# Joint estimates of heterozygosity and runs of homozygosity for modern and ancient samples 

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## 1 Supplementary Results

### 1.1 Simulated data

### 1.1.1 Local estimates of heterozygosity

## ROHan local h estimates at $\mathrm{Ne}=3000$ at 3 X



Figure 1: Comparison between the simulated local rates of heterozygosity versus the predicted one on windows of $1 \mathrm{Mbp}, N_{e}=3000$, a coverage of 3 X using A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña. The red dot represents the maximum-likelihood point estimate, the black whiskers represent the $95 \%$ confidence interval and the dark blue crosses represent the simulated value.


Figure 2: Comparison between the simulated local rates of heterozygosity versus the predicted one on windows of $1 \mathrm{Mbp}, N_{e}=9000$, a coverage of 3 X using A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña. The red dot represents the maximum-likelihood point estimate, the black whiskers represent the $95 \%$ confidence interval and the dark blue crosses represent the simulated value.

## ROHan local h estimates at $\mathrm{Ne}=3000$ at 5 X



Figure 3: Comparison between the simulated local rates of heterozygosity versus the predicted one on windows of $1 \mathrm{Mbp}, N_{e}=3000$, a coverage of 5 X using A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña. The red dot represents the maximum-likelihood point estimate, the black whiskers represent the $95 \%$ confidence interval and the dark blue crosses represent the simulated value.

## ROHan local $h$ estimates at $\mathrm{Ne}=9000$ at 5 X



Figure 4: Comparison between the simulated local rates of heterozygosity versus the predicted one on windows of $1 \mathrm{Mbp}, N_{e}=9000$, a coverage of 5 X using A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña. The red dot represents the maximum-likelihood point estimate, the black whiskers represent the $95 \%$ confidence interval and the dark blue crosses represent the simulated value.

## ROHan local $h$ estimates at $\mathrm{Ne}=3000$ at 9 X



Figure 5: Comparison between the simulated local rates of heterozygosity versus the predicted one on windows of $1 \mathrm{Mbp}, N_{e}=3000$, a coverage of 9 X using A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña. The red dot represents the maximum-likelihood point estimate, the black whiskers represent the $95 \%$ confidence interval and the dark blue crosses represent the simulated value.


Figure 6: Comparison between the simulated local rates of heterozygosity versus the predicted one on windows of $1 \mathrm{Mbp}, N_{e}=9000$, a coverage of 9 X using A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña. The red dot represents the maximum-likelihood point estimate, the black whiskers represent the $95 \%$ confidence interval and the dark blue crosses represent the simulated value.
1.1.2 Global estimates of heterozygosity
1.1.2.1 ROHan

ROHan $\theta$ estimates at $\mathrm{Ne}=3000$


Figure 7: Simulated versus predicted genome-wide $\theta$ by ROHan for a simulated chromosome of 15 Mbp and an effective population size of 3000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

ROHan $\theta$ estimates at $\mathrm{Ne}=5000$


Figure 8: Simulated versus predicted genome-wide $\theta$ by ROHan for a simulated chromosome of 15 Mbp and an effective population size of 5000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

ROHan $\theta$ estimates at $\mathrm{Ne}=7000$


Figure 9: Simulated versus predicted genome-wide $\theta$ by ROHan for a simulated chromosome of 15 Mbp and an effective population size of 7000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

ROHan $\theta$ estimates at $\mathrm{Ne}=9000$


Figure 10: Simulated versus predicted genome-wide $\theta$ by ROHan for a simulated chromosome of 15 Mbp and an effective population size of 9000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

ROHan $\theta$ estimates at $\mathrm{Ne}=12000$


Figure 11: Simulated versus predicted genome-wide $\theta$ by ROHan for a simulated chromosome of 15 Mbp and an effective population size of 12000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.
1.1.2.2 ATLAS

ATLAS $\theta$ estimates at $\mathrm{Ne}=3000$


Figure 12: Simulated versus predicted genome-wide $\theta$ by ATLAS for a simulated chromosome of 15 Mbp and an effective population size of 3000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

ATLAS $\theta$ estimates at $\mathrm{Ne}=5000$


Figure 13: Simulated versus predicted genome-wide $\theta$ by ATLAS for a simulated chromosome of 15 Mbp and an effective population size of 5000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

## ATLAS $\theta$ estimates at $\mathrm{Ne}=7000$



Figure 14: Simulated versus predicted genome-wide $\theta$ by ATLAS for a simulated chromosome of 15 Mbp and an effective population size of 7000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

## ATLAS $\theta$ estimates at $\mathrm{Ne}=9000$



Figure 15: Simulated versus predicted genome-wide $\theta$ by ATLAS for a simulated chromosome of 15 Mbp and an effective population size of 9000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.


Figure 16: Simulated versus predicted genome-wide $\theta$ by ATLAS for a simulated chromosome of 15 Mbp and an effective population size of 12000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.
1.1.2.3 ANGSD

ANGSD $\theta$ estimates at $\mathrm{Ne}=3000$


Figure 17: Simulated versus predicted genome-wide $\theta$ by ANGSD for a simulated chromosome of 15 Mbp and an effective population size of 3000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

ANGSD $\theta$ estimates at $\mathrm{Ne}=5000$


Figure 18: Simulated versus predicted genome-wide $\theta$ by ANGSD for a simulated chromosome of 15 Mbp and an effective population size of 5000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

## ANGSD $\theta$ estimates at $\mathrm{Ne}=7000$



Figure 19: Simulated versus predicted genome-wide $\theta$ by ANGSD for a simulated chromosome of 15 Mbp and an effective population size of 7000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

ANGSD $\theta$ estimates at $\mathrm{Ne}=9000$


Figure 20: Simulated versus predicted genome-wide $\theta$ by ANGSD for a simulated chromosome of 15 Mbp and an effective population size of 9000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.


Figure 21: Simulated versus predicted genome-wide $\theta$ by ANGSD using transversions only for a simulated chromosome of 15 Mbp and an effective population size of 9000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

## ANGSD $\theta$ estimates at $\mathrm{Ne}=9000$



Figure 22: Simulated versus predicted genome-wide $\theta$ by ANGSD using options "-tole $10 \mathrm{e}-12$ maxIter 200 " for a simulated chromosome of 15 Mbp and an effective population size of 9000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of singlestranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.


Figure 23: Simulated versus predicted genome-wide $\theta$ by ANGSD using transversions and options "-tole $10 \mathrm{e}-12$-maxIter 200 " only for a simulated chromosome of 15 Mbp and an effective population size of 9000 . The dotted line represented the simulated rate of heterozygosity. The different subpanels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

ANGSD $\theta$ estimates at $\mathrm{Ne}=12000$


Figure 24: Simulated versus predicted genome-wide $\theta$ by ANGSD for a simulated chromosome of 15 Mbp and an effective population size of 12000 . The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.


Figure 25: Simulated versus predicted genome-wide $\theta$ by ANGSD when a certain subsampling of the data is performed. The length of the chromosome was subsampled as well as the coverage. The simulated values for $15 \mathrm{M}, 30 \mathrm{M}, 60 \mathrm{M}, 120 \mathrm{M}$ and 250 M are found in the upper portion followed by a subsampling of the coverage at 25X and finally, 10X.
1.1.3 Ignoring deamination from the computation

ROHan $\theta$ estimates while ignoring deamination at $\mathrm{Ne}=9000$


Figure 26: Simulated versus predicted genome-wide $\theta$ by ROHan while ignoring deamination in the computation for a simulated chromosome of 15 Mbp and an effective population size of 9000 . This was evaluated to verify whether ignoring the rates of deamination would have a significant impact. The dotted line represents the measured simulated rate of heterozygosity. The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different rates of simulated ancient DNA damage. A) no aDNA damage B) low rates of single-stranded damage from Ust'-Ishim C) high rates of double-stranded damage patterns from ATP2 D) medium rates of double-stranded damage from LaBraña.

### 1.1.4 Incorrectly inferring deamination rates

## Effect of incorrectly estimating damage rates on the $\theta$ estimate



Figure 27: Simulated versus predicted genome-wide $\theta$ by ROHan if the incorrect deamination rates are supplied. On a dataset of 15 Mbp , a effective population of 9000 was used and the high damage rates from the ATP2 sample were applied. The measured rates of damage were multiplied by a factor (ranging from 0.3 to 1.8 ) and ROHan was supplied these incorrect rates of damage. The dotted line corresponds to the simulated rate of heterozygosity. As expected, our results show that an overestimate of deamination rates (factor $>1.0$ ) causes an underestimate of $\theta$ and an underestimate of deamination rates (factor $<1.0$ ) causes an overestimate of $\theta$. However, our results show that underestimates ranging from $80 \%$ to $120 \%$ do not cause a significant error in the estimation of $\theta$. While there is a certain robustness to incorrect estimates of damage, care should be taken while estimating those rates and programs to do so are provided with the software package. Namely, script to mask potentially polymorphic positions is provided and is evaluated on simulated data on page 1.1.5.
1.1.5 Error in inferring deamination rates

| coverage | position from the 5 ' end |  |  |  |  |  |  |  |  |  |  | position from the 3' end |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Rn | -1 | -2 | -3 | -4 | -5 | -6 | -7 | -8 | -9 | -10 | RMSD |
| 1 | 0.57 | 0.52 | 0.54 | 0.51 | 0.51 | 0.50 | 0.49 | 0.51 | 0.46 | 0.50 | 0.07 | 0.54 | 0.54 | 0.52 | 0.50 | 0.50 | 0.52 | 0.51 | 0.50 | 0.49 | 0.51 | 0.07 |
| 2 | 0.74 | 0.70 | 0.71 | 0.69 | 0.68 | 0.67 | 0.68 | 0.68 | 0.66 | 0.67 | 0.04 | 0.73 | 0.73 | 0.72 | 0.71 | 0.71 | 0.72 | 0.72 | 0.71 | 0.69 | 0.70 | 0.04 |
| 3 | 0.81 | 0.78 | 0.78 | 0.77 | 0.76 | 0.76 | 0.76 | 0.76 | 0.74 | 0.75 | 0.03 | 0.81 | 0.82 | 0.81 | 0.80 | 0.80 | 0.80 | 0.80 | 0.81 | 0.79 | 0.79 | 0.03 |
| 4 | 0.85 | 0.82 | 0.81 | 0.80 | 0.80 | 0.79 | 0.80 | 0.80 | 0.78 | 0.79 | 0.03 | 0.84 | 0.86 | 0.85 | 0.84 | 0.84 | 0.85 | 0.84 | 0.85 | 0.83 | 0.84 | 0.02 |
| 5 | 0.86 | 0.84 | 0.83 | 0.82 | 0.81 | 0.81 | 0.82 | 0.82 | 0.80 | 0.81 | 0.02 | 0.86 | 0.88 | 0.86 | 0.86 | 0.86 | 0.86 | 0.86 | 0.87 | 0.85 | 0.86 | 0.02 |
| 6 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.82 | 0.82 | 0.82 | 0.81 | 0.82 | 0.02 | 0.87 | 0.89 | 0.88 | 0.87 | 0.87 | 0.88 | 0.87 | 0.88 | 0.87 | 0.88 | 0.02 |
| 7 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.83 | 0.83 | 0.82 | 0.81 | 0.82 | 0.02 | 0.88 | 0.89 | 0.88 | 0.87 | 0.87 | 0.88 | 0.88 | 0.88 | 0.87 | 0.88 | 0.02 |
| 8 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.83 | 0.82 | 0.82 | 0.81 | 0.82 | 0.02 | 0.88 | 0.89 | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 | 0.87 | 0.02 |
| 9 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.83 | 0.82 | 0.82 | 0.81 | 0.81 | 0.02 | 0.88 | 0.89 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.02 |
| 10 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.83 | 0.82 | 0.82 | 0.81 | 0.81 | 0.02 | 0.88 | 0.89 | 0.88 | 0.88 | 0.88 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.02 |
| 11 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.83 | 0.82 | 0.82 | 0.82 | 0.82 | 0.02 | 0.89 | 0.89 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.02 |
| 12 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.83 | 0.82 | 0.83 | 0.82 | 0.82 | 0.02 | 0.89 | 0.89 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.87 | 0.88 | 0.87 | 0.02 |
| 13 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.83 | 0.81 | 0.83 | 0.81 | 0.82 | 0.02 | 0.88 | 0.89 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.87 | 0.88 | 0.87 | 0.02 |
| 14 | 0.87 | 0.84 | 0.84 | 0.83 | 0.83 | 0.83 | 0.81 | 0.82 | 0.81 | 0.81 | 0.02 | 0.88 | 0.89 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.88 | 0.88 | 0.87 | 0.02 |
| 15 | 0.86 | 0.84 | 0.84 | 0.82 | 0.82 | 0.83 | 0.81 | 0.82 | 0.81 | 0.81 | 0.02 | 0.88 | 0.89 | 0.88 | 0.86 | 0.88 | 0.87 | 0.87 | 0.88 | 0.87 | 0.87 | 0.02 |
| 16 | 0.86 | 0.84 | 0.83 | 0.82 | 0.82 | 0.82 | 0.80 | 0.82 | 0.81 | 0.81 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.88 | 0.86 | 0.02 |
| 17 | 0.86 | 0.84 | 0.83 | 0.82 | 0.82 | 0.82 | 0.80 | 0.82 | 0.81 | 0.81 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.88 | 0.86 | 0.02 |
| 18 | 0.86 | 0.84 | 0.83 | 0.82 | 0.82 | 0.82 | 0.80 | 0.81 | 0.81 | 0.80 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.86 | 0.87 | 0.87 | 0.86 | 0.02 |
| 19 | 0.86 | 0.84 | 0.83 | 0.82 | 0.82 | 0.81 | 0.80 | 0.81 | 0.81 | 0.80 | 0.03 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.86 | 0.02 |
| 20 | 0.86 | 0.84 | 0.83 | 0.82 | 0.82 | 0.81 | 0.80 | 0.81 | 0.81 | 0.80 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.02 |
| 21 | 0.86 | 0.84 | 0.84 | 0.82 | 0.82 | 0.82 | 0.80 | 0.80 | 0.80 | 0.80 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.02 |
| 22 | 0.86 | 0.84 | 0.83 | 0.82 | 0.82 | 0.82 | 0.80 | 0.81 | 0.80 | 0.80 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.02 |
| 23 | 0.86 | 0.84 | 0.83 | 0.82 | 0.82 | 0.82 | 0.80 | 0.80 | 0.80 | 0.80 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.02 |
| 24 | 0.86 | 0.84 | 0.84 | 0.82 | 0.82 | 0.82 | 0.80 | 0.81 | 0.80 | 0.80 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.02 |
| 25 | 0.86 | 0.84 | 0.84 | 0.82 | 0.83 | 0.82 | 0.80 | 0.80 | 0.80 | 0.80 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.02 |
| 26 | 0.86 | 0.84 | 0.83 | 0.82 | 0.83 | 0.82 | 0.80 | 0.80 | 0.79 | 0.79 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.86 | 0.87 | 0.87 | 0.87 | 0.87 | 0.02 |
| 27 | 0.86 | 0.84 | 0.83 | 0.82 | 0.82 | 0.82 | 0.81 | 0.81 | 0.79 | 0.80 | 0.02 | 0.88 | 0.89 | 0.87 | 0.86 | 0.87 | 0.86 | 0.87 | 0.88 | 0.87 | 0.88 | 0.02 |

[^0]| coverage | position from the 5' end |  |  |  |  |  |  |  |  |  |  | position from the 3' end |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | RMSD | -1 | -2 | -3 | -4 | -5 | -6 | -7 | -8 | -9 | -10 | RMSD |
| 1 | 0.66 | 0.63 | 0.64 | 0.62 | 0.62 | 0.61 | 61 | 0.63 | 0.59 | 0.62 | 0.05 | 0.61 | 0.61 | 0.59 | 0.58 | 0.57 | 0.61 | 0.59 | 57 | 0.5 | 0.58 | 0.05 |
| 2 | 0.86 | 0.84 | 0.85 | 0.8 | 0.83 | 0.83 | 0.84 | 0.85 | 0.8 | 0.84 | 0.02 | 0.8 | 0.83 | 0.82 | 0.8 | 0.81 | 0.84 | 0.83 | 0.81 | 0.80 | 0.80 | . 02 |
| 3 | 0.95 | 0.93 | 0.93 | 0.94 | 0.92 | 0.93 | 0.94 | 0.94 | 0.93 | 0.94 | 0.01 | 0.92 | 0.92 | 0.93 | 0.93 | 0.92 | 0.93 | 0.93 | 0.92 | 0.92 | 0.91 | 0.01 |
| 4 | 0.99 | 0.98 | 0.97 | 0.98 | 0.97 | 0.97 | 0.98 | 0.99 | 0.99 | 0.99 | 0.00 | 0.96 | 0.97 | 0.97 | 0.98 | 0.96 | 0.98 | 0.97 | 0.97 | 0.96 | 0.95 | 0.00 |
| 5 | 1.01 | 1.00 | 0.99 | 1.00 | 0.99 | 0.99 | 1.02 | 1.01 | 1.01 | 1.02 | 0.00 | 0.98 | 0.99 | 0.99 | 0.99 | 0.99 | 1.00 | 0.99 | 0.99 | 0.98 | 0.98 | . 00 |
| 6 | 1.02 | 1.01 | 1.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.02 | 1.02 | 1.02 | 0.00 | 1.00 | 1.00 | 1.01 | 1.01 | 1.00 | 1.02 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 |
| 7 | 1.02 | 1.01 | 1.00 | 1.01 | 1.01 | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 0.00 | 1.00 | 1.00 | 1.01 | 1.01 | 1.00 | 1.02 | 1.01 | 1.00 | 1.01 | 1.00 | 0.00 |
| 8 | 1.02 | 1.01 | 00 | 1.01 | 01 | . 02 | 1.02 | 1.02 | . 02 | 1.03 | 0.00 | 1.01 | 1.00 | 1.02 | 1.01 | 1.01 | 1.02 | 1.01 | 1.0 | 1.01 | 0.99 | 0.00 |
| 9 | 1.02 | 1.01 | 1.00 | 1.01 | 1.01 | 1.02 | 1.02 | 1.02 | 1.03 | 1.02 | 0.00 | 1.01 | 1.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.01 | 1.00 | 1.02 | 1.00 | 0.00 |
| 10 | 1.02 | 1.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.01 | 1.02 | 1.03 | 1.02 | 0.00 | 1.01 | 1.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.01 | 1.00 | 1.01 | 1.00 | 0.00 |
| 11 | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 | 1.02 | 1.01 | 1.02 | 1.03 | 1.02 | 0.00 | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 | 1.02 | 1.01 | 1.00 | 1.02 | 0.99 | 0.00 |
| 12 | 1.01 | 1.01 | 1.01 | , 01 | 1.01 | 1.02 | 1.01 | . 03 | 1.03 | . 02 | . 00 | . 01 | 1.01 | 1.01 | 1.01 | 1.01 | 1.0 | 1.01 | 1.00 | 1.0 | 99 | 0.00 |
| 13 | 1.01 | 1.01 | 1.00 | 1.01 | 1.01 | 1.02 | 1.01 | 1.03 | 1.03 | 1.03 | 0.00 | 1.01 | 1.01 | 1.01 | 1.00 | 1.01 | 1.02 | 1.01 | 1.00 | 1.02 | 99 | 0.00 |
| 14 | 1.01 | 1.01 | 1.00 | 1.00 | 1.00 | 1.02 | 1.01 | 1.02 | 1.02 | 1.02 | 0.00 | 1.01 | 1.00 | 1.01 | 1.00 | 1.01 | 1.02 | 1.01 | 1.00 | 1.01 | 0.99 | 0.00 |
| 15 | 1.01 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 1.02 | 1.02 | 1.02 | 0.00 | 1.01 | 1.00 | 1.01 | 1.00 | 1.01 | 1.01 | 1.00 | 1.00 | 1.01 | 0.99 | 0.00 |
| 16 | 1. | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 0.99 | 1.01 | 1.02 | 1.02 | 0.00 | . 00 | 1.01 | 1.00 | 1.00 | 1.00 | 1.01 | 1.0 | 1.0 | 1.01 | 0.99 | 0.00 |
| 17 | 1.00 | 1.00 | 1.00 | 99 | 1.00 | . 01 | 99 | 1.01 | 1.03 | 1.02 | 0.0 | . 00 | 1.00 | 1.00 | 1.00 | 1.0 | 1.01 | 1.00 | 0.99 | 1.01 | 0.98 | 0.00 |
| 18 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.01 | 0.99 | 1.01 | 1.03 | 1.01 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 1.00 | 1.00 | 0.98 | 0.00 |
| 19 | , 0 | 1.00 | 1.00 | 0.99 | 0.99 | 1.00 | 0.99 | 1.00 | 1.02 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.01 | 1.00 | 0.99 | 1.00 | 0.99 | . 00 |
| 20 | 1.00 | 1.00 | 1.00 | 0.99 | 0.99 | 1.00 | 0.99 | 1.00 | 1.02 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 0.99 | 1.00 | 0.99 | 0.00 |
| 21 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.01 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 0.00 |
| 22 | , | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.01 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 0.99 | 1.00 | 0.99 | 0.00 |
| 23 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.01 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 0.99 | 1.00 | 0.99 | 0.00 |
| 24 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 0.00 |
| 25 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 0.00 |
| 26 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 |

[^1]| coverage | $\theta \times 10^{4}$ | $\theta_{\text {low }} \times$ <br> $10^{4}$ | $\theta_{\text {high }} \times$ <br> $10^{4}$ |
| :--- | :--- | :--- | :--- |
| 1 | 47.65 | 41.20 | 50.00 |
| 2 | 32.08 | 20.12 | 39.67 |
| 3 | 8.75 | 2.65 | 16.97 |
| 4 | 6.13 | 2.46 | 10.69 |
| 5 | 6.38 | 2.90 | 10.10 |
| 6 | 6.06 | 3.43 | 9.37 |
| 6 | 5.99 | 4.05 | 8.66 |
| 7 | 6.14 | 4.30 | 8.24 |
| 8 | 5.90 | 4.43 | 8.06 |
| 9 | 5.92 | 4.31 | 8.18 |
| 10 | 6.06 | 4.41 | 8.43 |
| 11 | 6.15 | 4.13 | 7.94 |
| 12 | 5.78 | 4.39 | 8.54 |
| 13 | 6.00 | 4.21 | 8.62 |
| 14 | 6.53 | 4.11 | 8.55 |
| 15 | 5.91 | 3.75 | 8.19 |
| 16 | 5.91 | 4.41 | 8.05 |
| 17 | 6.13 | 4.28 | 7.87 |
| 18 | 5.93 | 4.54 | 7.45 |
| 18 | 6.04 | 4.50 | 8.51 |
| 19 | 5.81 | 4.66 | 7.73 |
| 20 | 6.13 | 4.44 | 8.11 |
| 21 | 6.07 | 4.38 | 8.47 |
| 22 | 6.21 | 4.54 | 7.98 |
| 23 | 5.98 | 4.36 | 7.65 |
| 24 | 6.20 | 4.40 | 7.92 |
| 25 | 6.19 | 4.41 | 7.76 |
| 26 | 6.15 | 4.58 | 8.13 |
| 27 | 6.08 | 4.21 | 8.26 |

Table 3: Predicted $\theta$ using ROHan on simulated sample of 15 M using an effective population size of 9000 . The aDNA damage was simulated using the high rates of misincorporations from the ATP2 sample. The simulated $\theta$ for this dataset was of 6.19 segregating sites per $10^{4}$. Damage patterns were evaluated using a script provided with the software package which masks potentially polymorphic sites. The underestimate in estimating aDNA damage seen at coverage 1X-3X (see Supplementary Table 3) causes overestimates. Currently, our method cannot estimate substitutions due to aDNA damage highly deaminated samples at 1X-3X while masking potentially polymorphic positions.
1.1.6 Simulating multiple libraries with different damage rates

## Estimate of $\theta$ at various coverage for $\mathrm{Ne}=9000$

## with a mixed library



Figure 28: Simulated versus predicted genome-wide $\theta$ by ROHan for a simulated dataset which was composed of a $50 \% / 50 \%$ blend of a highly deaminated library from the ATP2 sample and a non-deaminated one. Damage rates were evaluated on this new dataset and were intermediate between the damage rates of the 2 original datasets.

### 1.1.7 Different window sizes



Figure 29: Effect of using different windows for the estimation of local heterozygosity on the estimate for the genome-wide estimate of $\theta$. No aDNA damage was added and an effective population size of 3000 was used. The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different window sizes A) 1 kbp B) 2.5 kbp C) 5 kbp D$) 1 \mathrm{Mbp}$.


Figure 30: Effect of using different windows for the estimation of local heterozygosity on the estimate for the genome-wide estimate of $\theta$. No aDNA damage was added and an effective population size of 9000 was used. The dotted line represented the simulated rate of heterozygosity. The different sub-panels represent different window sizes A) 1 kbp B) 2.5 kbp C) 5 kbp D$) 1 \mathrm{Mbp}$.

### 1.1.8 High sequencing error rate



Figure 31: Robustness of our methodology for inferring heterozygosity rates to a substantial increase in sequencing errors. We increased the amount of simulated sequencing errors 10 -fold to reach a probability of error of $1.6 \%$ (please refer to Appendix Table 1). The amount of ancient DNA damage was the same as in previous sections: A) no simulated damage due to deamination B) damage levels from the Ust'-Ishim sample, which contains a low rate of misincorporations and followed patterns corresponding to a single-stranded library building protocol C) damage levels from the APT2 sample, which contains a high rate of misincorporations and followed patterns corresponding to a double-stranded library building protocol D ) damage levels from the LaBraña sample, which contains a medium rate of misincorporations and followed patterns corresponding to a double-stranded library building protocol.

## ANGSD $\theta$ estimates at $\mathrm{Ne}=9000$ with high rate of sequencing errors



Figure 32: Robustness of ANGSD $\theta$ estimate to a substantial increase in sequencing errors without any additional simulated deamination. We increased the amount of simulated sequencing errors 10 -fold to reach a probability of error of $1.6 \%$ (please refer to Appendix Table 11). The results for this dataset without additional sequencing errors is found in Supplementary Figure 20A).

### 1.1.9 Identifying runs of homozygosity



Figure 33: Presence or absence of segregating sites on the simulated chromosomes using windows of A) 1 kbp B) 2.5 kbp C$) 5 \mathrm{kbp}$ using the inbreeding scenario 1 (inbreeding between siblings). For the evaluation of BCFtools/RoH, the lineages between the 16 chromosomes to form the grand-parents chromosomes and the 1000 chromosomes which provide allele frequencies is at 0 years.
1.1.9.1 ROHan


Figure 34: ROHan's accuracy in predicting the percentage of genomic regions in an ROH for a chromosome of 250 Mbp using A) inbreeding scenario 1 (inbreeding between siblings) B) inbreeding scenario 2 (inbreeding between a grandparent and a grandchild) C) using inbreeding scenario 3 (inbreeding between first cousins). As coverage increases, the greater the accuracy in predicting ROHs. ROHan was used with a window of 1 Mbp for the local heterozygosity estimates. The different dotted lines represent the measured percentage of genomic windows in an ROH at different genomic window sizes. The blue dots represent the maximum-likelihood point estimate, the black whiskers represent the $95 \%$ confidence interval.

Posterior HMM decoding using ROHan at coverage:0.9X


Figure 35: Posterior decoding using ROHan at different window sizes for the computation of local heterozygosity. The average simulated coverage was of 0.9 X . The window sizes were A) 100kbp B) 250 kbp C) 500 kbp D) 1 Mbp . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome.





Figure 36: Posterior decoding using ROHan at different window sizes for the computation of local heterozygosity. The average simulated coverage was of 2.1 X . The window sizes were A) 100kbp B) 250 kbp C) 500 kbp D) 1 Mbp . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome.


Figure 37: Posterior decoding using ROHan at different window sizes for the computation of local heterozygosity. The average simulated coverage was of 3.0 X . The window sizes were A) 100 kbp B) 250 kbp C) 500 kbp D) 1 Mbp . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome.


Figure 38: Posterior decoding using ROHan at different window sizes for the computation of local heterozygosity. The average simulated coverage was of 5.1 X . The window sizes were A) 100 kbp B) 250 kbp C) 500 kbp D) 1 Mbp . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome.

Posterior HMM decoding using ROHan at coverage:9.9X


Figure 39: Posterior decoding using ROHan at different window sizes for the computation of local heterozygosity. The average simulated coverage was of 9.9 X . The window sizes were A) 100 kbp B) 250 kbp C) 500 kbp D) 1 Mbp . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome.


Figure 40: Posterior decoding using ROHan at different window sizes for the computation of local heterozygosity. The average simulated coverage was of 15 X . The window sizes were A) 100kbp B) 250 kbp C) 500 kbp D) 1 Mbp . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome.


Figure 41: Posterior decoding using ROHan at different window sizes for the computation of local heterozygosity. The average simulated coverage was of 24.3 X . The window sizes were A) 100 kbp B) 250 kbp C) 500 kbp D) 1 Mbp . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome.

### 1.1.9.2 PLINK

1.1.9.3 BCFtools/RoH


Figure 42: Posterior decoding using PLINK at different levels of simulated coverage namely: A) 0.9X B) 2.1X C) 3.0X D) 5.1X E) 9.9X F) 15X G) 24.3 X . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome. The lineages of the 16 chromosomes to form the grand-parents' chromosomes and the 1000 chromosomes which provide the allele frequency were jointed at a time of 0 years.


Figure 43: Posterior decoding using BCFtools/RoH at different levels of simulated coverage namely: A) 0.9 X B) 2.1 X C) 3.0 X D) 5.1 X E) 9.9 X F) 15 X G) 24.3 X . Please refer to Supplementary Figure 33 for the distribution of the segregating sites on the chromosome. The lineages of the 16 chromosomes to form the grand-parents' chromosomes and the 1000 chromosomes which provide the allele frequency were jointed at a time of 0 years.


Figure 44: Presence or absence of segregating sites on the simulated chromosomes using windows of A) 1 kbp B) 2.5 kbp C) 5 kbp using the inbreeding scenario 1 (inbreeding between siblings). For the evaluation of BCFtools/RoH, the lineages of the 16 chromosomes to form the grand-parents' chromosomes and the 1000 chromosomes which provide the allele frequency were jointed at a time of 150 k years.


Figure 45: Posterior decoding using BCFtools/RoH at different levels of simulated coverage namely: A) 0.9 X B) 2.1 X C) 3.0 X D) 5.1 X E) 9.9 X F) 15 X G) 24.3 X . Please refer to Supplementary Figure 44 for the distribution of the segregating sites on the chromosome. The lineages of the 16 chromosomes to form the grand-parents' chromosomes and the 1000 chromosomes which provide the allele frequency were jointed at a time of 150 k years.


Figure 46: Presence or absence of segregating sites on the simulated chromosomes using windows of A) 1 kbp B) 2.5 kbp C) 5 kbp using the inbreeding scenario 1 (inbreeding between siblings). For the evaluation of BCFtools/RoH, the lineages of the 16 chromosomes to form the grand-parents' chromosomes and the 1000 chromosomes which provide the allele frequency were jointed at a time of 500 k years.

Posterior HMM decoding using BCFtools (lineages joined at:500k years)


Figure 47: Posterior decoding using BCFtools/RoH at different levels of simulated coverage namely: A) 0.9 X B) 2.1 X C) 3.0 X D) 5.1 X E) 9.9 X F) 15 X G) 24.3 X . Please refer to Supplementary Figure 46 for the distribution of the segregating sites on the chromosome. The lineages of the 16 chromosomes to form the grand-parents' chromosomes and the 1000 chromosomes which provide the allele frequency were jointed at a time of 500 k years.

### 1.2 Empirical data

1.2.1 Humans

| ID | population | coverage | $\theta$ | $\theta_{\text {low }}$ | $\theta_{\text {high }}$ | ROH (\%) | SGDP $\theta$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| HG00707 | Southern Han Chinese (CHS) | 4.5 | 0.000713603 | 0.000545385 | 0.000924186 | 0 | 0.000792535 |
| HG00708 | Southern Han Chinese (CHS) | 4.9 | 0.000759869 | 0.000607272 | 0.000915707 | 0 | 0.000792535 |
| HG02364 | Chinese Dai in Xishuangbanna, | 7.1 | 0.000810391 | 0.00069312 | 0.000932023 | 0 | $0.000793714-0.00082176$ |
| HG02367 | China (CDX) | 7.2 | 0.000805949 | 0.000687336 | 0.000930138 | 0.138074 | 0.000793714-0.00082176 |
| HG02085 | Kinh in Ho Chi Minh City, | 8.2 | 0.000840848 | 0.000732537 | 0.00094835 | 0 | 0.000806996-0.000828772 |
| HG02086 | Vietnam (KHV) | 7.9 | 0.000859125 | 0.000751194 | 0.000977007 | 0 | 0.000806996-0.000828772 |
| NA19068 | Jap | 9.2 | 0.000867054 | 0.000750137 | 0.000990337 | 0 | 0.000801412-0.000826611 |
| NA19070 | Jap | 6.3 | 0.000961556 | 0.000819523 | 0.00110828 | 0 | $0.000801412-0.000826611$ |
| HG01974 | Peruvians from Lima, Peru | 11.0 | 0.000883585 | 0.000779036 | 0.000984885 | 0 |  |
| HG01976 | (PEL) | 11.0 | 0.000970756 | 0.000862864 | 0.00107667 | 0 | 0.00070688-0.000756678 |
| HG03708 | Punjabi from Lahore, Pakistan | 7.1 | 0.000896297 | 0.000768666 | 0.00102456 | 0 |  |
| HG03709 | (PJL) | 7.6 | 0.00091677 | 0.000805633 | 0.00103292 | 0 |  |
| NA21137 | Gujarati Indian from Houston, | 12.7 | 0.00101854 | 0.000920601 | 0.0011136 | 0 | $0.000863594{ }^{1}$ |
| NA21141 | Texas (GIH) | 7.8 | 0.000909886 | 0.000799777 | 0.00102094 | 0.0690369 | 0.000863594 |
| HG04225 | Indian Telugu from the UK | 6.7 | 0.000910065 | 0.000782637 | 0.00104459 | 0 | 0.000826589-0.000887523 ${ }^{2}$ |
| HG04222 | (ITU) | 8.2 | 0.000924757 | 0.000818621 | 0.00103226 | 0.172592 | .00082658-0.00088752 |
| HG04171 | Bengali from Bangladesh (BEB) | 8.1 | 0.000927666 | 0.000815712 | 0.00104155 | 0 | 0.00088649-0.000887424 |
| HG04173 | Bengali from Bangladesh (BEB) | 5.7 | 0.00114297 | 0.000979405 | 0.00131118 | 0 | 0.00088649-0.000887424 |
| HG04038 | Sri Lankan Tamil from the UK | 5.4 | 0.00118858 | 0.000993661 | 0.00137724 | 0 |  |
| HG04039 | (STU) | 6.0 | 0.00117458 | 0.00101575 | 0.00133913 | 0 |  |
| HG03136 | Esan | 8.3 | 0.00123733 | 0.00111082 | 0.00135873 | 0 | . 001 |
| HG03139 | Esan | 7.3 | 0.00114978 | 0.00101661 | 0.00128663 | 0.0345185 | , 001 |
| HG02891 | Gambian in Western Divisions | 6.9 | 0.00117041 | 0.00102119 | 0.001323 | 0 |  |
| HG02895 | in the Gambia (GWD) | 5.9 | 0.00118227 | 0.00102562 | 0.00133952 | 0 |  |
| HG02537 | African Caribbeans in Barbados | 16.7 | 0.00123678 | 0.00113072 | 0.00134187 | 0 | NA |
| HG02536 | (ACB) | 5.1 | 0.00133456 | 0.00113154 | 0.00153834 | 0 | NA |

Table 4: Comparison between the genome-wide $\theta$ obtained by ROHan on lower coverage samples from the 1000 Genomes project Phase III Genomes Project Consortium et al., 2015] data and the heterozygosity estimates obtained by the Simons Genome Diversity Project Mallick et al., 2016


Figure 48: Local estimate of heterozygosity and HMM posterior decoding for chromosomes 11 for the HG04222 individual.

Predicted $\theta$ for the Vindija sample at different levels of subsampling


Figure 49: Global estimate of $\theta$ for the Vindija 33.19 sample at different rate of subsampling . The deamination rates were evalutated using the script provided with the software where potentially polymorphic positions are masked.

| coverage | position from the 5 ' end |  |  |  |  |  |  |  |  |  |  | position from the 3' end |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Rmsd | -1 | -2 | -3 | -4 | -5 | -6 | -7 | -8 | -9 | -10 | MSD |
| 1 | 0.77 | 0.73 | 0.73 | 0.76 | 0.76 | 0.74 | 0.75 | 0.75 | 0.70 | 0.71 | 0.03 | 0.81 | 0.75 | 0.74 | 0.74 | 0.75 | 0.73 | 0.72 | 0.72 | 0.71 | 0.69 | 0.03 |
| 2 | 0.91 | 0.90 | 0.90 | 0.93 | 0.93 | 0.93 | 0.94 | 0.92 | 0.89 | 0.89 | 0.01 | 0.94 | 0.92 | 0.92 | 0.91 | 0.92 | 0.90 | 0.90 | 0.89 | 0.91 | 0.87 | 0.01 |
| 3 | 0.96 | 0.96 | 0.97 | 0.99 | 1.01 | 1.00 | 0.99 | 0.98 | 0.96 | 0.95 | 0.01 | 0.97 | 0.98 | 0.98 | 0.98 | 0.98 | 0.99 | 0.96 | 0.97 | 0.98 | 0.95 | 0.00 |
| 4 | 0.98 | 0.99 | 1.00 | 1.02 | 1.03 | 1.02 | 1.01 | 1.00 | 0.99 | 0.97 | 0.00 | 0.99 | 1.00 | 1.01 | 1.00 | 1.01 | 1.04 | 0.99 | 1.01 | 1.01 | 0.98 | 0.00 |
| 5 | 0.98 | 1.00 | 1.01 | 1.03 | 1.04 | 1.03 | 1.02 | 1.02 | 1.00 | 1.00 | 0.00 | 1.00 | 1.00 | 1.01 | 1.01 | 1.03 | 1.04 | 1.00 | 1.02 | 1.03 | 1.01 | 0.00 |
| 6 | 0.99 | 1.01 | 1.02 | 1.03 | 1.05 | 1.03 | 1.02 | 1.02 | 1.00 | 1.00 | 0.00 | 1.00 | 1.01 | 1.01 | 1.02 | 1.03 | 1.04 | 1.00 | 1.02 | 1.03 | 1.00 | 0.00 |
| 7 | 0.99 | 1.01 | 1.03 | 1.03 | 1.05 | 1.03 | 1.02 | 1.01 | 1.01 | 0.99 | 0.00 | 1.00 | 1.01 | 1.01 | 1.02 | 1.03 | 1.04 | 1.00 | 1.04 | 1.04 | 1.01 | 0.00 |
| 8 | 0.99 | 1.01 | 1.02 | 1.03 | 1.04 | 1.02 | 1.01 | 1.01 | 1.01 | 1.01 | 0.00 | 1.00 | 1.01 | 1.01 | 1.02 | 1.03 | 1.03 | 1.00 | 1.03 | 1.03 | 1.01 | 0.00 |
| 9 | 0.99 | 1.01 | 1.02 | 1.03 | 1.05 | 1.03 | 1.01 | 1.01 | 1.00 | 1.00 | 0.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.03 | 1.02 | 1.00 | 1.02 | 1.03 | 1.00 | 0.00 |
| 10 | 1.00 | 1.01 | 1.02 | 1.02 | 1.04 | 1.03 | 1.02 | 1.01 | 1.00 | 1.00 | 0.00 | 1.00 | 1.01 | 1.01 | 1.02 | 1.02 | 1.02 | 1.01 | 1.02 | 1.03 | 1.00 | 0.00 |
| 11 | 0.99 | 1.01 | 1.02 | 1.02 | 1.04 | 1.03 | 1.02 | 1.01 | 1.00 | 1.00 | 0.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.02 | 1.02 | 1.00 | 1.04 | 1.02 | 0.98 | 0.00 |
| 12 | 0.99 | 1.01 | 1.02 | 1.03 | 1.04 | 1.03 | 1.01 | 1.02 | 1.00 | 1.00 | 0.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.02 | 1.02 | 1.00 | 1.03 | 1.02 | 0.99 | 0.00 |
| 13 | 0.99 | 1.00 | 1.02 | 1.02 | 1.04 | 1.03 | 1.02 | 1.01 | 1.00 | 1.00 | 0.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.01 | 1.01 | 1.00 | 1.03 | 1.02 | 0.99 | 0.00 |
| 14 | 0.99 | 1.00 | 1.01 | 1.02 | 1.04 | 1.03 | 1.02 | 1.01 | 1.00 | 1.00 | 0.00 | 1.00 | 1.01 | 1.01 | 1.02 | 1.02 | 1.01 | 1.00 | 1.02 | 1.02 | 1.00 | 0.00 |
| 15 | 0.99 | 1.00 | 1.01 | 1.01 | 1.03 | 1.02 | 1.02 | 1.01 | 1.01 | 0.99 | 0.00 | 1.00 | 1.01 | 1.01 | 1.02 | 1.01 | 1.01 | 1.00 | 1.01 | 1.01 | 1.00 | 0.00 |
| 16 | 1.00 | 1.00 | 1.01 | 1.01 | 1.03 | 1.02 | 1.01 | 1.01 | 1.00 | 0.99 | 0.00 | 1.01 | 1.01 | 1.01 | 1.02 | 1.01 | 1.01 | 0.99 | 1.01 | 1.01 | 0.99 | 0.00 |
| 17 | 1.00 | 1.00 | 1.00 | 1.01 | 1.02 | 1.01 | 1.00 | 1.01 | 1.00 | 0.99 | 0.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 1.01 | 1.00 | 1.01 | 1.01 | 0.99 | 0.00 |
| 18 | 1.00 | 1.00 | 1.00 | 1.01 | 1.02 | 1.01 | 1.00 | 1.00 | 0.99 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.01 | 0.99 | 1.01 | 1.00 | 1.01 | 1.01 | 0.99 | 0.00 |
| 19 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.01 | 0.99 | 1.01 | 0.99 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.01 | 1.00 | 1.01 | 1.01 | 0.99 | 0.00 |
| 20 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 0.99 | 1.00 | 0.99 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.00 | 1.01 | 1.00 | 0.99 | 0.00 |
| 21 | 1.00 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 0.99 | 1.00 | 0.99 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.00 | 1.00 | 1.01 | 1.00 | 0.00 |
| 22 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 0.99 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.00 | 1.01 | 1.00 | 0.99 | 0.00 |
| 23 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.00 | 1.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 |

[^2]

Figure 50: Global estimate of $\theta$ at a different rate of subsampling to simulate different depths of coverage for the following modern human individuals from the Simons Genome Diversity Project: A) Bergamo (LP6005441-DNA_B06) B) Czech (LP6005443-DNA_H05)C) Japanese (LP6005441DNA_G06) D) Karitiana (LP6005441-DNA_G06) and E) Yoruba (LP6005442-DNA_A02). As expected, the rate of heterozygosity is highest in the Yoruba followed by the Czech, Bergamo, Japanese and finally the Karitiana. Our estimates are on par with those reported in the original publication Mallick et al., 2016. Our subsampling reveals that our estimates are robust to a depth of 3-4X for these data.

### 1.2.2 Horses

| Used ID | Full sample name | population | age | publication of origin |
| :---: | :---: | :---: | :---: | :---: |
| Arab_0237A | Arab_0237A_SAMN02439777 | Arabian | modern | Metzger et al., 2014 |
| ARUS_0222A | ARUS_0222A_CGG101397 | Yakutian | 200 yrs | Librado et al., 2015 |
| ARUS_0223A | ARUS_0223A_Batagai | Wild horse from Batagai | 5.2 k yrs | Librado et al., 2015 |
| ARUS_0224A | ARUS_0224A_CGG10022 | Wild horse from Taymyr | 43 k yrs | Schubert et al., 2014 |
| ARUS_0225A | ARUS_0225A_CGG10023 | Wild horse from Taymyr | 16k yrs | Schubert et al., 2014 |
| Borly4_PAVH11 | Borly4_PAVH11_CGG_018171 | Pavlodar site (Kazakhstan) | 5 k yrs | Gaunitz et al., 2018 |
| Borly4_PAVH4 | Borly4_PAVH4_CGG_018157 | Pavlodar site (Kazakhstan) | 5 k yrs | Gaunitz et al., 2018 |
| Borly4_PAVH8 | Borly4_PAVH8_CGG_018165 | Pavlodar site (Kazakhstan) | 5 k yrs | Gaunitz et al., 2018 |
| Botai2 | Botai2_CGG_1_018174 | Botai Culture | 5.5 k yrs | Gaunitz et al., 2018 |
| Botai5 | Botai5_CGG_018177 | Botai Culture | 5.5 k yrs | Gaunitz et al., 2018 |
| Botai6 | Botai6_CGG_018178 | Botai Culture | 5.5 k yrs | Gaunitz et al., 2018 |
| Icel_0247A | Icel_0247A_IS074 | Icelandic | modern | Jäderkvist et al., 2014 |
| Icel_0144A | Icel_0144A_P5782 | Icelandic | modern | Jäderkvist et al., 2014 |
| Jeju_0275A | Jeju_0275A_SAMN01057172 | Jeju Pony | modern | Kim et al., 2013 |
| Mong_0215A | Mong_0215A_TG1111D2628 | Mongolian | modern | Do et al., 2014 |
| Mong_0153A | Mong_0153A_KB7754 | Mongolian | modern | Der Sarkissian et al., 2015 |
| Prze_0150A | Prze_0150A_KB3879 | Przewalski | modern | Der Sarkissian et al., 2015 |
| Prze_0151A | Prze_0151A_KB7674 | Przewalski | modern | Der Sarkissian et al., 2015 |
| Prze_0157A | Prze_0157A_SB293 | Przewalski | modern | Der Sarkissian et al., 2015 |
| Prze_0158A | Prze_0158A_SB339 | Przewalski | modern | Der Sarkissian et al., 2015 |
| Prze_0159A | Prze_0159A_SB4329 | Przewalski | modern | Der Sarkissian et al., 2015 |
| Prze_0160A | Prze_0160A_SB533 | Przewalski | modern | Der Sarkissian et al., 2015 |
| SCYT_I_Ch118 | I_Ch118_CGG_1_016176 | Scythian kurgan | 2.3 k yrs | Librado et al., 2017 |
| SCYT_E_Ch25 | E_Ch25_CGG_1_016172 | Scythian kurgan | 2.3 k yrs | Librado et al., 2017 |
| SCYT_F_Ch26 | F_Ch26_CGG_1_016173 | Scythian kurgan | 2.3 k yrs | Librado et al., 2017 |
| Shet_0249A | Shet_0249A_SPH020 | Shetland Pony | modern | Frischknecht et al., 2015 |
| Shet_0250A | Shet_0250A_SPH041 | Shetland Pony | modern | Frischknecht et al., 2015 |
| Stan_0081A | Stan_0081A_M5256 | Standardbred | modern | Der Sarkissian et al., 2015 |
| Thor_0290A | Thor_0290A_SAMN01047706 | Thoroughbred | modern | Do et al., 2014 |
| Thor_0145A | Thor_0145A_Twilight | Thoroughbred | modern | Wade et al., 2009 |
| Yaku_0170A | Yaku_0170A_Yak8 | Yakutian | modern | Librado et al., 2015 |
| Yaku_0171A | Yaku_0171A_Yak9 | Yakutian | modern | Librado et al., 2015 |
| Yaku_0163A | Yaku_0163A_Yak1 | Yakutian | modern | Librado et al., 2015 |

Table 6: Population of origin, coverage and inferred fraction of the genome to be an ROH for the different horse presented in the main manuscript.

| Sample name | global $\theta$ estimate |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
|  | accounting for deamination in modern |  | standard estimate |  |  |  |
|  | mid | low | high | mid | low | high |
| Prze_0150A_KB3879 | 0.00116396 | 0.00105745 | 0.00126795 | 0.00121625 | 0.00111703 | 0.00133975 |
| Prze_0158A_SB339 | 0.00130449 | 0.00119473 | 0.0014413 | 0.00136348 | 0.00124967 | 0.00148758 |
| Prze_0159A_SB4329 | 0.00137299 | 0.00123522 | 0.0015161 | 0.00151808 | 0.00137978 | 0.00165049 |
| Prze_0160A_SB533 | 0.00108715 | 0.00097201 | 0.00120968 | 0.00123092 | 0.00111888 | 0.00134607 |
| Icel_0144A_P5782 | 0.00132952 | 0.00110264 | 0.00158799 | 0.00167654 | 0.00154191 | 0.00181988 |
| Thor_0145A_Twilight | 0.00107117 | 0.000954924 | 0.0012049 | 0.00109072 | 0.000995605 | 0.00119876 |
| Yaku_0163A_Yak1 | 0.00165856 | 0.00147891 | 0.00183537 | 0.0018521 | 0.00167155 | 0.00199765 |

Table 7: Effect of accounting for ancient DNA damage in modern samples. The $\theta$ was computed by disallowing ROHs to provide a global average.

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[^0]:    Table 1: Error made at different rate of subsampling in the estimate by masking polymorphic positions of C to T substitution at the 5' end and the G to A substitutions for the simulated data using the high rates of misincorporations from the ATP2 sample.The estimate of substitutions was computed by masking potentially polymorphic and was performed using a script provided with the software package. The number reported is the ratio of the deamination rate found at that position to the one simulated. The consistent underestimate is likely due to mapping issues of the heavily deaminated aDNA fragments. RMSD stands for root-mean-square deviation.

[^1]:    Table 2: Error made at different rate of subsampling in the estimate of C to T substitution at the 5 ' end and the G to A substitutions for the simulated data using the high rates of misincorporations from the ATP2 sample. The estimate of substitutions was computed by masking potentially polymorphic and was performed using a script provided with the software package. The number reported is the ratio of the deamination rate found at that position to the one found at 27 X at the same position. RMSD stands for root-mean-square deviation.

[^2]:    Table 5: Error made in the estimate of C to T substitution for the Vindija 33.19 sample at different rate of subsampling. The number reported is the ratio of the deamination rate found to the one found at 24 X at the same position. The estimate of substitutions was computed by masking potentially polymorphic and was performed using a script provided with the software package. RMSD stands for root-mean-square deviation.

