\) indicates the average size of nuclear family, the second reports the average number of errors present in the generated haplotype configuration (original errors), the next four columns (and the last one) are defined as the rows of Table 1. The 7th column indicates the average number of errors present in the reconstructed haplotype configuration (computed errors). Precision and recall are defined as the proportion of original (computed, resp.) errors that have been correctly identified. Since MePhase was not able to solve all the instances within the 1-hour time limit, we also computed the measures obtained by reHC-star restricted to the subset of instances completed by MePhase.

| \(f\) | no. of errors | genot. imput. error rate | haplot. error rate | no. of comp. errors | precision | recall | avg. running time (sec) |
|-------|---------------|--------------------------|-------------------|---------------------|-----------|--------|------------------------|
| 3     | 17.1          | 719 0.234                | 0.044             | 0.024               | 21.1      | 0.503  | 0.376 5.8             |
| 4     | 17.1          | 713 0.092                | 0.017             | 0.007               | 17.1      | 0.751  | 0.627 16.0            |
| 5     | 16.1          | 687 0.048                | 0.008             | 0.003               | 15.8      | 0.854  | 0.788 83.1           |
| 6     | 13.8          | 495 0.022                | 0.003             | 0.001               | 13.6      | 0.926  | 0.921 1092.1          |
| Overall | 16.2        | 2614 0.106               | 0.019             | 0.009               | 17.2      | 0.739  | 0.630 234.6          |

| \(f\) | no. of errors | genot. imput. error rate | haplot. error rate | no. of comp. errors | precision | recall | avg. running time (sec) |
|-------|---------------|--------------------------|-------------------|---------------------|-----------|--------|------------------------|
| 3     | 17.1          | 720 0.123                | 0.025             | 0.014               | 16.8      | 0.663  | 0.643 15.2            |
| 4     | 17.1          | 720 0.072                | 0.016             | 0.009               | 21.0      | 0.654  | 0.745 19.5            |
| 5     | 16.1          | 720 0.049                | 0.011             | 0.007               | 21.6      | 0.629  | 0.765 19.7            |
| 6     | 13.8          | 720 0.024                | 0.006             | 0.004               | 22.0      | 0.678  | 0.841 22.6            |
| Overall | 16.2        | 2880 0.067               | 0.015             | 0.008               | 20.3      | 0.657  | 0.737 19.2            |

breeding animals. In our pedigree we have the genotypes of only 105 males (and no females). Consequently almost half of all genotypes are missing.

The aim of this part of the experimentation is twofold: (i) to validate the minimum-recombinant and minimum-error model implicitly assumed in the formulation of the \((r,e)\)-HC problem and, (ii) to empirically prove that reHC-star can handle large, real-world pedigrees with a lot of non-genotyped individuals.

We prepared 6 instances from the original data by selecting 6 subsets of 50 original markers spaced at different distances \(d\). The first subset has distance \(d = 1\) or, in other words, is composed by the first 50 original markers. The second subset has distance \(d = 2\) (i.e., we selected every other marker), the third has distance \(d = 10\) (i.e., we selected one marker every ten original subsequent markers), and the other three have distance \(d = 15, 20, 25\), respectively. The genotypes used in the experimentation have been obtained by restricting the original genotypes to the subsets of selected markers. This process simulates
Table 3. Summary of the minimum number of recombinations and errors needed to solve the livestock pedigree at 6 different marker distances $d = 1, 2, 10, 15, 20, 25$. The first two columns indicate the maximum recombinations and error rates given in input to \textsc{reHCstar}. Each other cell has either indicated the number of recombinations ($r$) and errors ($e$) in the resulting haplotype configuration or “no solution” if no solution exists within the given recombination and error rates or “timeout” if no solution has been found within the time limit. Blank cells indicate instances already solved with a more stringent choice of recombination and error rates.

| Recomb. rate $r_p$ | Error rate $e_p$ | Marker distances $d$ | 1 | 2 | 10 | 15 | 20 | 25 |
|-------------------|-----------------|---------------------|----|----|----|----|----|----|
| 0.0               | 0.0             | no sol.             | no sol. | no sol. | no sol. | no sol. | no sol. | no sol. |
| 0.0005            | 0.0             | $r = 4$            | $r = 5$ | no sol. | no sol. | no sol. | no sol. | no sol. |
| 0.001             | 0.0             | $r = 25$           | $r = 50$ | $r = 52$ | $r = 53$ |
| 0.0005            | 0.0005          | $e = 1$            | no sol. | no sol. | no sol. | no sol. | no sol. | no sol. |
| 0.0005            | 0.0001          | $e = 5$            | no sol. | no sol. | no sol. | no sol. | no sol. | no sol. |
| 0.0005            | 0.0005          | $e = 27$           | timeout | no sol. | timeout | timeout | timeout | timeout |
| 0.0005            | 0.0005          | $e = 45$           | timeout | $e = 94$ | timeout | timeout |
| 0.0005            | 0.0005          | $r = 0$, $e = 1$  | $r = 5$, $e = 0$ | no sol. | no sol. | no sol. | no sol. | no sol. |
| 0.0005            | 0.0005          | $r = 6$, $e = 20$ | timeout | timeout | timeout | timeout | timeout | timeout |
| 0.0005            | 0.0005          | $r = 0$, $e = 45$ | timeout | $r = 0$, $e = 94$ | $r = 6$, $e = 81$ |
| 0.001             | 0.0005          | $r = 12$, $e = 3$ | no sol. | no sol. | no sol. | no sol. | no sol. | no sol. |
| 0.001             | 0.0001          | $r = 11$, $e = 6$ | timeout | timeout | timeout | timeout | no sol. | no sol. |
| 0.005             | 0.0005          | $r = 40$, $e = 3$ | $r = 48$, $e = 3$ | $r = 50$, $e = 1$ |

genotypes collected at different “densities”. Since the closer the loci, the stronger their linkage, we expect that high-density genotypes (the ones with distance $d = 1$ and $d = 2$) can be solved with only a few recombinations and/or errors, while low-density genotypes should require a larger amount of recombinations and/or errors. To test this hypothesis, we ran \textsc{reHCstar} over the 6 instances with different maximum recombination and error rates in order to find the haplotype configuration with the smallest number of recombinations and errors.

The results of this experiment (Table 3) reveal a clear trend: genotypes with higher intra-marker distance (i.e., lower densities) require considerably more recombinations and/or errors than high-density genotypes. In fact, it was possible to find a haplotype configuration with a single genotyping error (and no recombination) that solves the instance with the highest density ($d = 1$), while 45 errors (or 50 recombinations) were needed in the solution of the medium-density instance ($d = 15$), and 89 errors (or 6 recombinations and 81 errors) were needed in the lowest-density instance ($d = 25$). Lower-density instances ($d \geq 15$) have not been solved within a time limit of 3 hours for some particular choices of maximum recombination and error rates.

Our overall conclusion is that the results of the three experimental parts support the soundness of the model and the feasibility of the approach. In fact, \textsc{reHCstar} has computed solutions with good accuracy on simulated instances
even in limit cases (pedigrees with many recombinations and errors and/or many untyped loci) and has found haplotype configurations with a few recombinations and errors on some real instances. The comparison with MePhase, revealed that reHCstar has a comparable accuracy, but it is much faster and better scales to large and complex pedigrees.

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Supplementary Material

Supplementary Table S.1. Summary of accuracy and performance obtained by reHCstar on randomly generated instances. Each table refers to a series of tests where only one parameter has been varied. The base values of the parameters are: pedigree size $n = 50$, genotype length $m = 50$, recombination probability $\theta = 0.005$, error probability $\varepsilon = 0.005$, and missing probability $\mu = 0.05$. The best result for each row is boldfaced.

(a) Increasing pedigree size ($n$)

| Pedigree size $n$ | 50  | 100 | 200 |
|-------------------|-----|-----|-----|
| No. of completed instances | 100/100 | 100/100 | 100/100 |
| Avg. genotype imputation error rate | 0.161 | 0.151 | 0.149 |
| Avg. haplotyping error rate | 0.043 | 0.039 | 0.033 |
| Avg. haplotyping error rate (wo/missing) | 0.037 | 0.034 | 0.027 |
| Avg. running time (in seconds) | 15.1 | 82.3 | 359.4 |
| Max. running time (in seconds) | 72.3 | 479.9 | 1129.3 |

(b) Increasing genotype length ($m$)

| Genotype length $m$ | 50  | 100 | 200 |
|---------------------|-----|-----|-----|
| No. of completed instances | 100/100 | 100/100 | 100/100 |
| Avg. genotype imputation error rate | 0.170 | 0.174 | 0.174 |
| Avg. haplotyping error rate | 0.043 | 0.049 | 0.063 |
| Avg. haplotyping error rate (wo/missing) | 0.038 | 0.045 | 0.059 |
| Avg. running time (in seconds) | 17.0 | 93.6 | 565.6 |
| Max. running time (in seconds) | 85.7 | 770.4 | 3127.5 |

(c) Increasing recombination probability ($\theta$)

| Recombination probability $\theta$ = | 0.0 | 0.005 | 0.01 | 0.02 |
|-------------------------------------|-----|-------|-----|-----|
| No. of completed instances | 100/100 | 100/100 | 100/100 | 100/100 |
| Avg. genotype imputation error rate | 0.172 | 0.166 | 0.166 | 0.166 |
| Avg. haplotyping error rate | 0.040 | 0.047 | 0.052 | 0.072 |
| Avg. haplotyping error rate (wo/missing) | 0.040 | 0.047 | 0.052 | 0.072 |
| Avg. running time (in seconds) | 5.0 | 7.0 | 13.0 | 10.5 |
| Max. running time (in seconds) | 14.7 | 50.1 | 75.7 | 62.6 |

(d) Increasing error probability ($\varepsilon$)

| Error probability $\varepsilon$ = | 0.0 | 0.005 | 0.01 |
|---------------------------------|-----|-------|-----|
| No. of completed instances | 100/100 | 100/100 | 100/100 |
| Avg. genotype imputation error rate | 0.172 | 0.166 | 0.166 |
| Avg. haplotyping error rate | 0.040 | 0.047 | 0.048 |
| Avg. haplotyping error rate (wo/missing) | 0.040 | 0.042 | 0.044 |
| Avg. running time (in seconds) | 12.8 | 28.9 | 74.2 | 74.2 |
| Max. running time (in seconds) | 55.5 | 109.8 | 485.2 |

(e) Increasing missing probability ($\mu$)

| Missing probability $\mu$ = | 0.0 | 0.05 | 0.1 | 0.2 |
|----------------------------|-----|-----|-----|-----|
| No. of completed instances | 100/100 | 100/100 | 100/100 | 100/100 |
| Avg. genotype imputation error rate | — | *0.167* | 0.183 | 0.207 |
| Avg. haplotyping error rate | 0.032 | 0.041 | 0.052 | 0.071 |
| Avg. haplotyping error rate (wo/missing) | 0.032 | 0.034 | 0.041 | 0.052 |
| Avg. running time (in seconds) | 16.6 | 15.3 | 17.9 | 14.0 |
| Max. running time (in seconds) | 79.7 | 76.0 | 89.0 | 69.2 |
Supplementary Figure S1. Pedigree graph of the real dairy cattle population used in the experimental evaluation of reHCstar.