Following guidelines review:

Does the title clearly reflect the content of the article? [X] Yes, [ ] No, [ ] I don't know

The title "Estimating allele frequencies, ancestry proportions and genotype likelihoods in the presence of mapping bias" accurately reflects the content of the article. It captures the main focus of the study, which is the impact of mapping bias on various population genomic analyses and the proposed methods to mitigate this bias.

Does the abstract present the main findings of the study? [X] Yes, [ ] No, [ ] I don't know

The abstract effectively summarises the key findings of the study, including:
1. The impact of mapping bias on allele frequency estimates and ancestry proportions.
2. The proposal of an empirical adjustment to genotype likelihoods to mitigate mapping bias.
3. Comparison of different methods for estimating ancestry proportions under various scenarios.
4. The effectiveness of the adjusted genotype likelihood approach in mitigating mapping bias.

Are the research questions/hypotheses/predictions clearly presented? [X] Yes, [ ] No, [ ] I don't know

The introduction clearly presents the research questions and objectives of the study. The authors effectively outline the problem of mapping bias in population genomic analyses (lines 1-6) and introduce their proposed solution of adjusting genotype likelihoods.

Does the introduction build on relevant research in the field? [X] Yes, [ ] No, [ ] I don't know

The introduction provides a comprehensive overview of the relevant research in the field, citing numerous studies that have addressed mapping bias in various contexts (lines 1-6, 19-23). The authors also discuss different strategies proposed to mitigate mapping bias (lines 23-30), effectively situating their work within the existing literature.

Are the methods and analyses sufficiently detailed to allow replication by other researchers? [ ] Yes, [X] No, [ ] I don't know

The methods section provides detailed descriptions of the simulation procedures, data analysis, and software used. The authors include specific parameters for their simulations, detail the process of generating sequencing data, and describe the methods used for estimating admixture proportions. However, full reproducibility hinges on access to the pseudo-haploid calls, which are inherently random. To ensure complete replicability of the results, it is essential that the authors either share the file containing all pseudo-haploid calls or provide the seeds used in their software (if applicable). This level of detail should allow for replication by other researchers.

Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No, [ ] I don't know

The methods and statistical analyses appear appropriate for addressing the research questions. The authors use a combination of simulations and empirical data analysis, which is a robust approach for testing their hypotheses. They provide a clear rationale for their choice of methods and describe the statistical analyses in sufficient detail.

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [ ] Yes, [ ] No, [X] I don't know

The study does not present negative results that would necessitate a power analysis or equivalence testing. The results generally support the authors' hypotheses about the impact of mapping bias and the effectiveness of their proposed correction method.

Are the results described and interpreted correctly? [X] Yes, [ ] No, [ ] I don't know

The results are described and interpreted with appropriate caution and consideration of limitations. The authors present their findings clearly, using figures and tables to support their interpretations. They discuss the impact of mapping bias on allele frequency estimates and ancestry proportion estimates in a balanced manner, acknowledging both the strengths and limitations of their approach.

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No, [ ] I don't know

The authors provide a balanced discussion of the strengths and limitations of their study. They acknowledge that their simulations may underestimate the effect of mapping bias in real-world scenarios (lines 315-324) and discuss the limitations of their approach in fully removing mapping bias effects (lines 373-376).

Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No, [ ] I don't know

The conclusions are well-supported by the results presented in the study. The authors do not overstate their findings and provide appropriate context for their conclusions. They emphasise the modest but significant effect of mapping bias on ancestry estimates and discuss the implications of their findings for future research in population genomics.

Addition comments:

MAJOR COMMENTS:

- The simulation of ancient DNA (aDNA) reads was conducted meticulously, adhering to established standards and protocols to generate data closely resembling authentic aDNA datasets. However, this approach does not fully capture the complexity of real-world scenarios, where aDNA samples typically undergo various laboratory treatments. Notably, treatments such as UDG (Uracil-DNA Glycosylase) or partial UDG are commonly applied but were not simulated in this study. Incorporating these treatments into the simulated dataset would enhance its fidelity to real-life aDNA data used in research.

  Despite this limitation, the findings presented in the paper remain significant and worthy of publication. Nonetheless, simulating these laboratory treatments would further improve the dataset's quality and increase its relevance to real-world aDNA analysis.

- Regarding reproducibility, it is crucial that the authors share either the pseudo-haploid calls files or the software seeds (if applicable). This step is essential because these calls are inherently random, and without this information, it would be impossible for other researchers to replicate the study's results precisely.

MINOR COMMENTS:

- The authors' selection of Finnish (FIN) and Yoruba (YRI) populations might need further explanation. While one can infer that this choice aims to contrast reference bias between European and African genetic backgrounds, this rationale is not explicitly stated in the manuscript. Given that the human reference genome was primarily derived from European and African-American individuals (Green et al., 2010), it would be valuable to include a third population with a distinct genetic background, such as an East Asian population. This addition would provide a more comprehensive assessment of the new methods' performance across diverse ancestries. This suggestion is particularly relevant considering that Figure S3 examines variants attributed to East Asian ancestry.

- The manuscript would benefit from a brief discussion (or comparison with other methods , maybe put a figure in SI?) of the computational requirements for implementing the proposed genotype likelihood correction method. This information would be valuable for researchers considering adopting this approach in their own work (which for example is one of the main struggles with pangenome graphs).

- Line 34: Typo - "apporach" should be "approach"
- Line 52: Typo - "not to call genotypes all sites" should likely be "not to call genotypes at all sites"

- Line 74: Redundant wording - "We simulate data sequencing data with realistic ancient DNA damage.." Remove the first "data"
- Line 107: Inconsistent number formatting - "for ten FIN individuals and 10 YRI individuals" Should be either "ten" and "ten" or "10" and "10"
- Line 308: Incorrect idiom - "as face values" should be "at face value"
- Line 400: Formatting error in reference - "O?Sullivan" should be "O'Sullivan"
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In conclusion, this manuscript presents a significant contribution to the field of population genomics, particularly in addressing mapping bias in short-read sequencing data, with a focus on ancient DNA (aDNA) research. The authors' novel approach to mitigating mapping bias through empirical adjustment of genotype likelihoods is well-executed and shows promise. While I cannot provide expert feedback on the population genetics analysis pipeline, the methods appear sound and well-documented. However, the study could benefit from additional analyses, such as including an East Asian population and simulating UDG-treated reads (e.g., using tools like https://github.com/sbg/Mitty). These additions, along with addressing the previously mentioned typos and ensuring reproducibility by providing necessary files or software seeds, would further strengthen the paper. Despite these suggested improvements, the current results are sufficient and scientifically sound. If the reproducibility issues are addressed and typos corrected, the paper is suitable for publication