

1 **The evolutionary history of Neandertal and Denisovan** 2 **Y chromosomes**

3
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27 **Abstract**

28 Ancient DNA has allowed the study of various aspects of human history in unprecedented detail.
29 However, because the majority of archaic human specimens preserved well enough for genome
30 sequencing have been female, comprehensive studies of Y chromosomes of Denisovans and
31 Neandertals have not yet been possible. Here we present sequences of the first Denisovan Y
32 chromosomes (*Denisova 4* and *Denisova 8*), as well as the Y chromosomes of three late
33 Neandertals (*Spy 94a*, *Mezmaiskaya 2* and *El Sidrón 1253*). We find that the Denisovan Y
34 chromosomes split around 700 thousand years ago (kya) from a lineage shared by Neandertal and
35 modern human Y chromosomes, which diverged from each other around 370 kya. The
36 phylogenetic relationships of archaic and modern human Y chromosomes therefore differ from
37 population relationships inferred from their autosomal genomes, and mirror the relationships
38 observed on the level of mitochondrial DNA. This provides strong evidence that gene flow from
39 an early lineage related to modern humans resulted in the replacement of both the mitochondrial
40 and Y chromosomal gene pools in late Neandertals. Although unlikely under neutrality, we show
41 that this replacement is plausible if the low effective population size of Neandertals resulted in an
42 increased genetic load in their Y chromosomes and mitochondrial DNA relative to modern
43 humans.

44

45 **Introduction**

46 Ancient DNA (aDNA) has transformed our understanding of human evolutionary history,
47 revealing complex patterns of population migration, turnover and gene flow, including admixture
48 from archaic humans into modern humans around 55 thousand years ago (kya) (1–4). The majority
49 of insights into the relationships between archaic and modern humans have been based on
50 autosomal sequences, which represent a composite of genealogies of any individual’s ancestors.
51 Although mitochondrial DNA (mtDNA) and Y chromosomes only provide information about
52 single maternal and paternal lineages, they offer a unique perspective on various aspects of
53 population history such as sex-specific migration and other cultural phenomena (5–7).
54 Furthermore, because of their lower effective population size (N_e) compared to autosomal loci,
55 coalescent times of mtDNA and Y chromosomes sampled from two populations provide an upper
56 bound for the last time they experienced gene flow. In this respect, the mtDNA and autosomal
57 sequences of Neandertals, Denisovans and modern humans have revealed puzzling phylogenetic
58 discrepancies. Their autosomal genomes show that Neandertals and Denisovans are sister groups
59 that split from a common ancestor with modern humans between 550–765 kya (8). In contrast,
60 with the TMRCA with modern humans of 360–468 kya, mtDNAs of Neandertals are more similar
61 to the mtDNAs of modern humans than to those of Denisovans (9). Intriguingly, ~400 ky old early
62 Neandertals from Sima de los Huesos were shown to carry mitochondrial genomes related to
63 Denisovan mtDNAs, which is concordant with the autosomal relationships between these groups
64 of archaic humans (10, 11). Based on these results it has been suggested that Neandertals originally
65 carried a Denisovan-like mtDNA which was later completely replaced via gene flow from an early
66 lineage related to modern humans (9, 11).

67 Y chromosomes of Neandertals and Denisovans could provide an important additional
68 source of information about population splits and gene flow events between archaic and modern
69 humans or populations related to them. However, with the exception of a small amount of
70 Neandertal Y chromosome coding sequence (118 kb, (12)), none of the male Neandertals or
71 Denisovans studied to date have yielded sufficient amounts of endogenous DNA to allow
72 comprehensive studies of entire Y chromosomes.

73 **Specimens, DNA capture and genotyping**

74 Previous genetic studies identified two male Denisovans, *Denisova 4* (55–84 ky old) and *Denisova*
75 *8* (106–136 ky old) (13, 14), and two male late Neandertals, *Spy 94a* (38-39 ky old) and
76 *Mezmaiskaya 2* (43-45 ky old) (15) (Figure 1A). To enrich for hominin Y chromosome DNA of
77 these individuals, we designed DNA capture probes targeting ~6.9 Mb of the non-recombining
78 portion of the human Y chromosome sequence (Figure 1C, Supplementary Information). Using
79 these probes, we performed hybridization capture on selected single-stranded DNA libraries from
80 *Denisova 4*, *Denisova 8*, *Spy 94a*, and *Mezmaiskaya 2* (Supplementary Information). The captured
81 DNA molecules were sequenced from both ends, overlapping reads were merged and aligned to
82 the human reference genome (hg19/GRCh37) (Supplementary Information). Reads of at least 35
83 base-pair (bp) in length that aligned uniquely to the capture target regions were retained for further
84 analysis.

85 Of the total ~6.9 Mb of Y chromosome capture target regions, we generated 1.4X for
86 *Denisova 4*, 3.5X for *Denisova 8*, 0.8X for *Spy 94a* and 14.3X for *Mezmaiskaya 2* (Figure 1B,
87 Table S4.1). In addition, we sequenced 7.9X coverage of a smaller subset of the Y chromosome
88 of the ~46-53-ky-old *El Sidrón 1253* Neandertal from Spain (Figure 1B, Table S4.1) (16) by
89 capturing a set of previously generated double-stranded, UDG-treated libraries (17) using a ~560

90 kb capture array designed to study modern human Y chromosome variation (5). This data provides
91 an opportunity for validating our results with different sample preparation and capture strategies.

92 To call genotypes for the captured archaic human Y chromosomes, we leveraged the
93 haploid nature of the human Y chromosome and implemented a consensus approach that requires
94 90% of the reads observed at each site to agree on a single allele, restricting to sites covered by at
95 least three reads (Supplementary information; Table S5.1). This approach minimizes the impact of
96 aDNA damage on genotyping accuracy (Figure S5.1) while allowing for a small proportion of
97 sequencing errors, contamination or misalignment (Supplementary Information). To obtain a
98 reference panel of modern human Y chromosomes for the analyses below, we applied the same
99 genotype calling procedure to a set of previously published modern human Y chromosomes (6,
100 18, 19) (Supplementary Information).

101 **Archaic Y chromosome phylogeny**

102 To determine the relationships between Denisovan, Neandertal and modern human Y
103 chromosomes we constructed a neighbor-joining tree from the alignment of Y chromosome
104 genotype calls (Supplementary Information). Unlike the rest of the nuclear genome, which puts
105 Denisovans and Neandertals as sister groups to modern humans (2), we found that the Denisovan
106 Y chromosomes form a separate lineage that split before Neandertal and modern human Y
107 chromosomes diverged from each other (Figure 2A, 100% bootstrap support for both ancestral
108 nodes). Notably, all three late Neandertal Y chromosomes cluster together and fall outside of the
109 variation of present-day human Y chromosomes (Figure 2A, 100% bootstrap support), which
110 includes the African Y chromosome lineage A00 known to have diverged from all other present-
111 day human Y chromosomes around 250 kya (6).

112 **Ages of Y chromosomal ancestors**

113 To estimate the time to the most recent common ancestor (TMRCA) of archaic and modern human
114 Y chromosomes we followed an approach similar to that taken by Mendez *et al.*, expressing this
115 TMRCA relative to the deepest known split within present-human Y chromosomes (African Y
116 chromosome lineage A00, (6, 12), Supplementary Information). This has the advantage of not
117 relying on private mutations on the archaic human branch which makes it robust to low coverage
118 and aDNA damage which vary significantly between samples (Figure 2A, Table S4.1, Figure
119 S5.1).

120 We first calculated the mutation rate in the total 6.9 Mb target regions to be 7.34×10^{-10} per
121 bp per year (bootstrap CI: $6.27\text{-}8.46 \times 10^{-10}$; Figure S7.1, Table S7.2, Supplementary Information).
122 Using this mutation rate, we estimated the TMRCA of the African A00 lineage and a set of non-
123 African Y chromosomes from the SGDP panel (6, 19) at ~249 kya (bootstrap CI: 213-293 kya;
124 Figure S7.1, Table S7.2, Supplementary information). These estimates are consistent with values
125 inferred from larger-scale studies of present-day human Y chromosomes (6, 18), suggesting that
126 the Y chromosomal regions we defined for capture are not unusual in terms of their mutation rate.

127 Second, assuming the A00 divergence time of 249 kya, we inferred TMRCAs between
128 archaic Y chromosomes and present-day non-African Y chromosomes for each archaic individual
129 at a time (Figure S7.4, Table S7.3, Supplementary information). We found that the two Denisovan
130 Y chromosomes (*Denisova 4* and *Denisova 8*) split from the modern human lineage around 700
131 kya (CI: 607-833 kya for the higher coverage *Denisova 8*, Figure 2B, Table S7.3). In contrast, the
132 three Neandertal Y chromosomes split from the modern human lineage about 350 kya: 353 kya
133 for *Spy 94a* (286-449 kya), 369 kya for *Mezmaiskaya 2* (326-419 kya) and 339 kya for *El Sidrón*
134 *1253* (275-408 kya) (Figure 2B, Table S7.3). Additionally, we used the proportions of sharing of

135 derived alleles with the high-coverage Mezmaiskaya 2 Y chromosome to estimate the TMRCA of
136 the three Neanderthal Y chromosomes at around 100 kya (Figure S7.14 and S7.15). We validated
137 the robustness of all TMRCA estimates by repeating the analyses using filters of varying levels of
138 stringency and different genotype calling methods (Figures S7.9, S7.11, S7.13). Similarly,
139 although we detected some evidence of capture bias in the data (Figure S4.5), we observed no
140 significant differences between capture data and shotgun sequences or between individuals
141 showing different read length distributions, indicating that the effect of technical biases on our
142 inferences is negligible (Figure S7.11).

143 The Denisovan-modern human Y chromosome TMRCA estimates are in good agreement
144 with population split times inferred from autosomal sequences, suggesting that the differentiation
145 of Denisovan Y chromosomes from modern humans occurred through a simple population split
146 (20). In contrast, the Neanderthal-modern human Y chromosome TMRCA estimates are significantly
147 younger than the inferred population split time (Figure 3A) and consistent with a time window for
148 gene flow from a lineage related modern humans into Neandertals inferred from mtDNA (9) and
149 autosomal sequences (21, 22).

150 **Disagreement with the previous Y chromosome TMRCA**

151 Our estimates of the Neanderthal-modern human TMRCA, including those obtained using the larger
152 amount of data we generated from the same individual (TMRCA of ~339 kya, Figure 2B), are
153 substantially younger than the previous estimate of ~588 kya from the *El Sidrón 1253* Neanderthal
154 (12). The previous estimate was based on ~3X coverage of 118 kb of exome capture sequence and,
155 due to the limited amount of data, used SNPs supported even by single reads (12, 17). Although
156 the TMRCA inference procedure used by (12) does not rely directly on the counts of private
157 mutations on the archaic lineage (Figure S7.4, Supplementary Information), it can still be affected

158 by erroneous genotype calls, which can lead to shared derived variants being converted to the
159 ancestral state, increasing the apparent TMRCA. Indeed, when we applied our stricter filtering
160 criteria to the *El Sidrón 1253* data analyzed previously, we arrived at TMRCA estimates for *El*
161 *Sidrón 1253* that are consistent with the other Neandertals in our study (Figure S7.12).

162 **The probability of replacement**

163 The phylogenetic relationships of archaic and modern human Y chromosomes are similar to the
164 observations made from mtDNA genomes (9, 10), suggesting that both mtDNA and Y
165 chromosomes of early Neandertals have been replaced via gene flow from an early lineage related
166 to modern humans, possibly as a result of the same population contact (Figure 3A, (9)). Although
167 such contact has been proposed, previous work suggests gene flow from modern humans into
168 Neandertals on the order of only a few percent (21, 23). Assuming neutrality, the fixation
169 probability of a locus is equal to its initial frequency in a population (24). Therefore, the joint
170 probability of both Neandertal mtDNA and Y chromosome replacements by their modern human
171 counterparts under neutrality is even lower. However, several studies have suggested that due to
172 their low N_e and reduced efficacy of purifying selection, Neandertals may have accumulated an
173 excess of deleterious variation compared to modern humans (25, 26) and sequencing of the exomes
174 of three Neandertals directly demonstrated that they carried more deleterious alleles than present-
175 day humans (17). To explore the dynamics of introgression into Neandertals, we simulated the
176 frequency trajectories of a non-recombining, uniparental locus under a model of purifying
177 selection (Supplementary Information, (27)). We simulated lower N_e on the Neandertal lineage
178 after its split from modern humans and accumulation of deleterious variants on both lineages
179 across a grid of several relevant parameters (such as the time of the split between both populations
180 or the amount of sequence under negative selection). For each combination of parameters, we then

181 calculated the ratio of fitnesses of average Neandertal and average modern Y chromosomes
182 produced by the simulation, and traced the trajectory of introgressed modern human Y
183 chromosomes in Neandertals over 100 ky following 5% admixture (Figures S8.1 and S8.2,
184 Supplementary information).

185 We found that even a small reduction in fitness of Neandertal Y chromosomes compared
186 to modern human Y chromosomes has a strong effect on the probability of a complete replacement
187 by introgressed modern human Y chromosomes (Figure 3B). Specifically, even a 1% reduction in
188 Neandertal Y chromosome fitness increases the probability of replacement after 20ky to ~25% and
189 a 2% reduction in fitness increases this probability to ~50%. This fitness reduction measure is an
190 aggregate over all linked deleterious mutations on the Y chromosome and integrates a number of
191 biological parameters, only a subset of which we consider here (Figure S8.1 and S8.3).
192 Importantly, we note that although we simulated introgression of Y chromosomes, the abstract
193 measure of fitness reduction of a non-recombining, uniparental locus can also be generalized to
194 the case of mtDNA introgression (Figure 3B).

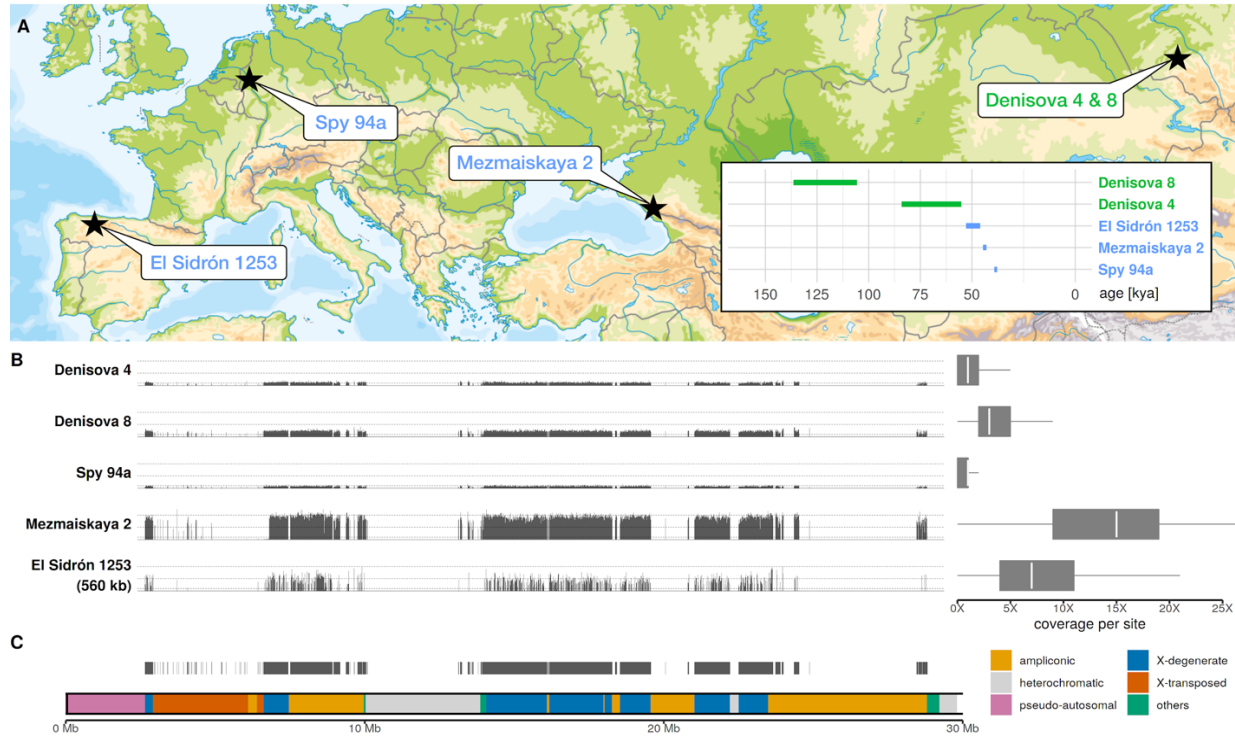
195 These results show that a model of higher genetic load in Neandertals is compatible with
196 an increased probability of replacement of Neandertal mtDNA and Y chromosomes with their
197 introgressed modern human counterparts. Furthermore, given the crucial role of the Y
198 chromosome in reproduction and fertility, and its haploid nature, it is possible that deleterious
199 mutations or structural variants on the Y chromosome have a dramatically larger impact on fitness
200 than we considered in our simulations (28).

201 **Conclusions**

202 Our results show that the Y chromosomes of late Neandertals represent an extinct lineage related
203 to modern human Y chromosomes that introgressed into Neandertals some time between ~370 kya

204 and ~100 kya. The presence of this Y chromosome lineage in all late Neandertals makes it unlikely
205 that genetic changes that accumulated in Neandertal and modern human Y chromosomes prior to
206 the introgression lead to incompatibilities between these groups of humans. We predict that the
207 ~400 ky old Sima de los Huesos individuals, who are early Neandertals but carry a Denisovan-like
208 mtDNA (10, 11), should also carry a Y chromosome lineage more similar to Denisovans than to
209 later Neandertals. Although complete replacement of mtDNA and Y chromosomes might seem
210 surprising given that limited modern human gene flow has been detected in the genomes of late
211 Neandertals (15, 21–23), mitochondrial-autosomal discrepancies are predicted by population
212 genetic theory, and are relatively common during interspecific hybridization in the animal
213 kingdom (29–31). Our simulations show that differences in genetic loads in uniparental loci
214 between the two hybridizing populations is a plausible driver of this phenomenon.

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219 **Figure 1. Geographical locations, ages and sequencing coverage of the male archaic humans**

220 **in our study. (A)** Locations of archaeological sites where the five archaic human specimens have

221 been found. Estimates of the ages shown as an inset (14–16). **(B) Left** - Spatial distribution of

222 sequencing coverage for each archaic human Y chromosome along the ~6.9 Mb of capture target

223 regions. The heights of the thin vertical bars represent average coverage in each target region. The

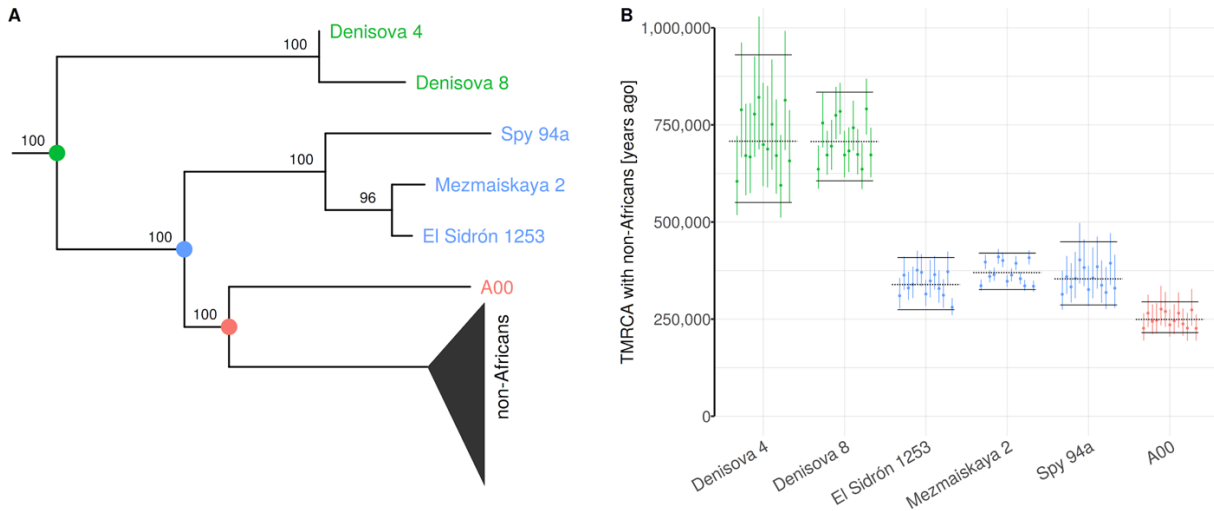
224 chromosomal coordinates are aligned to match the Y chromosome structure depicted in panel C.

225 **Right** - Distribution of coverage across all target sites for each archaic Y chromosome on the left.

226 **(C)** Genomic structure of the portion of the human Y chromosome targeted for capture. Thin black

227 vertical lines show the position of individual target capture regions. The coordinates of Y

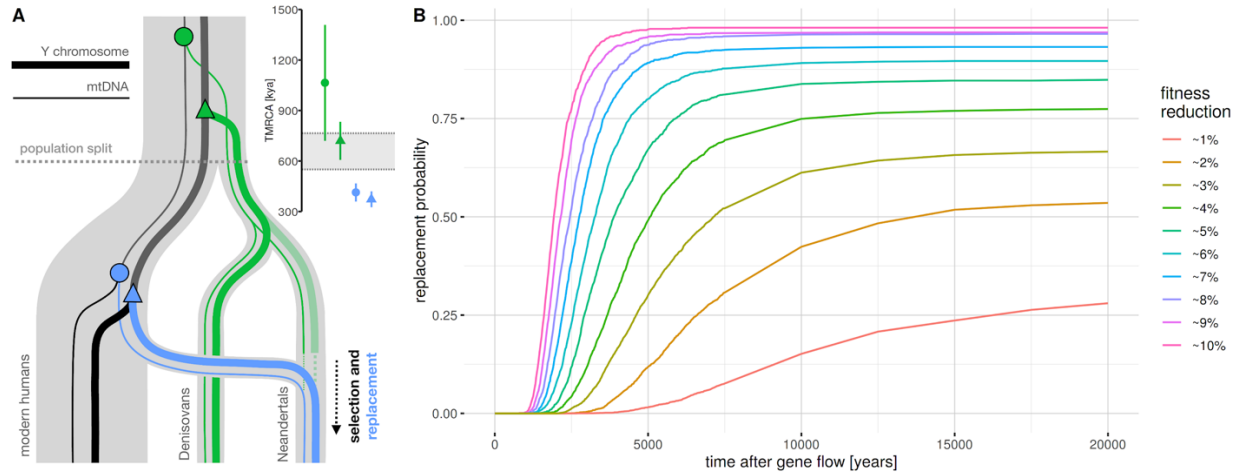
228 chromosome regions were taken from (32).



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232 **Figure 2. Phylogenetic relationships between archaic and modern human Y chromosomes.**

233 **(A)** Neighbor-joining tree based on the alignment of Y chromosome genotype calls, excluding C-
234 to-T and G-to-A polymorphisms to mitigate the effects of aDNA damage. Numbers next to the
235 internal nodes show bootstrap support for the three major clades (green - Denisovans, blue -
236 Neandertals, red – deeply divergent African lineage A00) based on 100 bootstrap replicates. The
237 tree was rooted using a chimpanzee Y chromosome as the outgroup. We note that the terminal
238 branch lengths are not informative about the ages of specimens due to differences in sequence
239 quality (Figure 1A). **(B)** Distributions of the times to the most recent common ancestor (TMRCA)
240 between Y chromosomes listed along the x-axis and a panel of 13 non-African Y chromosomes.
241 Each dot represents a TMRCA estimate based on a single non-African Y chromosome, with error
242 bars showing approximate 95% C.I. based on resampling of branch counts (Supplementary
243 Information). Black horizontal lines show the mean TMRCA calculated across the full non-African
244 panel (dotted lines) with bootstrap-based 95% C.I. (solid lines).



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247 **Figure 3. Proposed model for the replacement of Neandertal Y chromosomes and mtDNA.**

248 **(A)** Schematic representation of the relationships between archaic and modern human mtDNA

249 (thin lines) and Y chromosomes (thick lines) based on current phylogenetic inferences, with the

250 hypothesized time-window for selection. The semi-transparent Neandertal lineage indicates an as

251 yet unsampled, hypothetical Y chromosome which was replaced by an early lineage related to

252 modern human Y chromosomes. Positions of relevant most recent common ancestors with modern

253 human lineages are shown for mtDNA (circle nodes) and Y chromosomes (triangle nodes). Inset

254 shows TMRCA estimates for the four nodes in the diagram: Y chromosome TMRCAs as estimated

255 by our study, mtDNA TMRCAs estimates from the literature (9, 10). The grey horizontal bar

256 highlights the 95% C.I. for the population split time between archaic and modern humans (8). **(B)**

257 Probability of replacement of a non-recombining, uniparental Neandertal locus as a function of

258 time after gene flow, assuming a given level of fitness burden relative to its modern human

259 counterpart. Trajectories are based on forward simulations across a grid of parameters (Figure

260 S8.1-S8.3, Supplementary Information). Modern human introgression was simulated in a single

261 pulse at 5%.

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310 supervised the project. M.P., J.K. wrote and edited the manuscript with input from all co-authors.

311

312 Data and materials availability: All sequence data are available from the European Nucleotide
313 Archive under accession numbers xxx (*Mezmaiskaya 2*), xxx (*Spy 94a*), xxx (*Denisova 4*), xxx
314 (*Denisova 8*) and xxx (*El Sidrón 1253*).

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316 Complete source code for data processing and simulations, as well as Jupyter notebooks with all
317 analyses and results can be found at <https://github.com/bodkan/archaic-ychr>. All data is available
318 from <https://bioinf.eva.mpg.de/archaic-ychr>.

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Supplementary Information

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The evolutionary history of Neandertal

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and Denisovan Y chromosomes

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17 Complete source code for data processing and simulations, as well as Jupyter notebooks
18 with all analyses can be found at <https://github.com/bodkan/archaic-ychr>. All data is
19 available from <https://bioinf.eva.mpg.de/archaic-ychr>.

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46 1. Y chromosome DNA capture design

47 To design a set of DNA capture probes, we identified regions of the human Y
48 chromosome that are uniquely mappable with short sequence reads. Starting from the
49 entire human Y chromosome reference sequence (version *hg19*), we removed regions
50 that overlap those found by the Tandem Repeats Finder (1) and those identified by a
51 previously described mappability track as regions that may result in ambiguous alignment
52 of short reads (so called “*map35_50%*” filter, (2)). We then removed any regions that were
53 shorter than 99 bp of continuous sequence. In total, this process yielded 6,912,728 bp
54 (~6.9 Mb) of the Y chromosome suitable for use as an ancient DNA capture target.

55 We designed 52 bp oligonucleotide probes by tiling the identified 6.9 Mb of target
56 sequence with 52 bp fragments in steps of 3 bp. This resulted in 2,049,846 individual
57 oligonucleotide probes. To verify that the probe sequences are unique genome-wide, we
58 aligned each probe to the complete *hg19* reference sequence and confirmed that they all
59 aligned only to their expected position on the Y chromosome with mapping quality of at
60 least 30. The files containing the coordinates of target regions, as well as the coordinates
61 and sequences of all capture probes, including 8 bp adapters, are freely available from
62 <https://bioinf.eva.mpg.de/archaic-ychr>.

63

64 Following the approach taken by Fu *et al.* (3), 60 bp oligonucleotides containing the probe
65 sequences as well as an 8 bp universal linker sequence were synthesized on three One
66 Million Feature Arrays (Agilent Technologies), converted into probe libraries and
67 amplified. Single-stranded biotinylated DNA probes were generated using a linear
68 amplification reaction with a single biotinylated primer (3).

69

70 We also co-analyzed data from two additional captures carried out previously: (i) ~120 kb
71 of Y chromosome sequence from the *El Sidrón 1253* Neandertal that was targeted as a
72 part of an exome capture study (4) and has been analyzed previously (5), and (ii) a larger
73 amount of data (~560 kb) from the same *El Sidrón 1253* individual which we captured
74 using probes designed for a previously published set of Y chromosome target regions (6).
75 The file containing the coordinates of target regions and coordinates and sequences of
76 all capture probes, including 8 bp adapters, is freely available from
77 <http://bioinf.eva.mpg.de/archaic-ychr>.

78

79 The features of our new capture design, as well as a comparison with the Y chromosome
80 target regions on the exome capture (4, 5) and the ~560 kb capture (6) are reported in
81 Table S1.1.

82

83

84

target	total [bp]	# of regions	min [bp]	median [bp]	mean [bp]	max [bp]
entire mappable Y	6,912,728	15,903	99.0	240.0	434.7	9,425.0
~560 kb capture (*)	573,657	1,251	60.0	151.0	458.6	3899.0
~560 kb capture	556,259	1,779	1.0	119.0	312.7	2,829.0
exome subset	118,643	2,519	1.0	3.0	47.1	1,257.0

85

86 **Table S1.1. Characteristics of the three sets of Y chromosome capture targets**

87 **analyzed in our study.** “*Exome subset*” refers to a Y chromosome subset of the exome

88 capture sequence generated by Castellano *et al.* and analyzed by Mendez *et al.* (called

89 “filter 1”) (4, 5), “~560 kb capture” refers to target regions originally designed for studying

90 present-day human Y chromosome variation (6), star (*) signifies statistics before

91 intersecting the original set of target regions with the “*map35_50%*” filter (2), “*entire*

92 *mappable Y*” represents capture regions targeting the entire mappable portion of the

93 human Y chromosome designed for our study.

94

95

96

97 2. Sampling, DNA extraction, library preparation and capture

98 Samples of 15.4 mg and 14.9 mg of tooth powder from *Denisova 8* were used for DNA
99 extraction using a silica-based method (7) with modifications as described in (8). Ten mg
100 of the tooth powder from *Denisova 4* were used for a silica-based DNA extraction that is
101 optimized for the recovery of extremely short DNA fragments (9). Four samples of
102 Mezmaiskaya 2 bone powder, ranging between 3.2 mg and 17.5 mg were treated with
103 0.5% hypochlorite solution to minimize microbial and present-day human DNA
104 contamination (8) before DNA was extracted either manually (7) or on an automated liquid
105 handling platform (Bravo NGS workstation B, Agilent Technologies) (10). See Table S2.1
106 for an overview of the DNA extracts and libraries generated in this and previous studies
107 and the experimental conditions used.

108

109 In addition to existing single-stranded libraries for *Spy94a* and *Mezmaiskaya 2*
110 (11), new single-stranded DNA libraries for *Mezmaiskaya 2*, *Denisova 4* and *Denisova 8*
111 were prepared from DNA extracts made for this study (Table S2.1). Two of the single-
112 stranded DNA libraries for *Denisova 8* (A9461 and A9462) were prepared manually using
113 10 μ L of each extract as an input (12). All other single-stranded DNA libraries were
114 prepared using either 10 μ L or 30 μ L of extract as input (13) on an automated liquid
115 handling platform (Bravo NGS workstation B, Agilent Technologies) (14). All new libraries
116 were prepared without UDG treatment (non-UDG treated libraries).

117 In order to monitor the efficiency of library preparation, a control oligonucleotide
118 was spiked into each aliquot of a DNA extract used for library preparation (9). Quantitative
119 PCR was used to determine the total number of unique library molecules and the number

120 of oligonucleotides that were successfully converted to library molecules (9, 13) (Table
121 S2.1). Each library was tagged with two unique index sequences (15) and amplified into
122 plateau with AccuPrime Pfx DNA polymerase (Life Technologies) (16) according to the
123 modifications detailed in (8). Fifty microlitres (half of the total volume) of each of the
124 amplified libraries were purified on an automated liquid handling platform (Bravo NGS
125 workstation B, Agilent Technologies) using SPRI beads (14). A NanoDrop 1000
126 Spectrophotometer (NanoDrop Technologies) was used to determine the concentrations
127 of the purified libraries.

128 In solution hybridization capture of the Y chromosome was performed in two
129 successive rounds of capture as described previously (3), using the Y chromosome probe
130 set designed in the present study and single-stranded libraries prepared in this and
131 previous studies. In addition, we performed hybridization capture on 40 double-stranded
132 libraries prepared in a previous study from the *El Sidrón 1253* Neandertal (see Table S1
133 in (4)) using a smaller ~560 kb Y chromosomal probe set that was also designed
134 previously (6).

135

136 3. Sequencing and data processing

137 3.1. Newly generated archaic human Y chromosomes

138 All captured libraries were sequenced on the Illumina HiSeq 2500 platform in a double
139 index configuration (2x76 cycles) (15), and base calling was done using Bustard
140 (Illumina). Adapters were trimmed and overlapping paired-end reads were merged using
141 *leeHom* (17). The Burrows-Wheeler Aligner (BWA) (18) with parameters adjusted for
142 alignment of ancient DNA (“-n 0.01 -o 2 -l 16500”) was used to align the sequenced
143 fragments to the human reference genome version hg19/GRCh37. Only reads showing
144 perfect matches to the expected index sequence combinations were retained for
145 subsequent analyses. PCR duplicates were removed using the *bam-rmdup* program,
146 which can be downloaded in source form from <https://github.com/mpieva/biohazard-tools>.
147 DNA fragments that were at least 35 base pairs (bp) long and had a mapping quality of
148 at least 25 were extracted using *samtools* (19). Each processed and filtered BAM file (one
149 for each archaic human Y chromosome) was intersected with a BED file of the appropriate
150 Y chromosome target (full ~6.9 Mb capture, ~120 kb exome capture or ~560 kb capture).

151 3.2. Previously published archaic human sequences

152 In addition to the new capture data generated here, we analyzed previously published
153 shotgun sequences of the *Spy 94a* and *Mezmaiskaya 2* individuals (11), as well as exome
154 capture data of the *El Sidrón 1253* individual (4). For comparisons to our capture data,
155 we generated BAM files for *Spy 94a* and *Mezmaiskaya 2* shotgun sequences and the *El*
156 *Sidrón 1253* exome capture by filtering the published data to minimum read length of 35

157 bp and mapping quality 25, keeping only sequences aligned to the set of appropriate
158 target capture regions (~6.9 Mb capture target for *Spy 94a* and *Mezmaiskaya 2*, ~118 kb
159 capture target for *El Sidrón 1253*, *Table S1.1*).

160 3.3. Previously published modern human sequences

161 For comparisons with modern human Y chromosomes, we downloaded 19 BAM files of
162 African and non-African Y chromosomes published by the Simons Genome Diversity
163 Project (SGDP) (20), two Y chromosomes representing the African A00 lineage (21) and
164 the Y chromosome of a ~45,000-year-old hunter-gatherer *Ust'-Ishim* (22). Because the
165 two individuals from which the A00 Y chromosomes were sequenced are closely and
166 each is only about half of the coverage of the other modern human Y chromosomes
167 (Table S4.3), we followed the approach of the original A00 publication and merged the
168 two A00 Y chromosomes into a single BAM file (21, 23). All individual BAM files (one for
169 each modern human Y chromosome) were then filtered to retain reads with a minimum
170 length of 35 bp and mapping quality of at least 25, and alignment to the appropriate set
171 of Y chromosome target capture regions (Table S1.1).

172

173 4. Coverage and measures of ancient DNA quality

174 4.1. Coverage

175 Sequencing coverage was calculated using *bedtools* (24). To get coverage for a given
176 individual in a given set of target regions, we ran the command `bedtools coverage -`
177 `a <BED> -b <BAM> -d`, which reports the coverage for each position in a BED file in
178 the last column of its output. We removed sites with coverage higher than the 98%
179 quantile of the entire distribution in each of the individuals in our study. Figure 1B (spatial
180 distribution and overall distribution) and Tables S4.1, S4.2 and S4.3 summarise the
181 values of coverage at sites with less than 98% quantile of the overall distribution in a
182 sample.

183

individual	mean coverage	target
Spy 94a	0.8	6.9 Mb
Denisova 4	1.4	6.9 Mb
Denisova 8	3.5	6.9 Mb
El Sidrón 1253	7.9	560 kb
Mezmaiskaya 2	14.3	6.9 Mb

184

185 **Table S4.1. Mean coverage of archaic human Y chromosomes sequenced in this**

186 **study.** Sites with coverage higher than 98% quantile of the entire distribution were

187 excluded from the calculation.

188

189

name	mean coverage	target	study
Spy 94a (shotgun)	0.5	6.9 Mb	Hajdinjak <i>et al.</i> , 2018
Mezmaiskaya 2 (shotgun)	0.8	6.9 Mb	Hajdinjak <i>et al.</i> , 2018
El Sidrón 1253 (capture)	3.2	118 kb	Castellano <i>et al.</i> , 2014, Mendez <i>et al.</i> , 2016

190

191 **Table S4.2. Mean coverage of previously published archaic human Y chromosome**

192 **sequences.** The coverage reported for *Spy 94a* and *Mezmaiskaya 2* shotgun sequence

193 data is that of sequences overlapping the 6.9 Mb Y capture regions. The *El Sidrón 1253*

194 libraries were captured using an exome capture array (4, 5) and the coverage reported

195 here is for the ~118 kb exome target capture regions. For each individual, sites with

196 coverage higher than 98% quantile of the entire distribution were excluded from the

197 calculation.

198

199

name	mean coverage	target	study
A00-1	8.8	6.9 Mb	Karmin <i>et al.</i> , 2015
A00-2	12.0	6.9 Mb	Karmin <i>et al.</i> , 2015
S_Mandenka-1	16.3	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Yoruba-2	17.0	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Finnish-2	17.0	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Punjabi-1	17.1	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Sardinian-1	18.1	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Dai-2	19.8	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Gambian-1	20.1	6.9 Mb	Mallick <i>et al.</i> , 2016
Ust'-Ishim	20.1	6.9 Mb	Fu <i>et al.</i> , 2014
S_Mbuti-1	20.3	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Dinka-1	20.8	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Han-2	20.8	6.9 Mb	Mallick <i>et al.</i> , 2016
A00	20.9	6.9 Mb	merged A00-1 and A00-2
S_BedouinB-1	21.7	6.9 Mb	Mallick <i>et al.</i> , 2016
S_French-1	21.9	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Karitiana-1	22.2	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Turkish-1	22.5	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Saami-2	22.6	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Ju_hoan_North-1	22.7	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Papuan-2	23.2	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Thai-1	25.1	6.9 Mb	Mallick <i>et al.</i> , 2016
S_Burmese-1	29.2	6.9 Mb	Mallick <i>et al.</i> , 2016

200

201 **Table S4.3. Mean coverage of modern human Y chromosomes in capture target**

202 **regions.** Coverage is reported using sequences within the 6.9 Mb target capture regions.

203 For each individual, sites with coverage higher than 98% quantile of the entire distribution

204 were excluded from the calculation.

205

206

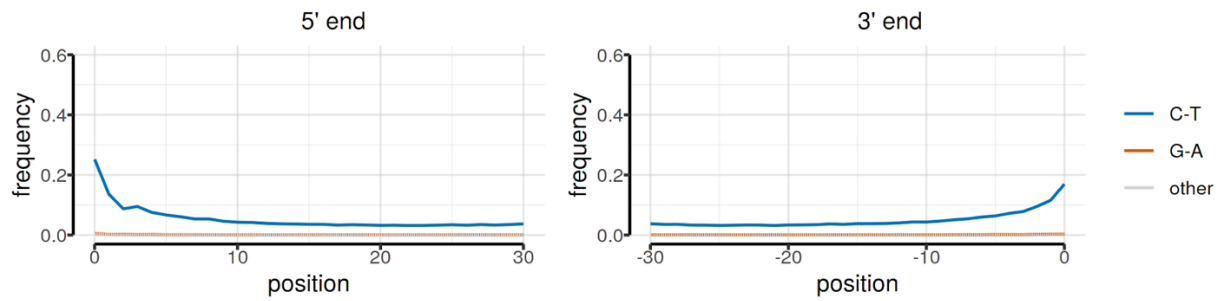
207 4.2. Patterns of ancient DNA damage

208 To check for the presence of genuine ancient DNA sequences, we looked for an
209 increased rate of deamination-induced substitutions, an important signature of ancient
210 DNA damage (25). We counted substitution frequencies for each individual BAM file (one
211 BAM file per individual Y chromosome) and found that molecules from single-stranded
212 libraries that were not treated by uracil-DNA glycosylase (UDG) enzyme (those from *Spy*
213 *94a*, *Mezmaiskaya 2*, *Denisova 4* and *Denisova 8*) show highly elevated frequencies of
214 C-to-T substitutions towards the ends of molecules, as well as C-to-T substitutions
215 throughout the molecules (Figure S4.1). As is characteristic of double-stranded libraries
216 treated with the UDG enzyme, the deamination substitution frequency signal in the
217 capture data from *El Sidrón 1253* UDG-treated libraries is much less pronounced and
218 present only at the terminal positions of DNA fragments as both C-to-T and G-to-A
219 substitutions (Figure S4.2). For comparison, Figure S4.3 shows DNA damage patterns
220 from previously published shotgun sequences of *Spy 94a* and *Mezmaiskaya 2* individuals.

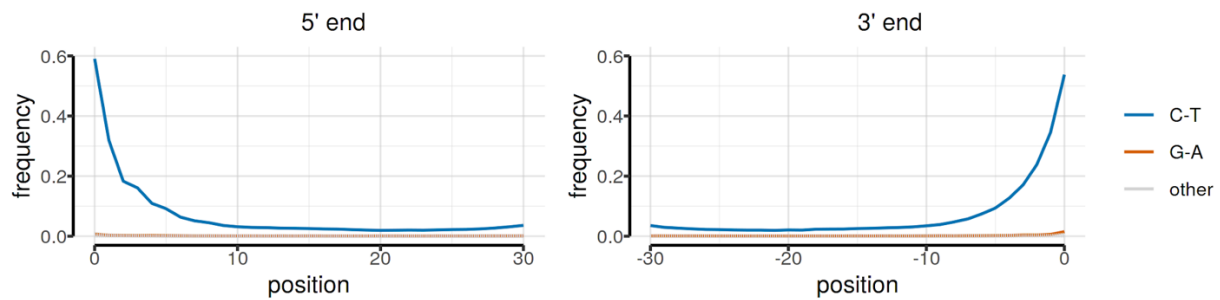
221

222

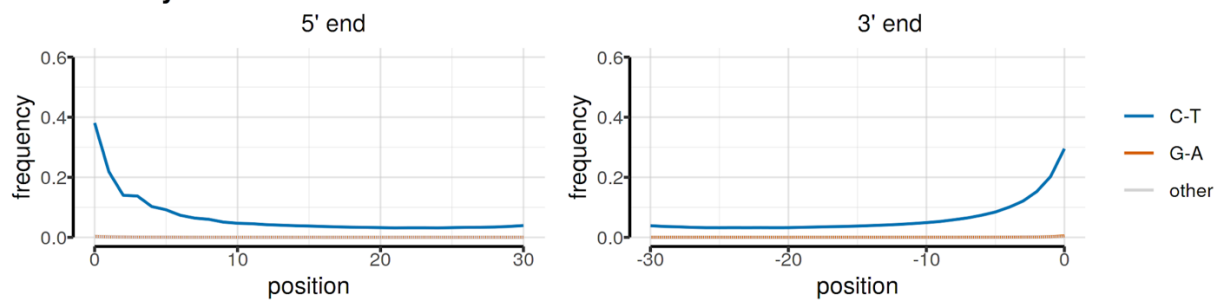
Denisova 4



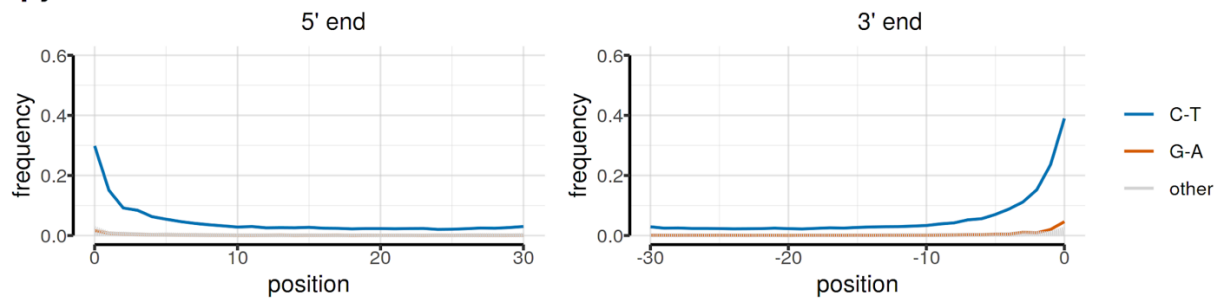
Denisova 8



Mezmaiskaya 2



Spy 94a



223

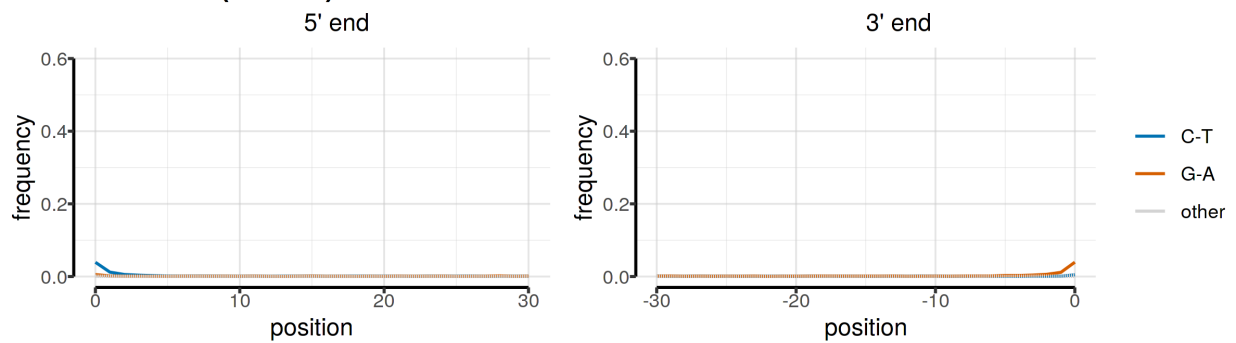
224 **Figure S4.1. Patterns of ancient DNA damage in non-UDG-treated sequences**
225 **captured using the 6.9 Mb capture.**

226

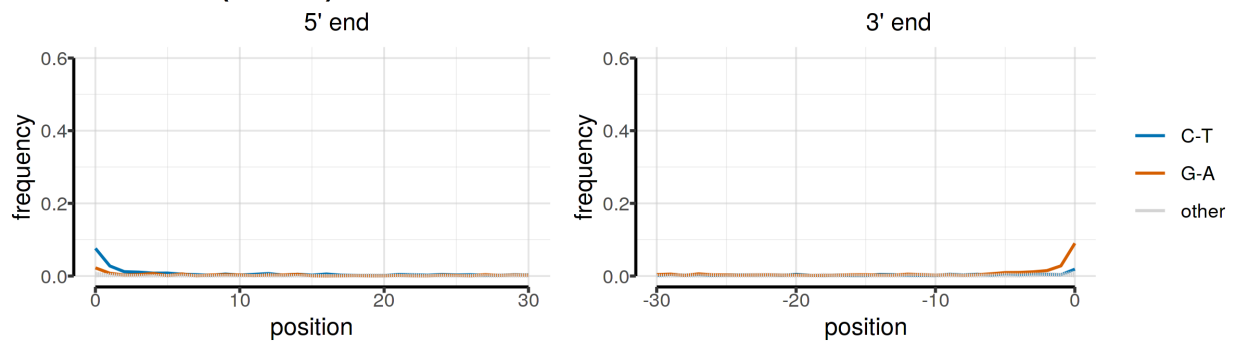
227

228

El Sidrón 1253 (560 kb)



El Sidrón 1253 (118 kb)



229

230 **Figure S4.2. Patterns of ancient DNA damage in UDG-treated sequences from the**

231 ***El Sidrón 1253* individual. Top row shows deamination patterns in the 560 kb capture**

232 **generated for our study (SI 1), bottom row shows deamination patterns in previously**

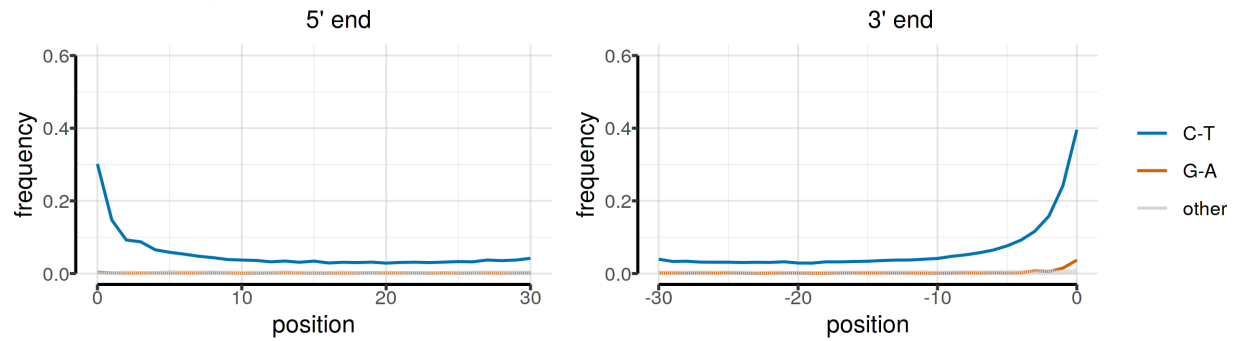
233 **published Y chromosome sequences from the exome capture of the same individual (4,**

234 **5).**

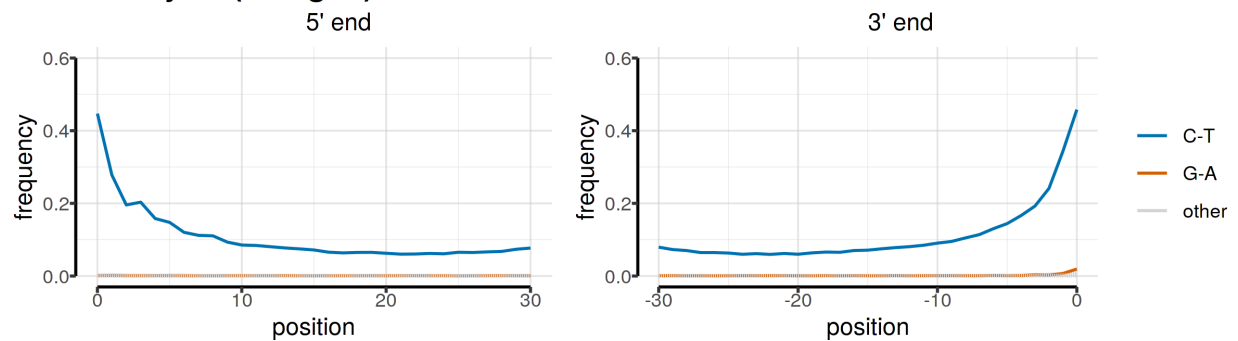
235

236

Spy 94a (shotgun)



Mezmaiskaya 2 (shotgun)



237

238 **Figure S4.3. Patterns of ancient DNA damage in non-UDG-treated shotgun**
239 **sequences of *Spy 94a* and *Mezmaiskaya 2*.** Figures show results from previously
240 published sequence data (11), using sequences within the 6.9 Mb Y chromosome capture
241 target.

242

243

244

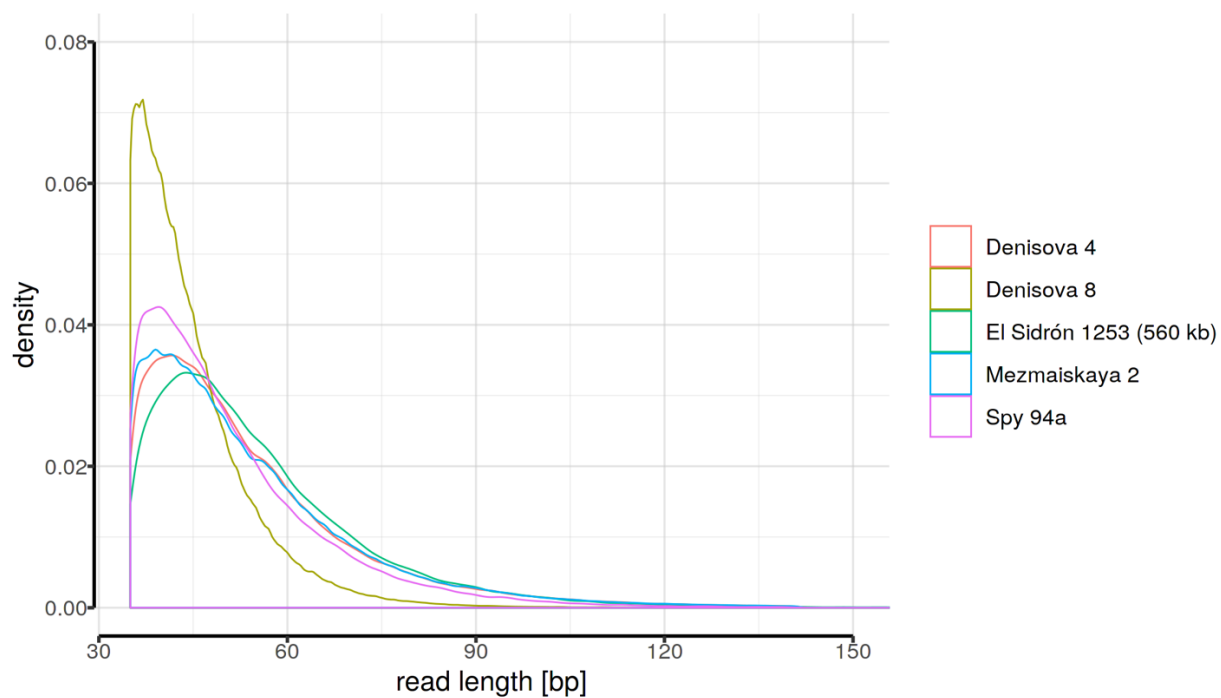
245 4.3. Read length distribution

246 We calculated read lengths for each final processed BAM file using *samtools view* and
247 *awk*. As expected for ancient sequences, archaic human Y chromosome fragments are
248 very short (Figure S4.3, Table S4.4). We note that *Denisova 8* shows an even more
249 extreme reduction in read length compared to the other captured archaic human Y
250 chromosomes (Figure S4.3, Table 4.4), consistent with the fact that the *Denisova 8*
251 specimen is possibly nearly twice as old as the other archaic humans in our study (Figure
252 1A).

253

254

255



256

257 **Figure S4.3. Distributions of read lengths calculated using sequences within the**

258 **6.9 Mb target regions.**

259

260

individual	mean [bp]	median [bp]
Denisova 8	44.8	42
Spy 94a	51.2	47
Mezmaiskaya 2	54.1	49
Denisova 4	54.1	50
El Sidrón 1253 (560 kb)	55.3	51

261

262 **Table S4.4. Mean and median values of read length distributions in Figure S4.3.**

263

264

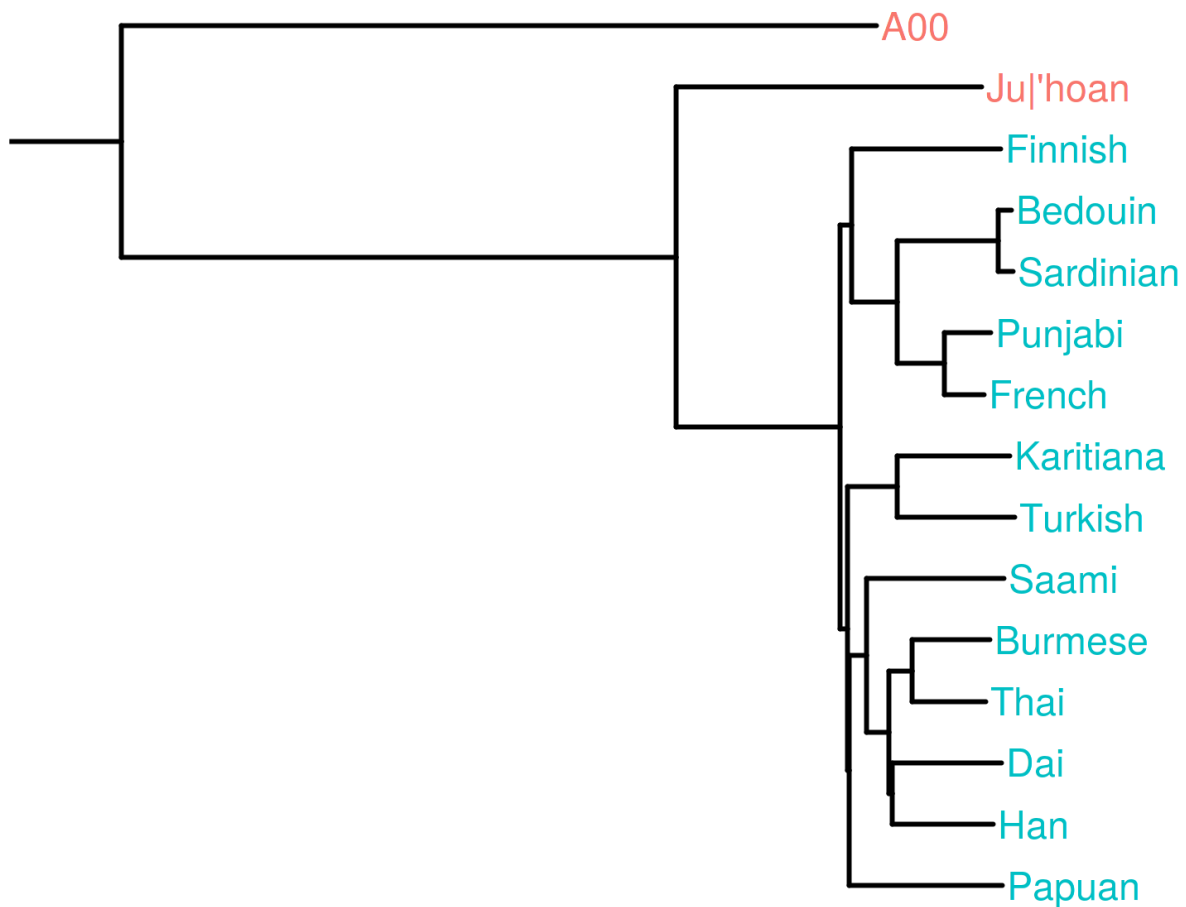
265 4.4. Modern human contamination

266 Our consensus-based genotype calling strategy (described in SI 5) is designed to remove
267 the effect of modern human contamination under the assumption that contaminant reads
268 at a given position never represent more than 90% of the total number of reads. To
269 validate that this approach achieves the desired effect, we assessed the frequency of
270 modern human-derived SNPs at positions informative about modern human
271 contamination in the final archaic human Y chromosome genotype calls.

272 To define these informative positions, we used genotypes of present-day human
273 Y chromosomes and identified ancestral states by determining which sites carry the same
274 allele in chimpanzee and two present-day African lineages *A00* and *S_Ju_hoan_North-1*
275 (20, 21) (red branches in Figure S4.4). We then further restricted these sites to those in
276 which a different allele is observed in all 13 non-African individuals from the SGDP panel
277 (20). These represent alleles derived on the non-African Y chromosome lineage (blue
278 branches in Figure S4.4). This conditioning led to a total of 268 informative positions.
279 Given that all archaic human Y chromosomes are expected to carry the ancestral state
280 at these sites because they all fall basal to modern human Y chromosomes (Figure 2A),
281 observing a derived allele at any of these informative sites implies the presence of a
282 modern human contaminant allele, double mutation or an erroneous SNP call. We note
283 that although the 13 non-African Y chromosomes that we used to define the potential
284 ‘contaminant-derived states’ may not represent the true contaminant population, the
285 contaminating population would still share the same derived states due to the non-
286 recombining nature of human Y chromosomes.

287 Using this set of 268 informative positions, we found that the five archaic human Y
288 chromosomes carry the ancestral state at all informative positions except for a single
289 position in the *Spy 94a* individual which shows a derived allele out of the total 16
290 informative sites available (Table S4.5). This shows that the consensus genotype calling
291 method is efficient in mitigating the effect of modern human contaminant reads on the
292 final set of Y chromosome genotype calls.

293



294

295 **Figure S4.4. Phylogenetic tree demonstrating the definition of positions**
296 **informative about modern human contamination.** Red branches represent lineages
297 which we required to carry one state (together with the chimpanzee) at positions where
298 the blue lineages carry a different state. Therefore, red branches represent the ancestral
299 states and blue branches represent the derived state. The tree was rooted with a
300 chimpanzee Y chromosome as an outgroup (cropped for plotting purposes).

301

individual	ancestral count	derived count	total	derived/total
Spy 94a	15	1	16	0.0625
Mezmaiskaya 2	189	0	189	0.0000
Denisova 4	14	0	14	0.0000
Denisova 8	90	0	90	0.0000
El Sidrón 1253 (560 kb)	29	0	29	0.0000

302

303

304 **Table S4.5. Counts and proportions of potential ‘contaminant-derived’ non-African**

305 **alleles in all archaic human Y chromosomes.**

306

307 4.5. Capture bias and reference (mapping) bias

308 Because the probes we designed for Y chromosome DNA enrichment are based on the
309 human reference genome sequence (SI 1), we were concerned about the effect of
310 capture bias on our inferences, specifically on the observed differences in divergence
311 times between Denisovan and Neandertal Y chromosomes with respect to present-day
312 humans (Figure 2). An earlier study of reference bias in published aDNA data sets has
313 found minor but significant allelic imbalances at heterozygous sites from a baseline
314 expectation of 50% ratio between reference and alternative alleles (26). Because this
315 approach is not applicable for haploid Y chromosomes, we instead looked for departures
316 of the observed number of sites without any genomic coverage from the theoretical
317 expectation.

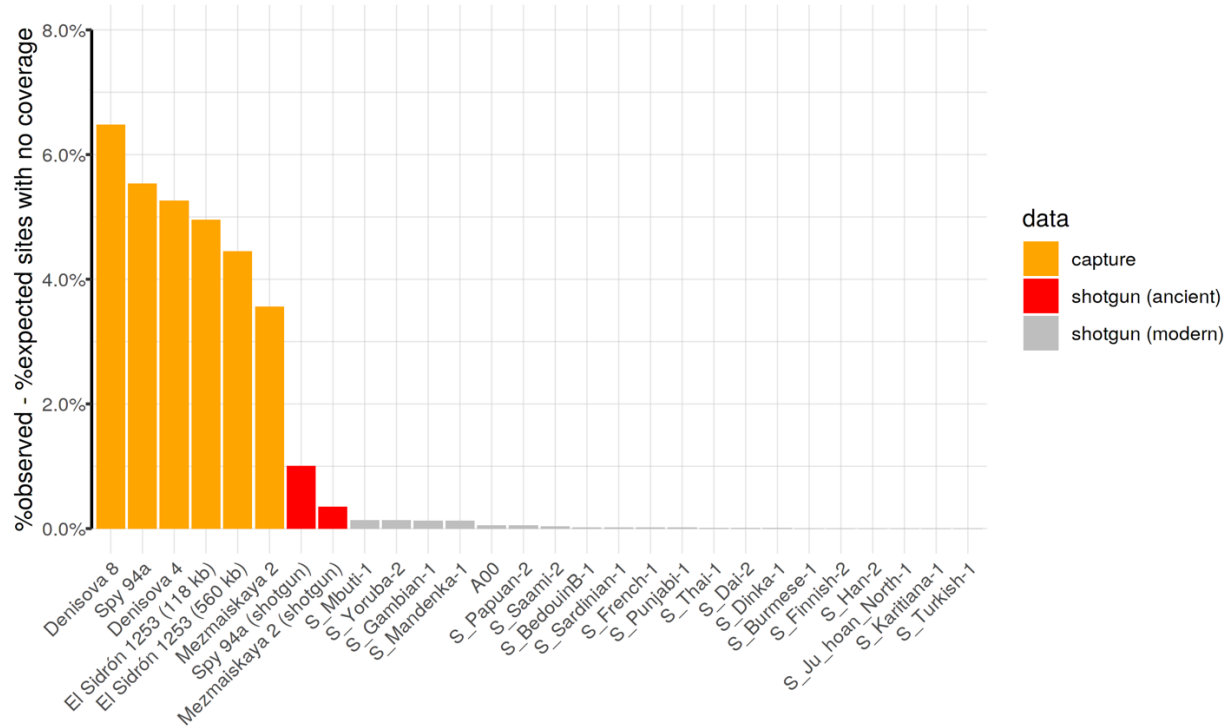
318 To build an intuition about this expectation, let's first consider a case of a truly
319 random distribution of sequencing reads in a complete absence of capture or reference
320 bias. In such a situation, the count of reads observed at any site can be modeled as a
321 random variable which follows a Poisson distribution with a parameter λ , where λ
322 represents the average coverage observed across all sites. In a mathematical notation,
323 letting X be this count of reads: $X \sim Poisson(\lambda)$. Then, given some value of λ , the
324 expected proportion of sites that are not covered by any sequencing reads can be
325 expressed as $Poisson(X = 0, \lambda)$, i.e. as the probability of observing zero reads at any site
326 given the overall average coverage of λ . As an example, assuming 1-fold sequencing
327 coverage we would expect to see $Poisson(X = 0, \lambda = 1) = 0.3678794 \sim 37\%$ of the target
328 sites not to be covered by any read at all, just by random chance. Importantly, however,
329 capture bias or reference bias will manifest by some regions of the genome being

330 underrepresented in terms of captured molecules or mapped reads. Therefore, the
331 presence and magnitude of this bias in a given DNA enrichment experiment can be
332 detected by estimating the difference between the proportion of sites without any
333 sequencing coverage from the theoretical Poisson expectation.

334 The results, shown in Figure S4.5 and Table S4.6, demonstrate that there is both
335 reference and capture bias in our data and offers several interesting insights. First, we
336 see a comparable effect of bias in all capture data (4-6% departure from the theoretical
337 Poisson expectation) regardless of which capture array was used for the enrichment
338 procedure (i.e., the full 6.9 Mb capture array, the 560 kb capture array or the exome
339 capture array, Figure S4.5). Furthermore, comparisons of capture and shotgun
340 sequences of *Spy 94a* and *Mezmaiskaya 2* show that the majority of bias must be due to
341 the capture procedure itself (i.e. failure to capture molecules). This is because the
342 underlying true biological divergences of *Spy 94a* and *Mezmaiskaya 2* to the reference
343 genome (which cause a failure to map reads due to an increased number of substitutions
344 – i.e., a reference bias) must be the same for both capture and shotgun sequences from
345 these individuals. Crucially, however, despite the differences in bias between capture and
346 shotgun sequences, both datasets lead to the same estimates of TMRCA with present-
347 day human Y chromosomes (Figure S7.11). Furthermore, although we see dramatically
348 different phylogenetic relationships of Denisovan and Neandertal Y chromosomes with
349 respect to modern humans (Figure 2A), both groups of archaic human capture sequences
350 display comparable magnitudes of both sources of bias (Figure S4.5). Therefore,
351 although undoubtedly present, capture and reference biases cannot result in the

352 observed differences in divergence times between archaic human Y chromosomes and
353 present-day human Y chromosomes (Figure 2).

354



355

356 **Figure S4.5. Differences between expected and observed counts of sites without**
357 **any sequencing coverage.** Exact counts are reported in Table S4.6. Data were filtered
358 according to the criteria described in SI 4.1.

359

360

361

name	coverage	observed	expected	difference	data
Denisova 8	3.4820355	0.09555	0.03074	0.06481	capture
Spy 94a	0.8253622	0.49341	0.43808	0.05533	capture
Denisova 4	1.3717806	0.30622	0.25365	0.05257	capture
El Sidrón 1253 (118 kb)	3.2121215	0.08978	0.04027	0.04951	capture
El Sidrón 1253 (560 kb)	7.9165032	0.04479	0.00036	0.04443	capture
Mezmaiskaya 2	14.3494002	0.03561	0.00000	0.03561	capture
Spy 94a (shotgun)	0.5052873	0.61337	0.60333	0.01004	shotgun (ancient)
Mezmaiskaya 2 (shotgun)	0.8248822	0.44174	0.43829	0.00345	shotgun (ancient)
S_Yoruba-2	16.9616115	0.00129	0.00000	0.00129	shotgun (modern)
S_Mbuti-1	20.3360989	0.00129	0.00000	0.00129	shotgun (modern)
S_Mandenka-1	16.3312730	0.00127	0.00000	0.00127	shotgun (modern)
S_Gambian-1	20.1210272	0.00127	0.00000	0.00127	shotgun (modern)
A00	20.8671570	0.00050	0.00000	0.00050	shotgun (modern)
S_Papuan-2	23.1529894	0.00048	0.00000	0.00048	shotgun (modern)
S_Saami-2	22.6298230	0.00033	0.00000	0.00033	shotgun (modern)
S_BedouinB-1	21.7140764	0.00016	0.00000	0.00016	shotgun (modern)
S_Sardinian-1	18.1189362	0.00015	0.00000	0.00015	shotgun (modern)
S_Punjabi-1	17.1469680	0.00013	0.00000	0.00013	shotgun (modern)
S_French-1	21.9031161	0.00013	0.00000	0.00013	shotgun (modern)
S_Thai-1	25.1203313	0.00010	0.00000	0.00010	shotgun (modern)
S_Dai-2	19.8120096	0.00004	0.00000	0.00004	shotgun (modern)
S_Dinka-1	20.7590166	0.00004	0.00000	0.00004	shotgun (modern)
S_Finnish-2	17.0155033	0.00003	0.00000	0.00003	shotgun (modern)
S_Han-2	20.7680004	0.00003	0.00000	0.00003	shotgun (modern)
S_Karitiana-1	22.1963029	0.00003	0.00000	0.00003	shotgun (modern)
S_Turkish-1	22.4638799	0.00003	0.00000	0.00003	shotgun (modern)
S_Ju_hoan_North-1	22.7025717	0.00003	0.00000	0.00003	shotgun (modern)
S_Burmese-1	29.2087547	0.00003	0.00000	0.00003	shotgun (modern)

362

363 **Table S4.6. Proportions of expected and observed sites without any coverage.**

364

365

366

367

368 5. Genotype calling

369 5.1. Consensus genotype calling

370 The haploid nature of the human Y chromosome alleviates many issues inherent to
371 genotype calling of diploid genomes. Most importantly, given that only one allele is
372 expected to be present at each site of a non-recombining portion of the Y chromosome,
373 observing more than one allele at a site must be the result of sequencing errors, DNA
374 damage, contamination, or misalignment of reads. While such issues present a significant
375 problem for calling diploid genotypes, by making it challenging to distinguish true
376 heterozygous calls from erroneously called heterozygous genotypes (27, 28), they are
377 less of an issue for haploid genotyping.

378 To call genotypes of the archaic and modern human Y chromosomes in our study,
379 we applied a conservative approach to produce a consensus of sequencing reads. For
380 each Y chromosome BAM file, we performed a pileup of reads at each site (disabling
381 base quality recalibration), filtering out reads with mapping quality less than 25, ignoring
382 bases with base quality less than 20 and removing reads carrying indels at a pileup
383 position. Then, under the assumption that alleles introduced due to DNA damage,
384 sequencing errors, misalignments, or contamination will be in a minority at each site, we
385 called the allele supported by 90% of the reads in a pileup as the haploid genotype for
386 that site. For further analyses, we additionally restricted to genotype calls supported by
387 at least three reads, as described in section 5.3. This genotype calling procedure has
388 been implemented as part of a functionality of a program which is freely available for
389 download at <https://github.com/bodkan/bam-caller>.

390

391 5.2. Genotype calling using *snpAD*

392 The consensus genotype calling approach described in the previous section is quite
393 conservative and does not incorporate an explicit model of DNA damage and sequencing
394 errors. To validate the robustness of our consensus-based results, we compared them to
395 genotype calls generated using *snpAD*, an aDNA-specific genotype caller (28). A major
396 caveat of this approach is the fact that *snpAD* has been designed for calling diploid
397 genotypes and accurate results requires at least 4X genomic coverage (28). Therefore,
398 its genotype calling model has not been tested on low coverage, haploid chromosomes
399 such as those generated in our study. While recognizing these limitations, we used *snpAD*
400 to call genotypes of all four archaic Y chromosomes captured for the 6.9 Mb target regions
401 (*Denisova 4*, *Denisova 8*, *Spy 94a* and *Mezmaiskaya 2*), discarded any sites which were
402 called as heterozygous (likely the result of errors, contamination or aDNA damage), and
403 converted all homozygous genotype calls to a haploid state.

404 In accordance with *snpAD*'s more sophisticated model of aDNA damage patterns,
405 we have found that the number of successfully genotyped sites is higher than those
406 generated by our simpler consensus-based genotype calling approach, but only
407 marginally so (Table S5.1). Furthermore, although the rates of C-to-T and G-to-A SNP
408 frequencies observed in the final set of genotype calls of the high coverage *Mezmaiskaya*
409 2 is very close to the baseline expectation for present-day DNA, the remaining low
410 coverage archaic Y chromosomes are still affected by aDNA damage and show an
411 excess of falsely called genotypes (Figures S5.1 and S5.2). We note that this is not
412 unexpected, because the coverage of these individuals is much lower than what is
413 recommended by for *snpAD* (28). Overall, we did not observe significant differences

414 between *snpAD*-based and consensus-based genotypes in terms of the inferred times to
415 the most recent common ancestor (TMRCA) and, in fact, we found that both lead to the
416 same conclusions (Figure S7.11). Based on these analyses we concluded that our
417 conservative 90% cutoff for consensus genotype calling method is appropriate and
418 decided to use it for all analyses.
419

420 5.3. Minimum coverage filtering

421 The majority of libraries analyzed in our study have not been treated with the uracil-
422 deglycosylase (UDG) enzyme (SI 2). Unlike UDG-treated libraries, non-UDG libraries
423 retain an increased deamination signal throughout the molecules (Figures S4.1 and S4.2)
424 which poses a significant challenge for distinguishing false substitutions caused by aDNA
425 damage from true polymorphisms (28).

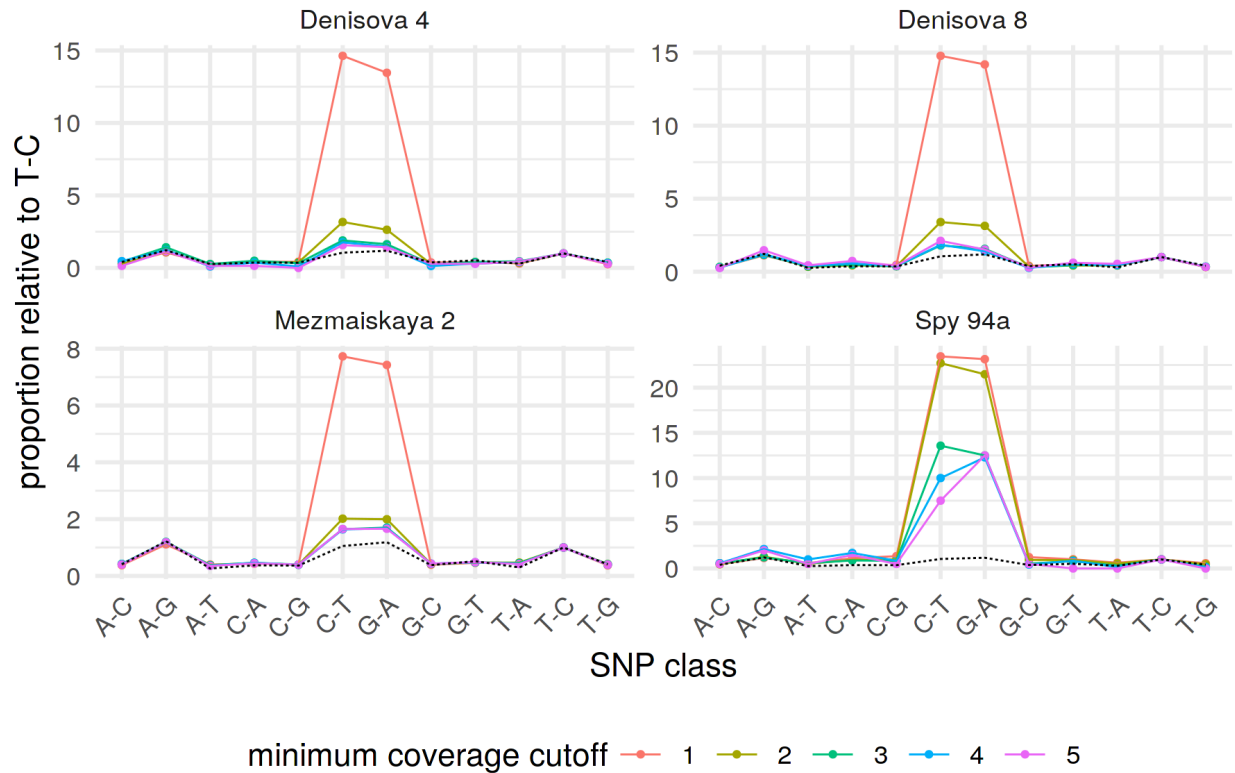
426 For a given sequencing read carrying a putative substitution, it is not
427 straightforward to decide whether this substitution represents a true polymorphism or
428 error. Given enough sequencing coverage, this issue can be mostly overcome by
429 observing a sufficient number of bases from reads that do not carry a deamination-
430 induced substitution, integrating evidence from multiple reads at a site (28). However, as
431 our data is of relatively low coverage (Figure 1B, Table S4.1), we were concerned by
432 selecting an appropriate lower coverage cutoff to minimize the impact of false
433 polymorphisms on our inferences. Specifically, if the same nucleotide is observed in a
434 majority of reads mapped to the same genomic position, it is unlikely that this would be
435 the result of aDNA damage, sequencing errors or contamination, as these occur mostly
436 at relatively low frequencies in the individuals in our study (Figure S4.1 and Table S4.5).
437 To get a sense of the frequency of calling false polymorphisms as a function of coverage,
438 we calculated the proportions of observed genotypes in each archaic human Y
439 chromosome given a certain coverage filtering cutoff and compared those to the baseline
440 expectation for present-day human DNA. As expected, allowing SNPs supported by only
441 one read leads to a significant excess of C-to-T and G-to-A SNP (Figure S5.1), a
442 consequence of the presence of aDNA damage (Figure S4.1). We found that increasing

443 the minimum coverage cutoff to two reads causes the rate of aDNA-induced SNPs to drop
444 significantly towards the baseline expectation, but going beyond requiring the support of
445 three reads for each SNP does not lead to further improvement in accuracy of genotype
446 calling (Figure S5.1). Because of this and because each additional increase in required
447 minimum coverage is at the expense of the final number of available sites, we settled on
448 a minimum coverage cutoff of 3 reads.

449 It is important to note that despite the residual presence of false aDNA
450 substitutions in the final set of filtered genotype calls, manifesting as increased
451 frequencies of C-to-T and G-to-A SNPs compared to present-day DNA (Figure S5.1),
452 comparisons of archaic-modern human TMRCA estimates obtained using the full set of
453 genotype calls and those based on genotypes restricted to non-C-to-T/G-to-A SNPs did
454 not reveal any significant differences (Figure S7.9). This is partially due to very low rates
455 of residual false SNPs that pass through the filtering, but mostly because our TMRCA
456 estimators are quite insensitive to private mutations on the archaic lineage (SI 7). A
457 second validation of our coverage filter follows from the fact that the two Denisovan and
458 all three Neandertal Y chromosomes lead to the same TMRCA estimates with modern
459 human Y chromosomes despite differences in coverage and rates of aDNA damage
460 (Figures 1B, 2B and S5.1). Both of these factors would affect genotyping accuracy if not
461 handled appropriately, and would introduce noise in TMRCA estimates estimated for
462 individual Y chromosomes.

463 Complete counts of Y chromosome positions passing the filters for all individuals
464 are reported in Tables 5.1 and 5.2 (counts for archaic and modern human individuals,

465 respectively, in 6.9 Mb target regions) and Tables 5.3 and 5.4 (counts for archaic and
466 modern human individuals, respectively, in 560 kb target regions).



467

468 **Figure S5.1. Frequencies of observed polymorphisms normalized by the observed**

469 **frequency of T-C polymorphism.** SNP classes were counted for each archaic human Y

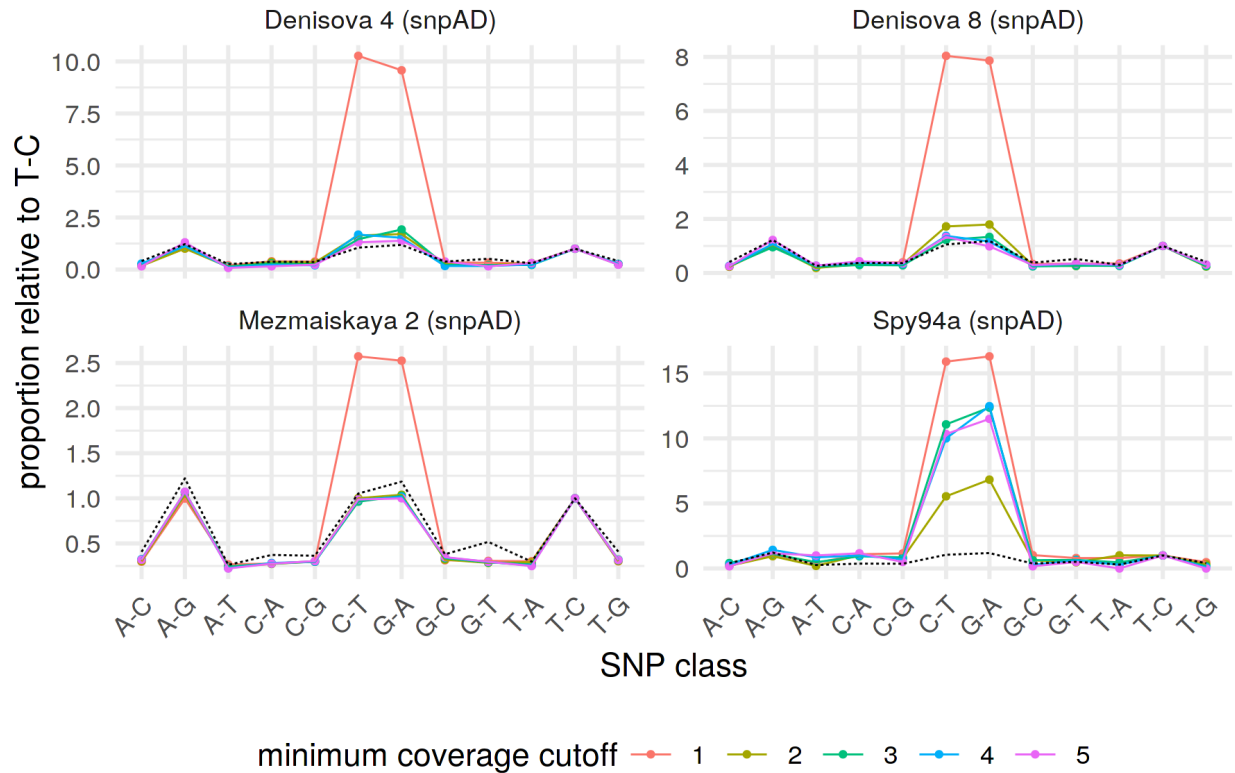
470 chromosome (one panel each) and all counts were normalized by dividing them with

471 observed counts of T-C SNPs. Dotted line shows an expectation based on SNP

472 proportions observed in Y chromosomes of the following SGDP individuals: *S_French_1*,

473 *S_Papuan_2*, *S_Burmese_1*, *S_Thai_1*, *S_Sardinian_1*.

474



475

476 **Figure S5.2. Frequencies of observed polymorphisms normalized by the observed**

477 **frequency of T-C polymorphism.** SNP classes were counted for each archaic human Y

478 chromosome (one panel each) and all counts were normalized by dividing them with

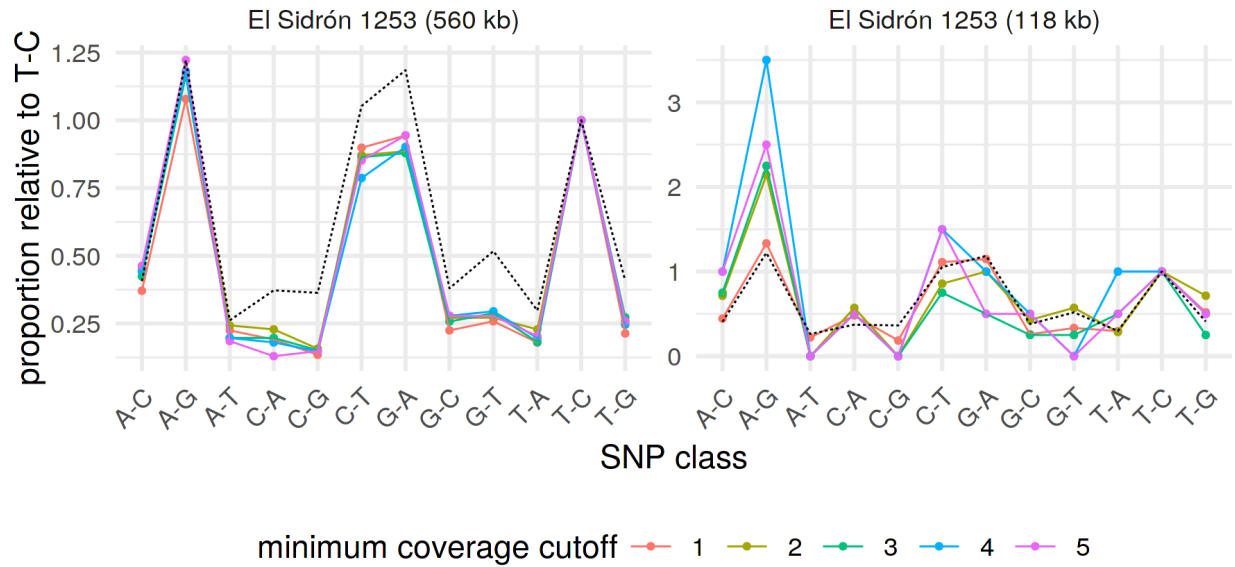
479 observed counts of T-C SNPs. Dotted line shows an expectation based on SNP

480 proportions observed in Y chromosomes of the following SGDP individuals: *S_French_1*,

481 *S_Papuan_2*, *S_Burmese_1*, *S_Thai_1*, *S_Sardinian_1*.

482

483



484

485 **Figure S5.3. Frequencies of observed polymorphisms normalized by the frequency**

486 **of T-C polymorphism.** SNP classes were counted for each archaic human Y

487 chromosome (one panel each) and all counts were normalized by dividing them with

488 observed counts of T-C SNPs. Dotted line shows an expectation based on SNP

489 proportions observed in Y chromosomes of the following SGDP individuals: *S_French_1*,

490 *S_Papuan_2*, *S_Burmese_1*, *S_Thai_1*, *S_Sardinian_1*.

491

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496

name	all sites		excluding C-to-T/G-to-A	
	count	proportion	count	proportion
Spy 94a (shotgun)	2,664,787	38.5%	2,636,405	38.1%
Spy 94a	3,502,380	50.7%	3,470,671	50.2%
Spy 94a (snpAD)	3,557,124	51.5%	3,522,759	51.0%
Denisova 4	4,731,302	68.4%	4,705,860	68.1%
Denisova 4 (snpAD)	4,824,242	69.8%	4,798,037	69.4%
Denisova 8	5,851,332	84.6%	5,828,356	84.3%
Denisova 8 (snpAD)	6,260,242	90.6%	6,236,272	90.2%
Mezmaiskaya 2 (shotgun)	3,822,106	55.3%	3,762,768	54.4%
Mezmaiskaya 2	6,348,948	91.8%	6,346,684	91.8%
Mezmaiskaya 2 (snpAD)	6,669,912	96.5%	6,667,441	96.5%

497

498 **Table S5.1. Counts of sites for each archaic human Y chromosome in 6.9 Mb**
 499 **capture regions which passed the filtering for minimum depth of 3 reads in addition**
 500 **to other filtering and genotype calling criteria.** Multiple records for the same individual
 501 indicate different versions of the data (shotgun sequences as opposed to capture) or
 502 different ways of calling genotypes (consensus genotype calling or genotype calling using
 503 snpAD). Reported are numbers for all sites and for sites excluding C-T and G-A
 504 polymorphisms. The proportions are calculated relative to the total number of available
 505 sites (6,912,728; SI 1).

506

name	all sites		excluding C-to-T/G-to-A	
	count	proportion	count	proportion
A00-1	6,852,077	99.12%	6,851,175	99.11%
A00-2	6,867,616	99.35%	6,866,716	99.33%
A00	6,873,078	99.43%	6,872,184	99.41%
Ust'-Ishim	6,888,071	99.64%	6,887,986	99.64%
S_Yoruba-2	6,897,101	99.77%	6,896,820	99.77%
S_Mandenka-1	6,898,162	99.79%	6,897,879	99.79%
S_Mbuti-1	6,898,694	99.80%	6,898,400	99.79%
S_Gambian-1	6,899,210	99.80%	6,898,929	99.80%
S_Papuan-2	6,904,393	99.88%	6,904,208	99.88%
S_Thai-1	6,905,023	99.89%	6,904,846	99.89%
S_BedouinB-1	6,905,031	99.89%	6,904,820	99.89%
S_Saami-2	6,905,291	99.89%	6,905,102	99.89%
S_Sardinian-1	6,905,558	99.90%	6,905,352	99.89%
S_Ju_hoan_North-1	6,906,228	99.91%	6,905,872	99.90%
S_Karitiana-1	6,906,409	99.91%	6,906,258	99.91%
S_Dai-2	6,906,414	99.91%	6,906,248	99.91%
S_Burmese-1	6,906,438	99.91%	6,906,260	99.91%
S_Dinka-1	6,906,507	99.91%	6,906,225	99.91%
S_French-1	6,906,914	99.92%	6,906,738	99.91%
S_Punjabi-1	6,907,065	99.92%	6,906,885	99.92%
S_Finnish-2	6,907,088	99.92%	6,906,889	99.92%
S_Han-2	6,907,603	99.93%	6,907,417	99.92%
S_Turkish-1	6,907,751	99.93%	6,907,717	99.93%

507

508

509 **Table S5.2. Counts of sites for each modern human Y chromosome (consensus**
510 **genotype calls of shotgun data) in 6.9 Mb capture regions which passed the**
511 **filtering for minimum depth of 3 reads in addition to other filtering and genotype**
512 **calling criteria.** Reported are numbers for all sites and for sites excluding C-T and G-A
513 polymorphisms. The proportions are calculated relative to the total number of available
514 sites (6,912,728).

515

name	all sites		excluding C-to-T/G-to-A	
	count	proportion	count	proportion
Spy 94a	275,626	49.55%	272,798	49.04%
Denisova 4	408,775	73.49%	406,508	73.08%
Denisova 8	484,377	87.08%	482,608	86.76%
Mezmaiskaya 2	509,145	91.53%	508,962	91.50%
El Sidrón 1253 (560 kb)	530,172	95.31%	530,046	95.29%

516

517

518 **Table S5.3. Counts of sites for each archaic human Y chromosome (in 560 kb**
519 **capture regions) which passed the filtering for minimum depth of 3 reads in**
520 **addition to other filtering and genotype calling criteria.** Reported are numbers for all
521 sites and for sites excluding C-to-T and G-to-A polymorphisms. The proportions are
522 calculated relative to the total number of available sites (556,259).

523

name	all sites		excluding C-to-T and G-to-A	
	count	proportion	count	proportion
A00-1	552,557	99.33%	552,456	99.32%
S_Yoruba-2	553,203	99.45%	553,112	99.43%
S_Mandenka-1	553,348	99.48%	553,257	99.46%
S_Gambian-1	553,365	99.48%	553,275	99.46%
S_Mbuti-1	553,381	99.48%	553,288	99.47%
A00-2	553,422	99.49%	553,321	99.47%
A00	553,618	99.53%	553,517	99.51%
Ust'-Ishim	554,575	99.70%	554,550	99.69%
S_Thai-1	555,830	99.92%	555,787	99.92%
S_Dinka-1	555,842	99.93%	555,785	99.91%
S_BedouinB-1	555,850	99.93%	555,806	99.92%
S_Sardinian-1	555,855	99.93%	555,813	99.92%
S_Burmese-1	555,862	99.93%	555,818	99.92%
S_Dai-2	555,864	99.93%	555,819	99.92%
S_Ju_hoan_North-1	555,870	99.93%	555,818	99.92%
S_Karitiana-1	555,876	99.93%	555,848	99.93%
S_Saami-2	555,927	99.94%	555,891	99.93%
S_French-1	555,942	99.94%	555,902	99.94%
S_Finnish-2	555,945	99.94%	555,906	99.94%
S_Papuan-2	555,951	99.94%	555,918	99.94%
S_Punjabi-1	555,958	99.95%	555,920	99.94%
S_Turkish-1	555,971	99.95%	555,966	99.95%
S_Han-2	556,022	99.96%	555,978	99.95%

524

525 **Table S5.4. Counts of sites for each modern human Y chromosome (in 560 kb**

526 **capture regions) which passed the filtering for minimum depth of 3 reads in**

527 **addition to other filtering and genotype calling criteria. Reported are numbers for all**

528 sites and for sites excluding C-to-T and G-to-A polymorphisms. The proportions are
529 calculated relative to the total number of available sites (556,259).

530

531 6. Inferring phylogenetic relationships

532 To resolve the phylogenetic relationships of each archaic human Y chromosome to all
533 other Y chromosome sequences, we merged the VCF files with genotype calls from each
534 individual (including the chimpanzee) into a single VCF file and converted the genotypes
535 to the FASTA format using a custom Python script (available on our Github repository:
536 <https://github.com/bodkan/archaic-ychr>). To mitigate biases introduced by low coverage
537 and characteristics of aDNA damage (29), we excluded all C-to-T and G-to-A
538 polymorphisms and applied the same filters for each individual as for all other analyses
539 in our study. Finally, we excluded monomorphic sites and sites carrying private changes
540 on the chimpanzee lineage to reduce the size of the final alignment file.

541 To construct a neighbor-joining phylogenetic tree (Figure 2A), we utilized the
542 functionality provided by R packages *ape* and *phangorn* (30, 31). First, we calculated the
543 distance matrix between all Y chromosome pairs in the FASTA file with the function
544 `dist.dna`, using the model of simple pairwise differences (`model = "raw"`) and
545 excluding sites with missing data specific to each pair (`pairwise.deletion = TRUE`).
546 We then provided this distance matrix to the `nj` function and rooted the resulting
547 neighbor-joining tree using the function `midpoint` from the *phangorn* package. Bootstrap
548 confidence numbers for the neighbor-joining tree were calculated using *ape*'s
549 `boot.phylo` function over 100 replicates. After inspecting the resulting phylogenetic
550 tree, we found that the private branch leading to the *Denisova 4* had a negative length
551 (value = -0.00088). Given that negative branch lengths are a relatively common artefact
552 of the neighbor-joining algorithm and do not affect the reliability of the generated tree we
553 followed the recommendation to set the branch length to zero (32). We note that this does

554 not have any impact on our conclusions, because the change involves a private branch
555 whose length is not meaningful given the discrepancies between sample dates and
556 implied tree tip dates (Figures 1A and 2A, (29)). The final trees were annotated and
557 plotted using the R package *ggtree* (33).

558

559

560 7. Estimating the TMRCA of archaic and modern human Y 561 chromosomes

562 Given that most of the Y chromosome capture data analyzed in our study is of relatively
563 low coverage (Figure 1B, Table S4.1), care needs to be taken when estimating
564 phylogenetic parameters such as the time to the most recent common ancestor (TMRCA).
565 Similarly, low coverage and the associated reduction in the accuracy of genotype calls
566 render the inferred aDNA branch lengths unreliable (29). Any phylogenetic method of
567 choice must be therefore robust to sequencing errors and incorrect branch lengths. We
568 also observe discordances between sample dates and implied molecular tip dates, likely
569 due to residual genotype calling errors (compare Figure 1A vs Figure 2A). We therefore
570 estimated TMRCA between archaic and modern human Y chromosomes using a
571 method inspired by the analysis of the *El Sidrón 1253* Neandertal coding sequence (5).
572 Instead of using polymorphisms on the archaic human lineage, this method relies on first
573 estimating the TMRCA of a pair of high-coverage African and non-African Y
574 chromosomes ($TMRCA_{AFR}$) which is then used to extrapolate the deeper divergence time
575 between archaic and modern human Y chromosomes. We describe the method in the
576 sections below, detailing our modifications and improvements.

577

578

579 7.1. TMRCA of Africans and non-Africans $TMRCA_{AFR}$

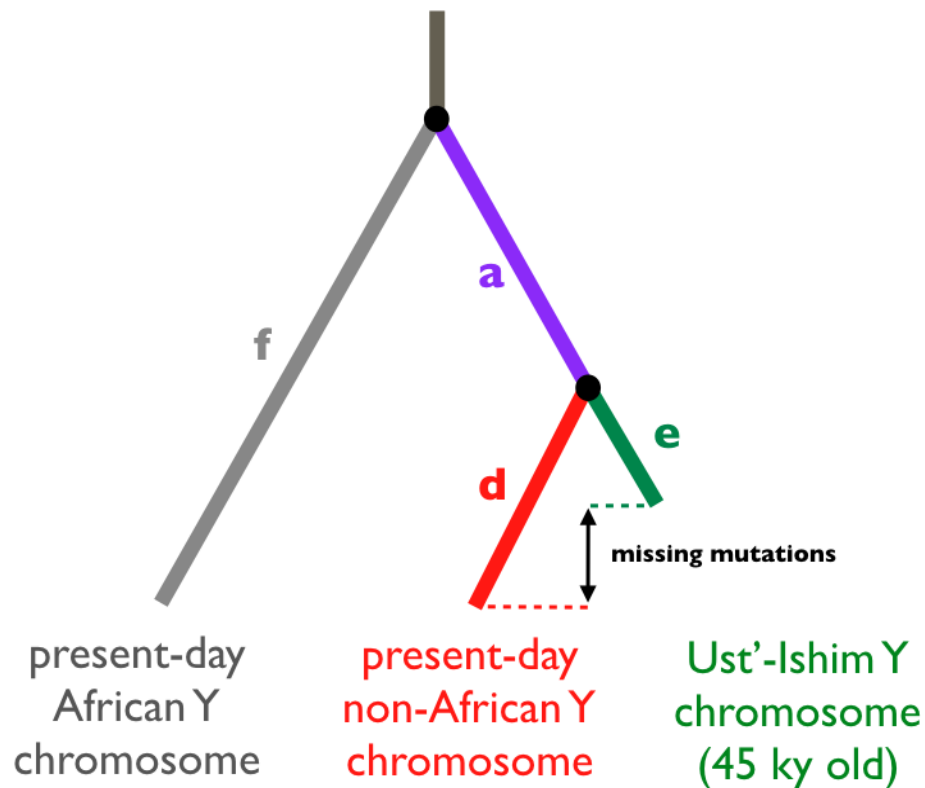
580 The original study by Mendez *et al.* estimated the TMRCA between the A00 African Y
581 chromosome lineage and the hg19 Y chromosome as a representative of non-African Y
582 chromosomes (5, 21). In order to get a better sense of the uncertainty and noise in our
583 TMRCA estimates, we expanded the present-day Y chromosome reference panel to 13
584 non-African and 6 African Y chromosomes from the SGDP data set (Table S7.1) (20).

585 In the first step, we estimated mutation rate in the 6.9 Mb capture target using the
586 high-coverage Y chromosome of *Ust'-Ishim*, a 45,000 years old hunter-gatherer from
587 Siberia (22). We counted derived mutations missing on the *Ust'-Ishim* branch compared
588 to those observed in the panel of 13 non-African Y chromosomes, and used this branch-
589 shortening to calculate mutation rate assuming generation time of 25 years (Figure S7.1,
590 Table S7.2). In the second step, we counted mutations accumulated on an African lineage
591 and a non-African lineage since their split from each other and calculated the TMRCA of
592 both (in units of years ago) using the mutation rate estimated in the first step. Importantly,
593 we discovered that the branch-lengths in Africans are as much as 13% shorter compared
594 to non-Africans (Figure S7.3), which is consistent with significant branch length variability
595 discovered in previous studies and suggested to be a result of various demographic and
596 selection processes (35, 36). To keep our methodology consistent throughout our
597 analyses, we estimated the TMRCA of African and non-African Y chromosomes as the
598 length of the non-African Y lineage (sum of branch lengths $a + d$ in Figure S7.1).
599 Encouragingly, we found that our mutation rate and $TMRCA_{AFR}$ point estimates (Table
600 S7.2) match closely those based on a large panel of present-day Y chromosomes (21).
601 Most importantly, using the A00 lineage as a representative of the deepest known split

602 among present-day human Y chromosomes we inferred a $TMRCA_{A00}$ of ~249 years ago
603 (point estimate based on an estimated mutation rate of 7.34×10^{-10} per bp per year), which
604 is comparable to the $TMRCA_{A00}$ of ~254 years ago estimated by Karmin et al., 2015.
605 Therefore, our more restricted 6.9 Mb capture target gives TMRCA estimates consistent
606 with those obtained from the full Y chromosome shotgun data (21).

607

608



609

610 **Figure S7.1. Schematic of the branch-counting method to estimate the mutation**

611 **rate and split times of African and non-African Y chromosomes.** Accurate knowledge

612 of the age of the *Ust'-Ishim* individual (22) makes it possible to estimate the mutation rate

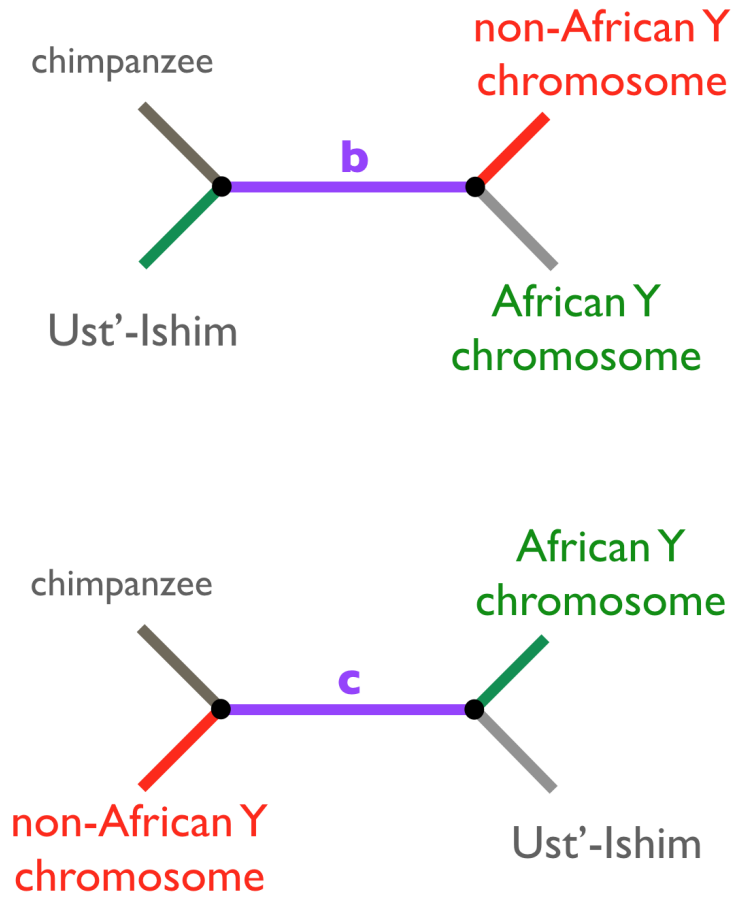
613 within the 6.9 Mb target capture regions. We use the number of mutations missing on the

614 *Ust'-Ishim* lineage since this individual died (45 kya) and compare it to another non-

615 African Y chromosome, i.e. the quantity $d - e$. We used this mutation rate to calculate the

616 TMRCA between a pair of non-African and African Y chromosomes as the total length of

617 the branches $a + d$.

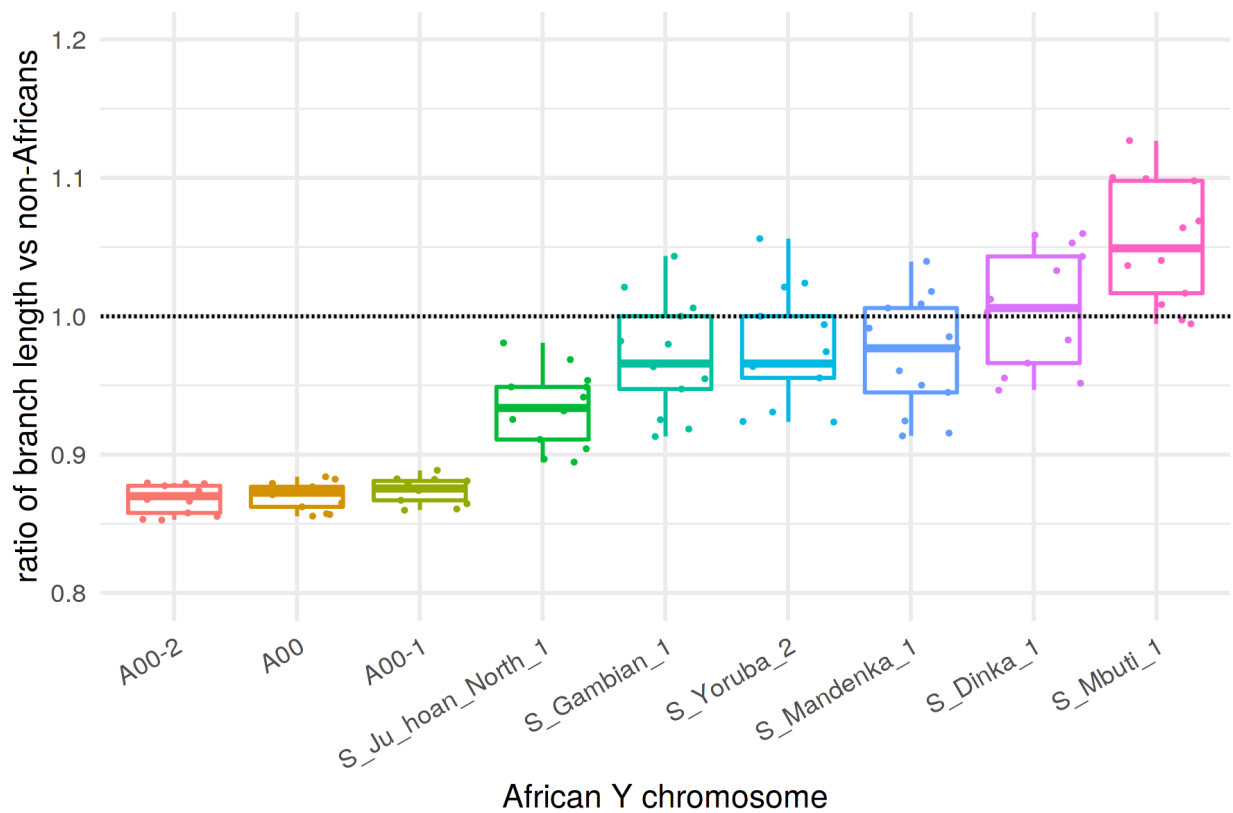


618

619 **Figure S7.2. Two alternative site patterns which are discordant with the true**
620 **phylogenetic relationship.** Given that the true phylogenetic relationship is that shown in
621 Figure S7.1, these alternative branch patterns must be a result of double mutations or
622 genotype calling errors.

623

624



625

626 **Figure S7.3. Branch length differences between African Y chromosomes and a**

627 **panel of 13 non-African Y chromosomes.** Ratios were calculated by creating an

628 alignment of chimpanzee, African and non-African Y chromosomes and taking the ratio

629 of the number of derived alleles observed in an African (x-axis) and the number of derived

630 alleles in each of the individual non-Africans (dots, Table S7.1). “A00” represents a merge

631 of sequences of two lower coverage Y chromosomes, A00-1 and A00-2 (Table S4.3).

632

633

individual	haplogroup
S_Finnish-2	I1a1b5
S_Sardinian-1	J1b2b
S_BedouinB-1	J1b2b
S_Punjabi-1	J2a1
S_French-1	J2a1b1
S_Turkish-1	R1b1a2a1a2c1g
S_Karitiana-1	Q1a2a1a1
S_Saami-2	N1c1a1a2a
S_Burmese-1	O3a1c2
S_Thai-1	O3a2c
S_Dai-2	O2a1
S_Han-2	O1a1
S_Papuan-2	S
S_Dinka-1	E2a
S_Gambian-1	E1b1a1a1f
S_Ju_hoan_North-1	B2b1b
S_Mandenka-1	E1b1a1a1g1
S_Mbuti-1	E1b1a1a1g1
S_Yoruba-2	E1b1a1a1f1b1
A00	A00
A00-1	A00
A00-2	A00

634

635

636 **Table S7.1. Haplogroups of present-day human Y chromosomes used in our**

637 **reference panel.** Haplogroup names were taken from the SGDP annotation table (20).

638 Haplogroups of the African panel are highlighted in gray. The A00 individual represents

639 a merge of lower coverage sequences of two individuals, here named A00-1 and A00-2

640 (Table S4.3).

641

642

643
644

African	a	b	c	d	e	f	total	mutation rate (bp ⁻¹ × year ⁻¹)	TMRCA _{AFR} (years ago)
A00	1060.2	1.2	8.3	214.5	14.2	1103.1	6,064,477	7.34e-10	249211.63
A00-2	1054.5	1.2	8.2	214.7	14.6	1092.7	6,045,632	7.35e-10	247074.54
A00-1	1004.2	1.2	8.1	207.2	14.2	1054.1	5,855,414	7.32e-10	246756.41
S_Ju_hoan_North_1	261.5	2.7	8.5	216.3	14.5	439.2	6,100,839	7.35e-10	98443.62
S_Mbuti_1	116.5	1.2	8.9	217.5	14.5	344.7	6,123,504	7.37e-10	76782.21
S_Dinka_1	110.8	2.1	8.7	217.4	14.5	321.2	6,090,467	7.40e-10	71573.49
S_Mandenka_1	120.9	1.2	8.8	218.3	14.5	321.0	6,123,094	7.39e-10	71226.46
S_Gambian_1	118.8	1.2	8.5	218.1	14.5	320.2	6,096,582	7.42e-10	71127.40
S_Yoruba_2	118.9	1.2	8.7	217.6	13.5	320.9	6,121,558	7.41e-10	71119.46

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Table S7.2. Branch counts and estimates of mutation rate and TMRCA between an African lineage and a panel of 13 non-African Y chromosomes. All quantities represent averages across all non-African Y chromosomes (Table S7.1). Counts in columns *a* to *f* represent counts of site patterns as shown in Figures S7.1 and S7.2. “Total” represents the number of sites out of the total 6.9 Mb of target sequence available for the analysis. The last two columns represent the inferred mutation rate based on the *Ust’-Ishim* branch-shortening and the average TMRCA between a given African and a panel of non-Africans calculated from the length of the *a* + *d* branch as shown in Figure S7.1.

658 7.2. Archaic human-modern human TMRCA ($TMRCA_{archaic}$)

659
660 Having estimated $TMRCA_{AFR}$ (section 7.1), we can express the TMRCA of archaic and
661 modern human Y chromosomes ($TMRCA_{archaic}$) as a factor of how much older is
662 $TMRCA_{archaic}$ compared to $TMRCA_{AFR}$ (Figure S7.4). In mathematical terms, if we call the
663 scaling factor α (following the terminology of (5)), we can write

$$664 \qquad TMRCA_{archaic} = \alpha \times TMRCA_{AFR} . \qquad (1)$$

665

666 In the remainder of this section, we present two ways of calculating α , first using the
667 original approach of Mendez *et al.* and then using a more straightforward method.

668

669 **Mendez et al. approach to calculate α**

670

671 Based on Figure S7.4, an alternative way to express $TMRCA_{archaic}$ in addition to
672 equation (1) is

$$673 \qquad TMRCA_{archaic} = T_{shared} + TMRCA_{AFR} . \qquad (2)$$

674

675 The expressions (1) and (2) define a system of two equations and three variables, which
676 can be solved for T_{shared} to get

$$677 \qquad T_{shared} = \alpha \times TMRCA_{AFR} - TMRCA_{AFR} = TMRCA_{AFR} \times (\alpha - 1). \qquad (3)$$

678
679

680

681 Mendez *et al.* found an expression for α by considering a ratio of time shared by hg19
682 and A00 Y chromosomes after their split from the *El Sidrón 1253* Neandertal (T_{shared}
683 above) and private branch lengths of both (Figure S7.4), arriving at the following
684 expression:

$$685 \quad \frac{T_a}{T_a + T_d + T_e} = \frac{T_{shared}}{T_{shared} + 2 T_{AFR}} = \frac{T_{AFR} (\alpha - 1)}{T_{AFR} (\alpha - 1) + 2 T_{AFR}} = \frac{\alpha - 1}{\alpha + 1}. \quad (4).$$

686

687 Assuming mutation rate constancy on different lineages, α can be found by solving the
688 following equation

$$689 \quad \frac{a}{a + d + e} = \frac{\alpha - 1}{\alpha + 1},$$

690 which leads to

$$691 \quad \alpha = \frac{2 a + d + e}{d + e}. \quad (5)$$

692

693 Using this expression for α and the values of $TMRC A_{AFR}$ estimated in section 7.1, we can
694 calculate $TMRC A_{archaic}$ for each pair of archaic and non-African Y chromosomes using
695 equation (5) (Figure 7.9B).

696

697 **A more robust α statistic**

698 While investigating the effect of minimum coverage filtering on genotype calling accuracy
699 (section 5.3), we discovered a concerning dependence of the apparent branch lengths on
700 the choice of the minimum coverage cutoff. Under normal conditions, the relative
701 proportions of branch lengths a , d and e (Figure S7.4) should remain constant regardless
702 of coverage. This is crucial because the α estimator proposed by Mendez *et al.* is
703 expressed in terms of proportions of lengths of all three of these branches (equation (5)).

704 Strikingly, we found that although the proportions of a and d branch lengths remain
705 relatively stable even for extremely strict coverage filters, the relative length of the e
706 branch (given by the proportion of derived mutations on the private African branch) has
707 increasing tendency (Figures S7.6 and S7.7). Furthermore, although this effect is most
708 pronounced in low coverage samples (Figures S7.6 and S7.7), it is clearly present even
709 in the high coverage *Mezmaiskaya 2*, although at much higher coverage cutoffs (Figure
710 S7.8). Therefore, the issue is clearly not sample-specific but is a common artifact caused
711 by pushing the minimum required coverage close to, or even beyond, the average
712 coverage. Restricting to sites with high number of aligned reads leads to enrichment of
713 regions of lower divergence from the reference sequence, distorting the normal
714 proportions of derived mutations observed on different branches of the tree.

715

716 We note that there is a more straightforward way to express the scaling factor α :

$$717 \quad \alpha = \frac{a + d}{d}. \quad (6)$$

718 This follows trivially from the definition of α as the factor of how much deeper
719 $TMRC A_{archaic}$ is compared to $TMRC A_{AFR}$ and, unlike the original formulation of α
720 (equation (5)), has the advantage of not relying on the relative length of the African branch
721 e . This is important not only because of discordant branch proportion patterns (Figures
722 S7.6-S7.8) but also due to known unequal branch lengths observed in African and non-
723 African Y chromosome lineages (Figure S7.3, (36, 37)).

724

725 For completeness, we note that in a situation without any bias we can assume $e \approx d$.

726 Substituting for e in equation (5) then gives

727
$$\alpha = \frac{2a + d + e}{d + e} \approx \frac{2a + 2d}{2d} \approx \frac{a + d}{d}.$$

728

729 Therefore, under ideal conditions, both approaches to calculate α (equations (5) and (6))
730 are mathematically equivalent.

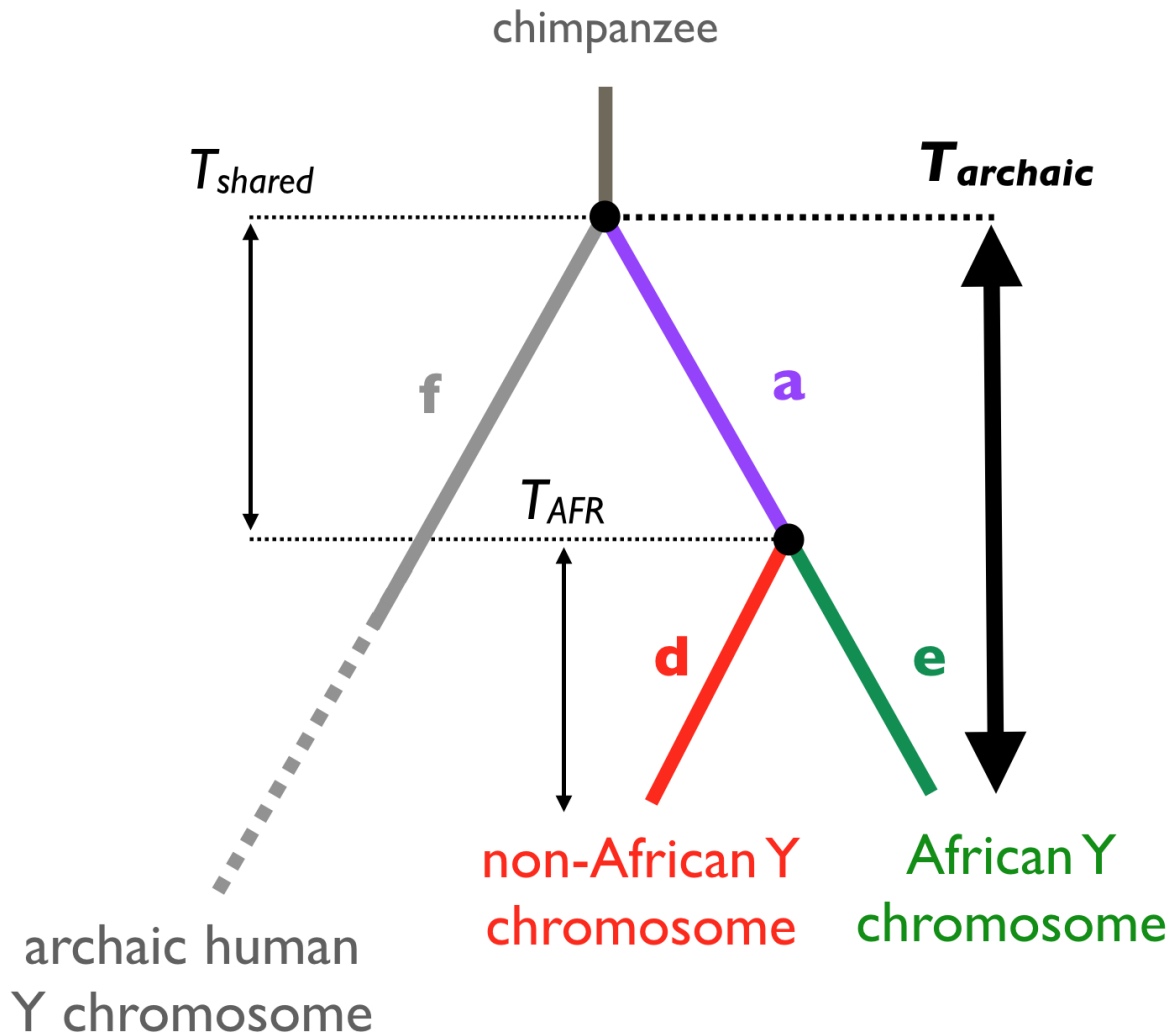
731

732 Using the new expression for α and the values of $TMRCA_{AFR}$ estimated in section 7.1, we
733 can estimate $TMRCA_{archaic}$ for each pair of archaic and non-African Y chromosomes
734 using equation (5) (Figure 2A, Table S7.3). By comparing TMRCA results based on the
735 two formulations for α , we found similar estimates for most of the archaic human Y
736 chromosomes in our study (Figures S7.9 and S7.10). The only exception are the two
737 Denisovan Y chromosomes, for which we infer slightly higher TMRCA with modern
738 humans using the new α estimation procedure compared to the formulation based on the
739 original method (Figures S7.9 and S7.10). This is a consequence of an increased
740 proportion of the e branch relative to the d branch in *Denisova 4* and *Denisova 8* at the
741 chosen minimum coverage filter which is evident in Figure S7.7. Because the original
742 method of Mendez *et al.* relies on the e count of the derived African alleles (equation (5)),
743 this leads to a slight decrease in the value of the α factor and, consequently, to a lower
744 inferred TMRCA value. In contrast, our new formulation of α (equation (6)) is robust to
745 this artifact and the inferred values of TMRCA are not affected.

746

747 Finally, we want to emphasize that although the analyses of branch length discrepancies
748 discussed in this section were mostly based on results obtained using the A00 Y
749 chromosome lineage, the issues we discovered are not specific to a particular choice of

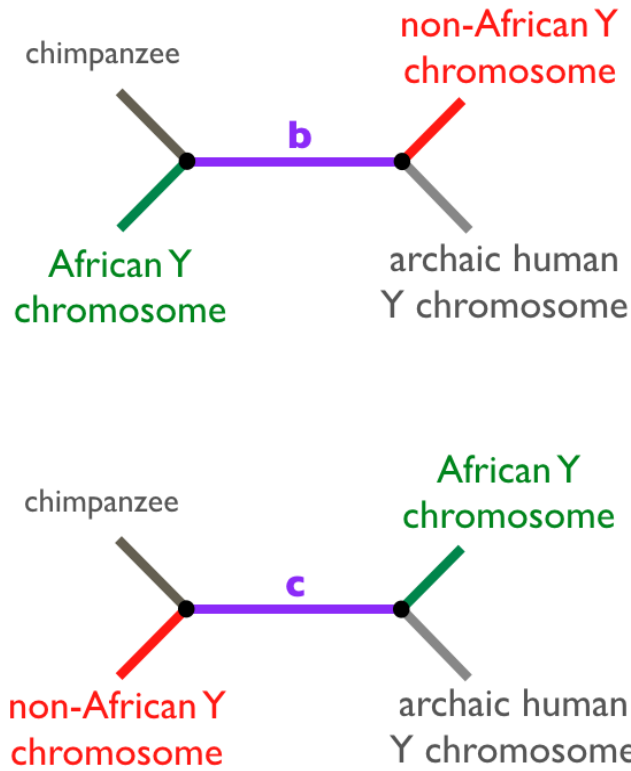
750 an African Y chromosome (Figure S7.8A-C). However, comparisons of TMRCA estimates
751 for the low coverage samples with those obtained for the high coverage samples (which
752 do not show any biases at coverage cutoffs used throughout our study) clearly show that
753 the inferences are most stable when the A00 lineage is used in the calculation of the α
754 scaling factor, even for the samples with lowest coverage (Figure S7.13). Therefore, all
755 main results in our study are based on calculations using the A00 high coverage Y
756 chromosome.
757



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759

760 **Figure S7.4. Branch-counting method to estimate the TMRCA of archaic and**
761 **present-day human Y chromosomes.** As explained in section 7.2, we can decompose
762 the quantity of interest (T_{ARCH} , thick arrow) using two quantities T_{shared} and T_{AFR} and
763 express it simply as a factor of T_{AFR} , which can be accurately estimated using high-quality
764 modern human Y chromosome sequences (section 7.1).

765



766

767

768 **Figure S7.5. Alternative tree topologies with two additional possible branch counts**

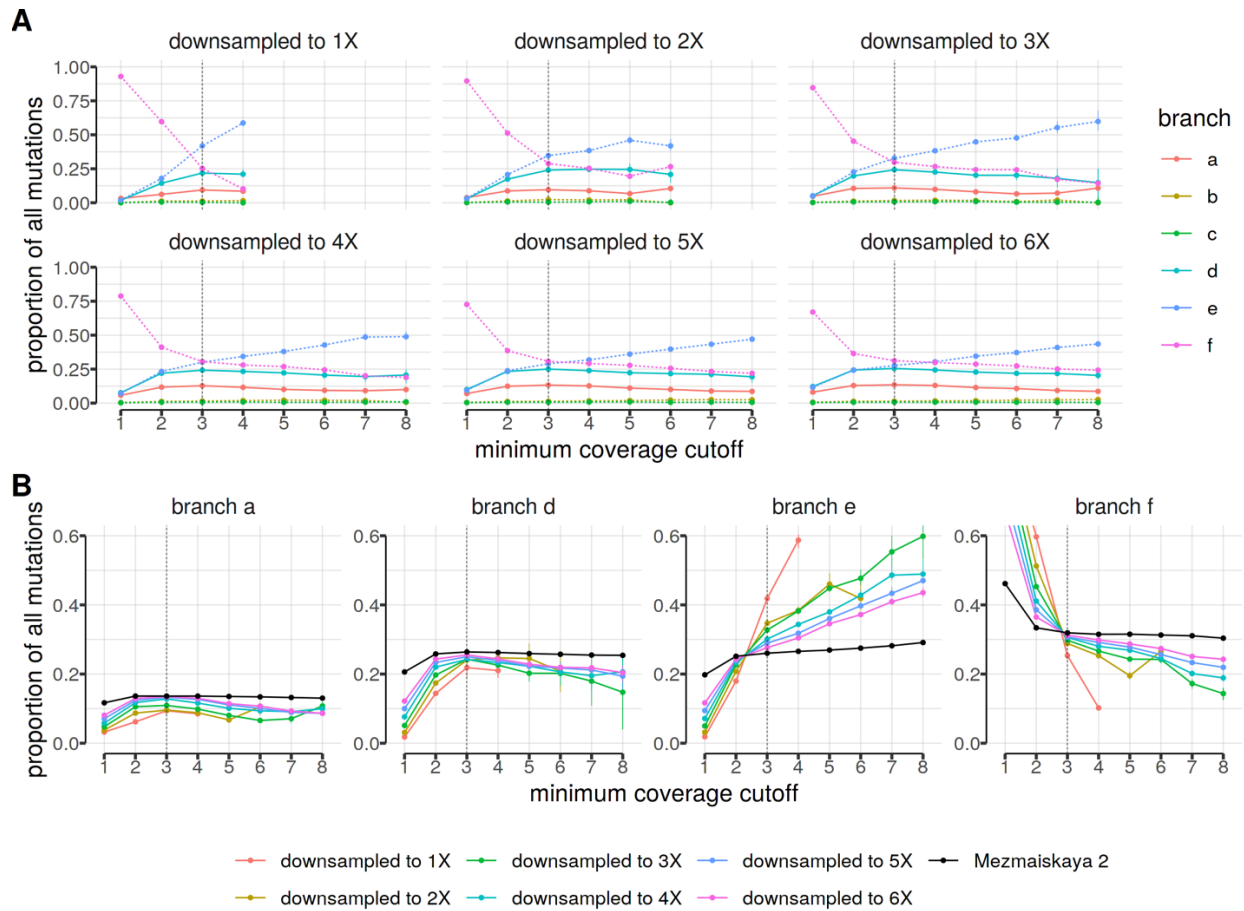
769 ***b* and *c*.** These topologies are incongruent with the true phylogenetic trees and the branch

770 counts *b* and *c* are most likely a result of back mutations or sequencing errors.

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775 **Figure S7.6. Relative proportion of branch lengths in downsampled *Mezmaiskaya***

776 **2 data as a function of minimum coverage cutoff. (A)** Panels show results for 14.3X

777 *Mezmaiskaya 2* downsampled down to 1X, 2X, ... 6X coverage. **(B)** Same as in panel (A)

778 but partitioned per branch. Black solid lines show expectations based on the full

779 *Mezmaiskaya 2* data. Increased relative proportions of the *f* branch lengths are due to

780 false polymorphisms at low coverage cutoffs. Branch length proportions (labeled as in

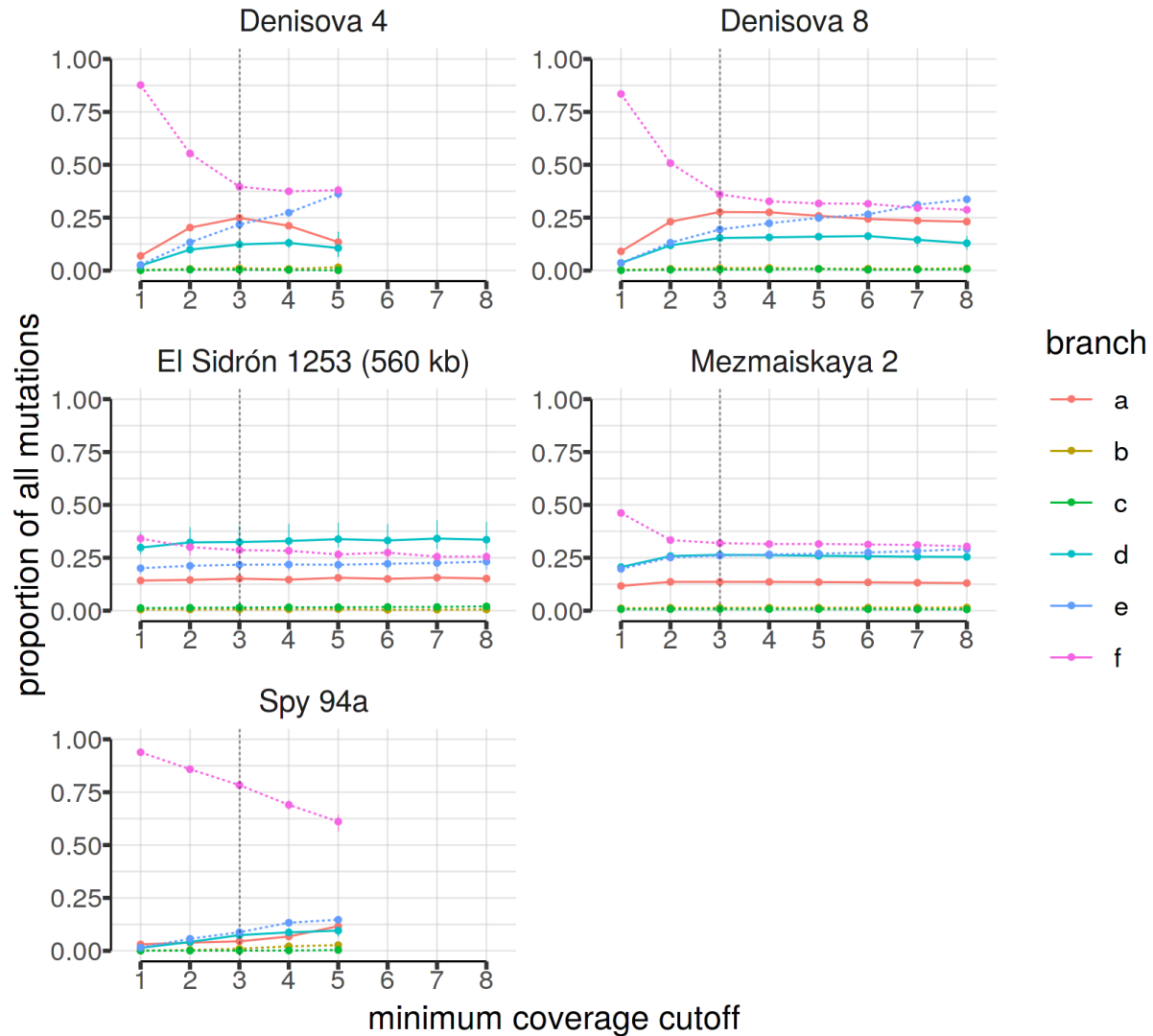
781 Figure S7.4) were calculated as $\frac{a}{N}, \frac{b}{N}, \dots, \frac{f}{N}$, where $N = a + b + \dots + f$. Vertical dotted lines

782 indicate a 3X lower coverage cutoff used throughout our study (section 5.3). Branch

783 counts were averaged over pairs of 13 non-Africans and the A00 African Y chromosome.

784 Analysis is based on all classes of polymorphisms.

785



786

787 **Figure S7.7. Relative proportion of branch lengths as a function of minimum**

788 **coverage support required for each genotype call.** Branch length proportions (colored

789 lines) were calculated as $\frac{a}{N}, \frac{b}{N}, \dots, \frac{f}{N}$, where $N = a + b + \dots + f$. Vertical dotted lines

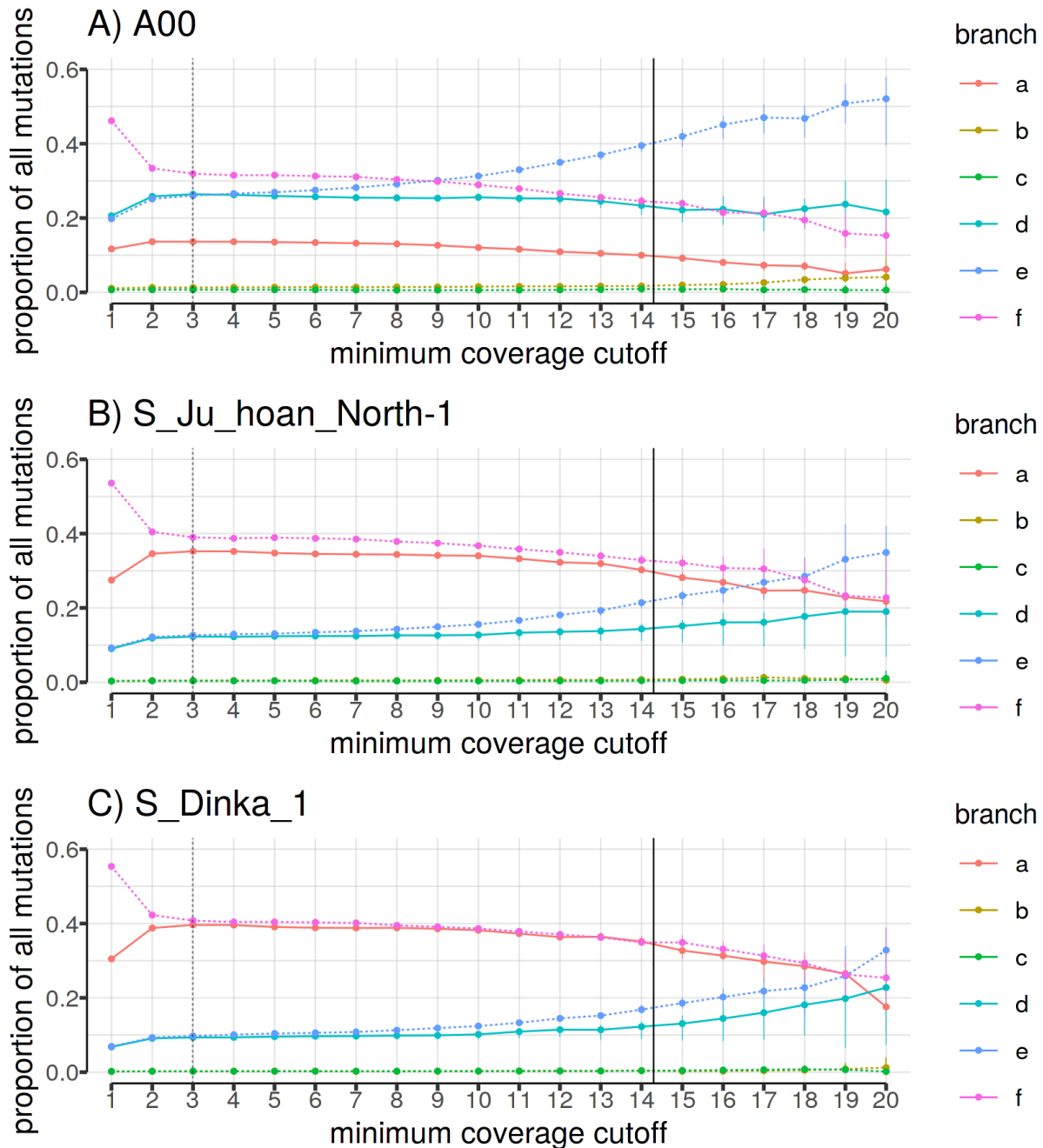
790 indicate a 3X lower coverage cutoff used throughout our study (section 5.3). With the

791 exception of *El Sidrón 1253*, all panels show results for the 6.9 Mb capture data. Analysis

792 is based on all polymorphisms.

793

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796 **Figure S7.8. Relative proportions of branch lengths in the 14.3X *Mezmaiskaya 2***

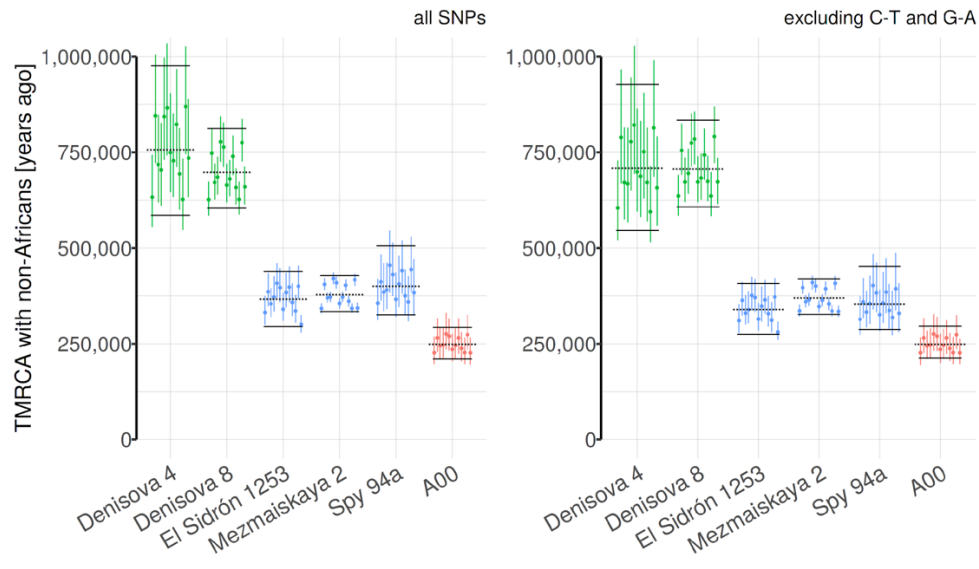
797 **capture data as a function of minimum coverage support required for each**

798 **genotype call. *Mezmaiskaya 2* was chosen for this example because its high coverage**

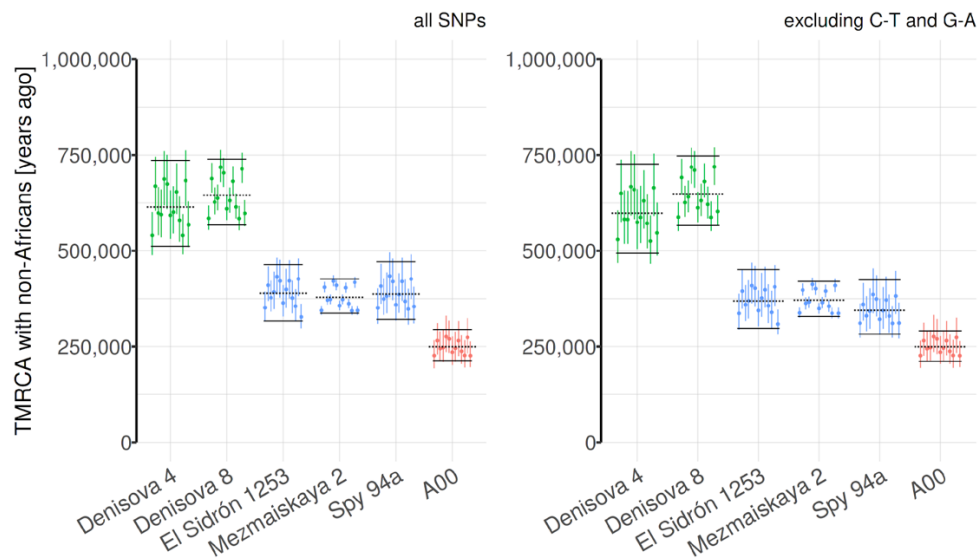
799 **makes the branch proportion patterns stand out more clearly. Panels (A), (B) and (C)**

800 show results based on three different African Y chromosomes used to define branch e
801 (Figure S7.4). Branch length proportions (colored lines) were calculated as $\frac{a}{N}, \frac{b}{N}, \dots, \frac{f}{N}$,
802 where $N = a + b + \dots + f$, using the complete 6.9 Mb capture data of *Mezmaiskaya 2*.
803 Vertical dotted lines indicate a 3X lower coverage cutoff used throughout our study
804 (section 5.3). Analysis is based on all polymorphisms. Vertical solid lines indicate the
805 average coverage in the *Mezmaiskaya 2* individual (14.3X).
806

A) New TMRCA statistic



B) TMRCA statistic of Mendez et al.



807

808 **Figure S7.9. Comparison of TMRCA estimates obtained using the new statistic and**

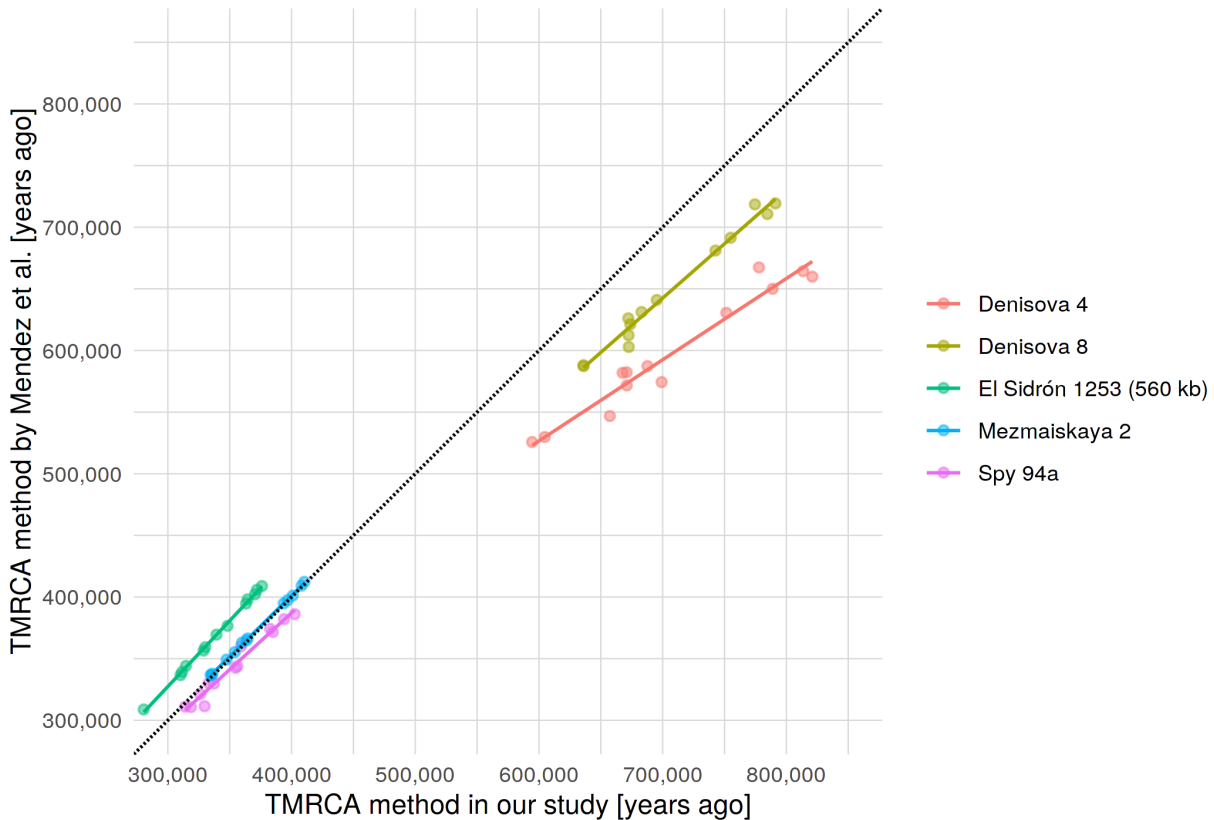
809 **the original approach used in the analysis of the *El Sidrón 1253* Neanderthal. (A)**

810 **Estimates using our new, more robust TMRCA estimate. (B) Original calculation**

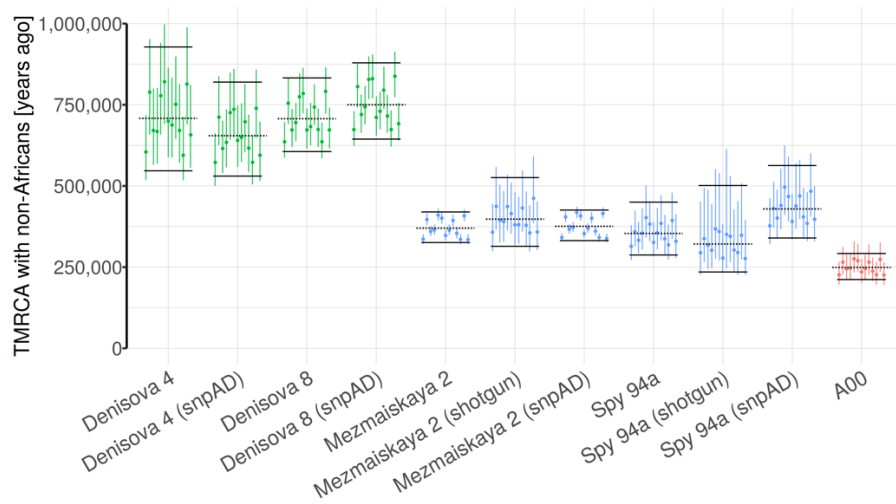
811 **proposed by *Mendez et al.*, 2016 (5). Shown are estimates based on all polymorphisms**

812 **(left panels) and excluding C-to-T or G-to-A polymorphisms which are more likely to be**

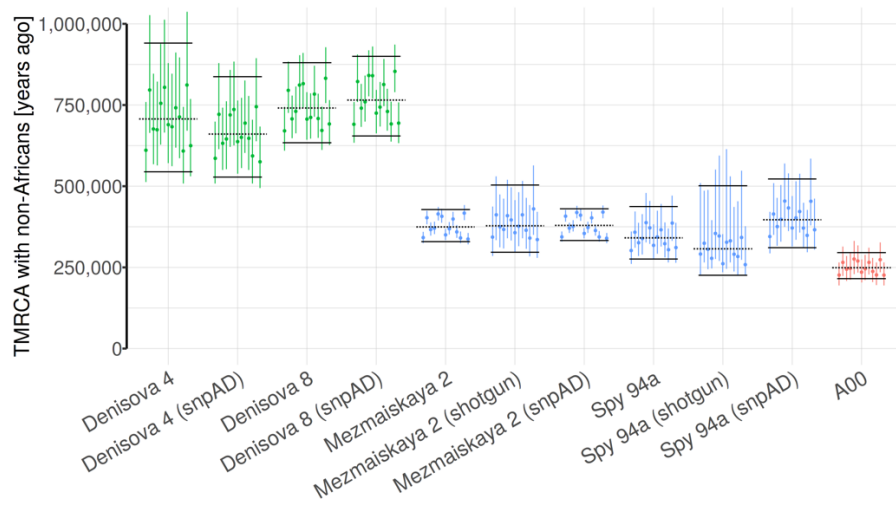
813 **caused by aDNA damage (right panels).**



A) 50% mappability filter



B) 100% mappability filter



825

826 **Figure S7.11. Detailed evaluation for potential technical biases in our TMRCA**

827 **estimates.** Shown are TMRCA results based on different versions of the data (shotgun

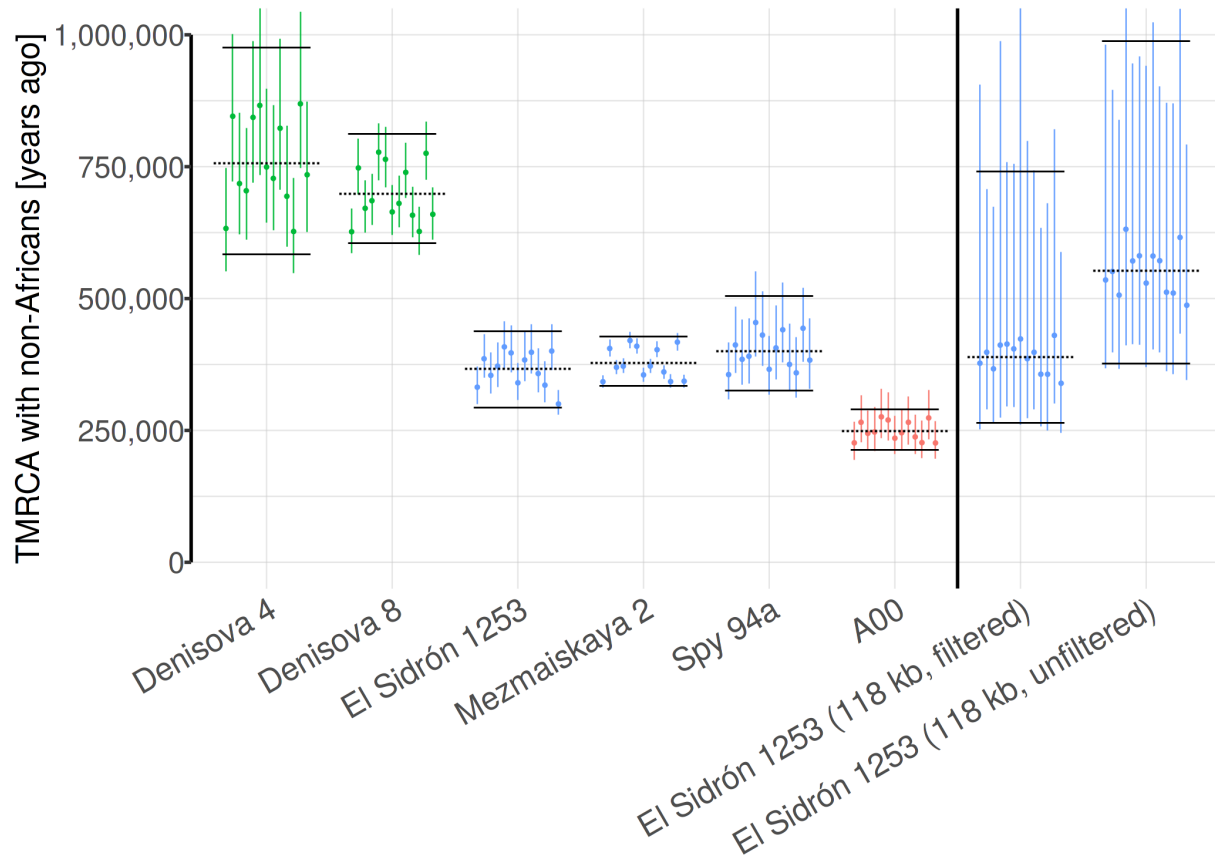
828 or capture) or genotype calling methods (snpAD or consensus-based genotype calling

829 method - which is the default). Panels (A) and (B) show results based on two versions of

830 mappability filters - less strict ("*map35_50%*") and more strict ("*map35_100%*") filters

831 described in the Altai Neandertal study (2). Analysis is based on all polymorphisms except

832 C-to-T and G-to-A changes.



833

834 **Figure S7.12. TMRCA between the *El Sidrón 1253* and modern human Y**

835 **chromosomes.** TMRCA estimates obtained for new capture sequence from the *El Sidrón*

836 *1253* Neanderthal (~370 kya) differ significantly from the previously published results

837 based on the 118 kb of the coding capture sequence (~600 kya, “118 kb, unfiltered”

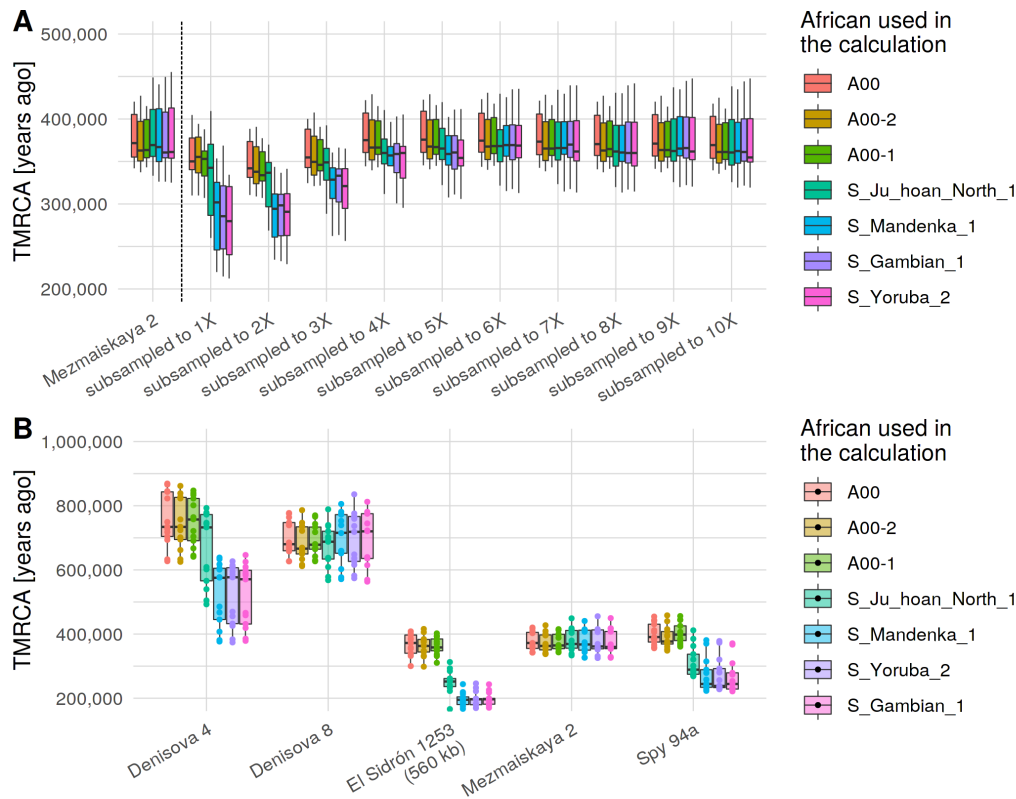
838 column on the right from the vertical line). We found that applying stricter filtering criteria

839 results in the same TMRCA values we obtain for the new capture data (“118 kb, filtered”

840 column on the right from the vertical line). Analysis is based on all polymorphisms.

841

842



843

844 **Figure S7.13. Estimates of $TMRCA_{archaic}$ for different African Y chromosomes used**

845 **in the calculation. (A)** Results for the high-coverage 14.3X *Mezmaiskaya 2* capture data

846 (left of the vertical line) and its subsets generated by downsampling. A00-based TMRCA

847 estimates are quite stable across the entire range of coverage and match those for the

848 full data. In contrast, estimates based on other, less divergent, African Y chromosomes

849 are heavily biased, and this bias is especially strong for low coverage samples. **(B)** A00-

850 based estimates for *Denisova 4*, *El Sidrón 1253* and *Spy 94a* match those for their higher

851 coverage counterparts (*Denisova 8* and *Mezmaiskaya 2*, respectively) as is required by

852 the topology of the phylogenetic tree (Figure 2A). However, estimates based on other

853 African individuals show the same bias shown for low coverage samples in panel (A).

854 Dots show TMRCA estimates based on 13 non-African individuals. Both analyses are

855 based on all polymorphisms.

856

857

name	a	b	c	d	e	f	total	TMRCA [years ago]	TMRCA (lower CI)	TMRCA (upper CI)
Denisova 4	142.2	2.0	2.2	77.8	125.8	165.9	1,084,363.6	708,133.1	549,422.5	930,979.7
Denisova 8	583.5	8.8	8.5	318.0	410.4	583.9	3,372,262.6	706,874.9	607,187.2	833,211.4
Mezmaiskaya 2	301.8	13.8	16.4	625.1	609.1	651.9	5,349,303.5	369,637.7	326,137.1	419,311.0
Spy 94a	17.4	4.5	0.2	42.0	49.5	87.6	510,735.5	353,265.5	286,250.7	449,185.5
El Sidrón 1253	27.9	0.0	2.5	78.8	38.2	62.3	414,420.4	339,207.2	274,711.4	408,161.1

858

859 **Table S7.3. Observed branch counts and estimates of TMRCA between archaic and**

860 **modern human Y chromosomes.** All quantities represent averages across a panel of

861 13 non-African Y chromosomes (Table S7.1) and are based on A00-based estimates of

862 mutation rate and $TMRCA_{AFR}$ (section 7.1). Counts in columns *a* to *f* represent counts of

863 site patterns as shown in Figures S7.4 and S7.5. “Total” represents the number of sites

864 out of the total 6.9 Mb of target sequence available for the analysis.

865

866 7.3. TMRCA of *Mezmaiskaya 2* and *Spy 94a*

867 The split time of Neandertal and modern human Y chromosomes estimated in the
868 previous section provides an upper bound for the last time the two populations
869 experienced gene flow. Similarly, the deepest divergence in late Neandertal Y
870 chromosomes represents a lower bound, as the introgressed Y chromosome lineage
871 must have already been present in Neandertals prior to this diversification.

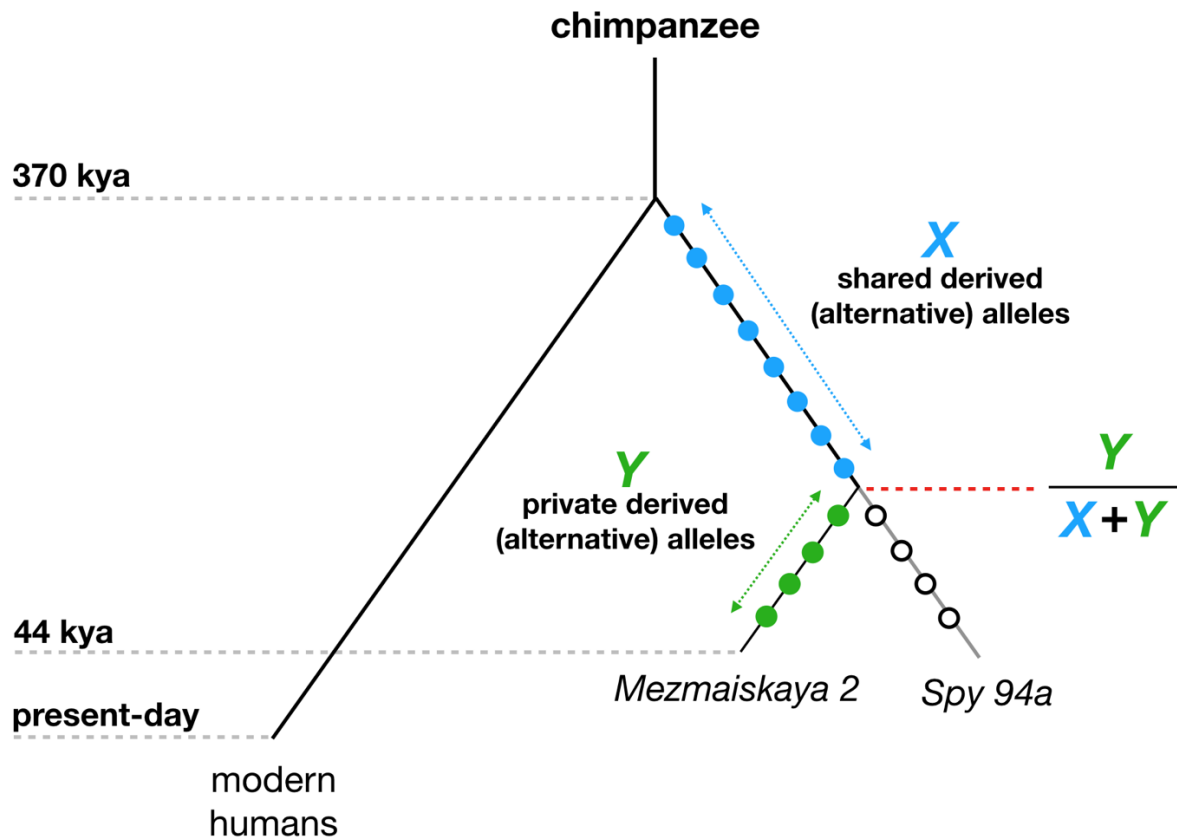
872 To estimate the deepest TMRCA of the known Neandertal Y chromosomes (*i.e.*
873 the TMRCA of *Mezmaiskaya 2* and *Spy 94a*, Figure 2A), we first defined a set of sites in
874 the ~6.9 Mb capture target regions which carry a reference allele in the chimpanzee, A00
875 and French Y chromosomes (the ancestral state) and an alternative allele (the derived
876 state) on the branch leading to the high-coverage Y chromosome of *Mezmaiskaya 2*
877 (Figure S7.14), using the standard filtering used in previous sections (minimum three
878 reads covering each genotyped site, section 5.3). We can calculate the approximate
879 length of this branch using the TMRCA of *Mezmaiskaya 2* and modern human Y
880 chromosomes (~370 kya, Table S7.3) and the known age of *Mezmaiskaya 2* (~44 kya,
881 (11)) as 370 kya – 44 kya = 326 ky (Figure S7.14). We can then estimate the split time
882 between *Mezmaiskaya 2* and *Spy 94a* Y chromosomes using the proportion of
883 *Mezmaiskaya*-derived sites which show the ancestral allele in *Spy 94a* (Figure S7.14).
884 Specifically, if we let Y be the number of ancestral alleles observed in *Spy 94a* and $X + Y$
885 be the total number of sites with genotype calls in *Spy 94a* at positions derived in
886 *Mezmaiskaya 2*, we can express the TMRCA of Y chromosomes of the two Neandertals
887 simply as

888
$$\frac{Y}{X+Y} \times 326 + 44 \text{ kya} \quad (6)$$

889 (Figure S7.14). We maximized the amount of data available for the analysis by merging
890 the capture data with previously published shotgun sequences (11) and evaluated the
891 robustness of the results to different genotype filtering and classes of polymorphisms. To
892 estimate confidence intervals (C.I.), we re-sampled the X and Y counts from Poisson
893 distributions with expected values given by the observed counts (Figure S7.14),
894 calculated the TMRCA on the re-sampled counts using equation (6) and then took the
895 2.5% and 97.5% quantiles of this simulated TMRCA distribution to arrive at the
896 approximate range of 95% C.I.

897 The TMRCA of *Mezmaiskaya 2* and *Spy 94a* are consistently around ~100 kya
898 regardless of the filtering criteria used (individual point estimates and 95% confidence
899 intervals shown in Figure S7.15 and Table S7.4). Together with the TMRCA of Neandertal
900 and modern human Y chromosomes, this suggests that the gene flow from an early
901 population related to modern humans is likely to have happened some time between ~100
902 kya and ~370kya. We note that this time window is significantly wider than the one
903 inferred based on a much more extensive set of available Neandertal mtDNA genomes
904 (219-468 kya) (38). However, it is likely that future sampling of Neandertal Y chromosome
905 diversity will reveal more basal Y chromosome lineages as has been the case for
906 Neandertal mtDNA (38).

907



908

909

910 **Figure S7.14. Estimating the TMRCA of Neandertal Y chromosomes.** Filled circles

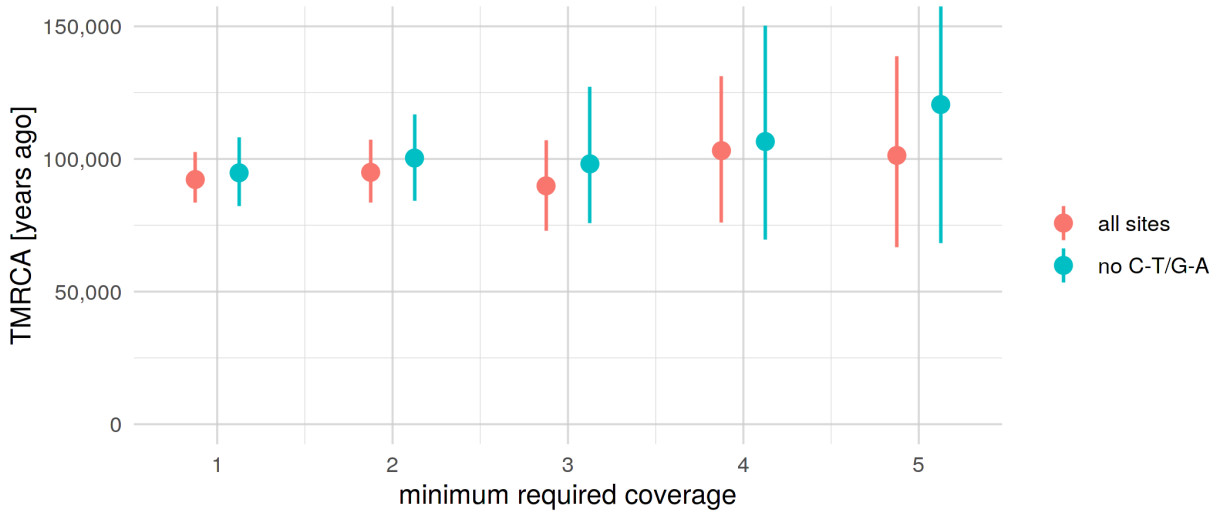
911 represent a set of derived (alternative) alleles on the high-coverage *Mezmaiskaya 2*

912 lineage, and are defined as sites at which *Mezmaiskaya 2* Y chromosome carries a

913 different allele than chimpanzee, A00 and French Y chromosomes. Empty circles

914 represent a subset of such sites which show the ancestral (reference) state in *Spy 94a*.

915



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918 **Figure S7.15. TMRCA of *Mezmaiskaya 2* and *Spy 94a*.** Informative positions (derived

919 alleles in *Mezmaiskaya 2*) were defined using genotype calls in *Mezmaiskaya 2* which

920 passed the standard filtering used throughout our study (minimum coverage of at least

921 three reads, maximum coverage less than 98% quantile of the total coverage distribution).

922 We called the genotypes in the *Spy 94a* Y chromosome at these positions and calculated

923 the TMRCA using the equation (6). We tested the robustness of the estimate to genotype

924 calling errors using different minimum coverage filters for *Spy 94a* (x-axis) and two sets

925 of polymorphisms (colors).

926

927

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REF count	ALT count	proportion REF	TMRCA	TMRCA (lower CI)	TMRCA (upper CI)	minimum coverage	calculated on
71	469	0.1315	92250.32	83814.88	102157.6	1	all sites
51	314	0.1397	94962.33	83315.75	107309.6	2	all sites
20	141	0.1242	89862.87	74453.89	108808.4	3	all sites
13	66	0.1646	103130.37	77184.06	129130.5	4	all sites
7	37	0.1591	101332.33	67250.74	141779.7	5	all sites
43	266	0.1392	94775.67	82515.76	108105.1	1	no C-T/G-A
32	173	0.1561	100347.68	83703.34	117665.3	2	no C-T/G-A
13	74	0.1494	98152.86	75463.15	125756.6	3	no C-T/G-A
7	33	0.1750	106565.56	69559.13	149511.3	4	no C-T/G-A
5	18	0.2174	120510.02	67229.10	180578.4	5	no C-T/G-A

929

930

931

932 **Table S7.4. Point estimates and 95% C.I. for the TMRCA of *Mezmaiskaya 2* and *Spy***

933 **94a Y chromosomes as shown in Figure S7.15.**

934

935

936 7.4. Confidence intervals

937 Under the assumption that mutations on each branch of a tree (Figures S7.1 and S7.4)
938 accumulate independently, the observed counts of mutations can be understood as
939 realizations of independent Poisson processes (mutation counts in Tables S7.2 and
940 S7.3). To quantify the uncertainty in our TMRCA estimates, we used a simulation-based
941 bootstrapping approach. For each set of branch lengths used to calculate scaling factor
942 α (branches a, d and e , Table 7.3), we generated 1000 sets of simulated counts by
943 randomly sampling from a Poisson distribution with the parameter λ set to values
944 observed from the data. In other words, we simulated “trees” implicitly by generating a
945 set of Poisson-distributed branch lengths. We then used the simulated counts to estimate
946 the corresponding TMRCA values, obtaining a distribution of TMRCA consistent with the
947 observed data. Finally, we took the lower 2.5% and upper 97.5% quantiles of the
948 simulated distribution as the boundaries of bootstrap-based 95% confidence intervals.

949 To estimate the confidence interval for TMRCA across the whole panel of 13 non-
950 African Y chromosomes (black dotted horizontal lines in TMRCA figures in our study such
951 as Figure 2B), we followed the same procedure but pooled all simulated counts together
952 (i.e., 1000 simulated counts for each of the 13 Y chromosomes). Then, we took the lower
953 2.5% and upper 97.5% quantiles of TMRCA estimates calculated from the pooled counts.

954

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957 8. Simulations of introgression under purifying selection

958 To investigate the expected frequency trajectories of Y chromosomes introgressed from
959 modern humans into Neandertals, we adapted a modeling approach previously used to
960 study negative selection against Neandertal DNA in modern humans (39, 40). Briefly, this
961 model assumes lower effective population size (N_e) in Neandertals than modern humans
962 as inferred from comparisons of whole-genome sequences (2). Under nearly-neutral
963 theory, such differences in N_e are expected to increase the genetic load in Neandertals
964 compared to modern humans through an excess of accumulated deleterious mutations
965 due to lower efficacy of purifying selection. Therefore, after introgression from
966 Neandertals into modern humans, Neandertal haplotypes would be under stronger
967 negative selection compared to modern humans haplotypes, causing a rapid decrease in
968 proportion of genome-wide Neandertal ancestry (39, 40).

969 In the context of evidence for Neandertal Y chromosome replacement in our study,
970 we were particularly interested in the dynamics of introgression in the opposite direction,
971 from modern humans into Neandertals. Specifically, given that nearly-neutral theory
972 predicts that Neandertal Y chromosomes would carry a higher load of deleterious
973 mutations compared to modern human Y chromosomes, how much is natural selection
974 expected to favor introgressed modern human Y chromosomes compared to their original
975 Neandertal counterparts?

976

977 To address this question, we used a forward population genetic simulation framework
978 SLiM (version 3.3) (41) to build an approximate model of modern human and Neandertal
979 demographic histories, following a strategy we used to study the long-term effects of

980 Neandertal DNA in modern humans (40). To simulate differences in N_e of both
981 populations, we set $N_e = 10,000$ in modern humans and $N_e = 1,000$ in Neandertals after
982 the split of both lineages from each other at 600,000 years ago. Given the non-
983 recombining nature of the human Y chromosome, we implemented a simplified model of
984 a genomic structure in which the only parameter of interest is the total amount of
985 sequence under selection (“functional” sequence, ranging from 100 kb to 2 Mb in steps
986 of 100 kb), and set the recombination rate to zero. Furthermore, because the amount of
987 deleterious variation accumulated on both lineages is directly related the time they have
988 been separated from each other, we simulated gene flow from modern humans into
989 Neandertals between 150,000 to 450,000 years ago in steps of 25,000 years (this time
990 range for gene flow encompasses the times inferred by (38, 42, 43) and our own study),
991 assuming a fixed split time of 600,000 years ago. We ran 100 independent replicates for
992 each combination of parameters described above, including an initial burn-in phase of
993 70,000 generations ($7 \times$ ancestral N_e of 10,000) to let the simulations reach the state of
994 mutation-selection-drift equilibrium.

995 In our previous study, we found evidence for different modes of selection in
996 different classes of functionally important genomic regions, suggesting that the fitness
997 consequences of mutations vary significantly according to the position of their occurrence
998 (40). Realistic modeling of negative selection and introgression would thus require precise
999 information about the distributions of fitness effects (DFE), dominance and epistasis for
1000 coding, non-coding and regulatory regions. Unfortunately, with the exception of DFE of
1001 amino acid changing *de novo* mutations affecting autosomal genes (44, 45), little is known
1002 about fitness consequences of non-coding and regulatory mutations on the Y

1003 chromosome. Furthermore, the impact of Y chromosome structural variation in the
1004 context of male fertility is highly significant, but still relatively poorly understood in terms
1005 of its DFE (46, 47). These issues, as well as the large parameter space of all relevant
1006 demographic and selection factors make analyzing the model dynamics quite challenging.
1007 To make our results easier to interpret, we scored each simulation run (i.e., each
1008 introgression frequency trajectory) with the ratio of fitness values of the average
1009 Neandertal Y chromosome and the average modern human Y chromosome generated
1010 by the simulation, just prior to the introgression. This way we collapse many potential
1011 parameters into a single relevant measure (how much worse are Neandertal Y
1012 chromosomes compared to modern human Y chromosomes in terms of evolutionary
1013 fitness) while, at the same time, generalizing our conclusions to other potential factors we
1014 do not model explicitly.

1015 To calculate the fitness of simulated Y chromosomes, we used the fact that
1016 mutations in SLiM behave multiplicatively, i.e. each mutation affects any individual's
1017 fitness independently of other mutations. Following basic population genetics theory (48),
1018 if we let the fitness of an individual be W and the fitness of each mutation be w_i , we can
1019 define W as $W = \prod w_i = \prod (1 - s_i)$, where i runs across all mutations carried by this
1020 individual and s_i is the selection coefficient of the i -th deleterious mutation. We can then
1021 transform multiplicative interaction into log-additive interaction by

$$1022 \quad W = \prod w_i = e^{\sum \log w_i} = e^{\sum \log(1-s_i)} \approx e^{\sum(-s_i + \frac{s_i^2}{2})} \approx e^{-\sum s_i}, \quad (7)$$

1023 using simple rules of Taylor expansion under the assumption that we are dealing with
1024 weakly deleterious mutations whose selection coefficients (s_i) are expected to be very
1025 small (48).

1026

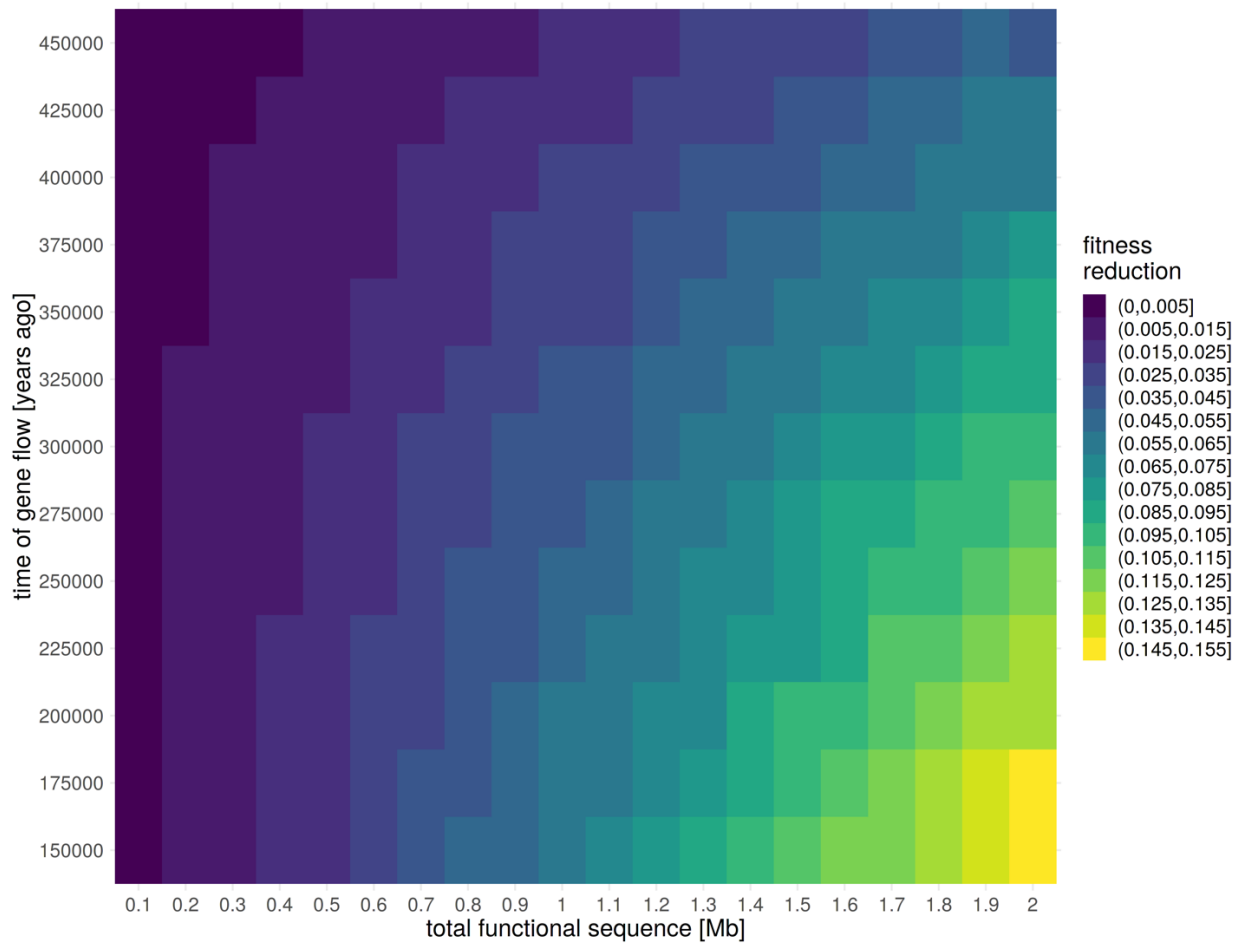
1027 In practice, we let each simulation replicate run until modern human introgression into
1028 Neandertals, at which point we saved all Neandertal and modern human Y chromosomes
1029 and their mutations to a SLiM population output file. After introgression, which we
1030 simulated at 5%, we tracked the frequency of modern human Y chromosomes in
1031 Neandertals for 100,000 years, saving the frequency values at regular time intervals to
1032 another output file.

1033 For the downstream analyses, we calculated the fitnesses of all Neandertal and
1034 modern human Y chromosomes from the population output files saved in the first step
1035 using equation (7) and calculated the ratio of the mean fitness values in both populations
1036 – this measure quantifies the expected decrease in fitness of Neandertal Y chromosomes
1037 compared to modern human Y chromosomes. The distribution of simulated fitness ratios
1038 across a two-dimensional parameter grid is shown in Figure S8.1. As expected, longer
1039 times of separation between Neandertals and modern humans and larger amounts of
1040 mutational target sequence increases the average genetic load of Neandertals. To
1041 analyze the dynamics of introgression in Neandertals over time, we scored the simulated
1042 modern human Y chromosome frequency trajectories with the calculated fitness decrease
1043 obtained from the simulation (Figure S8.2). Finally, we estimated the expected probability
1044 of replacement of the original Neandertal Y chromosomes over time by counting the
1045 proportion of the simulated trajectories (over all trajectories in each fitness bin) in which
1046 the introgressed modern human Y chromosomes reached fixation in each time point
1047 (Figure S8.2). These trajectories of replacement probabilities are shown in Figure 3B.

1048 Similarly to Figure S8.1, Figure S8.3 shows these probabilities expanded from the single
1049 fitness reduction score back on the two-dimensional parameter grid.

1050 We note that although the simulation setup presented here is specific to Y
1051 chromosomes, the conclusions based on the abstract measure of fitness reduction can
1052 be generalized even to the case of mtDNA introgression (Figure 3B).

1053



1054

1055

1056 **Figure S8.1. Expected decrease in fitness of an average Neandertal Y chromosome**

1057 **compared to an average modern human Y chromosome.** Fitness decrease values

1058 were averaged over 100 independent simulation replicates on a grid of two parameters

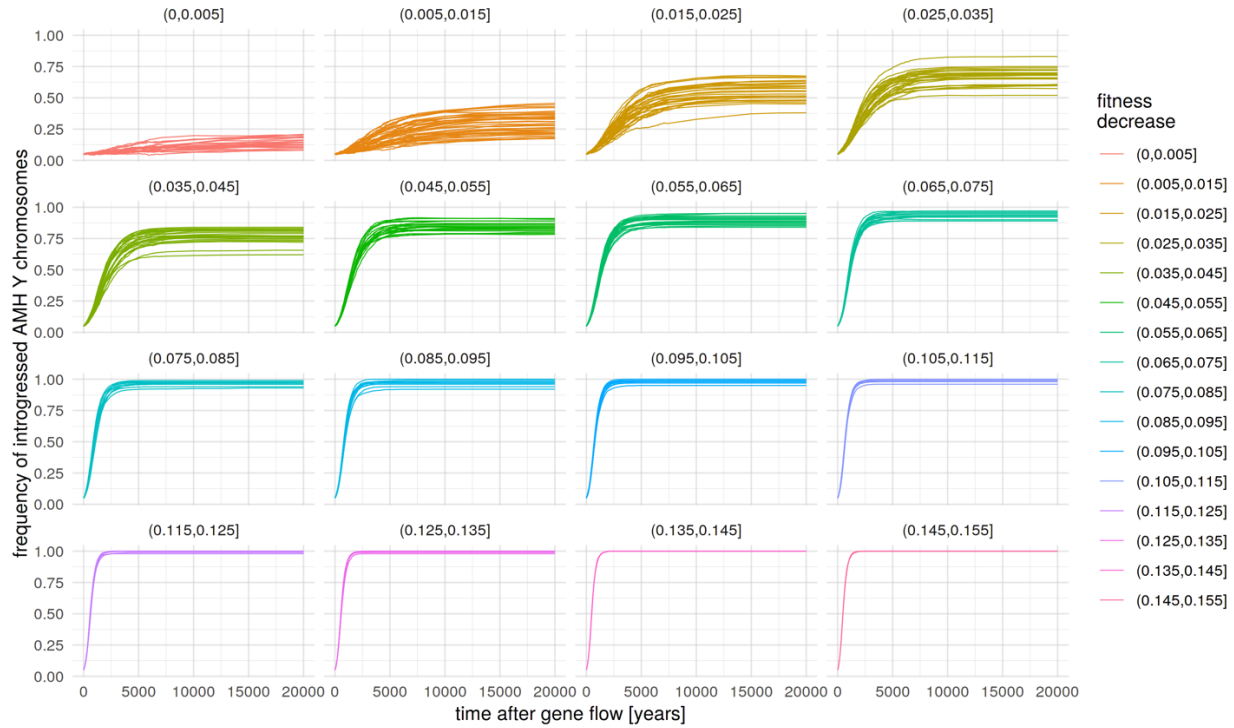
1059 as the ratios of the mean fitness of Neandertal Y chromosomes to the mean fitness of

1060 modern human Y chromosomes (calculated using equation 7). Lighter colors represent

1061 lower fitness of Neandertal Y chromosomes compared to modern human Y

1062 chromosomes.

1063



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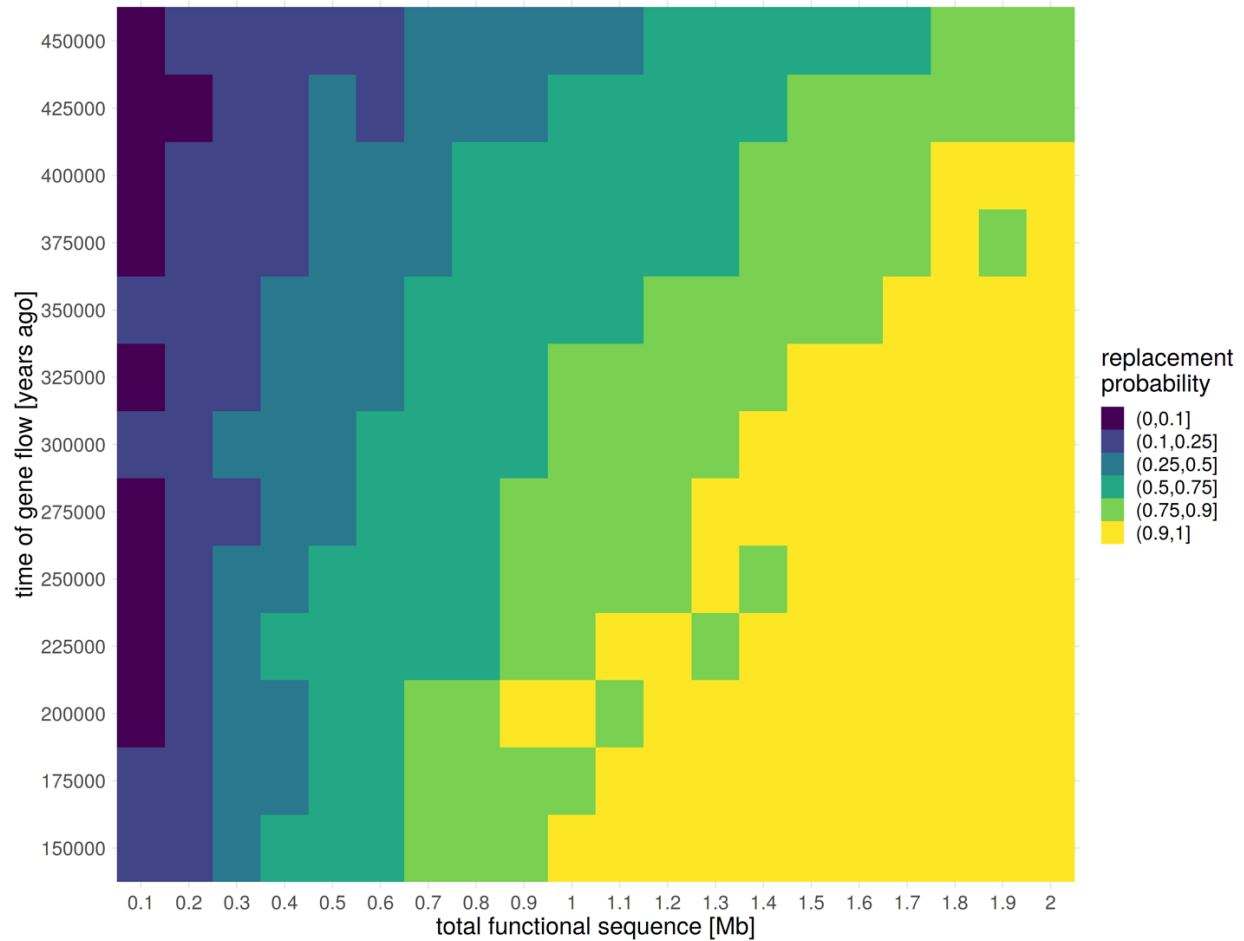
1066 **Figure S8.2. Frequency trajectories of introgressed modern human Y**

1067 **chromosomes in Neandertals, partitioned by the fitness decrease of Neandertal Y**

1068 **chromosomes compared to modern human Y chromosomes.**

1069

1070



1071

1072

1073 **Figure S8.3. Probability of replacement of a Neandertal Y chromosome at 20**

1074 **thousand years after gene flow from modern human.** Probabilities represent the

1075 proportion of introgressed modern human Y chromosome trajectories that reached

1076 fixation in the Neandertals after 20 thousand years after gene flow, out of the total 100

1077 simulation replicates performed for each combination of two-dimensional parameters

1078 (Figure S8.2).

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- 1081
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1132

Specimen	MPI Sample ID	Amount powder	Extraction performed in this/previous study	Pre-treatment of the bone powder with 0.5% hypochlorite solution	Volume of extraction buffer added	Volume of extraction buffer purified	Volume of extract produced	Extract ID	Extraction protocol and binding buffer used	Purification method	Library preparation performed in this/previous study	Library ID	P7 index	P5 index	Input volume in library preparation	Library prep. protocol	UDG treatment	#Library molecules obtained	#Spike-in control molecules obtained
Denisova 8	SP2812	15.4 mg	This	no	500 µl	500 µl	50 µl	E4231	5M40 (Dabney et al., 2013)	Manual R	This	A9461	414	34	10 µl	Gansauge and Meyer, 2013; manual	no	1,29E+09	4,27E+05
												D6448	346	18	10 µl	Gansauge et al. 2017; automated	no	1,55E+09	6,84E+04
												G7359	418	168	10 µl	Gansauge et al. 2017; automated	no	8,65E+08	4,29E+05
												G8329	435	183	10 µl	Gansauge et al. 2017; automated	no	1,27E+09	7,18E+05
												G8330	436	184	10 µl	Gansauge et al. 2017; automated	no	8,43E+08	4,30E+05
												A9662	415	35	10 µl	Gansauge and Meyer, 2013; manual	no	6,12E+09	4,86E+05
Denisova 4	SP2976	10 mg	This	no	500 µl	500 µl	50 µl	E6746	2M70 (Glocke and Meyer, 2013)	Manual R	This	D6449	347	19	10 µl	Gansauge et al. 2017; automated	no	6,93E+09	7,01E+04
												A11437	408	59	10 µl	Gansauge et al. 2017; automated	no	1,63E+09	7,83E+05
												A19116	388	11	10 µl	Gansauge et al. 2017; automated	no	2,67E+09	1,12E+06
												A19248	466	135	10 µl	Gansauge et al. 2017; automated	no	3,53E+09	1,38E+06
												A19443	319	57	10 µl	Gansauge et al. 2017; automated	no	3,09E+09	1,22E+06
												A19637	441	188	10 µl	Gansauge et al. 2017; automated	no	2,51E+09	9,26E+05
Spy 94a	SP3818	14.5 mg	Previous	yes	500 µl	500 µl	50 µl	E3342	5M40 (Dabney et al., 2013)	Manual R	Previous	R5556	432	62	10 µl	Gansauge and Meyer, 2013; manual	no	1,41E+08	1,00E+06
												A9416	410	10	10 µl	Gansauge and Meyer, 2013; manual	no	3,96E+07	3,14E+05
												A9417	411	11	10 µl	Gansauge and Meyer, 2013; manual	no	4,49E+07	4,43E+05
												A9418	412	12	10 µl	Gansauge and Meyer, 2013; manual	no	3,57E+07	4,13E+05
												A9419	413	13	10 µl	Gansauge and Meyer, 2013; manual	no	2,65E+07	3,04E+05
												A9180	428	14	15	Gansauge and Meyer, 2013; manual	no	1,09E+09	9,70E+05
Mezmaiskaya 2	SP2718	29 mg	Previous	yes	500 µl	500 µl	50 µl	E2830	5M40 (Dabney et al., 2013)	Manual R	Previous	A9252	442	32	10 µl	Gansauge and Meyer, 2013; manual	no	3,58E+08	5,65E+05
												A9253	443	33	10 µl	Gansauge and Meyer, 2013; manual	no	3,80E+08	7,35E+05
												A13742	492	182	30	Gansauge et al. 2017; automated	no	6,34E+08	1,13E+06
												A18478	445	123	30	Gansauge et al. 2017; automated	no	6,37E+08	1,68E+06
												A18563	423	170	30	Gansauge et al. 2017; automated	no	1,31E+09	1,02E+06
												A13743	488	157	30	Gansauge et al. 2017; automated	no	1,33E+09	1,04E+06
Mezmaiskaya 2	SP2718	15.8 mg	This	yes	300 µl	300 µl	30 µl	E10550	5M40 (Dabney et al., 2013)	Automated purification	This	A19113	387	33	30	Gansauge et al. 2017; automated	no	3,28E+09	6,10E+05
												A19113	387	33	30	Gansauge et al. 2017; automated	no	3,65E+08	4,10E+04
												A19245	490	136	30	Gansauge et al. 2017; automated	no	1,45E+09	3,20E+05
												A19440	389	80	30	Gansauge et al. 2017; automated	no	6,40E+07	1,16E+04
												A19634	430	153	30	Gansauge et al. 2017; automated	no	6,68E+07	1,07E+04
												A19634	430	153	30	Gansauge et al. 2017; automated	no	2,23E+09	5,49E+05
Mezmaiskaya 2	SP2718	17.5 mg	This	yes	300 µl	300 µl	30 µl	E13527	5M40 (Dabney et al., 2013)	Manual Q	This	A18865	399	147	30	Gansauge et al. 2017; automated	no	3,07E+09	1,53E+06
												A19114	341	93	30	Gansauge et al. 2017; automated	no	1,95E+09	1,06E+06
												A19246	480	152	30	Gansauge et al. 2017; automated	no	2,56E+09	8,90E+05
												A19441	382	84	30	Gansauge et al. 2017; automated	no	1,61E+09	3,57E+05
												A19635	420	111	30	Gansauge et al. 2017; automated	no	3,67E+08	4,72E+04
												A19635	420	111	30	Gansauge et al. 2017; automated	no	3,67E+08	4,72E+04

Manual R: Roche silica columns (High Pure Viral Nucleic Acid kit; Roche)
Manual Q: MinElute silica columns (Min Elute PCR purification kit; Qiagen)

Table S2.1. DNA library information.