An empirical evaluation of genotype imputation of ancient DNA

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Abstract

With capabilities of sequencing ancient DNA to high coverage often limited by sample quality or cost, imputation of missing genotypes presents a possibility to increase the power of inference as well as cost-effectiveness for the analysis of ancient data. However, the high degree of uncertainty often associated with ancient DNA poses several methodological challenges, and performance of imputation methods in this context has not been fully explored. To gain further insights, we performed a systematic evaluation of imputation of ancient data using Beagle v4.0 and reference data from phase 3 of the 1000 Genomes project, investigating the effects of coverage, phased reference, and study sample size. Making use of five ancient individuals with high-coverage data available, we evaluated imputed data for accuracy, reference bias, and genetic affinities as captured by principal component analysis. We obtained genotype concordance levels of over 99% for data with 1× coverage, and similar levels of accuracy and reference bias at levels as low as 0.75×. Our findings suggest that using imputed data can be a realistic option for various population genetic analyses even for data in coverage ranges below 1×. We also show that a large and varied phased reference panel as well as the inclusion of low- to moderate-coverage ancient individuals in the study sample can increase imputation performance, particularly for rare alleles. In-depth analysis of imputed data with respect to genetic variants and allele frequencies gave further insight into the nature of errors arising during imputation, and can provide practical guidelines for postprocessing and validation prior to downstream analysis.

Keywords: imputation; phasing; ancient DNA; low coverage; reference bias

Introduction

The possibility to sequence ancient DNA (aDNA) has increased capabilities to study archaeological remains and provided new insights into various aspects of human evolutionary history. Notable findings such as the detection of genetic introgression between anatomically modern humans and other hominins, confirmation of the African origin of modern humans, and an increased understanding of the spread of agriculture into Europe have been achieved through population genetic analyses of ancient and contemporary genomes (Nielsen et al. 2017).

Due to the age and varying preservation conditions that ancient samples may have been exposed to, aDNA has unique properties that pose methodological and computational challenges not present when working with data from present-day humans. Contamination of DNA from microbes and other nontarget sources can result in low proportions of endogenous DNA (Paabo et al. 2004; Prüfer et al. 2010), leading to limitations in sample availability that can cause sequencing to high coverage depth to be impossible or prohibitively expensive. Contamination also leads to issues regarding data authenticity. In addition, the DNA molecule is subject to degradation over time, which can cause errors in the sequencing pipeline (Brotherton et al. 2007; Sánchez-Quinto et al. 2012).

Although the identification of patterns of damage unique to aDNA has allowed for methods of data authentication and improved the process of reconstructing ancient genomes (Stiller et al. 2006; Briggs et al. 2007; Krause et al. 2010; Sawyer et al. 2012), these characteristics nonetheless cause biases in sequencing and mapping that can impact variant calling and other forms of downstream analysis (Prüfer et al. 2010; Ginolhac et al. 2011; Parks and Lambert 2015). Consequently, studies of aDNA samples are often limited to low- to moderate-coverage data with higher degrees of uncertainty than modern samples typically exhibit.

Genotype imputation is a powerful tool that can increase the information content in a sample by inferring unobserved genotypes. Imputation has been widely applied in various scenarios analyzing modern data, e.g. to increase power of inference in genome-wide association studies and to conform samples from different studies for merged analysis (Zeggini et al. 2008; Spencer et al. 2009; Marchini and Howie 2010). For aDNA, the possibility to increase information content of sparse and noisy data can potentially improve the quality of results as well as expand the range of analyses that are possible to perform.

Many common imputation methods for unrelated samples rely on sequential probabilistic models in which missing genotypes are inferred based on similarity to other individuals. The
estimation is founded on an assumption of the presence of short stretches of shared haplotypes that have been passed down from a distant common ancestor. Given the individual’s genotypes, its haplotype phase is inferred, allowing missing variants to be predicted based on similarity to other samples. Many methods are able to leverage the information in the study sample as well as an additional panel of phased reference haplotypes when performing the phase estimation.

Many widely employed tools, such as MACH (Li et al. 2010), IMPUTE2 (Howie et al. 2009), and PHASE (Stephens et al. 2001; Stephens and Scheet 2005), are based on variants of the “the product of approximate conditionals” (PAC) framework (Li and Stephens 2003). This model represents the sample sequence as an imperfect mosaic of the reference haplotypes, generally considering all possible transitions over the state space, with explicit modeling of mutation and recombination. A haplotype version of this framework is used in the software GLIMPSE (Rubinacci et al. 2021). A slightly different method is implemented in the software Beagle (Browning and Browning 2007), which is based on a model of local haplotype clusters based on similarity of the reference haplotypes at nearby markers. This results in a smaller state space with not all possible transitions considered at every position, reducing the computational burden. The effects of mutation and recombination are not explicitly modeled, but the change of cluster membership along the sequence can be seen as implicitly representing these biological processes.

The accuracy of genotype imputation depends on several factors, mainly related to the quality of the sample data and the properties of the phased reference panel. A larger sample size, as well as increased marker density and genotype accuracy generally results in better performance (Browning and Browning 2011). For data with high levels of uncertainty, imputation based on a probabilistic framework using genotype likelihoods rather than called genotypes may be beneficial (Browning and Yu 2009; Browning and Browning 2011; Nielsen et al. 2011), an option that is supported by some software tools, including Beagle v4.0. In Hui et al. (2020), a 2-step approach is introduced, in which genotype likelihood data is first used to obtain genotype probabilities based on a reference panel, and missing genotypes are subsequently imputed based on a subset of these which were able to be confidently called. They evaluate different pipelines on low coverage data below 1x and show that applying the 2-step method using Beagle v4.1 or GLIMPSE for calling genotypes and Beagle v5 for imputation gave similar overall accuracy as using the single-step methods Beagle v4.0 or GLIMPSE, but that the 2-step method gave more nuanced posterior genotype probabilities which allowed for a more informed postimputation filtering procedure.

Studies comparing different phased reference panels have yielded varying results, with some finding that highest performance is gained by using population-specific panels (Pistis et al. 2015; Mitt et al. 2017) and others indicating the benefits of a large reference with a high level of diversity, particularly for admixed populations with no clearly matching reference (Huang et al. 2009; Howie et al. 2011; Jostins et al. 2011). Using a phased genome reference panel from present-day individuals is currently the only option for ancient data, introducing a possible source of bias as it means that only variants that exist in the population today can be reproduced. Leveraging information in other ancient individuals by increasing study sample size may be a way to mitigate reference divergence, particularly as more sequenced ancient genomes become available, but benefits may be diminished in the context of sparse and uncertain data. The behavior of genotype likelihood-based imputation methods on data that exhibits the characteristic properties of aDNA discussed above has not been fully explored, particularly in combination with low coverage levels below 1x.

Genotype imputation has previously been performed on ancient human data in e.g. Gamba et al. (2014), Jones et al. (2015), Martiniano et al. (2017), Antonio et al. (2019), and Cassidy et al. (2020). In these studies, imputation was mainly performed using Beagle v4.0 and used to maximize the information content of ancient samples and allow for analyses such as Runs of Homozygosity (ROH) that require dense, diploid genotypes. Performance evaluation was mainly done by comparison of genotypes imputed from masked data to corresponding high-coverage calls, and showed satisfactory concordance to motivate the use of imputed data for downstream population-genetic analyses. The goal of this study is to complement and extend previous work by performing a systematic evaluation of a commonly used genotype likelihood-based imputation pipeline on ancient data. We investigate how the particular issues of sample quality and reference divergence associated with aDNA affect imputation, focusing on practical considerations regarding methodology and performance evaluation.

**Materials and methods**

**Data description and preprocessing**

**Ancient data**

The ancient genome data used in this study consisted of five individuals for which high-coverage data between 19x and 57x was available (ans17, LBK, Loschbour, sf12, and ne1), as well as a set of 61 individuals with low- to moderate-coverage ranging from 0.1x to 16x. See Supplementary Tables 1 and 2 for sample specificities and references to source publications.

The Genome Analysis Toolkit (GATK) v3.5.0 ( McKenna et al. 2010) tool UnifiedGenotyper was used to generate genotype likelihoods from alignment data for each of the ancient samples individually. The allele callset used was that of the 1000 Genomes phase 3 panel ( Auton et al. 2015), filtered to keep only autosomal, biallelic SNPs, resulting in a total of 77,818,182 markers. To avoid introducing a possible bias from nucleotide misincorporations due to postmortem damage, the generated VCF files were filtered to exclude all sites where the most likely genotype could have been inferred from a deaminated allele. For C → T deaminations, this was done by removing sites where the SNP was a C → T transition and the most likely genotype contained a T allele. The corresponding treatment was performed for G → A deaminations. The software bcftools v1.6 (Li et al. 2009) was used for filtering.

As in previous studies considering aDNA, assessment of imputation performance was done by comparison of imputation results to corresponding high-quality (HQ) genotypes. For this, the five individuals for which high-coverage data were available were used. The HQ genotypes considered as gold standard were called from the original high-coverage data, following the same pipeline as described in their respective publications (Supplementary Table 1). The called genotypes were filtered to keep only biallelic SNPs with a minimum depth of 15 and a QUAL score of at least 50. Heterozygote sites were further filtered for both alleles having a minimum allele depth of 25% of the total depth. Low-coverage data for each evaluation individual was generated by downsampling reads using Picard version 2.0.1 ( Broad Institute 2019), after which estimation of genotype likelihoods and filtering were performed in an identical manner to the method described above for the low- to moderate-coverage coverage levels.
individuals. The sparse data was used as input for imputation, and the resulting genotypes were compared to HQ data.

Reference panels

The reference material used for imputation was the 1000 Genomes phase 3 v5a panel of phased haplotypes and the GRCh37 genetic maps provided along with the Beagle software. The reference panel was filtered to only include biallelic SNPs, resulting in 27,904,756 markers over chromosomes 1–22. To evaluate the effects of the reference on imputation, two panels were considered: the entire data set of 2,504 samples, and a smaller one of 254 samples from European populations only. The two panels are denoted as “FULL” and “EUR,” respectively. The former was also used to estimate minor allele frequency (MAF) of SNPs when analysis of imputed data over the allele frequency spectrum was performed.

Imputation methodology

Imputation was performed using Beagle v4.0, with sample data split into segments of 50,000 markers with an overlap of 25,000, using genotype likelihoods as input. Version 4.0 was selected as the goal was to evaluate imputation based on probabilistic input in the context of extremely low coverages, and this is the latest version of the software that allows imputation to be performed based on genotype likelihoods. Further, as Beagle v4.0 uses a haplotype model that is constructed from both the phased reference panel as well as current estimates of the haplotypes of the study sample (Browning 2008), we also wanted to assess the effects of including other ancient individuals in the study sample, allowing the imputation to be informed by other low-coverage ancient data within the same probabilistic framework. The effects of including multiple ancient individuals in the imputation study sample were evaluated by performing imputation jointly as well as separately. In the first case, all 61 low-to-moderate coverage ancient individuals as well as the five evaluation individuals were included in the study sample to impute. In the second case, imputation was performed separately per study individual, meaning only the phased reference haplotypes were used in the genotype estimation.

Performance evaluation metrics

Genotype concordance

The main metric employed for assessing imputation accuracy was genotype concordance/discordance, defined as the fraction of genotypes that were imputed correctly/incorrectly. This was measured separately for each of the five evaluation individuals by comparison to the HQ genotypes derived from dense data. For each of the five evaluation individuals, we extracted the imputed markers for which there were corresponding HQ genotypes available, and divided them into two disjoint sets denoted as “overlapping” and “nonoverlapping,” based on whether or not the corresponding downsampled individual data had overlapping reads for the site or not. Supplementary Table 3 shows the sizes of these sets for each evaluation individual and coverage level.

Reference affinity

To assess whether imputed genotypes show a systematic bias toward the reference panel, the degree to which a sample showed an affinity toward the reference was compared between imputed and HQ genotypes. Here, reference affinity was measured as the fraction of markers that have the same genotype as the most frequently occurring one in the reference panel.

Principal component analysis

Principal component analysis (PCA) is a method of projecting data onto a basis that maximizes the variance of the data, possibly revealing previously unseen patterns or features. PCA can be used to reduce the dimensionality of data, e.g. for visualization purposes, and in the field of aDNA it is commonly used to show ancient samples in the context of modern variation. Here, it was used as a means of illustrating the difference between imputed and corresponding high-coverage genotypes.

PCA was performed on diploid genotypes, with a modern panel consisting of 429 European samples from the Human Origins data set of Patterson et al. (2012), filtered to remove variants with MAF under 1% or missing call rates exceeding 10%. To handle the fact that the ancient samples did not have observed genotypes for all sites used in the PCA, we used the method of known data regression (KDR) (Arteaga and Ferrer 2002). A reference PCA based on the modern panel was initially defined. Estimation of scores for each ancient sample based on this model proceeded by considering the data of the reference samples corresponding to observed ancient genotypes, and fitting a linear regression model to their original PCA scores in the reference model. The software SMARTPCA from EIGENSOFT 7.2.1 was used to define the reference PCA, and the Python library scikit-learn was used for solving linear least-squares problems.

Results

Effects of reference panel and study sample size

Imputation performance was assessed for three combinations of reference panel and study sample size, denoted as imputation configurations and shown in Table 1. In the first configuration, imputation was performed individually per evaluation individual, using the EUR phased reference panel. For configurations 2 and 3, all ancient individuals were included in the imputation study sample, using the EUR and FULL reference panels, respectively. All results in this section are for imputation performed on sample data with 1× coverage, and to perform a comprehensive evaluation of the effect of imputation configuration, no posterior filter was imposed on the imputed genotypes. Results are shown for each of the five evaluation individuals, with results split into overlapping and nonoverlapping marker sets.

Figure 1 shows genotype concordance for the three imputation configurations. Overall, concordance rates were similar between individuals and reached 0.99 in all cases. The results indicate that the larger reference panel as well as the inclusion of ancient individuals in the imputation study sample improved performance. For overlapping markers, concordance rates increased slightly from just under 0.987 to somewhat above. Concordance was lower among the nonoverlapping markers in general, and it was also among these that the effects of varying imputation configurations were the most pronounced.

To investigate imputation performance over the allele frequency range, the imputed markers were binned according to MAF, and the average genotype discordance was assessed per bin. Because there is a high risk of chance agreement between

| Configuration | Reference | Study sample |
|---------------|-----------|--------------|
| 1             | EUR       | Single       |
| 2             | EUR       | All ancient  |
| 3             | FULL      | All ancient  |
imputation run was performed using the five evaluation individuals and coverage level. A total concordance rate of 0.99 was reached around 0.25 coverage, and the evaluation was based on unfiltered results, with fully colored bars showing results for sites at which the downsampled data had overlapping reads, and shaded bars for nonoverlapping markers, i.e., sites at which all overlapping reads were removed by the downsampling process.

Homologous genotypes and the reference majority in the case of low MAF markers, only sites at which the HQ data were homologous were considered, thus measuring the ability of the imputation to recover heterozygotes. Results for imputation configurations 1–3 are shown in Fig. 2, and again indicate that using a larger phased reference panel and study sample size increases performance. The effects were particularly visible among nonoverlapping markers and in the low MAF ranges, where heterozygotes are the most difficult to recover.

Effects of coverage

Next, we assessed the effects of coverage on imputation. For every level $C \in \{0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00\}$, an imputation run was performed using the five evaluation individuals downsamplied to Cx. Imputation configuration 2 was used for all runs, and as in the previous section, no posterior filter was imposed on the resulting genotypes.

Figure 3 shows the concordance between imputed and HQ genotypes for all markers (A) and for heterozygotes (B), separated into overlapping and nonoverlapping marker sets for every evaluation sample and coverage level. A total concordance rate of 0.99 was reached around 0.25× for most individuals, with a planing out visible at 1× where levels around 0.9975 and 0.995 were obtained for overlapping and nonoverlapping sites, respectively. For heterozygote sites, concordance levels were below 0.975 throughout, reaching 0.95 at 1× and as low as 0.825 for the lowest coverage level of 0.1×.

To assess the level of systematic bias toward the variant that is most common in the reference, we compared the level of reference affinity of the imputed data to that of the corresponding HQ genotypes, considering these as a baseline for the similarity between the true genotypes and the reference. Figure 4 shows the difference in measured affinities between the HQ and imputed data for the nine coverage levels, over the allele frequency spectrum, averaged over the five evaluation individuals.

The negative values in the lower MAF ranges indicate that the imputed data shows a larger affinity toward the reference. While the extremely low coverages showed significantly higher levels of bias, the rates decreased and showed little variation for coverages 0.75× and higher. The results indicate a reduction in bias toward the reference with increasing MAF, showing little differences around MAF 0.3 for most coverage levels. A possible explanation for the positive difference at higher MAF values is that at these markers, imputation errors do not tend toward the reference majority as strongly as in the lower MAF ranges. Overall concordance is also lower at high MAF due to higher frequency of heterozygotes.

In-depth performance analysis

In this section, we present further evaluation of imputed genotypes to assess properties relevant to downstream analysis. We considered results of imputation configuration 3 on data with 1× coverage, and imposed a filter of minimum posterior genotype probability of 0.99 on the imputed data. First, performance for different genotypes was evaluated. Figure 5 shows concordance of sites split according to genotype in the high-coverage data. Although performance remained poorer for heterozygote sites, lower MAF markers, only sites at which the downsampled data had overlapping reads, and shaded bars for nonoverlapping markers, i.e., sites at which all overlapping reads were removed by the downsampling process.

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Finally, PCA was used to visualize and compare genetic affinities of imputed and high-coverage data. Figure 7 shows that, within the variation represented by the first two principal components, scores of imputed genotypes map closely to those of the HQ data, particularly for the individuals ans17, LBK, and ne1. As illustrated in e.g. Günther and Jakobsson (2019), considering subsets of SNPs introduces noise in the PCA projection, resulting in less accurate projections with higher degree of uncertainty compared to using all available data. In Fig. 7, this is visualized by the distance between low-coverage and HQ data points for each individual. For each of the five individuals, relative distances to the HQ data are smaller for the imputed genotypes than the low-coverage data, indicating that information lost by the downsampling process has been retained by imputation.

Discussion

This study provides a systematic investigation of the application of imputation to human aDNA. We have corroborated results from similar experiments showing that overall genotype concordance levels of over 0.99 can be reached for data with 1× coverage, and provided an in-depth analysis of the qualities of imputed genotypes. Investigation of performance at coverage levels tending to the ultra-low indicated that the quality of imputed genotypes began to plateau around 0.75–1×, and that common variants were more robust to different reference panels as well as less prone to reference bias overall. The results suggest a MAF threshold of around 0.1 for minimizing genotype discordance of heterozygote calls, and that in cases where rare alleles are of interest, an increased diversity and size of the phased reference as well as the imputation study sample are particularly beneficial. The fact that information from ancient individuals can be leveraged to improve imputation, along with the genotype discordance levels shown, may in some cases motivate the sequencing of more ancient samples to lower coverage as a cost-effective alternative to that of fewer samples to higher coverage.

The presented work provides a framework of practical considerations for performing imputation, as well as a basis for further investigations. A systematic evaluation of different imputation methods and adaptation of the statistical models to the context of sparse and uncertain data may increase performance for

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**Fig. 1.** Concordance of imputed genotypes for the three configurations in Table 1. Imputation was performed on data in which the five evaluation individuals were downsampled to 1× coverage, and the evaluation was based on unfiltered results, with fully colored bars showing results for sites at which the downsamleped data had overlapping reads, and shaded bars for nonoverlapping markers, i.e. sites at which all overlapping reads were removed by the downsampling process.
ancient samples. Further insights may also be gained by assessment of imputed data by means of different population genetic analyses, considering e.g. haplotype-based methods such as RoH as well as those based on allele frequencies.

For the five evaluation individuals considered, in-depth performance analysis showed lower performance for the hunter-gatherer genomes sf12 and Loschbour, both in terms of genotype concordance and similarity in PCA-space to HQ data. A possible explanation is that the reference panel of present-day individuals
used for imputation does not contain individuals with a genetic composition that is similar to these individuals. As discussed in e.g. Skoglund et al. (2014) and Günther and Jakobsson (2016), hunter-gatherer individuals have shown a particular genetic profile that is not represented in the genetic variation of modern-day people. The individuals LBK, ne1, and ans17, in contrast, are from early farming cultures, for which there are present-day European individuals who share a similar genetic make-up. Further investigations of the effects of sample ancestry and reference divergence will be required to customize imputation pipelines to individuals of varying genetic composition. As more sequenced ancient data becomes available, both imputation accuracy as well as the ability to assess various aspects of performance will be improved.

A possible source of bias in the evaluation of imputation performance is reference bias introduced by alignment and variant calling procedures on ancient sequence data. This can lead to alternate alleles being missed and heterozygous sites to be called as homozygous for the reference allele (Günther and Nettelblad 2019), causing ancient individuals to appear artificially similar to the modern reference genome. As imputation is expected to perform better for samples that are genetically close to the phased reference panel used, this could cause overestimated measures of accuracy. A possible means to mitigate this effect is the use of genotype callers that are tailored for aDNA such as snpAD (Prüfer 2018) and ATLAS (Link et al. 2017). As these callers also account for postmortem damage, the inclusion of transition-type polymorphisms in future imputation studies may also be motivated.

In this study, we have focused on a probabilistic imputation framework based on Beagle v4.0 that has been frequently used in the aDNA community. Other imputation pipelines have shown to give qualitatively similar results for imputing genotypes from ancient sequence data, and also indicated some performance trade-offs such as imputation accuracy at different parts of the allele frequency spectrum (Hui et al. 2020). Another relevant point is that more recent software for phasing and imputation such as GLIMPSE and Beagle v5 have increased focus on scalability to larger reference panels. Sample size, availability of computational resources and the type of downstream analysis intended are thus factors to consider in the selection of methodology for imputation of aDNA.

Data availability
The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article and in its online supplementary material.

Supplementary material is available at G3 online.

Acknowledgments
The authors acknowledge the use of computational resources provided by Swedish National Infrastructure for Computing (SNIC) at Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under project SNIC 2017/7-162.

Funding
CN acknowledges funding by Formas (grant number 2020-00712) and MJ acknowledges the Knut and Alice Wallenberg foundation.

Conflicts of interest
None declared.
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Communicating editor: A. Sethuraman