

CONGA: Copy number variation genotyping in ancient genomes and low-coverage sequencing data

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ABSTRACT

To date, ancient genome analyses have been largely confined to the study of single nucleotide polymorphisms (SNPs). Copy number variants (CNVs) are a major contributor of disease and of evolutionary adaptation, but identifying CNVs in ancient shotgun-sequenced genomes is hampered by typical low coverage ($<1\times$) and short fragments (<80 bps), precluding standard CNV detection software to be effectively applied to ancient genomes. Here we present CONGA, tailored for genotyping CNVs at low coverage. Simulations and down-sampling experiments suggest that CONGA can genotype deletions >1 kbps with F-scores >0.75 at $\geq 1\times$, and distinguish between heterozygous and homozygous states. We applied CONGA to genotype 10,002 outgroup-ascertained deletions across a heterogenous set of 71 ancient human genomes spanning the last 50,000 years, produced using variable experimental protocols. A fraction of these (21/71) display divergent deletion profiles unrelated to their population origin, but attributable to technical factors such as coverage and read length. The majority of the sample (50/71), despite originating from nine different laboratories and having coverages $0.44\times$ - $26\times$ (median $4\times$) and read lengths 52-121 bp (median 69), exhibit coherent deletion frequencies. Across these 50 genomes, inter-individual genetic diversity measured using SNPs and CONGA-genotyped deletions are strongly correlated. CONGA-genotyped deletions also display purifying selection signatures, as expected. CONGA thus paves the way for systematic CNV analyses in ancient genomes, despite the technical challenges posed by low and variable genome coverage.

Keywords Genomics · ancient DNA · CNV genotyping · deletion · low coverage whole genome sequencing

Introduction

Ancient genomics, the analysis of genetic material extracted from archaeological and paleontological remains, has become a major source of information for the study of population history and evolution over the last decade Skoglund and Mathieson (2018); Frantz *et al.* (2020); Shapiro and Hofreiter (2014); Marciniak and Perry (2017). While the number of published ancient genomes is exponentially growing, their analyses have yet been nearly exclusively limited to those of single-nucleotide polymorphisms (SNPs), while structural variations (SVs) in ancient genomes remain mostly ignored. Copy number variations (CNVs) are a common type of SVs and include deletions and duplications ranging from 50 bps to several megabasepairs. Although their number, by count, is much fewer than SNPs, the fraction of the genome affected by CNVs is well past that accounted for SNPs Conrad *et al.* (2010). Likewise, CNVs are a major contributor to phenotypic variation: they are frequently discovered as the basis of diverse biological adaptations Gonzalez *et al.* (2005); Perry *et al.* (2007); Xue *et al.* (2008); Chan *et al.* (2010); McLean *et al.* (2011); Hardwick *et al.* (2011); Kothapalli *et al.* (2016); Nuttle *et al.* (2016); Hsieh *et al.* (2019) as well as genetic diseases (reviewed in Zhang *et al.* (2009); Stankiewicz and Lupski (2010); Girirajan *et al.* (2011); Saitou and Gokcumen (2020)). This renders the study of CNVs in ancient genomes two-fold attractive. First, as CNVs frequently serve as genetic material for adaptation, their study in ancient genomes can allow detailed temporal investigation of adaptive processes. Examples include evolutionary changes in salivary amylase copy numbers in humans and in dogs, thought to represent

36 responses to a shift to starch-rich diets Mathieson and Mathieson (2018); Bergström *et al.* (2020). Second, large
37 deletions can be a major source of deleterious mutation load, and studying deletion frequencies in ancient genome
38 samples from extinct species or severely bottlenecked populations can inform about the genetic health of lineages. For
39 instance, a study on the last surviving mammoth population on Wrangel Island reported an excess of deletions in this
40 sample, which may have compromised the population's fitness Rogers and Slatkin (2017).

41 Despite this appeal, the impact of CNVs on evolutionary history and ancient phenotypes remains largely unex-
42 plored Frantz *et al.* (2020). The reason lies in the significant technical challenges in CNV detection posed by ancient
43 genomes. State-of-the-art methods for CNV discovery from shotgun genome sequencing data require at least mod-
44 erate depth of coverage Abyzov *et al.* (2011); Boeva *et al.* (2012); Smith *et al.* (2015); Alkan (2020) and read-pair
45 information Rausch *et al.* (2012); Layer *et al.* (2014); Chen *et al.* (2016); Eisfeldt *et al.* (2017); Soylev *et al.* (2017,
46 2019), or long reads Chaisson *et al.* (2015); Sedlazeck *et al.* (2018). However, due to the degraded and elusive nature
47 of ancient DNA, ancient genome data is frequently produced at low coverage ($<1\times$) and the molecules retrieved are
48 typically short, between 50-80 bps. Excess variability in genome coverage caused by taphonomic processes is another
49 potential issue. Although CNVs have been studied in a few relatively high coverage ancient genomes using CNV
50 discovery tools Green *et al.* (2010); Reich *et al.* (2010); Meyer *et al.* (2012); Rogers and Slatkin (2017); Bergström
51 *et al.* (2020), these methods are inapplicable to most ancient genome data sets, and so far, no specific algorithm for
52 CNV identification in ancient genomes has been developed and tested.

53 With the aim to fill this gap, here we present CONGA (Copy Number Variation Genotyping in Ancient Genomes and
54 Low-coverage Sequencing Data), a CNV genotyping algorithm tailored for ancient and other low coverage genomes,
55 which estimates copy number beyond presence/absence of events. We use simulations and down-sampling experiments
56 to assess CONGA's performance. Beyond simulations, we explore whether deletions can be reliably genotyped in
57 heterogeneous datasets composed of ancient genomes from different laboratories, where not only low coverage, but
58 also coverage variability caused by differences in taphonomy and experimental protocols may pose challenges. We
59 evaluate this by studying expected patterns of genetic drift and negative selection on CONGA-genotyped deletions.

60 Results

61 Motivation and overview of the algorithm

62 We developed CONGA to genotype given candidate CNVs in mapped read (BAM) files (Methods). The choice of
63 CNV genotyping over CNV discovery has obvious reasons: (a) CNV discovery using low coverage ancient genomes is
64 impractical; (b) for many species studied using ancient genomics, CNV reference sets based on high quality genomes
65 are already available (Supplemental Note S1); (c) variants in ancient genomes will largely overlap with present-day
66 variants in most cases; (d) genotyping has much shorter running times and lower memory usage than discovery. Indeed,
67 although algorithms for *de novo* SNP discovery exist Prüfer (2018); Link *et al.* (2017), most ancient genome studies to
68 date have chosen genotyping known variants because of low coverage and DNA damage Orlando *et al.* (2021). We
69 reasoned that it may be likewise possible to genotype CNVs in ancient genomes with high accuracy and in short running
70 times using depth of coverage and split-read information, despite low and variable coverage.

71 Briefly, CONGA first calculates the number of reads mapped to each given interval in the reference genome, which
72 we call "observed read-depth". It then calculates the "expected diploid read-depth", i.e., the GC-content normalized
73 read-depth given the genome average. Using these values, CONGA calculates the likelihood for each genotype by
74 modeling the read-depth distribution as Poisson, similar to common CNV callers Xie and Tammi (2009); Chiang *et al.*
75 (2009); Yoon *et al.* (2009). The genotypes can be homozygous CNV, heterozygous CNV, or no CNV. Using these
76 likelihoods CONGA then calculates a statistic we term the C-score, defined as the likelihood of a CNV being true (in
77 heterozygous or homozygous state) over it being false (no CNV). For genotyping duplications, CONGA also uses an
78 additional split-read step in order to utilize paired-end information. Briefly, it splits reads and remaps the split within
79 the genome, treating the two segments as paired-end reads Karakoc *et al.* (2012); Soylev *et al.* (2019). Either type of
80 signature, read-depth or paired-end, can be sufficient to call a duplication (Methods). The overall workflow is presented
81 in Figure 1.

82 Accuracy evaluation using simulated genomes and comparison with published algorithms

83 To evaluate the performance of CONGA we first simulated genomes with CNVs of ancient-like characteristics. We
84 employed VarSim Mu *et al.* (2015) to insert deletions and duplications into the human reference genome (GRCh37).
85 We used three different size intervals for CNVs: small (100 bps - 1000 bps), medium (1,000 bps - 10,000 bps) and
86 large (10,000 bps - 100,000 bps). We thus simulated three genomes, each with roughly 1,500 deletions and 1,500
87 duplications of a specific size range (see Supplemental Fig. S1 for the exact numbers and length distributions of CNVs

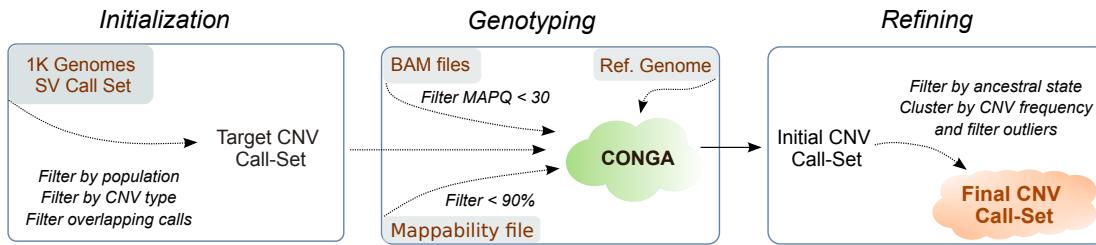


Figure 1: Overall workflow of CONGA. The first step involves initialization, where we create the input (reference) CNV file using the deletions and duplications of a high quality genome set. We apply our genotyping algorithm in the second step and create the initial CNV call set. We then perform a filtering and refining step, which is used to generate the final CNV call set.

88 inserted in each genome). We next used these genomes as input to the ancient read simulator Gargammel Renaud *et al.*
89 (2017), which generates paired-end short Illumina reads with varying fragment sizes (median 66 bps) and post-mortem
90 damage. The data was generated at various depths: $0.05\times$, $0.1\times$, $0.5\times$, $1\times$ and $5\times$ (Methods). We then used CONGA
91 to genotype CNVs across the simulated ancient genomes, using a candidate CNV call set. In order to assess specificity
92 and sensitivity, we also used a background (false) CNV list, prepared using published deletion and duplication calls
93 from modern-day human long-read sequencing datasets Audano *et al.* (2019); Chaisson *et al.* (2019); Zook *et al.* (2020);
94 Collins *et al.* (2020), as well as from African populations (AFR) from Phase 3 of the 1000 Genomes Project Sudmant
95 *et al.* (2015b). We mixed these false CNVs to the list of true CNVs with a ratio of approximately 10:1 ($\sim 15,000$
96 false events vs. $\sim 1,500$ true events) and used this mixed list as the candidate CNV call set to CONGA (Methods).
97 To assess the performance of CONGA in identifying CNVs, we further compared it with a CNV genotyping tool,
98 GenomeSTRiP Handsaker *et al.* (2011, 2015), and three of the widely used CNV discovery tools: CNVnator Abyzov
99 *et al.* (2011), FREEC Boeva *et al.* (2012) and mrCaNaVaR Alkan *et al.* (2009); Kahveci and Alkan (2018); Alkan
100 (2020).

101 Table 1 shows true and false predictions by CONGA, GenomeSTRiP, FREEC and CNVnator, as well as their true
102 positive rate (TPR), false discovery rate (FDR) and the F-score (F1) for identifying deletions and duplications of small,
103 medium and large size (as defined above). We report results with mrCaNaVaR separately in Supplemental Table S1.A
104 as this algorithm was specifically designed to target large duplications (>10 kbps) only.

105 Both genotypers, CONGA and GenomeSTRiP, achieved higher performance compared to the three CNV discovery
106 tools (Table 1; Supplemental Table S1.A). Although this may seem expected, the fact that our candidate CNV call set
107 included 10 times more false CNVs than true CNVs is notable, and indicates that both CONGA and GenomeSTRiP
108 achieve non-trivial performances in distinguishing true versus false CNVs in ancient genomes.

109 CONGA and GenomeSTRiP had comparable performances, although CONGA had lower FDR and slightly lower recall
110 (TPR) than the latter, leading to overall higher F-scores. We note that GenomeSTRiP was performed on each genome
111 independently here, and its performance when genotyping multiple genomes together could be higher Handsaker *et al.*
112 (2011). However, joint genotyping may also create biases in heterogeneous datasets (see Discussion).

113 We observed that all tools converge in performance as the coverage approaches depths of $5\times$, especially with large
114 CNVs. For small CNVs (<1 kbps), all tools under-performed, although CONGA predictions still had higher recall and
115 precision than the other tools (see Supplemental Fig. S2 for precision-recall curves).

116 The simulation results thus suggest that CONGA can efficiently and accurately genotype deletions and duplications of
117 length >1 kbps in ancient genomes at $\geq 0.5\times$ coverage, with higher overall accuracy compared to available discovery
118 and genotyping tools.

119 Copy number predictions of CNVs

120 Beyond the identification of deletion and duplication events, classifying individual genotypes as heterozygous or
121 homozygous CNVs could provide valuable information for population genetic analyses of CNVs. However, predicting
122 CNV copy numbers can be a significant challenge on low coverage genomes Kousathanas *et al.* (2017). We thus
123 assessed the performance of CONGA to determine the copy number of a CNV based on the likelihood model described
124 above using our simulation data. We focused on medium and large size CNVs given the weak performance of CONGA
125 on small CNVs. We note that CONGA only evaluates the possibility of homozygous duplications (ignores copy numbers

Table 1: Summary of simulation predictions by CONGA, GenomeSTRiP, FREEC and CNVnator.

	Cov.	CONGA					GenomeSTRiP					FREEC					CNVnator				
		T	F	TPR	FDR	F1	T	F	TPR	FDR	F1	T	F	TPR	FDR	F1	T	F	TPR	FDR	F1
Dels (small) <i>1810</i> <i>True</i>	.05×	1471	1887	0.81	0.56	0.57	829	6308	0.46	0.88	0.19	0	1221	0.00	1.00	-	3	47442	0.00	1.00	0.00
	.1×	1266	1440	0.70	0.53	0.56	851	4765	0.47	0.85	0.23	0	198	0.00	1.00	-	0	402	0.00	1.00	-
	.5×	1285	157	0.71	0.11	0.79	853	1549	0.47	0.64	0.41	0	6761	0.00	1.00	-	0	806	0.00	1.00	-
	1×	1410	46	0.78	0.03	0.86	888	719	0.49	0.45	0.52	0	1916	0.00	1.00	-	0	263	0.00	1.00	-
	5×	1593	8	0.88	0.00	0.93	917	89	0.51	0.09	0.65	20	392	0.01	0.95	0.02	341	493	0.19	0.59	0.26
Dups (small) <i>1751</i> <i>True</i>	.05×	601	548	0.34	0.48	0.41	829	3834	0.47	0.82	0.26	0	44	0.00	1.00	-	7	47700	0.00	1.00	0.00
	.1×	719	404	0.41	0.36	0.50	1048	2691	0.60	0.72	0.38	0	7	0.00	1.00	-	0	28699	0.00	1.00	-
	.5×	856	64	0.49	0.07	0.64	1077	686	0.62	0.39	0.61	0	3	0.00	1.00	-	0	9	0.00	1.00	-
	1×	1155	14	0.66	0.01	0.79	1127	311	0.64	0.22	0.71	0	555	0.00	1.00	-	0	884	0.00	1.00	-
	5×	1448	1	0.83	0.00	0.91	888	1270	0.73	0.05	0.82	35	77	0.02	0.69	0.04	2	0	0.00	0.00	0.00
Dels (med.) <i>1680</i> <i>True</i>	.05×	1136	1704	0.68	0.60	0.50	1430	5670	0.85	0.80	0.33	0	83	0.00	1.00	-	0	68	0.00	1.00	-
	.1×	1273	1308	0.76	0.51	0.60	1452	4383	0.86	0.75	0.39	0	237	0.00	1.00	-	1	216	0.00	1.00	0.00
	.5×	1423	171	0.85	0.11	0.87	1495	1467	0.89	0.50	0.64	239	6433	0.14	0.96	0.06	187	257	0.11	0.58	0.18
	1×	1506	53	0.90	0.03	0.93	1501	699	0.89	0.32	0.77	421	2135	0.25	0.84	0.20	330	257	0.20	0.44	0.29
	5×	1569	9	0.93	0.01	0.96	1510	102	0.90	0.06	0.92	929	485	0.55	0.34	0.60	949	423	0.56	0.31	0.62
Dups (med.) <i>1684</i> <i>True</i>	.05×	792	551	0.47	0.41	0.52	1104	3813	0.66	0.78	0.33	0	3	0.00	1.00	-	0	114	0.00	1.00	-
	.1×	950	422	0.56	0.31	0.62	1160	2701	0.69	0.70	0.42	0	3	0.00	1.00	-	0	102	0.00	1.00	-
	.5×	1340	60	0.80	0.04	0.87	1322	685	0.79	0.34	0.72	271	15	0.16	0.05	0.28	2	4	0.00	0.67	0.00
	1×	1451	11	0.86	0.01	0.92	1389	333	0.82	0.19	0.82	582	937	0.35	0.62	0.36	16	2	0.01	0.11	0.02
	5×	1553	1	0.92	0.00	0.96	1473	95	0.87	0.06	0.91	1000	329	0.59	0.25	0.66	105	2	0.06	0.02	0.12
Dels (large) <i>1385</i> <i>True</i>	.05×	1208	1812	0.87	0.60	0.55	1330	5891	0.96	0.82	0.31	0	87	0.00	1.00	-	84	131	0.06	0.61	0.11
	.1×	1251	1309	0.90	0.51	0.63	1337	4371	0.97	0.77	0.38	0	754	0.00	1.00	-	560	246	0.40	0.31	0.51
	.5×	1293	157	0.93	0.11	0.91	1335	1496	0.96	0.53	0.63	664	3136	0.48	0.83	0.26	1049	293	0.76	0.22	0.77
	1×	1299	53	0.94	0.04	0.95	1338	759	0.97	0.36	0.77	1239	156	0.89	0.11	0.89	1204	309	0.87	0.20	0.83
	5×	1299	4	0.94	0.00	0.97	1336	230	0.96	0.15	0.91	1260	154	0.91	0.11	0.90	1265	453	0.91	0.26	0.82
Dups (large) <i>1532</i> <i>True</i>	.05×	1263	563	0.82	0.31	0.75	1271	3900	0.83	0.75	0.38	0	6	0.00	1.00	-	4	354	0.00	0.99	0.00
	.1×	1327	366	0.87	0.22	0.82	1327	2855	0.87	0.68	0.46	0	0	-	-	-	455	315	0.30	0.41	0.40
	.5×	1420	58	0.93	0.04	0.94	1424	964	0.93	0.40	0.73	589	97	0.38	0.14	0.53	1039	77	0.68	0.07	0.78
	1×	1426	20	0.93	0.01	0.96	1445	623	0.94	0.30	0.80	1305	266	0.85	0.17	0.84	1216	94	0.79	0.07	0.86
	5×	1428	9	0.93	0.01	0.96	1447	454	0.94	0.24	0.84	1304	294	0.85	0.18	0.83	1350	165	0.88	0.11	0.89

The table shows CNV prediction performances of CONGA, GenomeSTRiP, FREEC and CNVnator on simulated genomes with depths 0.05×, 0.1×, 0.5×, 1× and 5×, for deletions (Dels) and duplications (Dups) of multiple CNV size intervals including 100 bps - 1 kbps (small), 1 kbps - 10 kbps (medium) and 10 kbps - 100 kbps (large). Here, **T** (True) and **F** (False) refer to correct and incorrect predictions respectively, **TPR** is true positive rate (or recall) and **FDR** is false discovery rate ($1 - Precision$) of each algorithm. **F1** (F-score), is calculated as $(2 \times Precision \times Recall) / (Precision + Recall)$. Bold values in each row represent the highest TPR, lowest FDR, or highest F1 across the tools. See Supplemental Table S1.A for details and mrCaNaVaR predictions for large variations. Commands that we used to run each tool are also given in Supplemental Material. The results here were generated using C-Score <0.5 for CONGA, while no read-pair or mappability filters were applied.

126 ≥ 3). Figure 2 shows CONGA’s copy number prediction performance for deletions and duplications using F-scores
 127 for each coverage tested. We found that F-scores were above 0.7 at coverages $\geq 0.5 \times$. Encouragingly, CONGA had
 128 comparable power in identifying heterozygous and homozygous events of size > 1 kbps (Supplemental Table S1.B).

129 Down-sampling experiments with real ancient genomes

130 We next studied the performance of CONGA in identifying CNVs at various depths of coverage using real ancient
 131 genome data. As no ground truth CNV call-set is available, we used the following approach: (i) we chose three
 132 published ancient genomes of relatively high coverage ($\geq 9 \times$), (ii) we genotyped CNVs using the full genome data with
 133 CONGA and using a modern-day human CNV call set as input, (iii) we down-sampled the ancient genome data to
 134 lower coverages, (iv) we assessed CONGA’s performance in genotyping the same CNVs at low coverage (Methods).

135 Specifically, we selected a ($\sim 23.3 \times$) ancient Eurasian genome (Yamnaya) de Barros Damgaard *et al.* (2018b), a
 136 13.1× ancient genome from Greenland (Saqqaq) Rasmussen *et al.* (2010), and a 9.6× ancient genome from Ethiopia
 137 (Mota) Llorente *et al.* (2015). The Yamnaya genome was only available as a BAM file, while the latter two were

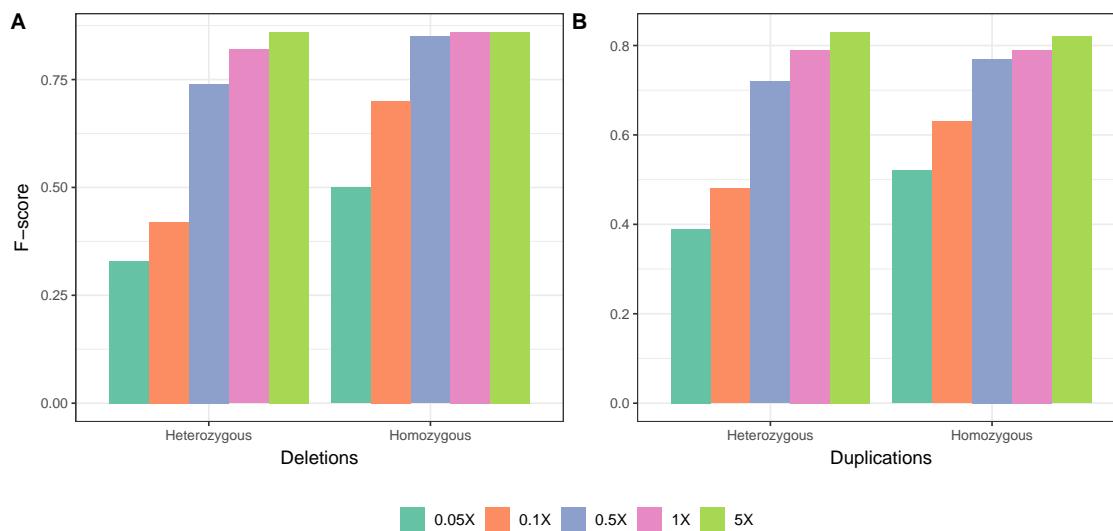


Figure 2: Performance (F-scores) of CONGA in predicting copy-numbers of (A) deletions and (B) duplications using merged sets of medium and large CNVs, at various coverage values.

138 available as FASTQ files, which we processed into BAM files (Methods). We used a list of modern-day human CNVs
139 as candidate CNV set ($n = 17,392$ deletions and $n = 14,888$ duplications) (Methods) as input to CONGA. We thus
140 genotyped between 688-1,581 deletions and 638-4,097 duplications across these three genomes using the full data. We
141 then down-sampled all three BAM files to various depths, and repeated the genotyping for each genome. We estimated
142 CONGA's TPR and FDR on down-sampled genomes by treating the CNVs genotyped using the full data as ground
143 truth (Methods).

144 CONGA displayed satisfactory performance in identifying deletions in all three genomes even at coverages around
145 $0.5\times$, with TPR of $>70\%$ and FDR of $<45\%$ (Figure 3, Supplemental Table S1.C). For duplications, however, CONGA
146 showed poor performance: at around $1\times$ coverage, duplication TPR was $>40\%$ in the Saqqaq and Mota genomes, and
147 only 22% in the Yamnaya genome. A detailed analysis of these results suggested that pre-publication quality filtering
148 of BAM files may have obliterated read-depth-based duplication signals in the data (Supplemental Note S2).

149 Overall, both our simulations and down-sampling experiments with real genomes suggest that CONGA can efficiently
150 genotype >1 kbps deletion events at depths of coverage of $0.5\times$, and even at $0.1\times$. CONGA could thus be applied on a
151 large fraction of ancient shotgun sequenced genomes available for deletion genotyping. In contrast, CONGA's low
152 performance in duplication genotyping in the down-sampled Yamnaya BAM data implies that identifying duplications
153 in published low coverage ancient genomes may not be feasible, as the data are mainly submitted in BAM format in
154 public repositories (see Discussion). We therefore limited downstream analyses on real ancient genomes to deletions
155 >1 kbps.

156 Analysis of 71 real ancient genomes and technical influences on deletion genotyping

157 Although CONGA's above performance in deletion genotyping was promising, heterogeneous sets of real ancient
158 genomes may pose additional challenges, as they are obtained from DNA samples of complex taphonomic history and
159 are produced via different experimental protocols. Hence, whether consistent biological signals may still be extracted
160 from low coverage genome sets remains unclear. To explore this, we genotyped deletions with CONGA across a
161 diverse sample of real ancient human genomes. We then studied their diversity with expectation that deletions, like
162 SNPs, should display genome-wide similarity patterns that reflect population origin, i.e., shared genetic drift, among
163 individuals Conrad and Hurles (2007); Levy-Sakin *et al.* (2019); Almarri *et al.* (2020).

164 We thus collected BAM files for 71 ancient human genomes belonging to a time range between c.2,800-45,000 years
165 Before Present (BP) (Supplemental Table S2) Rasmussen *et al.* (2014); Günther *et al.* (2015); Hofmanová *et al.* (2016);
166 Jones *et al.* (2015); Kılınç *et al.* (2016); de Barros Damgaard *et al.* (2018b); Gamba *et al.* (2014); González-Fortes *et al.*
167 (2017); de Barros Damgaard *et al.* (2018a); Keller *et al.* (2012); Sikora *et al.* (2019); Olalde *et al.* (2014); Lazaridis
168 *et al.* (2014); Antonio *et al.* (2019); Allentoft *et al.* (2015); Haber *et al.* (2019); Fu *et al.* (2014); Broushaki *et al.* (2016);
169 Seguin-Orlando *et al.* (2014); Jones *et al.* (2017); Haber *et al.* (2017); Raghavan *et al.* (2014); Martiniano *et al.* (2017);

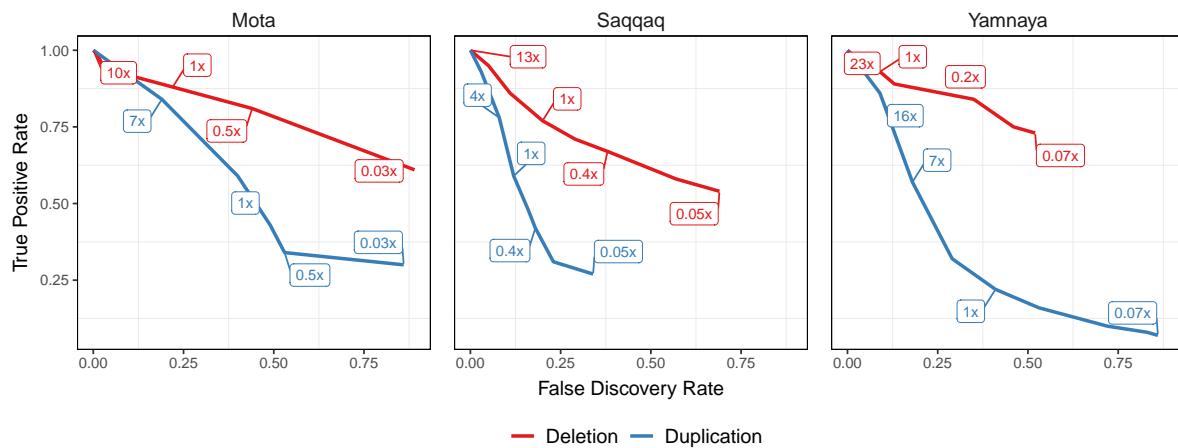


Figure 3: TPR vs FDR curves for deletion and duplication predictions of CONGA using Mota, Saqqaq and Yamnaya genomes down-sampled to various depths from their original coverages of $9.6\times$, $13.1\times$ and $23.3\times$, respectively. The numbers inside boxes show the down-sampled coverage values. We calculated TPR and FDR for down-sampled genomes assuming that our CONGA-based predictions with the original genomes (full data) reflect the ground truth. These predictions, in turn, were made using modern-day CNVs as candidate CNV list. The purpose of the experiment was to evaluate accuracy at lower coverage relative to the full data (Methods).

170 Krzewińska *et al.* (2018); Yaka *et al.* (2021). These were chosen to bear diverse characteristics, including a wide range
 171 in mean coverage ($0.04\times$ - $26\times$, median = $3.45\times$), population origin (West and East Eurasia and North America), the
 172 laboratory of origin (10 different laboratories), the use of shotgun vs. whole-genome capture protocols, or the use of
 173 uracil-DNA-glycosylase (UDG) treatment Rohland *et al.* (2015). For genotyping, we used a candidate CNV dataset of
 174 11,390 autosomal deletions (>1 kbps with mean 10,735 bps) identified among African populations (AFR) from Phase 3
 175 of the 1000 Genomes Project Sudmant *et al.* (2015b) (Methods). Our motivation for using an African sample here was
 176 to avoid ascertainment bias Clark *et al.* (2005) in studying deletion frequencies, as all of the 71 ancient individuals were
 177 non-African, and thus African populations represent an outgroup to our sample set. We further filtered these for high
 178 mappability (mean >0.9) and to be derived in the human lineage using chimpanzee and bonobo genomes to represent
 179 the ancestral state, leaving us with 10,002 deletion events (Methods).

180 Genotyping the 10,002 loci across 71 BAM files, we found 8,780 (88%) genotyped in at least one genome (as deletion
 181 or reference). Further, 5,467 (55%) loci genotyped as a deletion (in heterozygous or homozygous state) at least once.
 182 Across the 71 genomes, we detected a median number of 490 deletion events [396-2,648] again in either heterozygous
 183 or homozygous state.

184 We studied deletion copy number (frequency) variation across these 71 ancient genomes using a battery of heatmaps,
 185 hierarchical clustering, multidimensional scaling plots (MDS) and principal components analysis (PCA) (Supplemental
 186 Fig. S3; Supplemental Fig. S4). This revealed a minority of genomes exhibiting highly divergent frequencies, without
 187 obvious association with their population of origin. Given the close evolutionary relationship among Eurasian human
 188 populations, we reasoned that these divergent signals most likely originate from experimental artifacts, data processing
 189 artifacts, or variability of DNA preservation among samples. Supporting this, mean deletion frequencies across the
 190 71 genomes could be explained by laboratory-of-origin (Kruskall-Wallis test, $p = 0.08$). We identified a subset of
 191 21 divergent, or outlier genomes, and removing these also removed the laboratory-of-origin effect (Kruskall-Wallis
 192 test, $p = 0.22$; Supplemental Note S3). We could further recognize a number of attributes that could explain these
 193 divergent deletion profiles. First, the 21 divergent genomes had on average shorter read length compared to the rest
 194 (median = 57 vs. 69; Wilcoxon rank sum test $p < 0.001$; Supplemental Fig. S5A). One of these was the Iceman,
 195 with unusually short (50 bps) reads. Second, the coverage of the 21 divergent genomes was lower compared to the
 196 remaining 50 (median = 3.31 vs. 3.98; Wilcoxon rank sum test, $p = 0.014$; Supplemental Fig. S5B). For instance,
 197 all three genomes with $<0.1\times$ coverage in our dataset (ne4, ko2, and DA379) were among the outliers. The number
 198 of non-genotyped loci was likewise higher in the divergent group (median = 1509 vs. 1886; Wilcoxon rank sum test,
 199 $p = 5.39 \times 10^{-5}$; Supplemental Fig. S5C). UDG-treatment did not appear to be related to outlier behaviour (binomial
 200 test $p = 2.633 \times 10^{-9}$; Supplemental Fig. S5D). Meanwhile, Bon002, the only sample produced using whole-genome
 201 capture, was among the most extreme outliers, suggesting that the capture procedure distorts coverage. Consequently
 202 we removed these 21 genomes from further analyses.

203 **A comparison of deletion and SNP diversity across 50 ancient genomes**

204 The above filtering steps resulted in a dataset of 8,780 derived deletions genotyped in at least one of the 50 ancient
 205 Eurasian genomes, with 396-748 deletions (median = 467.5) detected in heterozygous or homozygous state per genome,
 206 and 29% detected in at least one genome.

207 We used this dataset to test three hypotheses: (i) that CONGA-called deletion diversity patterns should parallel SNP
 208 diversity patterns, reflecting shared demographic history (genetic drift and admixture) among genomes, (ii) that
 209 CONGA-called deletions should be evolving under some degree of negative selection (caused by gene expression
 210 alterations, exon loss, or frame-shifts), and (iii) that variation in deletion load among genomes may be correlated
 211 with variation in deleterious SNP load. We note that the first two patterns (hypotheses i and ii) have been previously
 212 described using large modern-day CNV datasets (see Conrad and Hurles (2007); Levy-Sakin *et al.* (2019); Almarri *et al.*
 213 (2020) for drift, and Conrad *et al.* (2010); Cooper *et al.* (2011); Sudmant *et al.* (2015a) for selection), and our goal here
 214 was mainly to perform a sanity check and assess CONGA's effectiveness in producing reliable biological signals.

215 To test the first hypothesis, we compared pairwise genetic distances among the 50 individuals (Figure 4A) calculated
 216 using either SNPs or deletion genotypes. For this, we collected 38,945,054 autosomal SNPs ascertained in African
 217 individuals in the 1000 Genomes Dataset and genotyped our 50 ancient genomes at these loci (Methods). We then
 218 calculated pairwise outgroup- f_3 statistics, a measure of shared genetic drift between a pair of genomes relative to an
 219 outgroup population Patterson *et al.* (2012). Using the Yoruba as outgroup, we calculated genetic distances for all
 220 pairs of ancient genomes as $(1 - f_3)$, using either SNPs or deletions. We observed strong positive correlation between
 221 the two resulting distance matrices (Spearman $r = 0.671$, Mantel test $p = 0.001$) (Figure 4B). Summarizing SNP-
 222 and deletion-based distances using multidimensional scaling (MDS) also revealed highly similar patterns, with clear
 223 clustering among west and east Eurasian genomes observed with either type of variation (Figure 4C, D). This result was
 224 encouraging in showing that diversity patterns based on deletion genotyping with CONGA in a heterogeneous sample
 225 of low coverage ancient genomes reveals expected signals of shared demographic history.

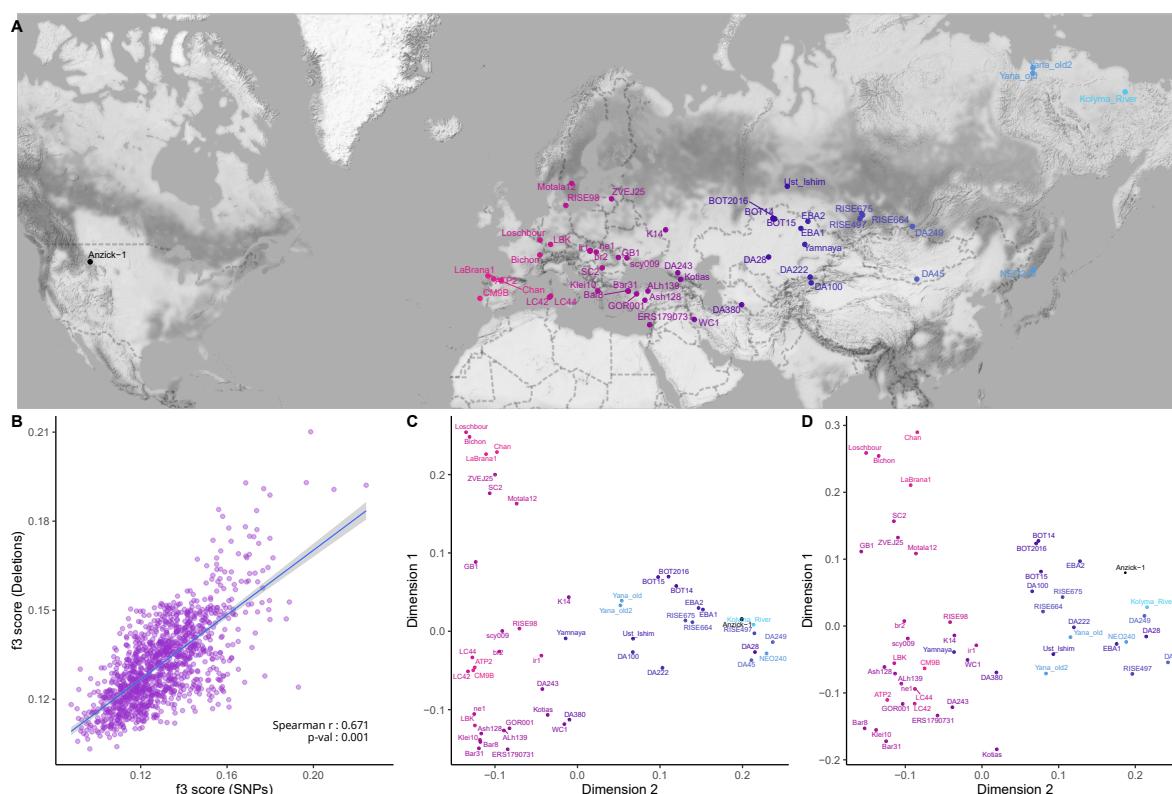


Figure 4: (A) Geographic locations of the 50 ancient individuals. (B) Comparison of genetic distances calculated using SNPs and deletions. We calculated the Spearman correlation coefficient between two matrices and then calculated Mantel test p-value using the "mantel" function in R package "vegan" (v2.5-7). (C) and (D) represent multidimensional scaling plots that summarize outgroup- f_3 statistics calculated across all pairs among the 56 ancient individuals using SNPs and deletions, respectively.

226 **Negative selection on deletion variants**

227 We next studied the impact of negative (purifying) selection on deletions by comparing the site-frequency-spectrum
228 (SFS) of autosomal deletions with those of SNPs. We used the 8,780 human-derived deletions and 32,304,437 human-
229 derived SNP alleles across the 50 ancient genomes (Methods). To allow comparison with the pseudo-haploidized SNP
230 genotype data, we randomly chose one allele per genome (i.e., deletion or no event) in the deletion dataset. Set side
231 by side with the SNP SFS, we observed an excess of singletons and a lack of fixed derived variants among deletions,
232 consistent with stronger negative selection on the latter (Figure 5A). The excess of undetected and singleton deletions
233 does not appear to be related to low recall, as both high and low coverage genomes show the same trend (Supplemental
234 Fig. S6).

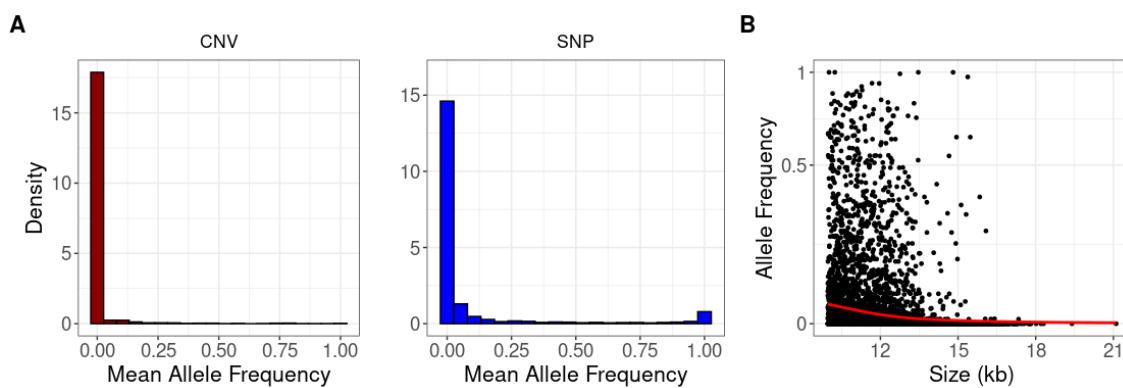


Figure 5: (A) The site-frequency-spectra of derived deletion alleles (on the left, $n=3,472$) and derived SNP alleles (on the right, $n=57,307$). The x-axes show mean allele frequency for each locus calculated using only those genomes where a locus has been observed (e.g. an allele observed in 10 out of 40 genomes will be represented as 25%). The two distributions are significantly different from each other (Kolmogorov-Smirnov test $p < 10^{-15}$). (B) The size distribution (in kbps) of the deletions versus mean allele frequency. The red line shows the fitting of smoothing spline and indicates a negative correlation (Spearman correlation $r = -0.33, p < 10^{-16}$). Both axes were \log_2 -scaled.

235 If deletions are under negative selection we may also expect longer deletions, or deletions containing evolutionary
236 conserved genes, to be segregating at lower frequencies. Indeed, we found that deletion allele frequencies were
237 negatively correlated with deletion size across the 50 genomes (Spearman correlation $r = -0.33, p < 10^{-16}$)
238 (Figure 5B). To test the second idea, we determined deletions overlapping Ensembl human genes. Overall, 26% of
239 the 8,780 derived deletions overlapped minimum one gene. We then collected mouse-human dN/dS ratios, an inverse
240 measure of protein sequence conservation (Methods). We found that deletions with lower (below-mean) allele frequency
241 had slightly lower dN/dS values compared to deletions with higher (above-mean) allele frequency (median = 0.086 vs.
242 0.097; Mann-Whitney U test, one-sided $p = 0.055$). These observations, along with the SFS comparison, follow the
243 notion that deletions are evolving under negative selection.

244 We further asked whether inter-individual variation in the total deletion mutation burden that we measure in our
245 dataset may be correlated with variation in the burden of functional deleterious SNPs based on their impact on protein
246 sequence. Demographic bottlenecks can theoretically cause variable levels of mutation burden –as deletions and/or as
247 SNPs– among ancient genomes, and these burden levels could be correlated especially if their phenotypic impacts are
248 comparable (see Discussion). To test this we collected (a) total deletion length and (b) the number of genes affected by
249 deletions, for each of the 50 ancient genomes (Supplemental Fig. S7). We further collected SIFT scores (an estimate of
250 how protein sequence would be affected by a SNP Ng and Henikoff (2003)) for $n=22,996$ SNPs in our dataset, predicted
251 to be "deleterious" or "tolerated", and used these to calculate a deleterious/tolerated ratio per genome (Methods;
252 Supplemental Fig. S8A). We then compared the deleterious/tolerated ratio-based burden levels with deletion-based
253 mutation burden levels (total length and number of genes), but found no significant correlation (Spearman $r = 0.09$ and
254 $r = -0.05$, respectively, $p > 0.5$; Supplemental Fig. S8B). This could be explained by high noise and lack of statistical
255 power, as well as differences in phenotypic impacts between deletions and SNPs (see Discussion). We also did not
256 observe any correlation between historical age and deletion frequencies in this sample (Supplemental Fig. S9).

257 **Time and memory consumption**

258 Finally, we examined time and memory requirements of CONGA. We first tested our performance of deletions with
259 BAM files of the 71 ancient genomes presented above. This finished in \sim 12 hours in total with as low as 2.2 GB of
260 peak-memory consumption. This is \sim 10 minutes per genome. In order to evaluate CONGA's performance with a
261 higher coverage genome sample, we ran 30 genomes (randomly selected 10 CEU, 10 YRI, 10 TSI) from the 1000
262 Genomes Project Phase 3, which had mean $7.4\times$ coverage Sudmant *et al.* (2015b). The analysis took just slightly
263 longer, \sim 15 minutes average per genome, with similar memory usage.

264 We also compared the time and memory requirements of CONGA, GenomeSTRiP, FREEC and CNVnator in Table 2.
265 In order to benchmark these tools, we used a $5\times$ simulated genome (the same genome with medium sized CNVs used
266 in the simulation experiments described above) with the same computing resources¹. CONGA has the lowest runtime
267 and memory footprint among the other tools.

268 In Supplemental Table S1.D we report the effects of parameter choices when using with CONGA on runtime and
269 memory usage. We note that using split-reads for duplication genotyping (intended for higher coverage genomes)
270 increases runtime and memory consumption significantly because here CONGA uses its own small-scale read mapper,
271 which creates a bottleneck.

272 We further provide a comparison of CONGA's performance on genomes of various depths of coverage in Supplemental
273 Table S1.D, calculated using the down-sampled $23\times$ Yamnaya genome (with coverages between $23\times$ and $0.07\times$).

Table 2: Time and Memory Consumption

Tools	Time (h:m)	Peak Memory Usage (GB)
CONGA	0:09	1.2
GenomeSTRiP	1:22	2.2
FREEC	0:39	7.1
CNVnator	0:32	14.1

Time and memory consumption of each algorithm for a simulated genome of $5\times$ depth of coverage with 1680 deletions and 1684 duplications. "Time" refers to wall clock time and "Peak memory usage" is the maximum resident set size. Note that GenomeSTRiP has two steps in its pipeline: preprocessing and genotyping. Here, time was calculated by summing the running times of each step, and memory by taking the maximum. For CONGA, we used default parameters used in the simulation experiments.

274 **Discussion**

275 Modern human genome sequencing experiments today typically reach coverages $>20\times$ and increasingly use long read
276 technology, and such experiments can employ diverse read signatures to reliably identify CNVs Alkan *et al.* (2011).
277 CONGA's approach that mainly relies on the read-depth signature is naive in comparison; however, using read-depth
278 appears as the main practical solution given the short fragment size and the predominance of low coverage (around or
279 $<1\times$) among ancient genome datasets.

280 **CONGA's overall performance and utility**

281 Despite these challenges, our experiments using simulated genomes and down-sampled real ancient FASTQ data showed
282 that CONGA can relatively efficiently genotype deletions and duplications of size >1 kbps at $1\times$ coverage, or even
283 lower. CONGA outperformed two "modern DNA" CNV discovery algorithms, FREEC and CNVnator, two methods
284 previously employed in ancient genome analyses Smith *et al.* (2017); Bhattacharya *et al.* (2018). CONGA exceeded both
285 tools in TPR and true negative rates, especially at coverages $<1\times$. This is unsurprising, as these tools were developed
286 for discovering novel CNVs in relatively high coverage genome data. Meanwhile, compared to GenomeSTRiP, a CNV
287 genotyper that also uses both different sources of information within a Bayesian framework Handsaker *et al.* (2011,
288 2015), CONGA performed better in achieving lower FDR rates at all coverages, while GenomeSTRiP had higher recall
289 at coverages $0.5\times$ or below. In time and memory use, CONGA surpassed all three tools.

290 In terms of deletion copy number estimates, CONGA again achieved acceptable accuracy (\sim 75% TPR and $<30\%$
291 FDR) in genomes of $0.5\times$ coverage. At lower depths of coverage and also when genotyping deletions <1 kbps, recall
292 and/or precision were weaker. CONGA's performance on duplications was also poor, as we discuss below.

¹Intel(R) Xeon(R) CPU E5-2640 v2 @ 2.00GHz: 2CPUs * 8 cores each=16 cores total and 216GB RAM

293 Overall, the relatively high accuracy at $\geq 0.5 \times$ coverage suggests that CONGA could be used to genotype deletions
294 across a considerable fraction of published shotgun sequenced ancient genomes. CONGA and GenomeSTRiP could
295 also be used in parallel, as they appear to complement each other in recall and specificity. Further, GenomeSTRiP
296 can be used in population samples for jointly genotyping low coverage genomes, which could potentially increase
297 performance. We caution, however, that joint genotyping can create ascertainment biases if coverage and ancestry
298 co-vary among jointly analysed genomes.

299 Beyond aDNA, CONGA is suitable for CNV analyses for any low depth whole-genome sequencing (WGS) experiment.
300 Such studies are increasing in number due to the trade-off between budget limitations and the wealth of genome-wide
301 information that can be used in population and conservation genetics (e.g. Vieira *et al.* (2016)).

302 **Caveats in duplication genotyping**

303 In simulated genome experiments, CONGA's performance in genotyping duplications was similar to that in deletions.
304 Beyond read-depth information (also used in genotyping deletions), duplication genotyping could also effectively benefit
305 from paired-end information from split reads. Using paired-end information alone yielded >0.65 F-Score for duplication
306 genotyping, though only at $5 \times$ coverage and with variants >10 kbps (Supplemental Table S1.E; Supplemental Fig.
307 S10). In down-sampling experiments, CONGA showed slightly lower performance in duplication genotyping than in
308 deletion genotyping when using two ancient genomes available as FASTQ files. However, CONGA's performance was
309 dramatically low on the $23 \times$ ancient BAM file, Yamnaya. This can be explained as follows (see Supplemental Note
310 S2): (i) The available Yamnaya data was processed in such a way that excess reads at duplicated loci, i.e. read-depth
311 information, was lost. (ii) Consequently, nearly all (97%) duplications CONGA genotyped in the original ($23 \times$)
312 BAM file were called only using paired-end information. (iii) Because paired-end information is rapidly lost with
313 decreasing coverage (as it requires reads overlapping breakpoints), and read-depth information was lacking, genotyping
314 duplications in this BAM files became infeasible at $<5 \times$ coverage.

315 The majority of shotgun ancient genomes in public databases are only published as BAM files. The majority of published
316 files are also at $<5 \times$ coverage. Hence, most published ancient shotgun genomes are not amenable to duplication
317 genotyping with CONGA. This is highly unfortunate, as gene duplications are a major source of evolutionary adaptation
318 that would be valuable to study also in ancient populations.

319 **Caveats in deletion genotyping**

320 Applying CONGA to genotype deletions on a heterogeneous set of real ancient shotgun genomes revealed conspicuous
321 technical influences on deletion genotyping, with a significant fraction of the 71 analysed genomes displaying outlier
322 behaviour in their deletion frequencies. We could notice technical particularities for the 21 genomes identified as
323 outliers, such as lower coverage, shorter read lengths, or the application of whole-genome hybridization capture. Our
324 results suggest that $0.4 \times$ coverage may be close to the lower threshold for deletion genotyping of >1 kbps events,
325 slightly higher than the threshold in our simulation results. We also find that whole-genome hybridization capture and
326 extra short reads (roughly ≤ 55 bp) compromise deletion genotyping, while UDG-treatment does not show a significant
327 effect. That said, we lack clear explanations for outlier deletion frequency patterns for some of these 21 genomes. For
328 instance, the genome SI-45 has coverage $>3 \times$ and an average read length of 60 bps, but nevertheless displays unusual
329 deletion patterns. We suspect that such unexpected patterns might reflect technical peculiarities in library preparation,
330 sequencing or data filtering. Unique taphonomic processes influencing DNA preservation and variability in coverage
331 may also be at play.

332 Such effects could be investigated by future studies compiling larger datasets with detailed experimental descriptions.
333 Meanwhile, our results point to the necessity of rigorous quality control and outlier filtering when calling deletions in
334 heterogeneous datasets, similar to practices traditionally adopted in transcriptome analyses. This is particularly essential
335 when combining genomes produced using different experimental protocols and sequencing platforms.

336 **Community recommendations for improving CNV analyses in ancient genomes**

337 The above observations mark the urgent need for new practices in producing and publishing ancient genomes to allow
338 reliable study of both deletions and duplications, beyond SNPs.

- 339 • Most published ancient genome data to date is SNP capture data, which is largely worthless for CNV analyses.
340 Our results underscore the long-term value of shotgun sequencing data over SNP capture, as well as whole-
341 genome capture.
- 342 • Publishing data as raw FASTQ files should be priority. The main motivation behind publishing BAM files
343 instead of raw data is to avoid publishing environmental DNA reads, which constitute a large fraction of

344 reads from shotgun sequenced aDNA experiments. Saving microbial (e.g. pathogenic) aDNA fractions
345 for investigations is another motivation. Nevertheless, our results show that raw FASTQ data is absolutely
346 necessary for duplication genotyping at low coverage and also helpful against biases in deletion genotyping.
347 In the long term, publishing raw data will be for the whole community's benefit.

348 • Sharing all details on DNA extraction, library construction, as well as the alignment and preprocessing steps
349 used in creating the exact version of datasets submitted to public databases is crucial for healthy reuse of the
350 data.

351 **Purifying selection and mutation loads in past populations**

352 Our analysis of >1 kbps deletions genotyped in 50 ancient genomes revealed how variation in deletion frequencies
353 reflect (a) demographic history, as reflected in strong correlation with SNP variation and spatial clustering, and (b)
354 negative selection, as reflected in a steeper SFS than of SNPs, lower frequencies of large deletions, and lower frequencies
355 of deletions overlapping conserved genes. These results show that CONGA can identify reliable biological signals in
356 technically heterogeneous and noisy datasets, which is a non-trivial outcome.

357 Beyond expected patterns, we also studied possible correlation between deletion loads and deleterious SNP loads
358 per genome across the 50 ancient individuals. High deleterious mutation loads could arise by relaxation of negative
359 selection due to strong bottlenecks, as suggested for Wrangel Island mammoths Rogers and Slatkin (2017) or for
360 dogs Marsden *et al.* (2016). Conversely, bottlenecks can cause high inbreeding levels, and this may lead the purging of
361 recessive deleterious variants, as recently described for a founder population of killer whales Foote *et al.* (2021). In
362 our dataset we found no significant relationship between deletion-related loads and deleterious SNP loads. This could
363 be due to lack of strong variability among Eurasian genomes in deleterious mutation burdens or due to low statistical
364 power, as we only use deletions segregating in Africa. The result could also reflect differences in dominance effects or
365 fitness effects between SNPs and deletions.

366 A full analysis of this question could be possible with the creation of a geographically comprehensive genomic time-
367 series, especially genomes of non-Eurasian populations with variable demographic histories. It would further require
368 CNV discovery in carefully processed high-coverage ancient genomes and subsequent genotyping on low coverage
369 data using CONGA. We hope that our study opens the way for such work, bringing deeper insight into the impacts of
370 selection and drift in humans and other species.

371 **Methods**

372 Among various approaches developed for CNV discovery using high throughput sequencing data, almost all use the
373 fact that read-depth, i.e., the density of reads mapped to the reference genome, will be on average lower in deleted
374 regions and higher in duplicated regions Alkan *et al.* (2011); Ho *et al.* (2020). The distance between paired-end reads,
375 their orientation, and split-read information (start and end of reads mapping to different locations) are further sources
376 of information used in determining CNVs. Although available CNV discovery algorithms generally perform well in
377 modern-day human genome sequencing data with high coverage, this is not necessarily the case for ancient genomes, as
378 well as other low coverage sequencing experiments (Supplemental Fig. S11, S12). The first reason is that the majority
379 of shotgun ancient genomes are produced at low coverage (typically <1×), which constrains the use of read-depth
380 information. Second, ancient DNA fragments are short and of variable size (typically between 50-100 bps) Shapiro and
381 Hofreiter (2014). Thus, paired-end information is absent, and available split-read information is also limited. Variability
382 in ancient DNA preservation and genome coverage Pedersen *et al.* (2014) is yet another noise source that is expected to
383 limit efficient CNV discovery. CONGA overcomes these limitations using genotyping instead of *de novo* discovery.
384 It estimates whether a candidate CNV, the location of which is provided as input, is present in a genome in BAM
385 format. It also estimates the genotype, i.e., the heterozygous or homozygous state. CONGA makes use of read-depth
386 information for deletions, and both read-depth and split-read information for duplications.

387 **Likelihood-based read-depth calculation for deletion and duplication genotyping**

388 The input to the algorithm is (1) a list of candidate CNV locations and CNV type, i.e., deletion or duplication, and (2) a
389 data set of reads aligned to the linear reference genome, e.g., using BWA Li and Durbin (2009), which should be in
390 BAM format.

391 In order to calculate the likelihood of a CNV at a given locus based on read-depth information, CONGA uses an
392 approach akin to Soylev *et al.* (2019). Let (S_i) be the i^{th} CNV in our CNV input list, defined by the breakpoint interval
393 (B_l, B_r) and the type of CNV: a deletion or duplication. At this locus, CONGA calculates the likelihood of the three
394 possible genotype states, k , given the read alignment data and CNV type. The genotype states are: no event ($k = 0$), a

395 heterozygous state ($k = 1$), or a homozygous state ($k = 2$). The likelihood, in turn, is calculated by comparing the
 396 observed (O_i) read-depth versus the expected (E_{ik}) read-depth within (B_l, B_r), given the three different genotypes.
 397 We detail the steps below.

398 1. We count the total number of mapped reads within that locus (falling fully within the interval (B_l, B_r)). This
 399 is the observed read-depth, (O_{RD}).
 400 2. We calculate expected read-depth under a "no event" scenario, i.e., representing the diploid state. Here we
 401 account for the GC bias in high-throughput sequencing data Smith *et al.* (2008), by using LOESS smoothing to
 402 normalize read-depth for GC content. Specifically, for each chromosome, we calculate the read-depth values
 403 per GC percentile for sliding windows of size 1,000 bps (step size = 1 bp). We then calculate the average
 404 read-depth per GC percentile. Then, using the chromosome-wide average GC value for the interval (B_l, B_r),
 405 we calculate the expected diploid read-depth, $E_{ik=0}$.
 406 3. We model the read-depth distribution as Poisson, using the expected read-depth values for $k = 0$, $k = 1$,
 407 $k = 2$. We calculate the probability $P(RD_{S_i} | state = k)$ as:

$$P(RD_{S_i} | state = k) = \frac{E_{ik}^{O_i} \times e^{-E_{ik}}}{O_i!},$$

408 where E_{ik} is the expected read-depth given $state = k$, and O_i is the observed read-depth at that specific locus.
 409 A typical autosomal human locus is diploid (has copy number = 2); therefore when there is no CNV event
 410 ($k = 0$), the expected value of O_i should be $E_{ik=0}$.

411 If a genome is homozygous for a deletion, we expect no reads mapping to the region, thus $O_i \sim E_{ik=2} = 0$. For
 412 heterozygous deletions, the expected number of mapped reads in that interval will be half of the expected diploid
 413 read-depth: $O_i \sim E_{ik=1} = E_{ik=0}/2$. For homozygous duplications, we expect $O_i \sim E_{ik=2} = E_{ik=0} \times 2$. For
 414 heterozygous duplications, we expect $O_i \sim E_{ik=1} = E_{ik=0} \times 1.5$.

415 4. We calculate a likelihood-based score, which we term the C-score, to estimate how likely locus S_i carries a
 416 non-reference variant in a genome, in either one copy or two copies. For this we use the calculated likelihoods
 417 for the three states. We define the C-score as the maximum of the likelihoods of (S_i) being present in
 418 heterozygous state ($k = 1$) or in homozygous state ($k = 2$) in that genome, over the likelihood of no event
 419 ($k = 0$). We use the log function to avoid numerical errors.

$$C-score(S_i) =$$

$$\frac{\max(\log(P(RD_{S_i} | k=1)), \log(P(RD_{S_i} | k=2)))}{\log(P(RD_{S_i} | k=0))},$$

420 The C-score is distributed between 0 and $+\infty$, with lower scores indicating higher likelihood of a true CNV
 421 event.

422 Results from our simulations and down-sampling experiments suggest that the relatively simple Poisson distribution
 423 can be effectively used to model copy number states, especially in the face of potentially non-independent errors due to
 424 ambiguous mapping of short and damaged reads or GC content heterogeneity. We note that alternative models have
 425 also been used for analysing CNVs in short read sequencing data, such as the negative binomial distribution Miller *et al.*
 426 (2011) or Gaussian mixed models Handsaker *et al.* (2011). We also note CONGA's our approach could be expanded in
 427 the future by including the evaluation of duplication events involving >2 copies, as in multicopy genes Sudmant *et al.*
 428 (2010).

429 Split-read and paired-end signatures for duplication genotyping

430 Beyond read-depth, information of paired-end reads or read fragments that do not linearly map to the genome can
 431 be used to identify CNVs. Ancient genomes are sometimes single-end and sometimes paired-end sequenced, but in
 432 the latter case, short overlapping reads are typically merged into a single read before alignment. Ancient genome
 433 data is thus practically single-read. However, the split-read method can be applied on single-read ancient genome
 434 data, which emulates paired-end information for genotyping duplications. This approach is visualized in Figure 6.
 435 We therefore designed CONGA to include both paired-end and single-end reads as input and to evaluate paired-end
 436 signature information.

437 First, assume a read of length L mapped to position pos_x in the reference genome, where pos_x is assumed to be one
 438 of the breakpoints of a putative CNV. There always exists a subsequence $\geq L/2$ that will have at least one mapping
 439 in the reference genome with some error threshold. Thus, we can split a read into two subsequences, assigning the

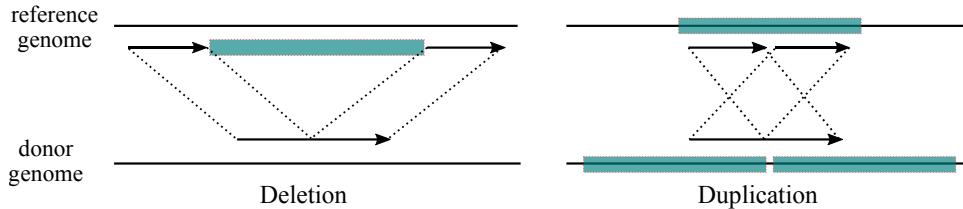


Figure 6: The figure shows our split-read approach to emulate paired-end using single-end reads. We use short-read Illumina mappings in a BAM file as input. We split each discordant read (whose mapping quality is larger than the given threshold and does not overlap with a known satellite) from the middle, keeping the initial mapping as one element and the other subsequence ("split segment") as the second element of a pair. We remap the split segment to the reference genome, and evaluate the position and the orientation of both reads to identify the presence of putative CNVs.

440 actual mapping to one of the pairs and remapping the other subsequence ("split segment") as a second pair. There
 441 are two possible split strategies: an even decomposition, where both subsequences are of equal lengths, or an uneven
 442 decomposition, where the subsequences are of unequal lengths. Given the infeasibility of testing each split position and
 443 the fact that ancient reads are typically already short, we follow Karakoc *et al.* (2012) and split the read from the middle
 444 to obtain two reads with equal lengths $L/2$. If a read overlaps a duplication breakpoint, and assuming that the expected
 445 position of the breakpoint will be uniformly distributed within the read, the split segment will map to the reference
 446 genome with insert size—the distance between the split-read pairs—greater than zero.

447 With this simple observation, the need to observe all possible breakpoints can be eliminated. Thus, given a single-end
 448 read Rse_i , we define $Rpe_i = (l(Rpe_i[pos_x : pos_x + RL/2]) \text{ and } r(Rpe_i[pos_y : pos_y + RL/2]))$, where pos_x is the
 449 initial mapping position of the single-end read, pos_y is the remapping position of the split read, RL is the length of the
 450 single-end read observed before the split, $l(Rpe_i[pos_x : pos_x + RL/2])$ is the left pair within pos_x and $pos_x + RL/2$
 451 and $r(Rpe_i[pos_y : pos_y + RL/2])$ is the right pair within pos_y and $pos_y + RL/2$ of the paired-end reads. We use this
 452 information as described in the following section.

453 Remapping paired-reads and utilizing paired-read information

454 According to our remapping strategy, we use a seed-and-extend approach similar to that implemented in mrFAST Alkan
 455 *et al.* (2009), where a read is allowed to be mapped to multiple positions. Our main concern here is that the split
 456 segment, due to its short length, can be mapped to unrealistically high numbers of positions across the genome. To
 457 overcome this problem we use the approach developed in TARDIS Soylev *et al.* (2017), allowing the split segment to
 458 be mapped only up to 10 positions within close proximity (15 kbps by default) of the original mapping position and
 459 applying a Hamming distance threshold for mismatches (5% of the read length by default).

460 Based on the distance between the reads (insert-size) and orientation, we then evaluate the type of putative CNV.
 461 As Figure 6C shows, if the split segment maps behind the initially mapped segment of the same pair to generate a
 462 reverse-forward mapping orientation, this would be an indication of a duplication.

463 In order to utilize this paired-read information, for each CNV locus used as input to our algorithm, we count the number
 464 of read-pair (i.e. split segments) that map around ± 5 kbps of the breakpoints. Each such read-pair is treated as one
 465 observation. We use these counts in combination with the C-score (read-depth information) to genotype duplications
 466 (see below). We do not use this read-pair information for genotyping deletions due to its low effectiveness in our initial
 467 trials (Supplemental Table S1.E).

468 Mappability filtering

469 The probability of unique alignment of a read of certain size varies across the genome, mainly due to repetitive
 470 sequences. Various algorithms estimate this probability, termed mappability, across the genome for k-mers of specific
 471 length Koehler *et al.* (2011); Derrien *et al.* (2012); Karimzadeh *et al.* (2018); Pockrandt *et al.* (2020). This is calculated
 472 by extracting k-mers of given length through the genome, remapping them to the reference genome, and measuring
 473 mappability as the proportion of unique mappings Karimzadeh *et al.* (2018). Because low mappability regions can
 474 be confounded with real deletions, we use mappability information to filter out CNV loci that could represent false
 475 positives.

476 CONGA accepts any mappability file in BED format, where values are distributed between 0 and 1. These can then be
 477 used to filter out CNVs for minimum mappability.

478 In our experiments, we used the 100-mer mappability data from the ENCODE Project ENCODE Project Consortium
479 (2012). Using this data, for each CNV event (S_i), we calculated the average mappability value within its breakpoints.
480 We used a minimum average mappability threshold of 0.9 for the CNV events we analyzed.

481 Our deletion frequency analysis results suggest that the strict filter should be used especially when analyzing data sets
482 of heterogeneous origin. This is because published BAM files frequently differ in mapping quality filters applied before
483 publishing (and these filters are usually not indicated), and such filtered BAM files will produce artificial deletion
484 signals at low mappability regions, while unfiltered BAM files will not.

485 **Simulation and down-sampling experiments**

486 **Simulating ancient genomes with implanted deletions and duplications**

487 Our goal here was to study the performance of CONGA on different sized deletions or duplications using simulated
488 genomes containing implanted CNVs and to determine thresholds for reliably calling these variants. We first employed
489 VarSim Mu *et al.* (2015) to simulate and insert deletions and duplications into the human reference genome GRCh37.
490 We repeated this three times, for small (100 bps - 1000 bps), medium (1000 bps - 10,000 bps), and large (10,000 bps -
491 100,000 bps) CNVs. As a result we generated three CNV-implanted genomes, with around 1500 deletions and 1500
492 duplications each (between 1385 and 1810). The CNVs were produced so that they were non-overlapping, and their
493 length distribution and exact counts are provided in Supplemental Fig. S1.

494 To evaluate specificity and sensitivity, we also included a background (false) CNV set in the experiment, which would
495 not be implanted but would be queried as part of the candidate list. This background set was prepared using recently
496 published deletion and duplication calls from human genome sequencing experiments Audano *et al.* (2019); Chaisson
497 *et al.* (2019); Zook *et al.* (2020); Collins *et al.* (2020) and also sequencing data from African populations (AFR) from
498 Phase 3 of the 1000 Genomes Project Sudmant *et al.* (2015b). We compiled a list of 17,392 deletions and 14,888
499 duplications that were non-overlapping and of size $>\sim 1000$ bps using BEDTools mergeBed Quinlan and Hall (2010).
500 When evaluating genomes with small CNVs (100 bps - 1,000 bps), we additionally included small CNVs from Chaisson
501 *et al.* (2019). Specifically we added 4,623 deletions and 3,750 duplications of size 100 bps - 1,000 bps to the above
502 background list.

503 In order to assess CONGA's performance, we added the true CNVs generated using VarSim to this background set (and
504 removed overlapping CNVs from the candidate genotype set), such that only $\sim 10\%$ of the input candidate CNV list
505 were true events. Finally, we determined how many of these true events could be correctly called by CONGA and other
506 software.

507 **Simulating ancient genome read data**

508 We used the above-described simulated genomes as input to Gargammel Renaud *et al.* (2017), which generates ancient-
509 like Illumina reads, i.e., short reads of variable size bearing postmortem damage (i.e., C-to-T transitions at read ends)
510 and including adapters. Gargammel can generate aDNA fragments following a size distribution given as input, and we
511 used a subset of Fu *et al.* (2014), which is default for this software. We used Gargammel to produce reads at various
512 depths of coverage: $0.05\times$, $0.1\times$, $0.5\times$, $1\times$ and $5\times$. We then removed adapters and merged overlapping reads Schubert
513 *et al.* (2016) to generate single-end Illumina reads. These reads had sizes ranging between 34 bps and 139 bps, with
514 average 69 bps and median 66 bps (these statistics were calculated using $1\times$ coverage data, but other data also had
515 similar distributions). We mapped the Gargammel-output reads back to the human reference genome (hg19, or GRCh37)
516 using BWA-aln Li and Durbin (2009) with parameters "-l 16500 -n 0.01 -o 2" (Supplemental Material). Note that
517 BWA-aln has been shown to be more accurate for short ancient reads than BWA-mem Oliva *et al.* (2021).

518 **Evaluation of CONGA, GenomeSTRiP, CNVnator and FREEC with simulated ancient genome data**

519 We ran CNVnator Abyzov *et al.* (2011), FREEC Boeva *et al.* (2012) and GenomeSTRiP Handsaker *et al.* (2011) on
520 the simulated genomes with parameters described in the Supplementary Information and CONGA with two values for
521 the C-score (<0.3 and <0.5). We used the above-described list of CNVs as the input candidate set for CONGA and
522 GenomeSTRiP.

523 To determine true calls, we used $>50\%$ reciprocal overlap for the two CNV events (the event in the input event set and
524 the called event) to be considered the same. This calculation was done using BEDTools Quinlan and Hall (2010). The
525 number of true CNVs were: 1810 deletions and 1751 duplications for 100 bps - 1000 bps; 1680 deletions and 1684
526 duplications for 1000 bps - 10,000 bps; and 1385 deletions and 1532 duplications for 10,000 bps - 100,000 bps.

527 **Down-sampling experiment with real ancient genomes**

528 We used three relatively high coverage ($\sim 23.3\times$, $\sim 13.1\times$ and $\sim 9.6\times$ respectively) genomes of a Yamnaya culture-
529 related individual from early Bronze Age Karagash (hereafter Yamnaya), Kazakhstan de Barros Damgaard *et al.* (2018b),
530 a Saqqaq culture-related individual from Bronze Age Greenland (hereafter Saqqaq) Rasmussen *et al.* (2010), and a
531 4500-year old East African hunter-gatherer individual from Mota Cave in Ethiopia (hereafter Mota) Llorente *et al.*
532 (2015). Using this data, and the above-described 17,392 deletions and 14,888 duplications of size >1 kbps (see above)
533 as input, we genotyped 2639 deletions and 1972 duplications in Yamnaya (deletion sizes: 1 kbps to 4 Mbps, median = 4
534 kbps, mean = 23 kbps; duplication sizes: 1 kbps to 28 Mbps, median = 14 kbps, mean = 80 kbps); 1581 deletions and
535 4097 duplications in Saqqaq (deletion sizes: 1 kbps to 5 Mbps, median = 5 kbps, mean = 17 kbps; duplication sizes: 1
536 kbps to 28 Mbps, median = 16 kbps, mean = 70 kbps); and 688 deletions and 638 duplications in Mota (deletion sizes:
537 1 kbps to 130 kbps, median = 4 kbps, mean = 7 kbps; duplication sizes: 1 kbps to 28 Mbps, median = 6 kbps, mean =
538 82 kbps).

539 We then randomly down-sampled the BAM files to various depths using Picard Tools Pic (2019): between 16-0.07 \times for
540 Yamnaya; 9-0.05 \times for Saqqaq; 7-0.03 \times for Mota. We note that this down-sampling procedure does not produce the
541 exact targeted depths, which is the reason why we obtain variable coverages in Fig. 3.

542 For calling deletions we used C-score <0.5 . For calling duplications, we called events that fulfilled either of the
543 following conditions (a) C-score <0.5 , or (b) C-score <10 and read-pair support >10 . Finally, treating the results of
544 the original data as the correct call-set, we calculated TPR (true positive rate) and FDR (false discovery rate) for
545 the down-sampled genomes. We considered CNVs with $\geq 50\%$ reciprocal overlap as representing the same event,
546 calculated using BEDTools Quinlan and Hall (2010).

547 **C-score and read-pair cutoffs and minimum CNV size**

548 We ran CONGA with a range of parameter values for the C-score [0.1-5] and for minimum read-pair support (from 0
549 support to >30), and using the above-described true event sets as the input candidate set involving medium and large
550 CNVs (1680 deletions and 1684 duplications for 1000 bps - 10,000 bps, and 1385 deletions and 1532 duplications for
551 10,000 bps - 100,000 bps).

552 We used simulation results (Supplemental Table S1.E) to choose an effective cutoff for calling CNVs. For both deletions
553 and duplications, we decided to use C-score <0.5 , which appears to yield a good trade-off between recall and precision.
554 Specifically, in simulations, this cutoff ensured an F-score of >0.5 at 0.1 \times for >1 kbps deletions, and superior F-scores
555 at higher coverages (Supplemental Fig. S13).

556 In addition, we observed that read-pair support >10 could be useful for identifying duplications in the absence of
557 read-depth support, but only when coverages were $\geq 1\times$ (Supplemental Table S1.E; Supplemental Fig. S10). Moreover,
558 read-pair support was not valid for detecting deletions.

559 We note that CONGA outputs the C-scores and read-pair counts for all input CNVs. Users can choose alternative
560 cutoffs to increase recall (higher C-scores) or precision (lower C-scores).

561 The simulation experiments showed that CONGA was not efficient in identifying events <1 kbps. CONGA therefore
562 ignores events <1 kbps under default parameters. This can be modified by the user if needed.

563 **Analysis of real ancient genomes**

564 **Ancient genome selection and preprocessing**

565 We selected 71 ancient shotgun or whole-genome captured genomes from individuals excavated in West and East
566 Eurasia and in North America (Supplemental Table S2). Our sample set belongs to a time range between c.2,800-45,000
567 years Before Present (BP). Samples from 10 different laboratories were selected in order to study the effects of different
568 data production protocols on deletion genotyping. We also chose genomes with a range of coverage levels (0.04 \times -26 \times ,
569 median = 3.45 \times) and that included both UDG-treated and non-UDG-treated libraries. The only capture-produced data
570 was Bon002 Kılınç *et al.* (2016), produced using whole-genome hybridization with myBaits (Arbor Biosciences, USA)
571 probes.

572 Selected ancient genomes were mapped to the human reference genome (hg19, or GRCh37) using BWA aln/samse
573 (0.7.15) Li and Durbin (2009) with parameters "-n 0.01, -o 2". PCR duplicates were removed using FilterUniqueSAM-
574 Cons.py Kircher (2012).

575 We also removed reads with $>10\%$ mismatches to the reference genome, those of size <35 bps, and with <30 mapping
576 quality (MAPQ).

577 **Candidate CNV call set for real ancient genomes**

578 Here our goal was to study properties of deletion variants in ancient genomes and to compare these with SNP variation
579 in terms of demographic history and purifying selection. Polymorphism data sets can suffer from ascertainment bias in
580 downstream evolutionary analyses Clark *et al.* (2005). A common practice to avoid this bias is to use SNPs ascertained
581 in a population that is an outgroup to the focal populations. We therefore used variants ascertained in modern-day
582 African populations for both calling SNP and deletion variants in our ancient genomes.

583 In order to create a candidate deletion call set to be used as input to CONGA, we downloaded deletions of size >1000
584 bps identified among 661 African population (AFR) genomes of the 1000 Genomes Project Phase 3 Sudmant *et al.*
585 (2015b). When a deletion was located inside the breakpoints of another deletion, we removed the internal one. In
586 addition, for pairs of deletions that had >50% overlap, we filtered out the smaller one. Finally, we filtered out deletion
587 loci with <50% average mappability (see above). This resulted in 11,390 autosomal >1000 bps deletions from 661
588 AFR genomes.

589 We filtered these deletions for high mappability (≥ 0.9 average mappability) and being derived in the human lineage
590 (see section "Ancestral state determination" below). This left us with 10,002 deletion loci.

591 **Deletion genotyping in ancient genomes**

592 We genotyped all the chosen 71 ancient genomes using the 11,390 AFR autosomal deletion data set (>1 kbps with
593 mean 10,735 bps). We used C-score <0.5 as cutoff for calling deletions, and >2 for calling the reference homozygous
594 genotype (0/0). To limit false negatives, C-scores between 0.5 and 2 were coded as missing (NA). Note that these
595 cutoffs can be modified by the user.

596 In total, 1,222 deletion loci (12%) out of 10,002 were missing across all the 71 genomes. Of the remaining, 5,467 were
597 genotyped as a deletion in heterozygous or homozygous state in at least one genome. Genotyping rates (non-missing
598 values) in the full dataset was overall 80.0%.

599 **Analyzing the ancient deletion dataset**

600 We generated a heatmap summarizing deletion copy numbers using the R "gplots" package "heatmap.2" function Warnes
601 *et al.* (2020). Further, we performed a principal components analyses (PCA) on the deletion copy number data set
602 (removing missing values) with 71, 60 (first outlier filter) and 50 (refined data set) ancient genomes (Supplemental Fig.
603 S4). PC1 and PC2 values were computed using the R "stats" package "prcomp" function using the default parameters R
604 Core Team (2020). On the same 3 genome sets, we likewise created multidimensional scaling plots (MDS) calculated
605 with parameter "k=2" with the R "cmdscale" function on a Euclidean distance matrix of deletion frequencies (without
606 removing NAs), and hierarchical clustering trees summarizing Manhattan distance matrices, calculated with the R "dist"
607 and "hclust" functions. This analysis revealed visible outliers in deletion frequency among samples, which we defined
608 as the "divergent" genome set (Supplemental Fig. S3A; Supplemental Note S3).

609 Based on this observation, we compared the total number of missing values, average read length, and coverage between
610 the divergent genome set (n=21) and the rest, which we refer to as the "coherent" set (n=50), using the Mann-Whitney
611 U test with the R "wilcox.test" function, and visualized the data with R utility function "boxplot" R Core Team (2020)
612 (Supplemental Fig. S5A, B, C, D). We likewise compared average deletion frequencies between UDG-treated and
613 untreated genomes using the Mann-Whitney U test.

614 **Creating and analyzing the refined deletion data set and the SNP data set**

615 **SNP genotyping in ancient genomes**

616 Following the same reasoning as above regarding ascertainment bias, we used an African population to create a SNP
617 genotyping set for calling SNPs in the ancient genomes. Specifically, we used the 1000 Genomes Yoruba data set,
618 which included a total of 38,945,054 autosomal bi-allelic SNPs (minor allele frequency > 0) in 661 African genomes
619 of the 1000 Genomes Project Phase 3 The 1000 Genomes Project Consortium (2015). First, all reads in all BAM
620 files were clipped (trimmed) using the trimBam algorithm implemented in BamUtil Jun *et al.* (2015). Following
621 standard practice Mittnik *et al.* (2018), we trimmed (a) the end 2 bases of each read for samples prepared with the
622 Uracil-DNA-glycosylase (UDG) protocol, and (b) the end 10 bases of each read for non-UDG samples.

623 Using these BAM files of the 50 ancient individuals and the above-described SNP list, we generated pseudo-haploid
624 SNP calls at these target SNP positions by randomly selecting one read and recording the allele carried on that read

625 as the genotype. This was performed using the pileupCaller software (<https://github.com/stschiff/sequenceTools>) on
626 samtools mpileup output (base quality>30 and MAPQ>30) Li *et al.* (2009).

627 Ancestral state determination

628 To polarize deletion and SNP alleles for being ancestral or derived in the human lineage, we mapped loci from hg19
629 (GRCh37) to panTro6 (chimpanzee) and to panPan2 (bonobo) using the UCSC Genome Browser tool "liftOver" with
630 default parameters Kent *et al.* (2002). For deletions, we filtered out deletions that did not fully map to either chimpanzee
631 or bonobo reference genomes, as these could represent derived insertions in the human lineage. The remaining deletions
632 could thus be inferred to be alleles that were derived in humans. For SNPs, we removed the positions not represent
633 in either chimpanzee or bonobo reference genomes and assigned the ancestral state as the Pan allele, only if both
634 chimpanzee and bonobo carried same allele. This left us with 32,344,446 SNP positions with derived allele information.

635 Creating the refined deletion data set

636 We removed 21 genomes identified as outliers in both heatmap, PCA and MDS analyses. Next, we genotyped the
637 8,780 AFR deletions in the remaining 50 genomes. We call this the "refined data set". After refining our data set,
638 we also checked its general properties. We plotted size distribution in logarithmic scale, deletion allele frequency
639 distribution and relative frequency distribution among observed heterozygous deletions over homozygous deletions
640 using R's "graphics" package hist function (Supplemental Fig. S14) R Core Team (2020). We also plotted relative
641 deletion (homozygous or heterozygous) frequencies of 8,780 deletions for each individual in our refined data set using
642 R's "graphics" package matplot function R Core Team (2020).

643 Genetic distance and selection analyses using deletions and SNPs

644 Here our goal was to calculate pairwise genetic distances among the 50 ancient genomes using deletion allele frequencies
645 and using SNPs, and further to compare the distances. We calculated distances using the commonly used outgroup- f_3
646 statistics, which measures shared genetic drift between two samples relative to an outgroup, and is implemented as
647 qp3pop in Admixtools v.7.0 Patterson *et al.* (2012). The outgroup- f_3 values were calculated for each pair of 50
648 individuals (a) in the deletion and (b) in the SNP data sets, using the African Yoruba as outgroup in both cases. To
649 convert the deletion data set to eigenstrat format, which Admixtools requires, we encoded the first nucleotide of each
650 deletion as the reference allele, and the alternative allele was randomly assigned among the remaining 3 nucleotides
651 using custom Python script. We thus calculated a pairwise similarity matrix for both data sets. Genetic distances
652 were calculated as $1-f_3$. Distances were then summarized using multidimensional scaling (MDS) with the "cmdscale"
653 function of R R Core Team (2020) (Figure 4C, D; Supplemental Fig. S4).

654 We further performed the Mantel test to compare the f_3 -based similarity matrices calculated using SNPs and deletions.
655 We used the "mantel" function in the R-package "vegan" with parameter "method=spearman" Oksanen *et al.* (2013).

656 Site frequency spectrum calculation for deletions and SNPs

657 Here our goal was to compare the SFS across deletions and SNPs called in ancient genomes. Because the ancient SNP
658 genotypes are pseudo-haploidized, we performed the same pseudo-haploidization process on the deletion data set. For
659 this, for any heterozygous call in the deletion data set, we randomly assigned either of the homozygous states, using the
660 R "sample" function (i.e., we converted 1's to 0's or 2's with 50% probability). We then counted derived alleles at each
661 locus, for deletions and for SNPs, and divided by the total number of genomes where an allele was observed at that
662 locus (i.e., removing the missing data). We plotted the site-frequency spectrum analysis on both deletions and SNPs
663 using R's "ggplot2" package geom_histogram function Wickham (2016). We also calculated the Spearman correlation
664 between the deletion size in logarithmic scale and the frequency using R's "stats" package "cor.test" function R Core
665 Team (2020). Further, we plotted the site-frequency spectrum analysis on deletions in high and low coverage genomes
666 using R's "ggplot2" package geom_histogram function Wickham (2016) (Supplemental Fig. S6). The threshold is
667 considered to be the median coverage ($3.98 \times$).

668 Evolutionary conservation

669 To measure evolutionary conservation for genes that overlapped deletions, we retrieved non-synonymous (dN) and
670 synonymous (dS) substitution rate estimates between human (GRCh37) and the mouse genome (GRCm38) per gene
671 from Ensembl (v75) via the R package "biomaRt" Durinck *et al.* (2005). We queried 18,112 genes with dN, dS values
672 and calculated the dN/dS ratio (or Ka/Ks) per gene. The ratio for genes with more than one dN or dS values were
673 calculated as the mean dN or dS per gene. We then intersected our deletions with the genes with dN/dS values using

674 BEDTools Quinlan and Hall (2010) and found 2,221 Ensembl (v75) human genes. Overall, 34% of the 10,002 derived
675 deletions overlapped with at least one gene. We then collected mouse-human dN/dS ratios (Methods) for these genes (n
676 = 2,221, 0-1.18, median = 0.09, mean = 0.13). For deletions overlapping with multiple genes, we calculated the mean
677 dN/dS per deletion. We then divided the deletions in our data set into two groups by the deletion allele frequency: high
678 versus low relative to the median. We plotted the dN/dS ratios of the deletion groups defined above using the R package
679 "ggplot2" and the "geom_boxplot" function Wickham (2016).

680 Comparison with SIFT predictions and temporal change

681 Here our goal was to study deleterious mutation loads per genome in the form of SIFT-predicted harmful SNPs and
682 CONGA-predicted deletions, across the 50 ancient genomes. We used SIFT predictions available in Ensembl (v75)
683 collected via the R package "biomaRt" Durinck *et al.* (2005, 2009). We retrieved SIFT predictions of "tolerated" and
684 "deleterious" impact and SIFT scores for all 1000 Genomes human SNPs from Ensembl, and subsetted the African
685 SNP set used for genotyping the ancient genomes. This resulted in 22,996 SNPs with SIFT predictions. Further, we
686 calculated a ratio representing the total number of SIFT-predicted "deleterious" SNPs over the number of "tolerated"
687 SNPs, for each of the 50 individuals. In addition, we calculated the total CONGA-predicted deletion length and the
688 total number of genes overlapping CONGA-predicted deletions per individual, ignoring homozygous or heterozygous
689 state. We plotted these three mutation load scores, i.e. SIFT-predicted deleterious/tolerated ratios per individual, the
690 number of affected genes, and the total deletion length, using R base function "plot" (Supplemental Fig. S7) R Core
691 Team (2020). We further estimated pairwise correlations between the three scores, fitting the values into a linear model
692 using the R "lm" function and calculating Spearman's rank correlation. We plotted the linear models using the R base
693 function "pairs" (Supplemental Fig. S8B) R Core Team (2020).

694 We finally tested whether the mean deletion allele frequency changed over time by fitting the values in a linear model
695 using the R "lm" function (Supplemental Fig. S9).

696 Software Availability

697 CONGA is implemented in C programming language and its source code is available under BSD 3-clause license
698 at <https://github.com/asylvz/CONGA>, as well as Supplemental Code. Simulated datasets and predictions of each
699 tool can be accessed through Zenodo (10.5281/zenodo.5555990). Mappability data was downloaded from <http://hgdownload.cse.ucsc.edu/goldenpath/hg19/encodeDCC/wgEncodeMapability/>

701 Competing interest statement

702 The authors declare no competing interests.

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707 Author Contributions

708 AS developed and implemented the algorithm, performed simulations and down-sampling experiments. SSC and DK
709 conducted technical and evolutionary analyses on real data. CA contributed to algorithm design. MS led the project and
710 coordinated the activities. All authors contributed to editing the manuscript and participated in weekly discussions.

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