

# 1 **Detecting ancient positive selection in humans using** 2 **extended lineage sorting**

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## 8 **ABSTRACT**

9 Natural selection that affected modern humans early in their evolution has likely shaped some of the  
10 traits that set present-day humans apart from their closest extinct and living relatives. The ability to  
11 detect ancient natural selection in the human genome could provide insights into the molecular basis  
12 for these human-specific traits. Here, we introduce a method for detecting ancient selective sweeps by  
13 scanning for extended genomic regions where our closest extinct relatives, Neandertals and  
14 Denisovans, fall outside of the present-day human variation. Regions that are unusually long indicate  
15 the presence of lineages that reached fixation in the human population faster than expected under  
16 neutral evolution. Using simulations we show that the method is able to detect ancient events of  
17 positive selection and that it can differentiate those from background selection. Applying our method  
18 to the 1000 Genomes dataset, we find evidence for ancient selective sweeps favoring regulatory  
19 changes and present a list of genomic regions that are predicted to underlie positively selected human  
20 specific traits.

21

## 1 INTRODUCTION

2 Modern humans differ from their closest extinct relatives, Neandertals, in several aspects, including  
3 skeletal and skull morphology (Weaver 2009), and may also differ in other traits that are not preserved  
4 in the archeological record (Laland et al. 2010; Varki et al. 2008). Natural selection may have played a  
5 role in fixing these traits on the modern human lineage. However, the selection events driving the  
6 fixation would have been restricted to a specific timeframe, extending from the split between archaic  
7 and modern humans ca. 650,000 years ago to the split of modern human populations from each other  
8 around 100,000 years ago (Prüfer et al. 2014). While methods exist, that can be used to scan the  
9 genome for the remnants of past or ongoing positive selection (Lemey et al. 2009; Nielsen et al. 2007),  
10 current methods have limited power to detect positive selection on the human lineage that acted during  
11 this older timeframe (see Sabeti et al. 2006 for a review on detection methods and their timeframes):  
12 an unusually high ratio of functional changes to non-functional changes, such as the dn/ds test,  
13 requires millions of years and often multiple events of selection to generate detectable signals  
14 (Kryazhimskiy and Plotkin 2008), while unusual patterns of genetic diversity between individuals and  
15 populations (e.g. extended homozygosity, Tajimas D, Fst) are most powerful during the selective  
16 sweep or shortly after (Oleksyk et al. 2010; Sabeti et al. 2006). A favorable substitution is not  
17 expected to leave a mark on linked neutral variation beyond 250,000 years in humans (Przeworski  
18 2002, 2003).

19 The genome sequencing of archaic humans (Neandertals and Denisovans) to high coverage (Meyer et  
20 al. 2012; Prüfer et al. 2014) has spawned new methods to investigate the genetic basis of modern  
21 human traits that are not shared by the archaics (Pääbo 2014). One method, called 3P-CLR, models  
22 allele frequency changes before and after the split of two populations using the archaic genomes as an  
23 outgroup (Racimo 2016). 3P-CLR outperforms previous methods in the detection of older event of  
24 selection (up to 150,000 years ago, Figure 2 from Racimo 2016) but has little power to detect events  
25 older than 200,000 years ago in modern humans. A second method applied an approximate Bayesian  
26 computation on patterns of homozygosity and haplotype diversity around alleles that reach fixation

1 (Racimo et al. 2014). Although, this approach expands our ability to investigate older time frames, this  
2 signal of selection also fades over time and events of positive selection older than 300kya become  
3 undetectable.

4 Based on a method introduced by Green et al. (2010), Prüfer et al. (2014) presented a hidden Markov  
5 model that identifies regions in the genome where the Neandertal and Denisovan individuals fall  
6 outside of present-day human variation (i.e. the archaic lineages fall basal compared to all present-day  
7 humans), and applied the model to detect selective sweeps on the modern human lineage. Regions that  
8 are unusually long are candidates for ancient selective sweeps as variants are likely to have swept  
9 rapidly to fixation, dragging along with them large parts of the chromosomes that did not have time to  
10 be broken up by recombination. While this method is, in principle, expected to be able to detect events  
11 as old as the modern human split from Neandertals and Denisovans, this power was never formally  
12 tested and it has several other shortcomings. First, the method was limited to modern human  
13 polymorphisms, ignoring the additional information given by fixed substitutions. Second, the method  
14 does not fit parameters to the data, but requires these parameters to be estimated through coalescent  
15 simulations.

16 Here, we introduce a refined version of this method, called ELS (Extended Lineage Sorting), that  
17 models explicitly the longer regions produced under selection, and includes the fixed differences  
18 between archaic and modern human genomes as an additional source of information. The ELS method  
19 also takes advantage of an Expectation-Maximization algorithm to estimate the model parameters  
20 from the data itself, making it free from assumptions regarding human demographic history.

21 To evaluate the power of the ELS method to detect ancient selective sweeps we tested its performance  
22 under scenarios of background selection and neutrality. Finally, we present an updated list of  
23 candidate regions that likely underwent positive selection on the modern human lineage since the split  
24 from the common ancestor with Neandertals and Denisovans.

## 1 RESULTS

### 2 Selection causes extended lineage sorting between closely related populations

3 The ancestors of modern humans split from the ancestors of Neandertals and Denisovans between  
4 450,000 and 750,000 years ago (Prüfer et al. 2014). Because the two newly formed descendant groups  
5 sampled the genetic variation from the ancestral population, a derived variant can be shared between  
6 some members of both groups, while other individuals show the ancestral variant. At these positions,  
7 some lineages from one group share a more recent common ancestor with some lineages in the other  
8 group than within the same group (Rosenberg 2002), a phenomenon called incomplete lineage sorting  
9 (Figure 1A).

10 Eventually, a derived allele may reach fixation as part of a region that has not been unlinked by  
11 recombination. In these regions all descendants will derive from one common ancestor and any lineage  
12 from the other population will constitute an out-group, i.e. all lineages are sorted. Because of  
13 recombination, the human genome is a mosaic of independent evolutionary histories and the process  
14 of lineage sorting is expected to randomly affect regions, until, ultimately, all lineages will be sorted.  
15 In the case of modern humans, only a fraction of the regions in the genome are expected to show  
16 lineage sorting (Prüfer et al. 2014), and the genome can be partitioned into regions where an archaic  
17 lineage falls either within the variation of modern humans (internal region) or outside of the human  
18 variation (external region) (Figure 1B).

19 While lineage sorting can occur under neutrality, selection on the modern human branch is expected to  
20 always lead to external regions as long as the selective sweep finished. In cases where the selective  
21 sweep is sufficiently strong, there will not be sufficient time for recombination to break the linkage  
22 with neighboring sites and a large region will reach fixation (extended lineage sorting, ELS, Figure  
23 1C). In contrast, selection on standing variation may fail to generate such large regions, since  
24 recombination can act on the haplotype(s) with the prospective advantageous variant before selection

1 sets in. We note that neither demography nor selection on the archaic lineage affect the lineage sorting  
2 within modern humans and thus the power to detect selective sweeps.

### 3 Expected Incomplete Lineage Sorting among Humans to Archaics

4 We used coalescent simulations to determine the incidence and expected length of regions resulting  
5 from incomplete lineage sorting in modern humans. Using a model of human demographic history  
6 (Yang et al. 2014), we estimated the fraction of lineage sorting in modern humans in regards to  
7 Neandertals and Denisovans. In simulations with 370 African chromosomes, and assuming a uniform  
8 recombination rate, about 10% of the archaic genome is more divergent than the time to the most  
9 recent common ancestor of all sampled human variation. The length of the external regions is expected  
10 to be about 0.0016 cM (95%-CI: 0.001-0.0095 cM; e.g. 1-9.5kb for a recombination rate of 1cM/Mb)  
11 with the longest regions in the order of 0.02 cM. In contrast, internal regions are expected to be 0.012  
12 cM long (95%-CI: 0.0097-0.07 cM).

### 13 Minimum Strength of Selection to Produce Detectable Sweep Signals

14 We investigated the range of selection coefficients that could have led to the fixation of a lineage after  
15 the split with the Archaic hominins, but before the differentiation of genetically modern humans about  
16 100–120 kyr ago (Li and Durbin 2011) by simulating mutations occurring at different times and  
17 evolving with different selection coefficients. While the simulations show that completed selective  
18 sweeps could have occurred with selection coefficients as low as 0.0005 (Figure 2A), the length  
19 distribution of haplotypes reaching fixation is indistinguishable from neutrality for selection  
20 coefficients under 0.001 (Figure 2, B and C). Under neutrality, the average length of external regions  
21 was 0.02 cM and remained below 0.03cM for most simulations with a selection coefficient of 0.001.  
22 In contrast, external regions longer than 0.1cM were observed for selection coefficients above 0.05.  
23 Therefore, detectable signals are expected to be biased towards strong events with a selection  
24 coefficient larger than 0.001.

## 1 Hidden Markov Model to Detect Extended Lineage Sorting

2 To detect regions of Extended Lineage Sorting, we modeled the changes of local genealogies along the  
3 genome with a hidden Markov model. We distinguish two types of genealogies, internal or external,  
4 depending on whether the archaic lineage falls inside or outside of the human variation respectively  
5 (Figure 3A). The model includes a third state corresponding to extended lineage sorting, and external  
6 regions produced by this state are required to be longer, on average, than those produced by the  
7 external state. The three states are inferred from the state of the archaic allele (ancestral or derived)  
8 either at a polymorphic position in modern humans or at a position where modern humans carry a  
9 fixed derived variant. In the following, we describe the different statistical properties expected for  
10 each type of genealogy.

11 We first consider external regions. At modern human polymorphic sites, the archaic genome is  
12 expected to carry the ancestral variant since the derived variant would indicate incomplete lineage  
13 sorting. To account for sequencing errors or misassignment of the ancestral state, we allow a  
14 probability of 0.01 for carrying the derived allele (see Material and Methods). At sites where the  
15 derived allele is fixed, the archaic genome will often carry the derived state, if the fixation event  
16 occurred before the split of the archaic from the modern human lineage, or, occasionally, the ancestral  
17 state, if the fixation event is more recent and occurred after the split.

18 For internal regions, the archaic is expected to share the derived allele at modern human fixed derived  
19 sites, but can carry the ancestral allele in our model to accommodate errors, albeit with low  
20 probability. In contrast, at sites that are polymorphic in modern humans, the probabilities of observing  
21 the ancestral or the derived allele in the archaic genome will depend on the age of the derived variant,  
22 with young variants being less likely to be shared compared to older variants. The frequency of the  
23 derived variant in the modern human population can be used as a proxy for its age and the emission  
24 probabilities in our model take the modern human derived allele frequency into account (see Material  
25 and Methods).

1 We modeled the transition probabilities between internal and external regions (related to the length of  
2 the regions) by exponential distributions. The extended lineage sorting state has the same chance of  
3 emitting derived alleles as the other external state but is required to have a larger average length. We  
4 used the Baum-Welch algorithm (Durbin et al. 1998), an Expectation-Maximization algorithm, to  
5 estimate the emission probabilities, and estimate the transition probabilities with a likelihood  
6 maximization algorithm.

## 7 Accuracy of Parameter Estimates and Inferred Genealogies

8 We first investigated the performance of the parameter inference on simulated data under neutral  
9 evolution. We found that the estimated probabilities for encountering ancestral/derived alleles in  
10 external and internal regions fit the simulated parameters well (on average less than  $\pm 0.08$  from  
11 simulated under all tested conditions) (Supplemental Figures S1 and S2), while the estimated length of  
12 internal and external regions deviate more from the simulated lengths (around 15% overestimate of the  
13 mean length, Supplemental Figure S3). However, we found that the model exhibits better accuracy in  
14 labelling the correct genealogies with the estimated length parameters compared to the simulated true  
15 values (Supplemental Figure S4). This difference seems to originate from the difficulty in accurately  
16 detecting very short external regions or internal regions with very few informative sites. We note that  
17 detecting selection is not affected by this problem since we are primarily interested in detecting long  
18 external regions. Including fixed differences improves the power to assign the correct genealogies  
19 compared to a version of the method without this additional source of information (Supplemental  
20 Figure S4).

21 We do not expect ELS regions to be detected in our neutral simulations and indeed we found that  
22 either the estimated proportion of ELS converged to zero or the maximum likelihood estimate for the  
23 length of ELS and external regions converge to the same value (49% and 51% of simulations  
24 respectively). A likelihood ratio test comparing a model without the ELS state to the full model with  
25 the ELS state also showed no significant improvement with the additional state in almost all neutral

1 simulations (only one likelihood ratio test out of 100 simulations showed a significant improvement  
2 after Bonferroni correction for multiple testing).

3 We then evaluated the accuracy of the ELS method to assign the correct genealogy to regions based on  
4 sequences obtained through coalescent simulations with selection (Figure 3, B and C). In these  
5 simulations, the underlying genealogy at each site along the sequences is known and can be compared  
6 to the estimates. To be conservative, we only focus on results with the smallest selection coefficient  
7 ( $s=0.005$ ) that produces regions long enough to be detectable. In Figure 3B we show the accuracy for  
8 labelling the extended lineage sorting regions dependent on the posterior probability cutoff for the  
9 ELS state. The results demonstrate that the model has sufficient power to accurately label sites that  
10 experienced selection with a coefficient  $s \geq 0.005$  and an occurrence of the beneficial mutation as long  
11 as 600,000 years ago.

12 We also used the simulations of positive selection events ( $s=0.005$ ) with two different times at which  
13 the beneficial mutation occurred, 300kya and 600kya, to test how often the beneficial simulated  
14 variant fall within a detected ELS region (Supplemental Table S1). To put this rate of true positives  
15 into perspective, we also counted how many ELS regions did not overlap the selected variant (false  
16 positives). A large fraction of selected mutations were detected (87-92%). However, we also found a  
17 substantial fraction of false positive ELS regions (10-11%). When restricting detected ELS regions to  
18 those that are longer than 0.025cM, we find less than 0.1% false positives compared to 65-68% true  
19 positives. Not all simulated regions with a selection coefficient of 0.005 produce ELS regions of this  
20 size, so that the rate of true positives for truly long regions is expected to be higher. For all following  
21 analysis, we used this minimal length cutoff of 0.025 cM.

## 22 Role of Background selection

23 Background selection is defined as the constant removal of neutral alleles due to linked deleterious  
24 mutations (Charlesworth et al. 1993). In regions of the genome that undergo background selection, a  
25 fraction of the population will not contribute to subsequent generations, causing a reduced effective



1 population size. As a consequence, remaining neutral alleles can reach fixation faster than under  
2 neutrality, potentially producing unusually long external regions that could be mistaken as signals of  
3 positive selection. We investigated the effects of background selection by running forward simulations  
4 with parameters that mimic the strength and extent of background selection estimated for the human  
5 genome (Messer 2013). While background selection simulations did produce some long outlier  
6 regions that fall outside the distribution observed in neutral simulations, most regions are still smaller  
7 than regions simulated with positive selection at a conservative selection coefficient of 0.005 (Figure  
8 4A). Indeed, among the 1160 external regions detected in our simulations of background selection  
9 ( $s=0.05$ , Figure 4A) only six were labeled as ELS and only three passed the minimal length filter of  
10 0.025 cM.

## 11 Candidate Regions of Positive Selection on the Human Lineage

12 To identify ancient events of positive selection on the human lineage, we applied the ELS method to  
13 African genomes from the 1000 genomes project (Abecasis et al. 2012). We disregarded non-African  
14 populations since Neandertal introgression in these populations could mask selective sweeps and lead  
15 to false negatives. A model with ELS fits the data significantly better than a model without the ELS  
16 state for all chromosomes and for both tested recombination maps ( $p$ -value  $< 1e-8$ , Supplemental  
17 Table S2).

18 We identified 81 regions of human extended lineage sorting for which both recombination maps  
19 support a genetic length greater than 0.025cM (average length: 0.05 cM). Depending on the  
20 recombination map, the longest overlap between the maps is 0.12 (African-American map) or 0.17  
21 (deCode map) cM long, which is three to four times longer than the longest regions produced under  
22 background selection in our simulations. An additional 233 regions are longer than 0.025cM according  
23 to only one recombination map, with 71% of those additional regions showing support for the ELS  
24 state using both recombination maps. This suggests that the variation in the candidate set mostly stems  
25 from uncertainty about recombination rates. We will refer to the set of 81 regions as the core set

1 (Supplemental File S1) and the set including the 233 putatively selected regions found with just one  
2 recombination map as the extended set (314 regions, Supplemental File S2).

3 For completeness, we also ran our model on the X chromosome and identified 12 additional  
4 candidates (43 if we consider candidates found with at least one recombination map), applying a more  
5 stringent length cutoff of 0.035 cM to account for the stronger effects of random drift on this  
6 chromosome (cf. Material and Methods). Interestingly, we also found a significant increase of  
7 posterior probabilities for selection within previously reported regions under potential recurrent  
8 selective sweeps in apes (Dutheil et al. 2015; Nam et al. 2015) (*Mann-Whitney U* one-sided test, *P*-  
9 value < 2.2e-16, Supplemental Table S3).

10 The detected selection candidate regions on the autosomes do not show a decrease in B scores  
11 (McVicker et al. 2009), a local measure of background selection strength, compared with random  
12 regions (Figure 4B; Wilcoxon rank sum test comparing the average B-scores with permuted regions,  
13 *P*-value=0.565, or comparing the lowest B-scores in our regions to permuted regions, *P*-value=0.504).  
14 This suggests that candidate regions are not primarily generated by strong background selection.

15 We compared our candidate regions to the top candidates of 8 previous scans for selection, including  
16 iHS, Fst, XP-CLR and HKA (Cagan et al. 2016; Chen et al. 2010; Hudson et al. 1987; Malécot 1948;  
17 Pybus et al. 2014; Voight et al. 2006; Wright 1951). Using the estimated TMRCA among Africans for  
18 each identified region/site, we found that our ELS scan identified significantly older events than other  
19 screens (Figure 5, *Mann-Whitney U* tests, Supplemental Table S4). We found 23 regions from the core  
20 set (detected by both recombination maps) overlapping with candidates from previous scans and 68 for  
21 the extended set (detected by at least one recombination map); neither overlap is more than expected at  
22 random (*P*-values are 0.06 and 0.595 respectively). In contrast, our candidate regions overlap more  
23 often candidate regions from 3P-CLR (Racimo 2016) and the ABC approach for detecting ancient  
24 selection (Racimo et al. 2014) than expected by chance (*P*-values<0.05; Supplemental Table S5).

## 1 Biological functions of the candidate regions

2 Since positive selection acts on advantageous phenotypes that are caused by changes to functional  
3 elements in the genome, we would expect that our candidate regions overlap functional elements in the  
4 genome more often than expected.

5 We first tested this hypothesis by counting the overlap between sweep candidate regions and protein  
6 coding genes (Ensembl release 82). We find no statistically significant overlap of ELS regions with  
7 protein coding genes compared to randomly placed regions of the same size ( $P$ -value = 0.671 and  
8 0.124, for core and extended set, respectively; Figure 6A). Previous work has identified 96 proteins  
9 that carry human fixed derived non-synonymous changes compared to Neandertal and Denisova,  
10 which constitute a particularly interesting subset of potentially functional changes to genes that may  
11 have been caused by selective sweeps (Prüfer et al. 2014). We found no overlap between these genes  
12 and the core set of sweep candidate regions that were identified by both recombination maps.  
13 However, when considering the extended set of sweep candidate regions, 11 regions overlapped such  
14 genes: *ADSL*, *BBIP1*, *ENTHD1*, *HERC5*, *KATNA1*, *KIF18A*, *NCOA6*, *PRDM10*, *SCAP*, *SLITRK1* and  
15 *ZNHIT2*. This overlap is significantly larger than expected by chance (only 2 genes are expected on  
16 average;  $P$ -value  $< 10^{-3}$ ). In all instances, the candidate regions contained at least one fixed amino acid  
17 change. Since fixed changes are part of the information used to infer external regions, it stands to  
18 reason that the presence of such a change may bias towards observing an overlap with candidate  
19 regions (72/81 core regions and 275/314 regions from the extended set contain fixed changes).  
20 However, we note that the overlap with fixed amino acid changes is also significantly larger than the  
21 overlap with other fixed changes (963 of 20347 fixed changes fall within candidate regions from the  
22 extended set; binomial  $P$ -value=0.006).

23 Phenotype may also be influenced by regulatory changes that affect gene expressions. Interestingly,  
24 we found a significant enrichment for regions overlapping enhancers and promoters ( $P$ -value $<0.001$

1 and  $P$ -value=0.002, respectively; see Figure 6A) when considering the extended set of 314 candidate  
2 regions. However, this enrichment was not significant for the smaller core set of candidates.

3 To further investigate the biological function of our regions, we tested for gene ontology enrichment in  
4 genes within the extended set of regions. No category showed significant enrichment when comparing  
5 to randomly placed regions of identical sizes in the genome (Material and Methods). We also assigned  
6 genes that overlap our extended dataset to tissues in which they show the significantly highest  
7 expression and found again no enrichment. In an attempt to include potential regulatory changes in the  
8 enrichment test, we assigned genes to candidate regions when a region fell upstream or downstream of  
9 a gene (see Material and Methods). Although many candidate genes that were annotated in this way  
10 were expressed highest in the brain or the heart (OR=2.10 for both tissues), this enrichment is not  
11 significant when correcting for gene length and multiple testing (FWER=0.336 and 0.997 respectively,  
12 Supplemental Table S7).

13 Additional work will be required to investigate the phenotypic consequences of changes in candidate  
14 regions for selection. To facilitate this work, we provide an annotated list of fixed or nearly fixed sites  
15 on the human lineage that fall within our candidate regions (Supplemental File S3).

16

## 17 **Overlap with Neandertal Introgression**

18 Introgression from Neandertals and Denisovans into modern humans occurred approximately 37,000  
19 to 86,000 years ago (Fu et al. 2014, 2015; Sankararaman et al. 2012, 2016). For those advantageous  
20 derived variants that arose on the modern human lineage prior to introgression, we would expect that  
21 selection may have acted against the re-introduction of the ancestral variant through admixture. We  
22 tested whether this selection may have affected the distribution of Neandertal introgressed DNA  
23 around fixed changes in candidate sweep regions. Out of a total of 963 fixed derived variants in  
24 Africans overlapping the extended set of sweep regions, 240 (25%) show the ancestral allele in non-  
25 Africans and show evidence for re-introduction by admixture using a map of Neandertal introgression

1 (Vernot and Akey 2014). This level of Neandertal ancestry is comparable to the genome-wide fraction  
2 of out-of-Africa ancestral alleles at African fixed derived sites (~26%; bootstrap  $P$ -value=0.583). We  
3 also find no significant reduction in frequency of Neandertal ancestry around candidate substitutions  
4 in sweep regions, when comparing one randomly sampled fixed African substitution per region against  
5 random regions matched for size and distance to genes (Supplemental Figure S6 and S7).

6 If selection against the re-introduction of an ancestral variant were very strong, selection may have  
7 depleted Neandertal ancestry in a large region surrounding the selected allele. Interestingly we find  
8 some of our sweep candidate regions that fall within the longest deserts of both Neandertal and  
9 Denisova ancestry (Table 1) (Vernot et al. 2016). A significantly high number of the core set of  
10 regions fall in these deserts (5/81 regions,  $P$ -value=0.024), while the extended set shows no significant  
11 enrichment (9/314 regions,  $P$ -value=0.205).

## 12 DISCUSSION

13 Many genetic changes set modern humans apart from Neandertals and Denisovans but their functions  
14 remain elusive. Most of these changes probably resulted in either no change to the phenotype or to a  
15 selectively neutral change. However, in rare instances selection may have favored changes modifying  
16 the appearance, behavior and abilities of present-day humans. Unfortunately, current methods to  
17 identify selection have limited power to detect such old events of positive selection (Przeworski 2002,  
18 2003; Sabeti et al. 2006).

19 Here, we introduce a hidden Markov model to detect ancient selective sweeps based on a signal of  
20 extended lineage sorting. Using simulations we were able to show that the method can detect older  
21 events of selection as long as the selected variant was sufficiently advantageous. The power to detect  
22 older events is due to the fact that the method increases in power with the number of mutations that  
23 accumulated after the sweep finished. We also showed that background selection can cause false  
24 signals and have chosen a minimum length cutoff on candidate regions. While this cutoff reduces the

1 number of false positives due to background selection, we note that this cutoff is expected to exclude  
2 *bona fide* events of positive selection, too.

3 We applied the ELS method to 185 African genomes, the Altai Neandertal genome and the Denisovan  
4 genome, and detected 81 candidate regions of selection when requiring a minimum genetic length  
5 supported by two independent recombination maps. The uncertainty in the recombination maps has a  
6 large effect on our results, as shown by the much larger number of 314 regions identified by either  
7 recombination map. Recombination rates over the genome are known to evolve rapidly (Lesecque et  
8 al. 2014) and of particular concern are recent changes in recombination rates that make some regions  
9 appear larger in genetic length than they were in the past. By comparing the current recombination  
10 rates in our regions to recombination rates in the ancestral population of both chimpanzee and humans  
11 (Munch et al. 2014), we identified some candidate regions that may have increased in recombination  
12 rates (Supplemental Table S7). However, it is currently impossible to date the change in  
13 recombination rates confidently and these candidate sweeps may post-date the change.

14 A particular strength of our screen for selective sweeps is the ability to detect older events, as  
15 indicated by the estimated power to detect simulated events of positive selection of old age and  
16 moderate strength. This sets the ELS method apart from previous approaches that made use of archaic  
17 genomes, which were geared towards detecting younger events with an age of less than 300,000 years  
18 ago (Racimo 2016; Racimo et al. 2014). Despite this difference, we found significant overlap between  
19 the ELS candidates and the candidates identified by these other approaches, while the overlap with  
20 other types of positive selection scans is smaller. Among our candidates, 55 are novel candidates (234  
21 if considering the extended set) that were not detected in any of the previous screens, including  
22 previous versions of the screen without fixed differences (Supplemental Figure S5).

23 While we find no difference in the fraction of genes in selected regions compared to randomly placed  
24 regions, we detect an enrichment for enhancers and promoter regions. This result is in agreement with  
25 the hypothesis that regulatory changes may play an important role in human-specific phenotypes  
26 (Carroll 2003; Enard et al. 2014; King and Wilson 1975), maybe more so than amino-acid changes

1 (Hernandez et al. 2011; see also Enard et al. 2014 and Racimo et al. 2014). Interestingly, several gene  
2 candidates falling within sweep regions play a role in the function and development of the brain. A  
3 particularly interesting observation is the potential selection on both the ligand *SLIT2* and its receptor  
4 *ROBO2*, which reside on chromosome 4 and 3 respectively (see Supplemental File S3 for an annotated  
5 list of changes in those genes). Members of the Roundabout (ROBO) gene family play an important  
6 role in guiding developing axons in the nervous system through interactions with the ligands SLITs.  
7 SLITs proteins act as attractive or repulsive signals for axons expressing different ROBO receptors.  
8 *ROBO2* has been further associated with vocabulary growth (St Pourcain et al. 2014), autism (Suda et  
9 al. 2011), and dyslexia (Fisher and DeFries 2002) and is involved in the development of neural circuits  
10 related to vocal learning in birds (Wang et al. 2015). Interestingly, *ROBO2* is also in a long desert of  
11 both Denisovan and Neandertal ancestry in non-Africans.

12 We also identified interesting brain-related candidates on the X chromosome, among them *DCX*, a  
13 protein controlling neuronal migration by regulating the organization and stability of microtubules  
14 (Gleeson et al. 1999). Mutations in this gene can have consequences for the expansion and folding of  
15 the cerebral cortex, leading to the “double cortex” syndrome in females and “smooth brain” syndrome  
16 in males (Gleeson et al. 1998).

17 We have presented a new approach to detect ancient selective sweeps based on a signal of extended  
18 lineage sorting. Applying this approach to modern human data revealed that selection may have acted  
19 primarily on regulatory changes. With population level sequencing of non-human species becoming  
20 more readily available we anticipate that this approach will help to reveal the targets of ancient  
21 selection in other species

## 22 MATERIALS AND METHODS

### 23 Data

24 We used 185 unrelated Luhya and Yoruba individuals from the 1000 Genomes Project phase I  
25 (Abecasis et al. 2012), corresponding to 370 sets of autosomes and 279 X chromosomes. From this

1 dataset, we extracted allele counts at single nucleotide polymorphism (SNP) sites using vcftools  
2 (Danecek et al. 2011). In order to add sites where all Africans differ from the common ancestor with  
3 chimpanzee, we first compiled a list of all sites where six high-coverage African genomes (Mbuti, San  
4 and Yoruban A and B-panel individuals from Prüfer et al. 2014) are identical. A site was regarded  
5 fixed different when the whole genome alignments of at least three out of four ape reference genome  
6 assemblies (chimpanzee (panTro3), bonobo (panPan1.1), gorilla (gorGor3) and orangutan (ponAbe2);  
7 lastz alignments to the human genome GRCh37/hg19 prepared in-house and by the UCSC genome  
8 browser (Speir et al. 2016)) had coverage and were different from the African allele, and when the site  
9 was not marked as polymorphic among the 1000 Genomes Luhya and Yoruba individuals.

10 Neandertal and Denisova alleles at polymorphic and fixed positions were extracted from published  
11 VCFs and positions were further filtered to sites passing the published map35\_100 filter for both the  
12 Denisova and Neandertal genotypes (Prüfer et al. 2014). Sites where either Neandertal or Denisova  
13 carried a third allele were disregarded.

14 Over all autosomes, 11 million SNPs passed the filters in addition to 6.6 million African fixed  
15 variants. For the X chromosome, pseudoautosomal regions, defined as chrX: 60,001-2,699,520, chrX:  
16 154,931,044-155,260,560 in hg19 coordinates (<http://www.ncbi.nlm.nih.gov/assembly/2758/>), were  
17 filtered out and around 315,000 SNPs as well as 248,000 African fixed variants remained for analysis.

18 Genetic distances between those positions were calculated using the African-American (Hinch et al.  
19 2011) and the DeCode (Kong et al. 2010) recombination maps (available in Build 37 from  
20 <http://www.well.ox.ac.uk/~anjali/>). Both maps were chosen since they estimate recombination rates  
21 from events that occurred within a few generations before present. Recombination maps based on  
22 older events (i.e. LD based map) can underestimate recombination rates in regions that underwent  
23 recent selective sweeps, potentially masking true signals.



1 Changes of recombination rates along the human lineage could also limit our power to detect selected  
2 regions, and we used an ancestral recombination map of the human-chimpanzee ancestor to annotate  
3 top candidate regions (Supplemental Table S7) (Munch et al. 2014).

#### 4 Hidden Markov model

5 We would like to estimate for each informative position the probabilities for the three possible  
6 genealogies external ( $E$ ), internal ( $I$ ) and extended lineage sorting ( $ELS$ ) given the observed data.  
7 Formally, and following the notation from Durbin et al. 1998, we calculate  $P(\pi_i = k|x)$  where  $i$   
8 denotes the position,  $k \in \{E, I, ELS\}$  and  $x$  is the sequence of observations with the  $i$ th observation  
9 denoted  $x_i$ . With the genetic distance  $d$  between consecutive sites and  $l_k$ , the average genetic length of  
10 a region in state  $k$ , we specify the transition probabilities between identical states as  $t_{k,k} = e^{-\frac{d}{l_k}}$ .  
11 Transitions from  $I$  to the states  $ELS$  and  $E$  depend on an additional parameter  $p$ , the proportion of  
12 transitions from  $I$  to  $ELS$ , and their probability is given by  $t_{I,ELS} = p \left(1 - e^{-\frac{d}{l_I}}\right)$  and  $t_{I,E} = (1 -$   
13  $p) \left(1 - e^{-\frac{d}{l_I}}\right)$ . Lastly, transitions from the two external states to internal have the probability  $t_{j,I} =$   
14  $1 - e^{-\frac{d}{l_j}}$ , with  $j \in \{E, ELS\}$ . By construction, transitions between  $E$  and  $ELS$  genealogies are not  
15 allowed: it would not be possible to detect such transitions as those two states have the same statistical  
16 properties.

17 The inference further requires the probability for observing an ancestral or derived allele in the archaic  
18 at a site  $i$  with a derived allele frequency  $f_i > 0$  in modern humans (noted  $x_i$ ) given that the true  
19 genealogy is  $k \in \{I, E, ELS\}$ :  $e_k(x_i) = P(x_i | \pi_i = k)$ . We assume that  $\forall x: e_{ELS}(x) = e_E(x)$ , i.e. that  
20 both external states give rise to ancestral and derived alleles in the archaic with equal probabilities  
21 given the same observation. Since external regions are not expected to give rise to derived sites when  
22 the derived allele is segregating in modern humans, the only sources for such an observation can be  
23 errors or independent coinciding identical mutations and we define an error rate for external regions:  
24  $\epsilon_E = e_E(x_i = derived, f_i < 1)$ . Similarly fixed derived sites are expected to show the derived allele

1 in the archaics if the local genealogy is internal and we define an error rate for internal regions:  $\epsilon_I =$   
2  $e_I(x_i = \text{derived}, f_i = 1)$ .

3 We compute the posterior probability  $P(\pi_i = k | x)$  that an observation  $x_i$  came from state  $k$  given the  
4 observed sequence  $x$  as:  $P(\pi_i = k | x) = \frac{P(x, \pi_i = k)}{P(x)}$ .  $P(x, \pi_i = k) = f_k(i)b_k(i)$  where  $f_k(i) =$   
5  $P(x_1 \dots x_i, \pi_i = k)$  and  $b_k(i) = P(x_{i+1} \dots x_L | \pi_i = k)$  are the output of the Forward and Backward  
6 algorithms respectively (Durbin et al. 1998; Rabiner 1989).  $P(x)$  corresponds to the likelihood of the  
7 data given our model and was also calculated from the Forward algorithm.

## 8 Parameter estimate

9 We used the Baum-Welch algorithm to estimate all emission probabilities with the exception of  $\epsilon_E$ ,  
10 the proportion of segregating sites derived in the archaic genome in external regions, due to limited  
11 accuracy in the estimates. We set this last parameter to a value of 0.01, a conservative upper limit on  
12 contamination and sequencing error in the two high-coverage archaic genomes. The Baum-Welch  
13 algorithm was run for a maximum of 40 iterations and the convergence criteria was set to a log-  
14 likelihood maxima difference of less than  $10^{-4}$ .

15 We estimated the remaining parameters (average lengths of regions and the proportion of transitions to  
16 the ELS state) using the derivative free optimization method COBYLA (Powell 1994) as implemented  
17 in the nlopt library (Steven G. Johnson, The NLOpt nonlinear-optimization package) to maximize the  
18 log-likelihood values calculated by the Forward algorithm. Convergence was attained in a maximum  
19 of 1000 evaluations and the log-likelihood maximization accuracy was set to  $10^{-4}$ . To test for  
20 convergence to local maxima, we ran the algorithm twice with different starting points and used the  
21 parameters of the run with the highest likelihood to run the re-estimation algorithm a third time  
22 starting with those parameters. All three runs gave similar results on all chromosomes.

## 23 Post-processing

24 The HMM was executed independently on all chromosomes for both Denisova and Neandertal and  
25 using the African-American and DeCode recombination maps. An external region was defined as a

1 stretch of high posterior probabilities ( $p \geq 0.7$ ) for the extended lineage sorting state that was  
2 uninterrupted by sites with a low probability ( $p \leq 0.1$ ). The two cutoffs on the posterior probabilities  
3 were determined by simulating sequences with positive selection ( $s=0.005$ , 500kya, see below). Sites  
4 that were simulated external in both Archaics were labeled as 1 and the remaining sites as 0. The  
5 HMM was then run on the simulations. By running a grid-search over possible cutoffs (step-sizes of  
6 0.05 for the two parameters) and labeling the HMM output accordingly, we identified the set of chosen  
7 parameters by minimizing the root mean square error  $\sqrt{\frac{\sum_i (t_i - o_i)^2}{n}}$  with  $n$  the number of labelled sites,  $t_i$   
8 the true label and  $o_i$  the observed label.

## 9 Simulations

10 We simulated sequences using a model of recent human demography to test the performance of our  
11 HMM under different scenarios of neutral evolution, positive selection or background selection. Each  
12 simulation consisted of one chimpanzee chromosome, one chromosome from each archaic hominin  
13 and 370 human chromosomes, matching the 185 Luhya and Yoruba individuals used in our analysis.  
14 For all simulations in this study, a constant mutation rate of  $1.45 \times 10^{-8}$  bp<sup>-1</sup>.generation<sup>-1</sup>, a constant  
15 recombination rate of 1cM.Mb<sup>-1</sup>.generation<sup>-1</sup> and a generation time of 29 years were assumed. We  
16 used estimates of population sizes from (Yang et al. 2014) and population split estimates from (Prüfer  
17 et al. 2014) as parameters for the simulated demography (Supplemental Information 1 and 2). Neutral  
18 simulations with these parameters using the coalescent simulator scrm (Staab et al. 2014) give a good  
19 match to our observed data when plotting derived allele frequency in modern humans against the  
20 proportion of derived alleles in the outgroup (Supplemental Figure S8).

21 We generated a total of 100 loci of 1Mb-long sequences under neutrality to investigate the accuracy of  
22 labeling external and internal regions using our HMM. To evaluate the length of external regions  
23 expected under neutrality for the chromosome X, we simulated 100 loci of 1Mb-long sequences under  
24 the demographic model shown in Supplemental Information 1 with the exception that all effective  
25 population sizes were reduced to 75% of the original value. To evaluate the accuracy of parameter

1 estimation, we additionally simulated splits of two populations (including an out-group individual)  
2 with a constant population size and different split times ranging from 400ky to 1My (step-size of  
3 50ky). For each condition, we generated 25 sets of 10 Mb each. In an additional set of 100 loci of  
4 1Mb, we introduced random errors by changing the state of the archaic allele with different rates in  
5 order to assess our error estimates.

6 To assess our power to detect events of positive selection, we explored selection coefficients ranging  
7 from 0.0005 to 0.1 and different times for the occurrence of the selected allele (every 100ky from  
8 200kya to 600kya) using the coalescent simulator msms (Ewing and Hermisson 2010). The selected  
9 mutation was introduced in the middle of the sequence and we assumed an additive effect of the  
10 selected mutation (i.e. the homozygous genotype has twice the advantage stated by the selection  
11 coefficient). We performed 2000 simulations of 100kb-long loci for which all demographic parameters  
12 match our neutral simulations as described above. We used the `-SForceKeep` switch to drop the  
13 simulation if the selected mutation was lost. As 100kb loci are too short to make reliable parameter  
14 inferences, we concatenated our simulated sequences, intermittently combining them with 1Mb-long  
15 neutral loci from the previous simulations to limit the extent of the sequence affected by positive  
16 selection.

17 To explore the power over different settings of divergence, we simulated a simple demographic model  
18 with constant population size and varying degrees of divergence between two populations (see  
19 Supplemental Table S8 and S9 for further details).

20 We investigated how background selection affects lineage sorting in and around a conserved region by  
21 performing forward in time simulations using SLiM (Messer 2013). The simulated locus of 500kb  
22 length contained a conserved region resembling an ‘average’ human gene (see pg. 19 of the  
23 documentary accompanying SLiM (Messer 2013)) and covered 100kb (20%) of the simulated locus.  
24 Mutations in the conserved region were assumed to be neutral (25%) or deleterious (75%), with the  
25 selection coefficients of the deleterious mutations drawn from a gamma distribution with mean  $s =$

1 -0.05 and shape parameter  $\alpha = 0.2$ . The deleterious mutations were assumed to be partially recessive  
2 with dominance coefficient  $h = 0.1$  for a set of 100 simulations. To explore the effect of the strength of  
3 selection on the results, we produced 2 other sets of 40 simulations each by varying the mean of the  
4 gamma distribution ( $s = -0.001$  and  $-0.1$ ).

## 5 Age Comparison with other Scans for Selection

6 To compare our sweep screen with previous scans, we downloaded candidate regions from the 1000G  
7 positive selection database (Pybus et al. 2014). Only candidates with a  $P$ -value lower than 0.001 were  
8 considered. We added to this set of regions the top reported regions from a HKA scan (Cagan et al.  
9 2016). Allele age estimates were obtained from ARGweaver (Rasmussen et al. 2014).

10  $F_{st}$ ,  $iHS$  and XP-EHH are site-based statistics which localise sites that may have been selected (Sabeti  
11 et al. 2007; Malécot 1948; Voight et al. 2006; Wright 1951), whereas selective scans such as CLR,  
12 XP-CLR, Tajima's  $D$ , Fay & Wu's  $H$  and HKA identify candidate regions (Chen et al. 2010; Fay and  
13 Wu 2000; Hudson et al. 1987; Kim and Stephan 2002; Tajima 1989). In order to compare the age of  
14 the selection events, we assumed that the selected variant in candidate regions was the site with the  
15 highest frequency. We note that this procedure will underestimate the age of events if the true selected  
16 site reached fixation, as often expected for our method; the comparison is thus conservative.

## 17 Annotations

18 We used the latest Ensembl gene annotation for hg19 (release 82) to identify protein-coding genes  
19 overlapping with our candidate regions. Based on this annotation, a regulatory region was defined as  
20 at least 5kb upstream and 1kb downstream of each gene. The regulatory region was extended until it  
21 reached a size of 1Mb or came within 5kb upstream or 1kb downstream of a neighboring gene. We  
22 additionally used a set of promoters and enhancers mapped by GenoSTAN in 127 cell types and  
23 tissues from the ENCODE and Roadmap Epigenomics projects (Zacher et al. 2016).

24 We used B-scores (McVicker et al. 2009) in hg19 coordinates constructed with UCSC's liftover tool  
25 to evaluate the extent of background selection in our candidate regions. We also compared our

1 candidate regions on the X chromosome with regions previously suggested to have experienced  
2 recurrent selective sweeps in apes (Dutheil et al. 2015; Nam et al. 2015). And, finally, we examined  
3 patterns of introgression in our candidate regions with two maps of Neandertal ancestry  
4 (Sankararaman et al. 2014; Vernot et al. 2014) and overlapped our regions with long deserts of  
5 Neandertal and Denisova ancestry from another recent study (Vernot et al. 2016).

6 To statistically test the overlap of our regions with these annotations, we permuted regions of similar  
7 physical sizes in the regions of the genome that passed our quality filters. Quality filtered regions that  
8 were smaller than the longest gap present in our candidate ELS regions were regarded as sufficiently  
9 short to not prohibit the placement of regions.

## 10 Gene ontology and gene expression analysis

11 We defined genes that show tissue-specific expression levels using the Illumina BodyMap 2.0 RNA-  
12 seq data (Derrien et al. 2012), which contains expression data from 16 human tissues. We computed  
13 differential expression for all genes between a given tissue and all other tissues pooled using the  
14 DESeq package (Anders and Huber 2010) and genes were defined to be expressed in a tissue-specific  
15 manner when their expression levels were significantly higher ( $P$ -value  $< 0.05$ ) in a given tissue  
16 compared to all other tissues. We tested for enrichment of candidate genes in the 16 sets of tissue-  
17 specifically expressed genes comparing to genes that were located outside of candidate regions using  
18 Fisher's exact test. We calculated family-wise error rates for each tissue by randomly placing regions  
19 of sizes similar to the candidate regions in the genome. We repeated this process 1000 times,  
20 performed the same enrichment analysis as described above and counted how often any tissue in the  
21 randomized sets yields a smaller or equal  $P$ -value than the  $P$ -value observed in the candidate regions  
22 for a given tissue. This strategy corrects for the difference in length of genes expressed in specific  
23 tissues. We performed a similar analysis for the gene ontology analysis using `func` and the  
24 hypergeometric test (Prüfer et al. 2007), again comparing the genes associated with the candidate  
25 regions to a thousand sets of random regions to calculate family-wise error rates.

## 1 DATA ACCESS

2 The software and input files used in this study have been made available through the website  
3 <http://bioinf.eva.mpg.de/ELS/> and <https://github.com/StephanePeyregne/ELS/>.

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## 11 DISCLOSURE DECLARATION

12 The authors declare no competing financial interests.

## 13 AUTHOR CONTRIBUTIONS

14 SP implemented the method. SP, MJB and MD analyzed data. SP, MJB, MD and KP interpreted the  
15 results. KP designed the study. SP and KP wrote the manuscript with input from all authors.

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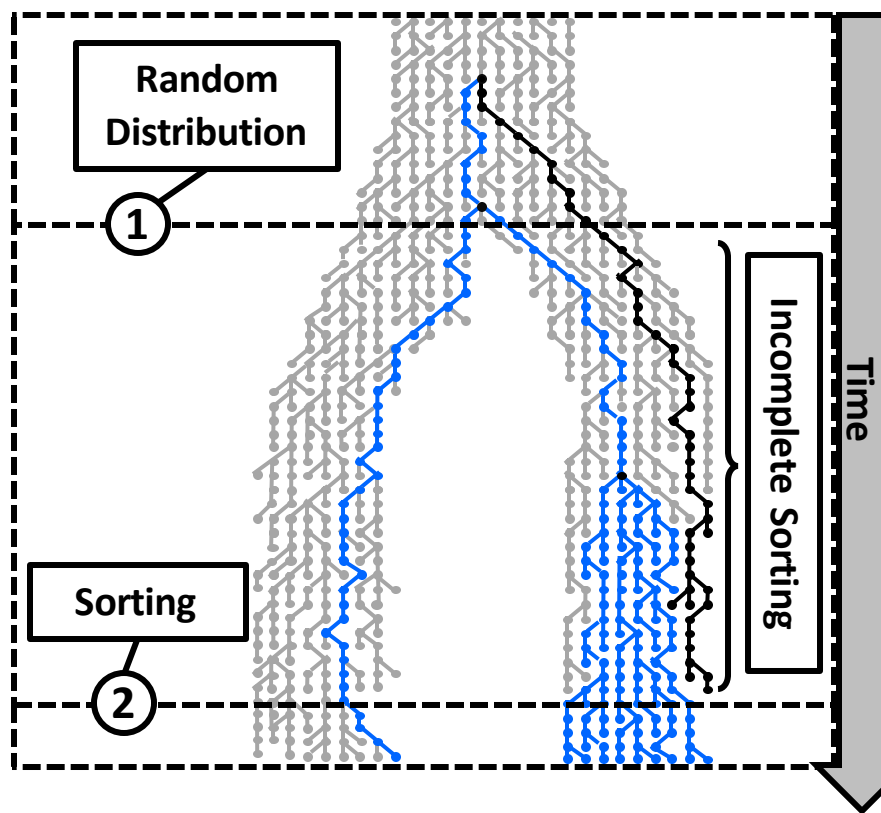
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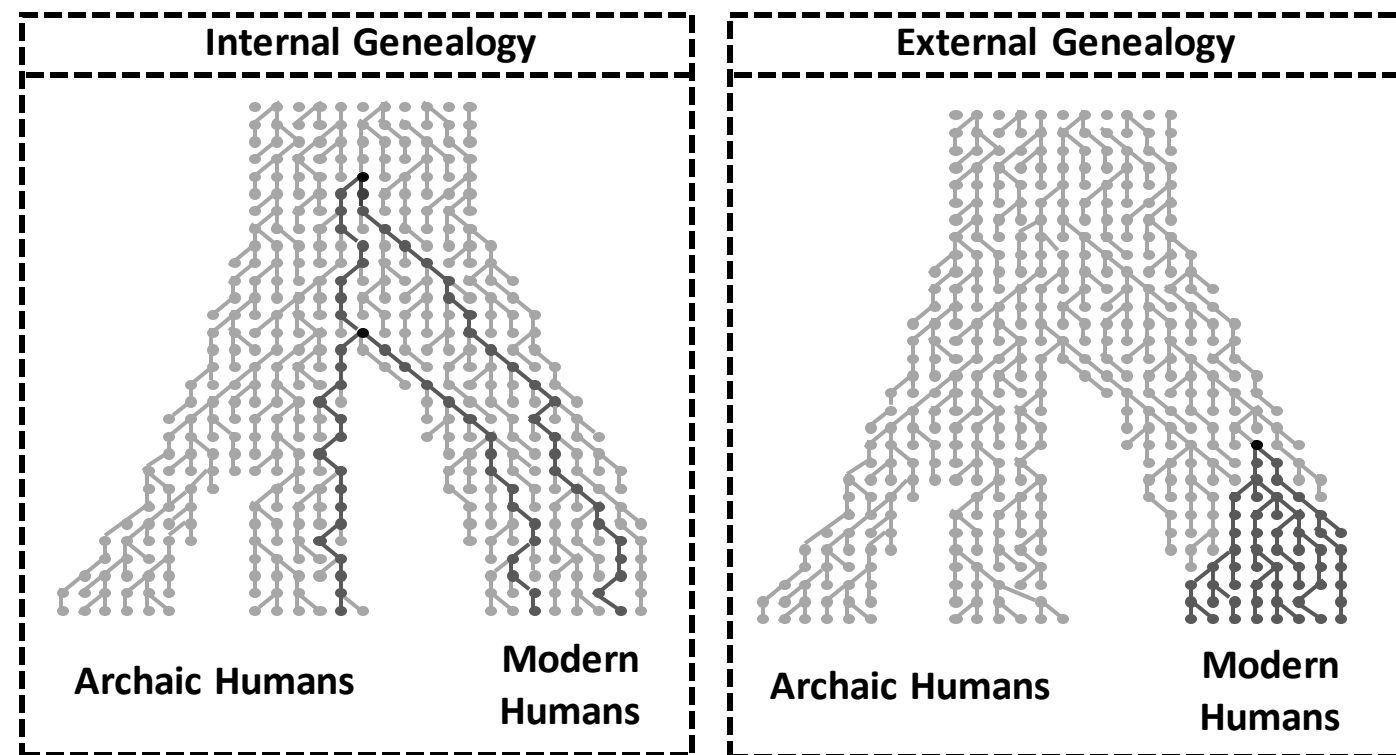
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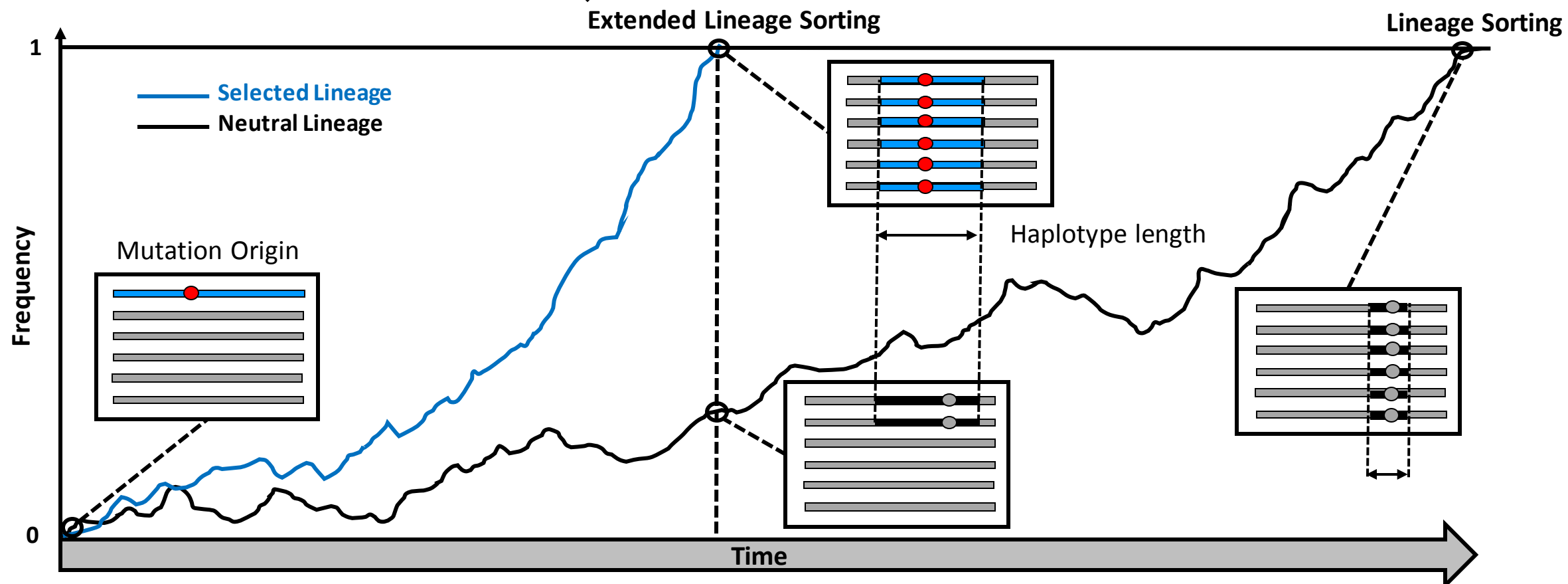
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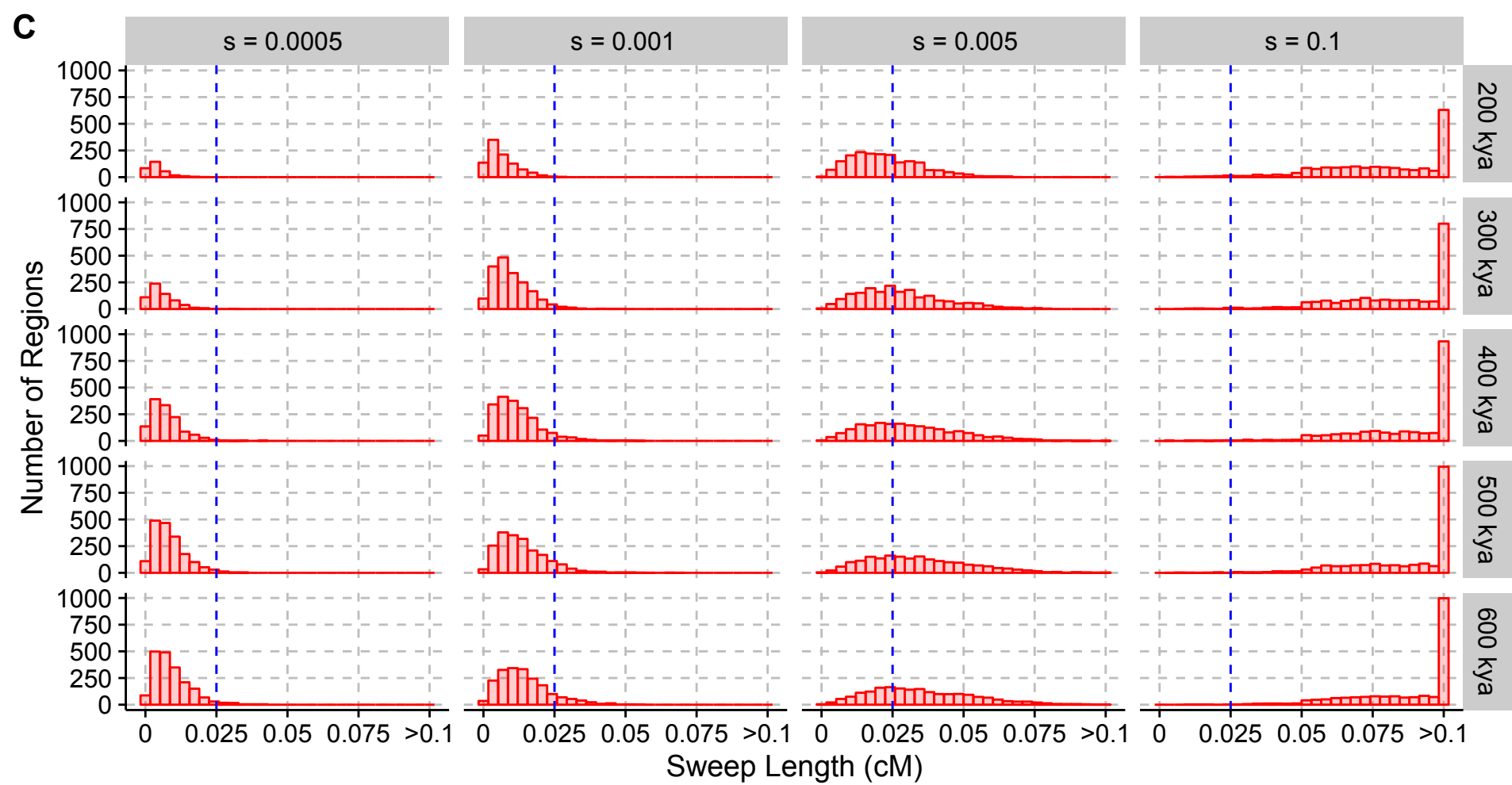
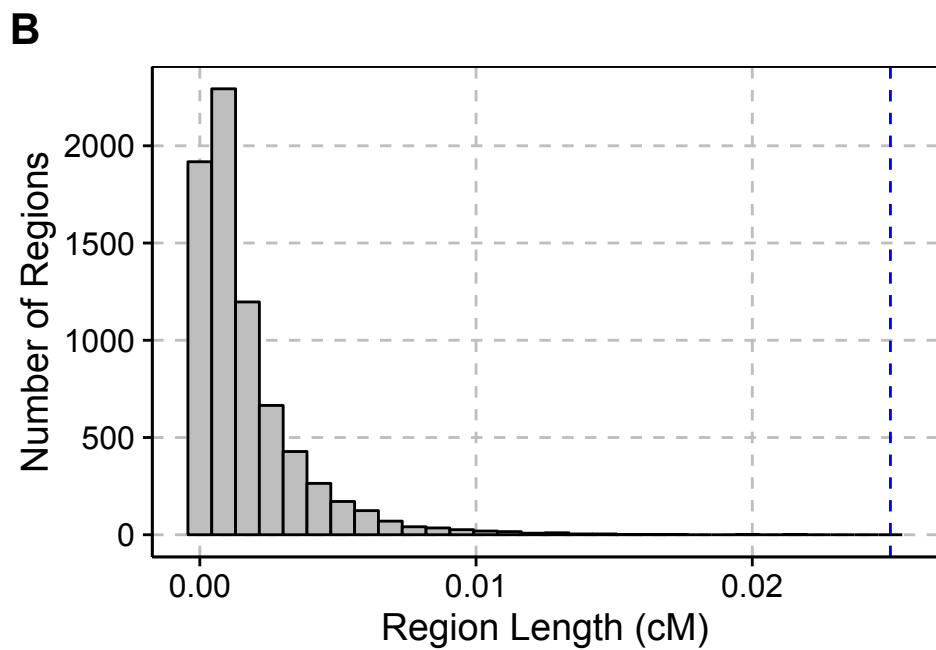
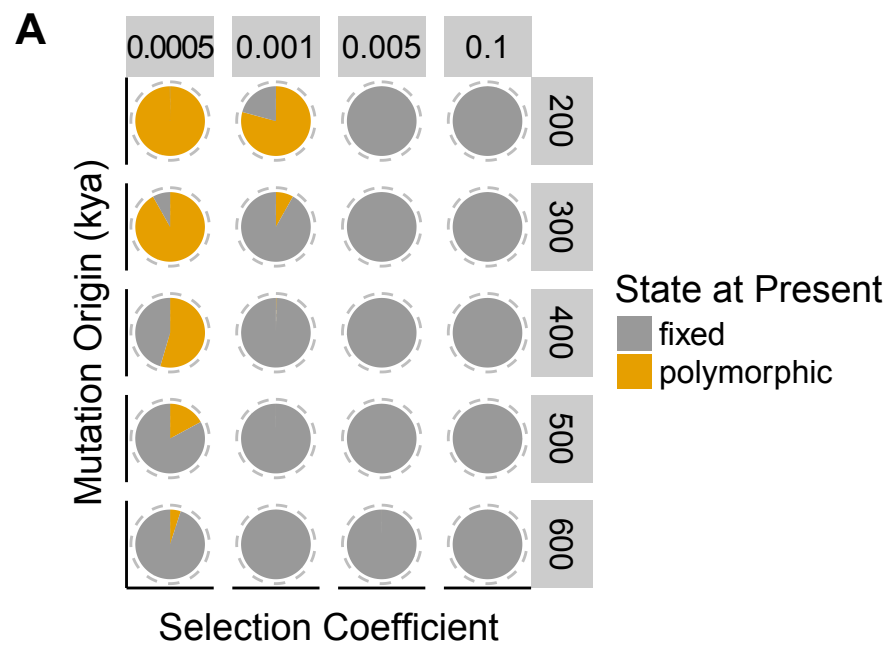


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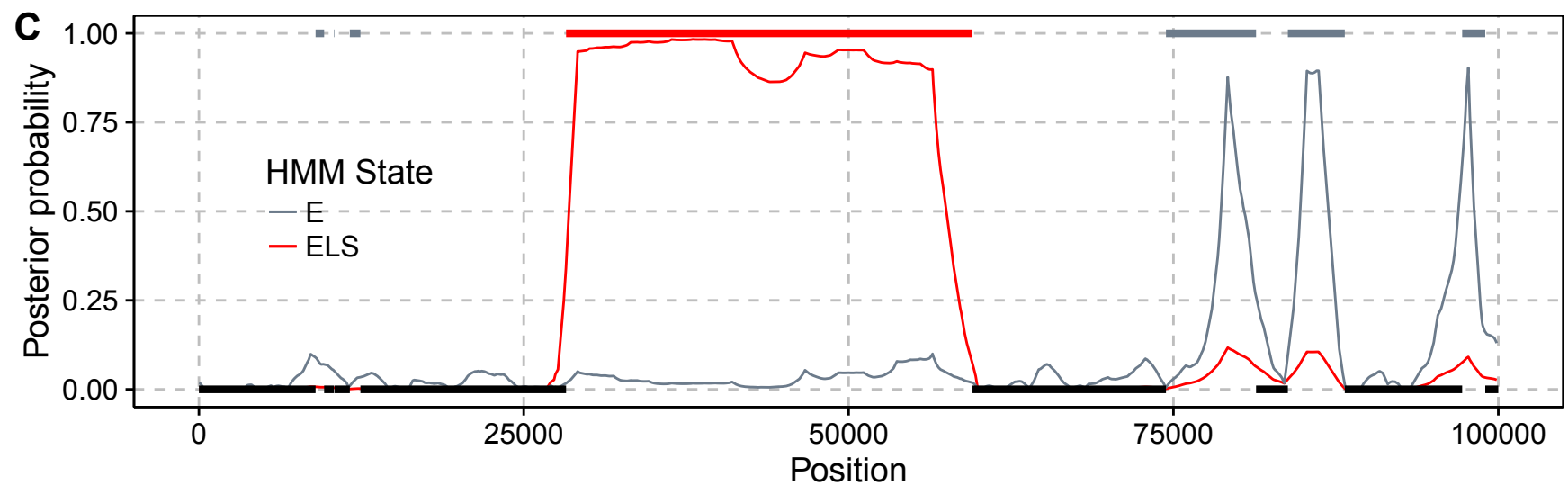
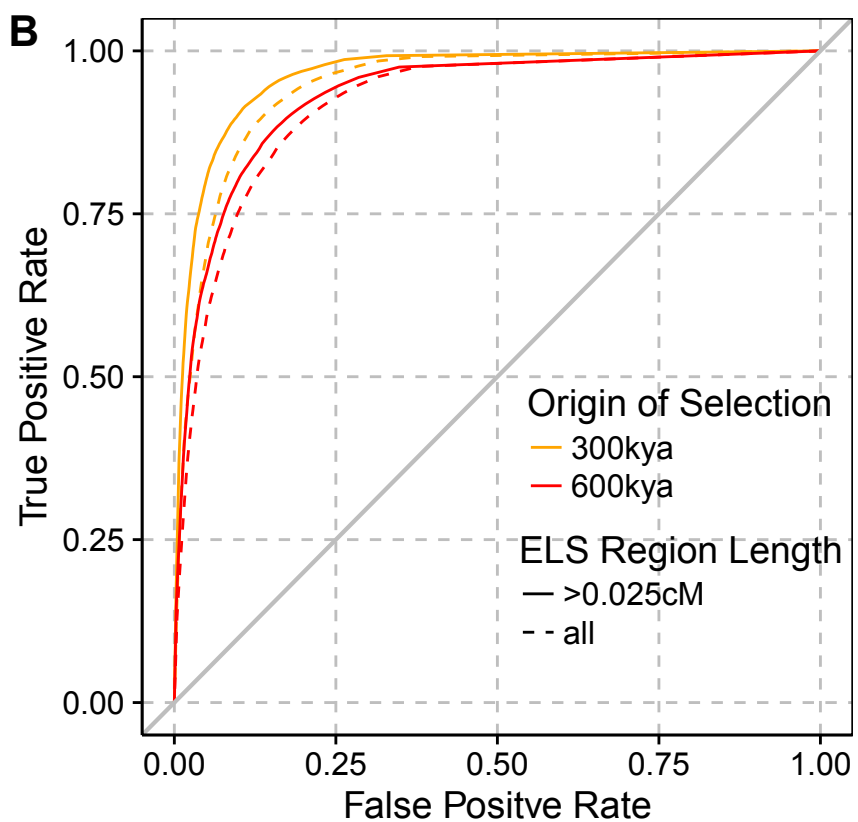
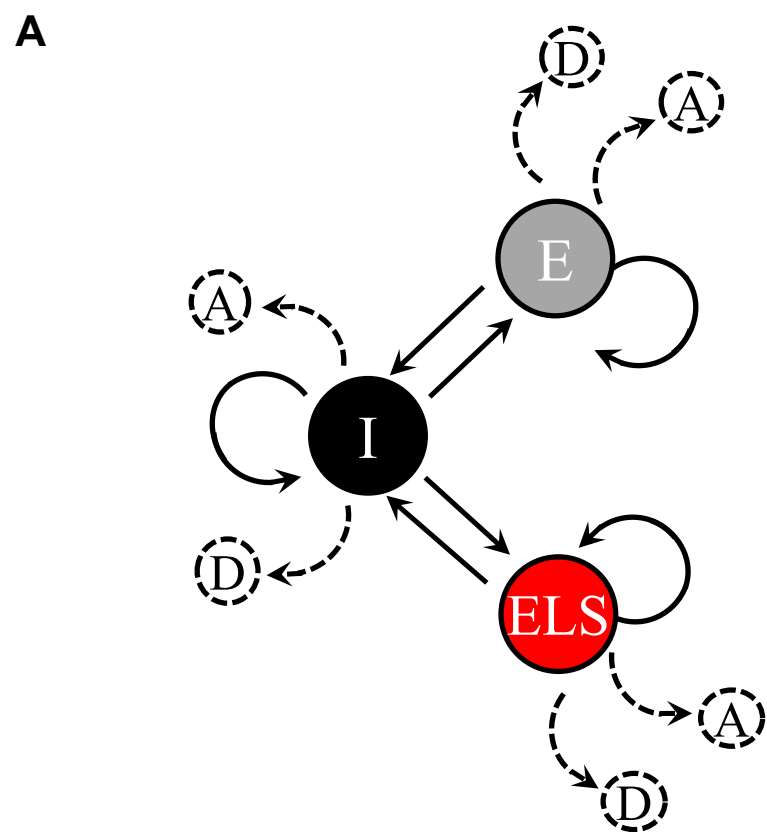


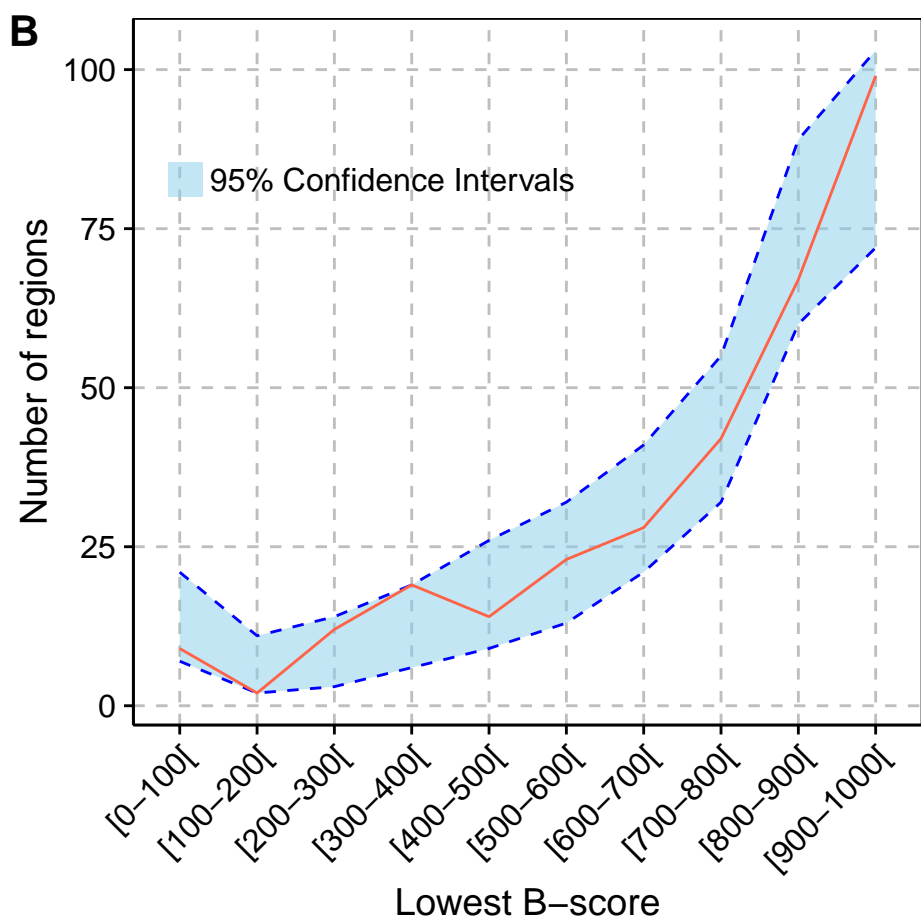
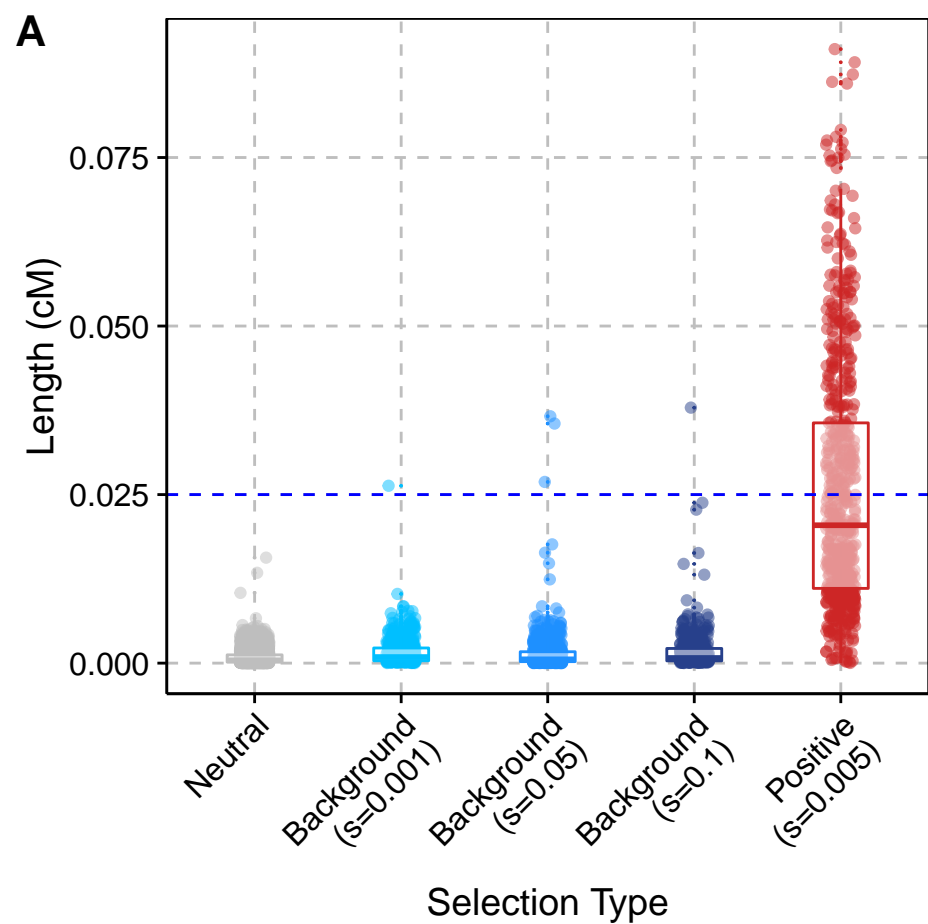
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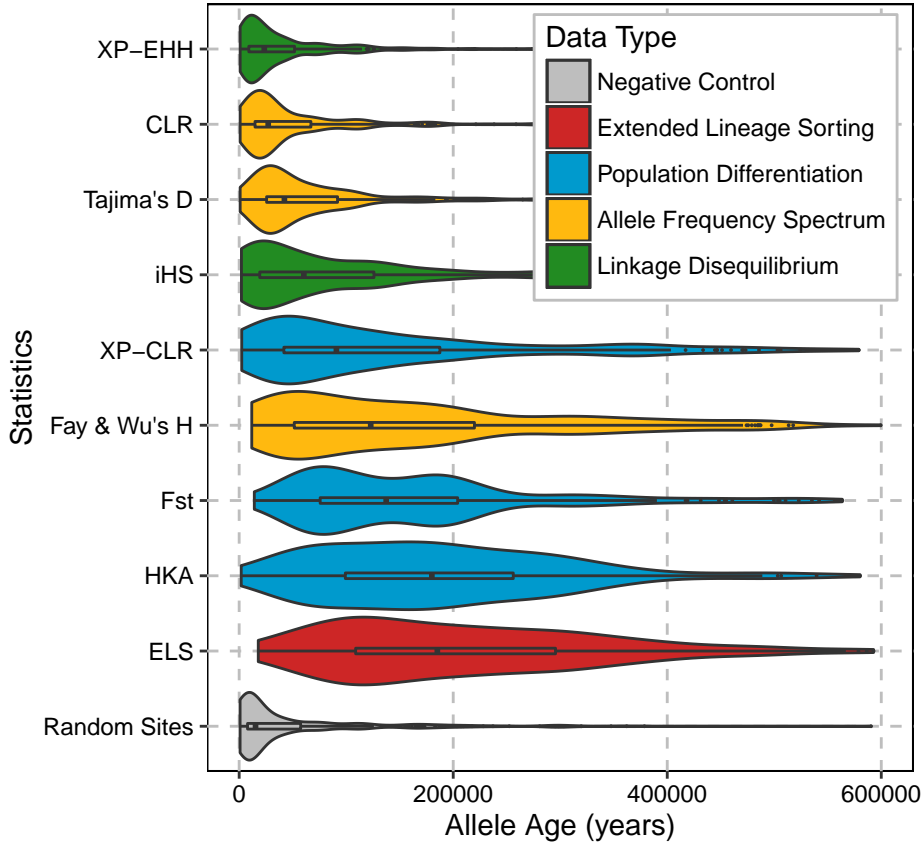












Number of Regions Overlapping  
at Least One Element

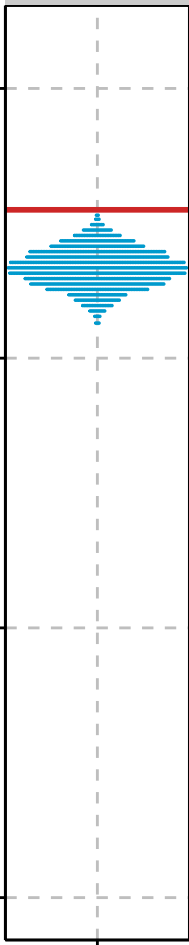
enhancers

300

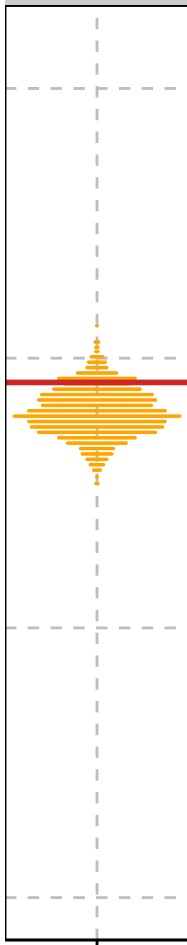
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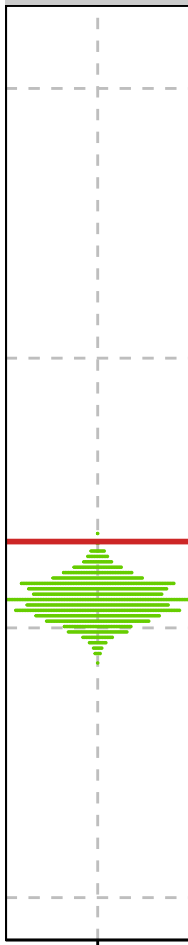
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genes



promoters



**Figure 1:** Illustration of the lineage sorting process. (A) Effects on the genealogy. The process starts with a random distribution of lineages when the ancestral population splits. The lineage in black is an out-group to lineages in blue, so that the blue lineages show a closer relationship between populations than to the black lineage (incomplete lineage sorting). When the blue lineages in the top population reach fixation (through a selective sweep for instance), any lineage from the other populations will constitute an out-group, thereby completing the sorting of lineages. (B) Two types of genealogies illustrating the possible relationships between an archaic lineage and modern human lineages. (C) Local effects in the genome at different time points. The curves represent the progression of lineage sorting for two independent regions, evolving under neutrality (black curve) and positive selection (blue curve), respectively. Longer fixation times are associated with more recombination so that neutrality produces smaller external regions.

**Figure 2:** (A) Fraction of selected alleles reaching fixation (grey) or segregating (orange) at present, depending on the strength of selection (columns) and the age of the mutation (rows, in kya) in our simulations. Events for which the selected variant was lost are not shown. (B) Distribution of the genetic length of external regions simulated under neutrality. (C) Distributions of the genetic length of external regions depending on the strength of selection (columns) and age of mutations in kya (rows). The blue line corresponds to the upper limit for the length of external regions produced under neutrality from (B).

**Figure 3:** (A) Graphical representation of the Extended Lineage Sorting Hidden Markov Model. States are depicted by nodes and transitions by edges. Each state emits an archaic allele as either derived, D, or ancestral, A, depending on the type of site in the modern human population (fixed or segregating at a given frequency). States are labelled I for Internal, E for External and ELS for Extended Lineage Sorting. (B) Receiver Operator Curves for varying cutoffs on the posterior probability of the ELS state and counting the number of sites in ELS regions that were correctly labeled. All bases

labelled ELS outside of simulated ELS regions are considered false positives. Sites in ELS regions with a posterior probability below the cutoff are considered false negatives. (C) Example of the labelling of a simulated ELS region. Horizontal bars indicate true external (top) and internal (bottom) regions. The posterior probability is shown in red for ELS regions and in grey for E regions. The region overlapping position 50,000 (red bar) is caused by a simulated selective sweep.

**Figure 4: Effects of background selection.** (A) Comparison of the length of ELS regions in simulations of different scenarios. For the distribution under background selection, the  $s$  parameter corresponds to the average selection coefficient from the gamma distribution (shape parameter of 0.2). We assumed that the deleterious mutations are recessive with dominance coefficient  $h=0.1$ . The horizontal blue line corresponds to the length cutoff applied to the real data. (B) Distribution of B-scores in the candidate sweep regions (red curve) compared to sets of random regions with matching physical lengths (blue area with dotted blue lines indicating the 95% confidence intervals over 1000 random sets of regions). The lowest B-score (i.e. stronger background selection) was chosen when a region overlapped several B-score annotations.

**Figure 5:** Distributions of estimated ages of the modern human segregating derived variants with the highest frequency in putatively selected regions or the age of the derived variants at sites identified by various genome-wide scans. Our candidate regions are labelled as ELS, for Extended Lineage Sorting, other candidate regions are from (Cagan et al. 2016; Pybus et al. 2014). The color coding indicates the type of signal detected by each method. Ages were estimated by ARGweaver (Rasmussen et al. 2014). We only report events between 0 and 600kya.

**Figure 6:** Enrichment for regulatory elements (enhancers,  $P$ -value $<0.001$ , protein-coding genes,  $P$ -value=0.124, and promoters,  $P$ -value=0.002) in the extended set of 314 candidate sweep regions. The distributions were obtained by randomly placing candidate regions in the genome to obtain lists of regions with similar physical length. The red lines represent the value observed in the real extended set.

**Table 1:** Genes from the core set of candidate regions overlapping with long deserts of Neandertal and Denisovan ancestry.

Chromosome	Start	End	Overlapping Genes	Overlapping Regulatory Domains
chr1	104000000	104154236	<i>AMY2B, RNPC3</i>	<i>COL11A1</i>
chr1	113429666	113560554	<i>SLC16A1</i>	<i>FAM19A3, LRIG2</i>
chr3	77027850	77033270	<i>ROBO2</i>	-
chr7	122320038	122379695	<i>RNF133, RNF148, CADPS2</i>	<i>TAS2R16</i>
chr10	107809941	107866217	-	SORCS1, SORCS3