



Robust inference of phenotypic traits from low-coverage ancient genomes

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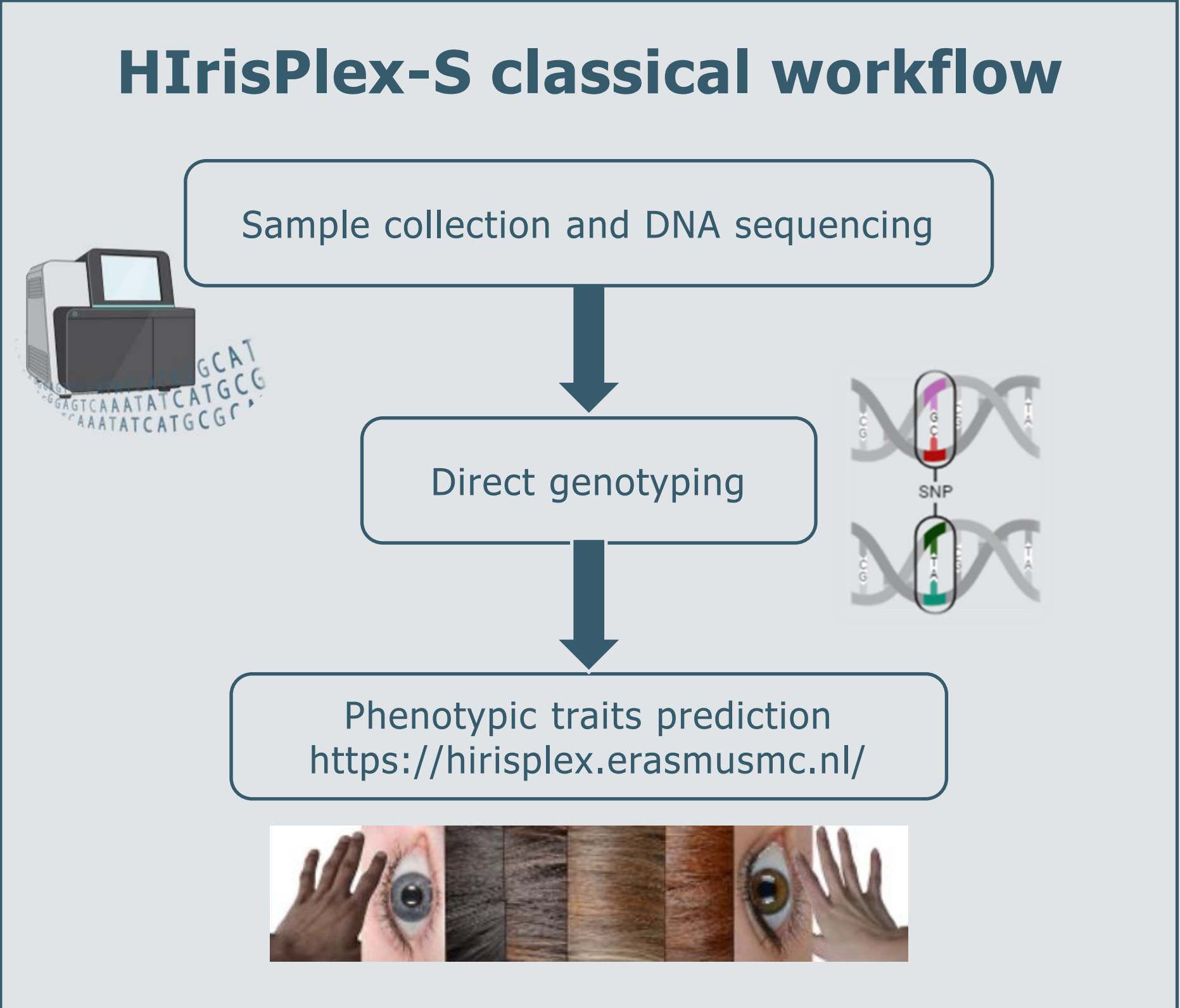
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Introduction

The HIRisPlex-S system, which analyses 41 pigmentation gene polymorphisms, estimates individual probabilities for three eye, four hair, and five skin colour categories. Originally developed for criminal investigations, this system has been extensively applied in recent years to predict phenotypes from ancient human skeletal remains. However, its accuracy with low-coverage ancient DNA data has not been thoroughly tested.

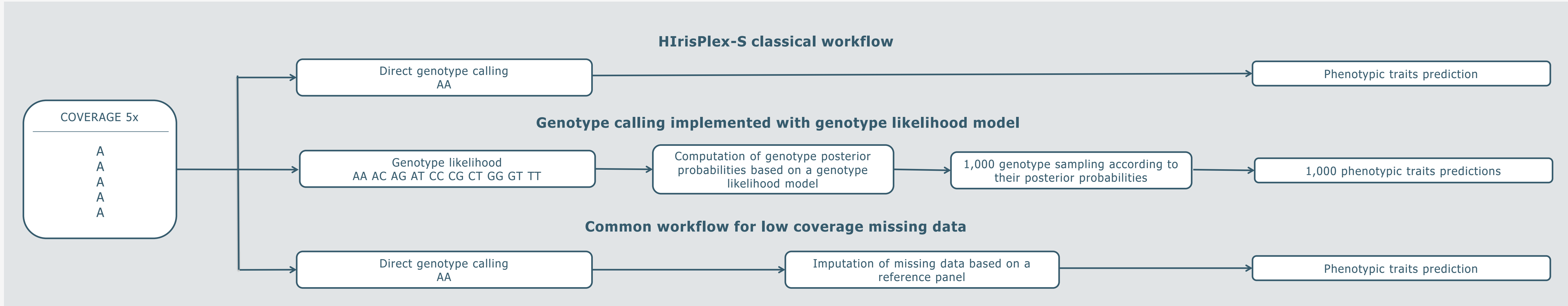
Our primary aims are to evaluate the effectiveness and robustness of the HIRisPlex-S system in predicting phenotypic traits from low-coverage ancient DNA data and to propose a novel methodology based on genotype likelihoods that explicitly addresses genotype calling uncertainty in such data.

Based on these evaluations, we inferred the geographical distribution of eye, hair, and skin pigmentation in 348 Eurasian individuals ranging from the Upper Palaeolithic to the Iron Age.

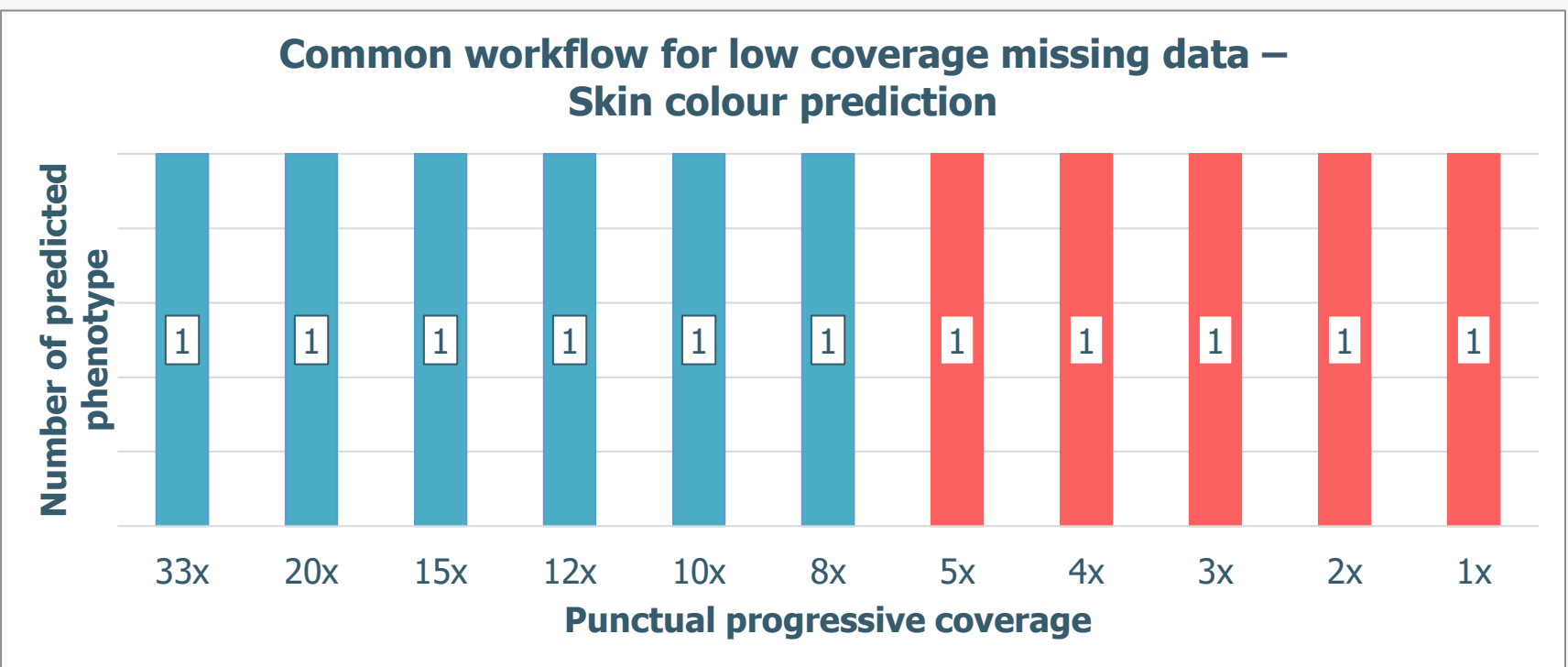
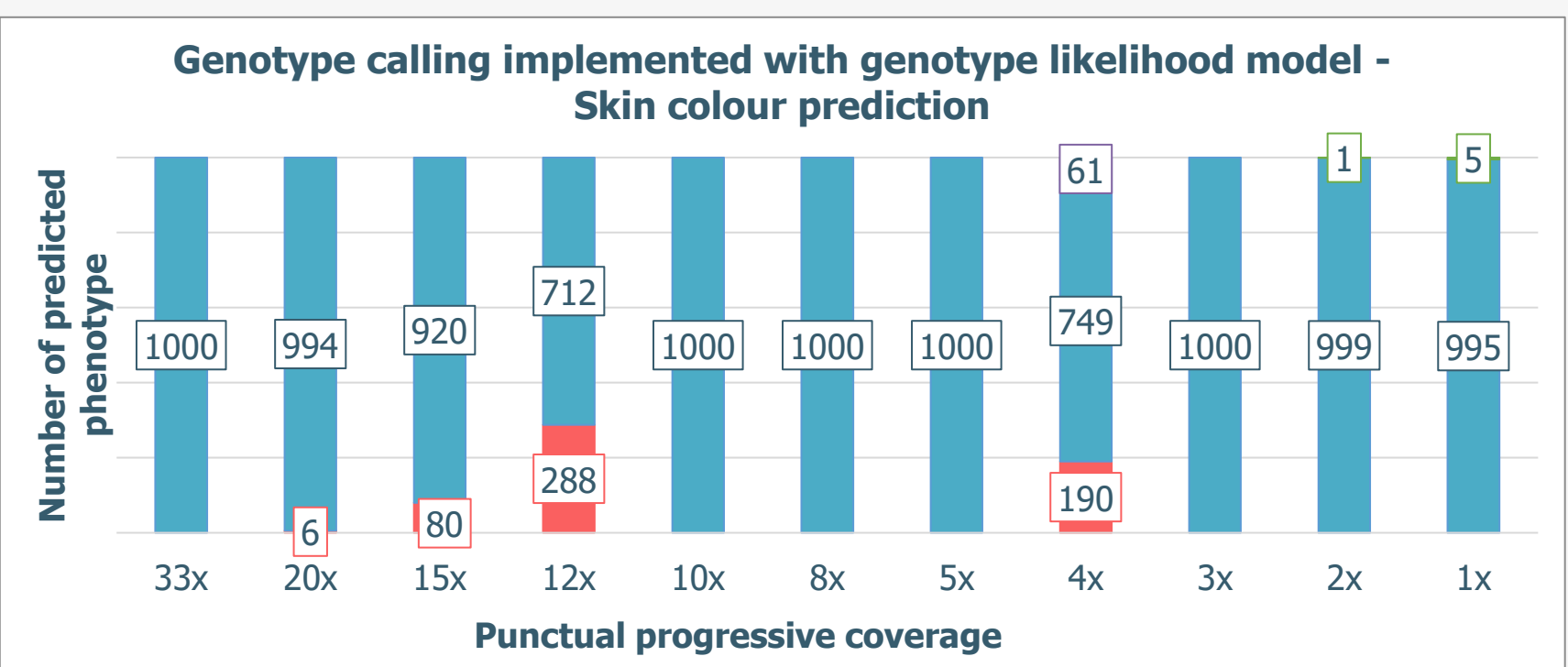
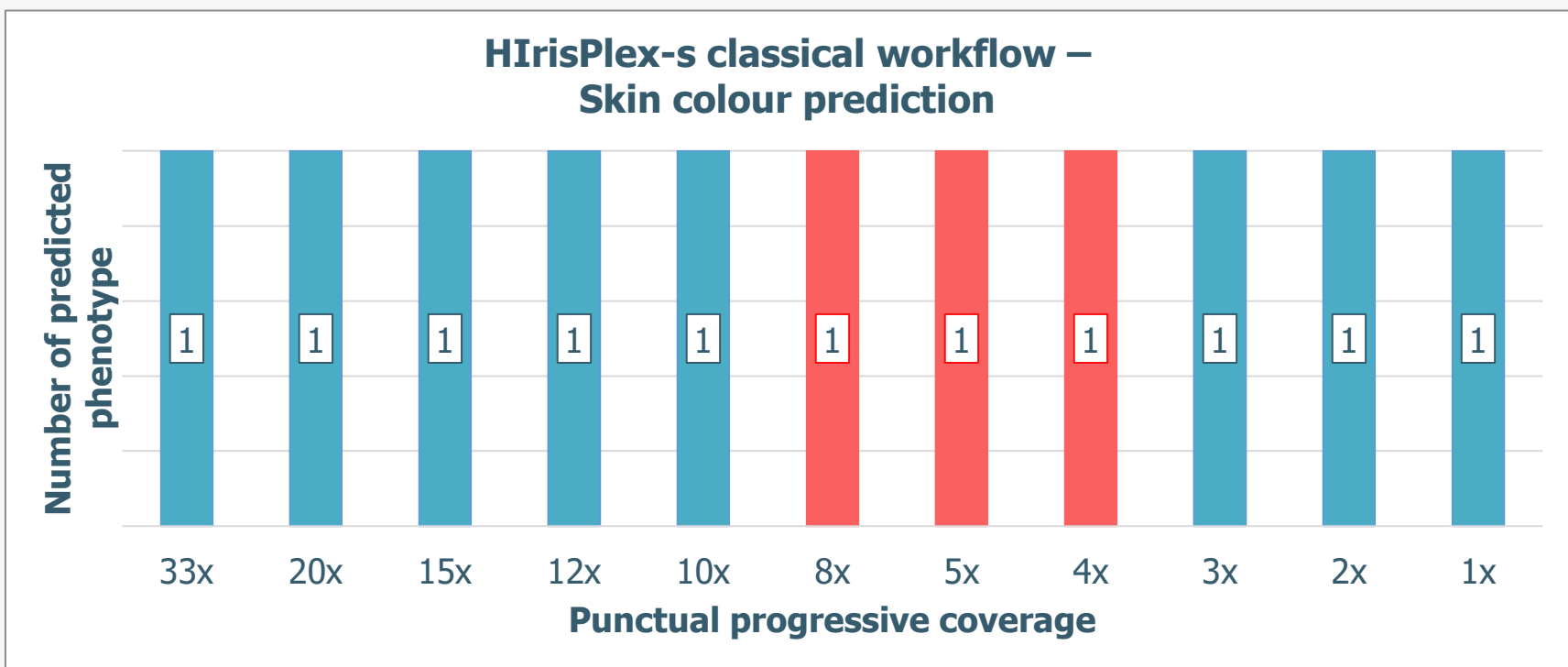


Methods

1. Test sample selection: Ust'-Ishim (Palaeolithic period), SF12 (Mesolithic period), and I5835 (Bronze Age).
2. Downsampling of the data from the maximum coverage at HIRisPlex-S positions across various coverage levels.
3. Genotype Analysis.
4. Phenotypic Prediction of 1,000 phenotypic traits.
5. Comparative Analysis against the standard HIRisPlex-S workflow and the imputation method.
6. Dataset Application.

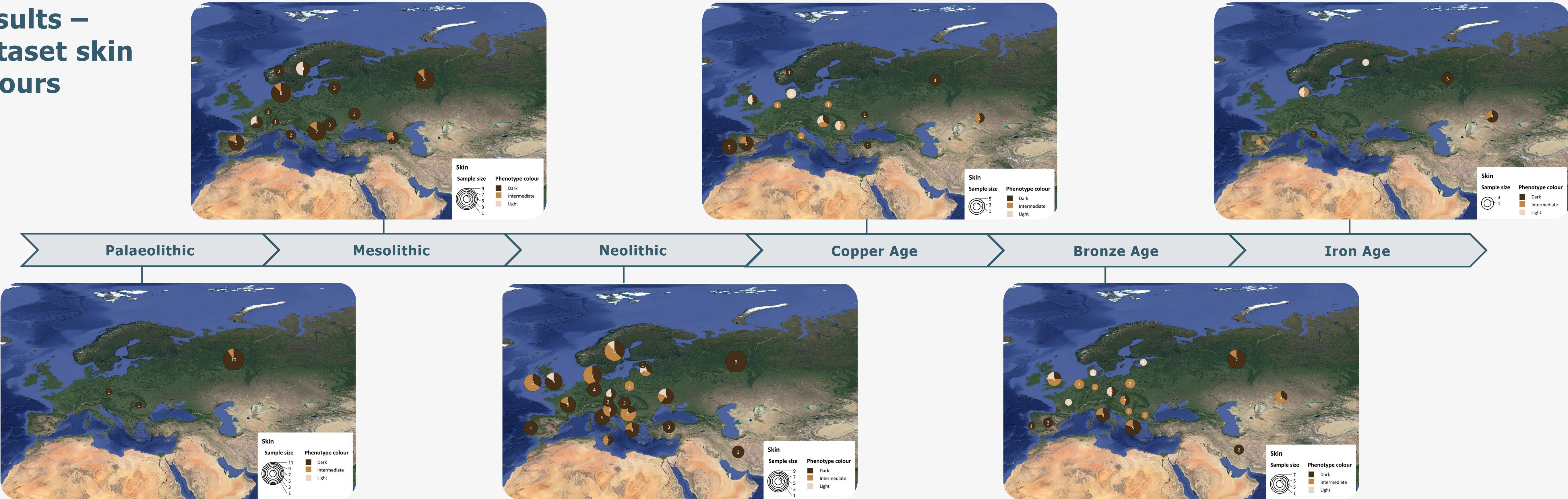


Results – Skin colour



Phenotype A Phenotype B Phenotype C Phenotype D Phenotype E Not predicted

Results – Dataset skin colours



Conclusions

- The HIRisPlex-S Protocol, when combined with genotype calling using a genotype likelihood model, demonstrates the highest robustness and accuracy in inferring phenotypic traits, particularly at lower coverage levels. This method accounts for all 10 possible genotypes, which can lead to various allelic combinations and, consequently, different inferred phenotypes. Unlike other methods that may fail or produce incorrect phenotypes, the novel methodology based on genotype likelihoods can accurately present the phenotype of interest, even at low percentages, while also providing alternative potential phenotypes and assessing the reliability of the prediction. Consequently, it offers more robust phenotypic inference, addresses call uncertainty, supports a greater number of predictions, and indicates when the phenotype cannot be determined with certainty.
- For the first time, a dataset encompassing both European and Asian samples from the Palaeolithic to the Iron Age has been utilized to predict phenotypic traits. The application of the protocol to this dataset of 348 Eurasian individuals revealed a complex evolution of physical traits. Dark phenotypes were initially predominant, while light phenotypes began to emerge during the Mesolithic period. The Neolithic period introduced greater phenotypic variability. Although dark phenotypes persisted through the Copper and Bronze Ages, intermediate and light variants increased, culminating in a heterogeneous phenotypic landscape by the Iron Age.