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Differences in local population history at the finest level: the case of the Estonian population

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1Title: Differences in local population history at the finest level: the case of the Estonian
2population

3Running title: Genetic structure of Estonia

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21Abstract

22Several recent studies detected fine-scale genetic structure in human populations. Hence,
23groups conventionally treated as single populations harbour significant variation in terms of
24allele frequencies and patterns of haplotype sharing. It has been shown that these findings
25should be considered when performing studies of genetic associations and natural selection,
26especially when dealing with polygenic phenotypes. However, there is little understanding of
27the practical effects of such genetic structure on demography reconstructions and selection
28scans when focusing on recent population history. Here we tested the impact of population
29structure on such inferences using high-coverage (~30X) genome sequences of 2,305
30Estonians. We show that different regions of Estonia differ in both effective population size
31dynamics and signatures of natural selection. By analyzing identity-by-descent segments we
32also reveal that some Estonian regions exhibit evidence of a bottleneck 10-15 generations
33ago reflecting sequential episodes of wars, plague, and famine, although this signal is
34virtually undetected when treating Estonia as a single population. Besides that, we provide a
35framework for relating effective population size estimated from genetic data to actual census
36size and validate it on the Estonian population. Our results suggest that the history of human
37populations within the last few millennia can be highly region-specific and cannot be properly
38studied without taking local genetic structure into account. Our approach to estimating the
39census population size may be widely used both to cross-check estimates based on
40historical sources as well as to get insight into times and/or regions with no other information
41available.

42

43Main text

44As more and more datasets including genetic data from hundreds and thousands of
45individuals become available it becomes apparent that most if not all human populations
46exhibit at least some degree of geography-driven genetic structure even at small scales (for
47some examples see¹⁻⁵). Many recent publications have shown the confounding effect of such
48population structure on studies of genetic associations and natural selection signals, mainly
49in the case of polygenic phenotypes⁶⁻⁹. Here we study the fine-scale genetic structure of the
50Estonian population and the local differences in recent demographic history and action of
51natural selection between genetically defined Estonian subgroups to gain a deeper
52understanding of the forces shaping this population structure and the consequences it has
53for population genetics analyses. In doing so we make use of high-coverage whole genome
54sequences from more than 2300 Estonian individuals generated within a different study¹⁰.

55Our exploratory principal component analysis (PCA) (Figure 1) shows the presence of
56genetic structure within Estonia with the main differentiation between South-East and North-
57East of the country in agreement with previous studies^{2,11,12}. To zoom-in into the fine-scale
58structure in Estonia, we used total genetic length of shared IBD segments detected with
59*IBDseq*¹³ as input for the fineSTRUCTURE¹⁴ clustering algorithm (Methods). We applied this
60approach to a subset of 468 individuals sampled in rural areas at the age of 50 or more, as
61this cohort is expected to be the least affected by recent migrations (Figure 2, S11:2.3). We
62refer to this subset as “R50+” throughout the text (Methods).

63IBD-based analysis (Figure 2) reinforces previous observations^{2,11,12}, including the strong
64differentiation between South-East and the rest of Estonia, and provides a deeper insight
65into Estonian genetic structure, showing that most of the revealed clusters are highly
66geographically localized. The sharing matrix provides additional details. First, off-diagonal
67sharing also reflects geography with clusters from the same area tending to have higher
68inter-cluster sharing. Second, intra-cluster sharing substantially varies among clusters,

69implying differences in effective population size (N_e), which is also supported by the results
70of homozygosity-by-descent analysis (Figure 3).

71In order to understand how gene flow barriers and/or differences in local population density
72shaped the IBD-sharing pattern in the R50+ dataset, we inferred migration surfaces using
73MAPS¹⁵. We used two windows of IBD segments length (in centimorgans, cM), 2-6 cM and
74more than 6 cM, which under a simplistic model of infinite population size have mean
75segment ages of 50 and 12.5 generations respectively¹⁵. Results for the two length bins
76generally agree with each other, suggesting higher levels of gene flow in the North along
77with a barrier separating South-East Estonia (SI1:2.4). A second barrier, separating the
78islands, especially Hiiumaa, from the mainland is also evident. This observation suggests
79that the population ancestral to modern South-East Estonians was partially isolated from the
80rest of the country at least since 50 generations ago. Interestingly, this genetic differentiation
81is consistent with linguistic data suggesting that the deepest split within the Finnic languages
82separates Southern Estonian from the other branches of the phylum that includes Northern
83Estonian¹⁶.

84As local differences in admixture with external populations may have played a role in
85creating the observed genetic structure within Estonia we looked at patterns of haplotype
86sharing between R50+ Estonians and different non-Estonian populations (Table SI2:3.1-I).
87Here we used a conventional CHROMOPAINTER/fineSTRUCTURE/GLOBETROTTER
88(CP/FS/GT) approach¹⁷ (Methods). Figure 4 shows the results of non-negative least squares
89(NNLS)¹, modelling each individual from the R50+ dataset as a result of admixture between
90non-Estonian groups revealed by CP/FS (Figure 4, SI1:3.1, Table SI2-3.1-IV).

91Admixture signals in Figure 4 show clear geographic patterns that match known historical
92evidence of external migration to Estonia, including Swedish settlements on the western
93coast and islands in 14-15th centuries and Finnish immigration to North-East Estonia in the
9417th century¹⁸. Comparing NNLS results between clusters from Figure 2 we found that some
95of them, such as NE_1 and NE_2, stand out in terms of sharing with external groups but
96most of the clusters have overlapping distributions of NNLS scores (SI1:3.1). A similar

97 pattern is observed in IBD-sharing patterns (SI1:3.2). These results suggest that admixture
98 with non-Estonian groups can only partially explain the fine genetic structure observed in
99 Figure 2.

100 We show that, despite the small territory it occupies, the Estonian population exhibits a
101 readily detectable genetic structure, reflected in patterns of IBD segments sharing (Figure 2)
102 and allele frequencies (Figure 1, Table SI2-2.3-III, Table SI2-2.3-IV). Next, we sought to
103 explore whether this differentiation has any effect on the reconstruction of demographic
104 processes, namely whether there are region-specific differences in effective population size
105 dynamics and action of natural selection. We hence applied *IBDNe*, which estimates
106 effective population size (N_e) in past generations¹⁹, and SDS (Singleton Density Score), a
107 tool for detecting signatures of natural selection²⁰, as both methods give insight into very
108 recent time periods, when regional differences in population history may be anticipated. For
109 both analyses, we used the entire dataset of 2,305 samples, for which clusters were inferred
110 using the same approach as for the R50+ subset (Figure 5).

111 We ran *IBDNe*¹⁹ on the four most distinct clusters from Figure 5, representing four regions of
112 Estonia: North-West, North-East, South-West and South-East and observed rather distinct
113 N_e trajectories (Figure 6a, SI1:4.2). In particular, all clusters (except for eSE_5) show
114 evidence of an effective population size decline between 10 and 20 generations ago, which
115 is not detected when the entire dataset is analyzed (Figure 6a). Overall, these results
116 suggest that population dynamics are region-specific and hence population-wide result may
117 depend on the sampling scheme. For a deeper understanding of this phenomenon and the
118 effects of other factors on *IBDNe* results, we applied *IBDNe* to genetic data, simulated under
119 various demographic scenarios (SI1:4.1). Furthermore, the same approach has been applied
120 to genotype data from the UK population, where regional differences in N_e dynamics are
121 observed as well (SI1:4.3).

122 Based on our simulations and MAPS results, we propose that most of the differences in N_e
123 dynamics between Estonian subpopulations may be attributed to different patterns of gene
124 flow and external admixture. South-West and North-West Estonia are characterized by an

125 overall high level of gene flow (SI1:2.4), leading to similar N_e trajectories that deviate only
126 during the last 20 generations (Figure 6, SI1:4.2) reflecting very recent differences in
127 population size dynamics and/or migration. This also brings about the idea that the strong
128 bottleneck in South-West could contribute to the observed population structure, in particular
129 leading to differentiation of South-West and its subgroups. On the other hand, South-East
130 Estonia has the most distinct N_e trajectory according to Figure 6a, having a substantially
131 lower long-term N_e compared to other regions. Together with MAPS results (SI1:2.4) this
132 might suggest a recent expansion of a previously small-size eSE_5-like population and its
133 admixture with other local subpopulations occupying South-East Estonia thus contributing to
134 other eSE groups. This, in turn, results in a rather recent increase in relative proportion of
135 individuals with eSE_5-like ancestry in the entire Estonian population affecting the N_e
136 reconstructions for the entire dataset (SI1:4.2).

137 Given our understanding of confounders of the observed regional N_e patterns, we exploited
138 the fine-grained temporal resolution enabled by *IBDNe* to infer changes in actual census
139 sizes (N_c) of the ancestors of contemporary Estonians, adapting previous theoretical work²¹
140 to empirical case of human populations (Methods). We applied equation [3] (Methods) to the
141 Estonian-wide N_e trajectory inferred using the Est1527 subset, which excludes clusters that
142 can be considered as outliers in terms of external admixture and/or N_e trajectory (SI1:4.4).
143 We then compared the inferred N_c with available historical estimates (Figure 6b) showing a
144 remarkable match between the two with the exception of the last three generations, for
145 which *IBDNe* estimates are extrapolated from preceding time points¹⁹. This match may be
146 attributed to i) our success in adequately controlling for events of recent gene flow and
147 population structure; ii) the relatively recent time intervals considered, which limits the range
148 of spatial interaction among the ancestors of contemporary Estonians. However, note that
149 the pronounced fluctuations in N_c reported by historians between 1500 and 1700 are only
150 very roughly approximated by the N_e -derived curve which, as expected²², provides only
151 relatively long-term harmonic average of N_e . Nevertheless, we suggest that after controlling
152 for confounders such as population structure and admixture and keeping in mind all the

153assumptions implied by the biological notion of N_e , our approach could be used to convert
154 N_e to human N_c at any time interval for which historical records are missing, including the
155ones provided by PSMC²³, which are beyond the scope of the current paper.

156We then questioned whether natural selection could have also acted differently within the
157Estonian population. In doing so, we applied singleton density score (SDS)²⁰ to the entire
158dataset of 2,305 samples as well as to two regional subsets, South-East Estonia (SE,
159consisting of 1,029 samples belonging to clusters eSE_1 - eSE_5 in Figure 7) and the
160remaining 1,276 samples from the rest of the country (nonSE) (Methods, SI1:5.1).

161First, we inspected the genome-wide distribution of positive SDS scores in the three
162datasets (Figure 7) for any evidence of recent selection acting at individual loci.

163Unlike other studies that used SDS^{20,24} we don't observe any hits with very low p-value
164(possible reasons are discussed in SI1:5.3). However, we see that the distribution of SDS
165scores differs between the three datasets (Figure 7, Table SI2-5.3-I). Whereas one genome-
166wide significant hit (rs75386033 and rs79907158 on chromosome 6) is detected in the SE,
167nonSE and the entire dataset had many more hits with p-values in the range between 5×10^{-8}
168and 1×10^{-5} (Figure 7, SI1:5.3, SI1:5.4, Table SI2-5.3-I). Whereas most of the top SDS signals
169do not overlap between the three datasets analyzed, one region on chromosome 10
170corresponding to the *WDFY4* gene appears in both SE and nonSE (Figure 7, Table SI2-5.3-
171I). It has been shown that *WDFY4* is involved in immune response toward viral and tumor
172antigens²⁵ as well as in autoimmune diseases²⁶⁻²⁸. Functional annotation of variants with
173positive SDS scores²⁹ coupled with enrichment test^{30,31} did not reveal any annotation
174category to be specific for a particular subset studied (SI1:5.3, SI1:5.4, Tables SI2-5.3-IV-V,
175Tables SI2-5.4-IV-VII). Likewise, alternative enrichment test employing the GWAS catalog
176showed that similar phenotype categories are present in the three tested datasets (SI1:5.3,
177SI1:5.4, Table SI2:5.3-III, Tables SI2:5.4-II-III).

178On the other hand, frequency differences of rs75386033 derived allele T (10.3% in SE vs
1796.1% in nonSE, Weir and Cockerham³² $F_{st}=0.0117$ corresponding to the 0.999 percentile of
180the genome-wide distribution (Figure S5.4-I) together with its low standardized SDS p-value

181form strong evidence for a recent frequency increase of the rs75386033 T allele in South-
182East Estonia. Both rs75386033 and rs79907158 lie within an intron of the *GRM1* gene,
183which is characterized by high levels of expression in the brain
184(<https://www.ncbi.nlm.nih.gov/gene/2911>). These SNPs themselves are not known to be
185associated with any phenotypes, however, there are some indications that variant rs362870
186which is in high linkage disequilibrium with rs75386033 and rs79907158, might be a cis-
187eQTL for the *EPM2A* gene (SI1:5, Table SI2:5-II), suggesting a plausible biological effect
188behind the frequency change. *EPM2A* gene is associated with Lafora disease which is a
189form of progressive myoclonus epilepsy^{33–35}. This gene codes for a protein called laforin
190which is involved in regulating glycogen synthesis and potentially prevents glycogen
191accumulation in neurons^{33–35}.

192Given the lack of information on the phenotypic effect of this *GRM1* allele and its modestly
193strong SDS signal, it is unclear whether the raise in frequency happened due to actual
194selection or because of random genetic drift especially given the fact that South-East
195Estonians exhibit signals of long-lasting low N_e and further differentiation into smaller
196subclusters (Figures 2 and 6). Nevertheless, differential SDS signals between the entire
197Estonian dataset and its subsets including *GRM1*, *WDFY4*, suggest that recent selection,
198restricted to regional subpopulations, may remain undetected if population-wide datasets are
199treated as a single entity.

200In conclusion, here we describe a dataset of more than 2300 high-coverage Estonian
201genomes from a population genetics perspective making it one of the smallest populations to
202date with such high-resolution data available. We show that the Estonian population, despite
203occupying a small area with no strong geographic barriers, is genetically structured and
204exhibits rather pronounced interregional differences with respect to recent admixture with
205neighbouring populations, population dynamics and potential action of natural selection.
206These observations together with results of other studies suggest that population
207stratification could be ubiquitous in human populations, and should be taken into account in
208any large-scale genetic study including reconstructions of recent population history. We also

209show that we are able to accurately link effective population size to actual census size based
210on some simple assumptions about human population age structure and reproduction
211patterns. We envisage future studies exploiting this framework to ultimately unlock the
212potential of genomic data to provide a reliable estimate of past human census size, hence
213informing other historical sciences such as the study of cultural evolution, history and
214archaeology.

215METHODS

216*Data reporting*

217No statistical methods were used to predetermine sample size. The experiments were not
218randomized and the investigators were not blinded to allocation during experiments and
219outcome assessment.

220*Whole Genome Sequencing data*

221We used whole genome sequences of the Estonian Biobank participants reported in Kals et
222al.¹⁰. We applied exactly the same criteria for filtering individuals (sequencing quality control
223filters, match between WGS and chip genotype, total number of SNVs, self-reported
224Estonian ethnicity etc., see Kals et al.¹⁰ for details) except for relatedness and singleton
225count (see below). For manipulations with vcf files bcftools-1.8³⁶ was used unless specified
226otherwise. Additionally to sample filtering applied by Kals et al.¹⁰, we removed seven
227samples with missing call over 3% as well as related individuals. To do so we used PLINK-
2281.9³⁷ and KING-2.1.6³⁸ to estimate relatedness coefficient and removed one individual from
229each pair with this value equal to 0.0442 or higher, corresponding to third degree relatives.
230This resulted in a dataset consisting of 2,305 individuals that was used for all downstream
231analyses.

232For analyses that require phased and/or imputed data (CHROMOPAINTER, SDS) phasing
233and imputation was done using Eagle v2.3³⁹ on the dataset consisting of 2,420 samples to

234 benefit from the presence of related individuals and subsequently relevant samples were
235 extracted.

236 All Estonian Biobank participants have signed a broad informed consent which allows
237 research in the fields of genetic epidemiology, disease risk factors and population history. All
238 work at Estonian Biobank is conducted according to the Estonian Human Gene Research
239 Act. The original study generating the WGS data¹⁰ was approved by the Research Ethics
240 Committee of the University of Tartu (application number 234/T-12).

241 *The 'Rural above 50 years old' (R50+) panel*

242 As information on parents' and grandparents' birthplace is mostly unavailable for the
243 samples used here, we subsetted the 2,305 dataset for individuals born in rural areas and
244 sampled at the age of 50 or older as we expect this cohort to be the least affected by recent
245 migration and long-distance marriages, hence expecting it to preserve the original genetic
246 structure. This resulted in a dataset of 474 individuals which we further pruned for PCA
247 outliers (see below) and samples with more than 10,000 singletons (SI1:1.1-SI1:1.3). Per-
248 sample number of singletons was estimated using vcfTools-0.1.14⁴⁰ on the entire (2,305
249 samples) non-imputed dataset. We ended up with a panel of 468 individuals, which we call
250 "R50+".

251 *Non-Estonian samples*

252 To place the Estonian population genetic variation in Eurasian context we compiled two
253 datasets containing the R50+ Estonian samples each and samples from various populations
254 predominantly representing West Eurasia. The first dataset used for PCA contained 59
255 samples from 17 populations sequenced on the Complete Genomics platform, 207 samples
256 from 8 populations sequenced using Illumina technology and 255 samples from 14
257 populations genotyped on Illumina arrays (Table SI2-1.2-I). Whole genome sequences were
258 pruned to keep positions matching those overlapping between genotyping arrays resulting in
259 approximately 450K SNPs.

260The second dataset used for CHROMOPAINTER/fineSTRUCTURE/GLOBETROTTER
261except for R50+ Estonians included 425 samples from 27 populations genotyped on Illumina
262arrays and 175 samples from seven 1000 Genome Project populations (CHB, FIN, GBR,
263GIH, IBS, TSI, YRI) (Table SI2-3.1-I). Whole genome sequences were pruned to keep
264positions matching those overlapping between genotyping arrays resulting in approximately
265500K SNPs.

266Principal component analysis

267We ran principal component analysis (PCA) for the entire Estonian dataset in two settings: **a)**
268with only the 2,305 Estonians and **b)** combining the 2,305 Estonians with 521 non-Estonian
269samples from 18 European populations (Table SI2-1.2-I). In both cases *smartPCA* from
270EIGENSOFT-7.2.0⁴¹ was used. In setting **a** we directly ran PCA on the dataset filtering for
271MAF below 0.01, no-call above 0.03 and positions in LD ($r^2 > 0.4$ within sliding windows of
272200 positions). Results obtained in setting **a** were used to identify Estonian samples with
273extreme position in the PCA plot to be removed from the R50+ panel and from the dataset
274used for SDS (S1:1.2). In setting **b** we used 255,536 bi-allelic SNPs that overlap between
275the different datasets and passed LD-pruning ($r^2 > 0.6$ within sliding windows of 1,000
276positions), MAF (< 0.05) and no-call (> 0.05) filters. We first calculated the principal
277components (PCs) based on all non-Estonian samples and then projected the Estonian
278individuals onto the first two PCs.

279CHROMOPAINTER/fineSTRUCTURE/GLOBETROTTER

280To study genetic similarities between Estonians and other European populations we used
281the CHROMOPAINTER/fineSTRUCTURE (CP/FS) pipeline¹⁷. It involves a chromosome
282“painting” procedure which represents each chromosome of an individual (the recipient) as a
283mixture of chunks received (copied) from every other individual in the dataset (donor). The
284number of chunks copied by a recipient from each of the donors makes a “copying vector”
285which are used in the FS algorithm to group individuals into populations.

286Initial chromosome painting parameters were estimated using 30% of the phased dataset of
2871068 Estonian and non-Estonian samples (Table S12-3.1-I). FS was run for 15 million MCMC
288iterations in two parallel runs to assess convergence. The tree-building step was performed
289using the approach from Leslie et al.¹ and the run with the highest observed posterior
290likelihood was used to cluster the samples into genetic groups. Inferred FS groups were
291further manually inspected and clustered into the higher-order FS populations (S1:3.2).
292These FS groups were used as surrogate populations to infer admixture with
293GLOBETROTTER and estimate ancestry profile with NNLS.

294Next, GLOBETROTTER (GT)¹⁷ was used to detect signals and dates of admixture for the
295Estonian groups defined using the approach described above. Unlike many other methods,
296GT allows the structure of unsampled source populations which were involved in the
297admixture event(s) to be assessed by modelling them as a mixture of sampled surrogate
298populations. GT inference was performed using a “regional” approach^{17,42}. Estonian clusters
299were only allowed to copy from external surrogates, but not from other Estonians.
300CHROMOPAINTER parameters were estimated for each Estonian target group individually
301and the average over all target populations was used to prepare input copying vectors for
302GT. Two separate runs, with and without standardization by “NULL” individual, were
303performed and consistency between runs was checked. To assess whether unbalanced
304surrogate population sample size could have biased our GT inference, we performed five
305additional GT runs by down sample both target and surrogate populations to 20 individuals.

306Finally, given complex admixture signal in Estonia, we implemented non-negative least-
307squares (NNLS) method¹. This allowed us to assign relative ancestral proportions to each
308individual in the R50+ panel using the non-Estonian surrogate groups identified by FS as
309sources. NNLS values for CP/FS Estonian groups were extracted from GT output while for
310individual samples these were calculated with an in-house R script. Obtained results were
311then summarized across Estonian parishes as well as across IBD/FS clusters.

312*Detecting segments identical-by-descent (IBD segments)*

313 To detect IBD segments in the Estonian dataset we applied *IBDseq* version r1206 (10) with
314 default settings (errormax=0.001, errorprop=0.25, r2window=500, r2max=0.15, minalleles=2,
315 lod=3.0) to the non-phased non-imputed dataset consisting of 2,305 Estonians. Choosing
316 *IBDseq* over *refined IBD*⁴³ here is justified by working with samples coming from a relatively
317 homogeneous population, which makes *IBDseq* frequency model applicable, while *IBDseq*
318 has the advantage of not requiring phasing as well as having sequencing errors and rare
319 alleles being explicitly accounted for. As *IBDseq* software reports only physical coordinates
320 of a segment's start and end we interpolated segments' genetic length in cM using GRCh37
321 recombination map (ftp://ftp.ncbi.nlm.nih.gov/hapmap/recombination/2011-01_phaseII_B37/)
322 using R⁴⁴. When working with the R50+ panel corresponding IBD segments were retrieved
323 from the general output obtained on the 2,305 dataset. Homozygosity-by-descent segments
324 were also inferred with *IBDseq*.

325 IBD segments between Estonians and non-Estonian individuals were detected by applying
326 *refined IBD* version 12Jul18.a0b⁴³ with default parameters except for length (window=40.0,
327 length=1.0, trim=0.15, lod=3.0) to the same dataset that was used for CP/FS/GT, as in this
328 case the dataset is highly structured. This was followed by applying the *merge-ibd* utility
329 version 12Jul18.a0b to merge together segments separated by at most 1 cM long gaps and
330 no more than 2 positions with genotypes discordant with IBD.

331 Both for *IBDseq* and *refined IBD/ibd-merge* results segments shorter than 2 cM were
332 discarded, as longer segments are detected with higher reliability.

333 MAPS

334 In order to evaluate the extent of gene flow across the whole country together with local
335 population densities, we estimated migration surfaces using MAPS¹⁵, which harnesses a
336 similarity matrix summarizing the total number of IBD segments shared in a given
337 population. In doing so, we used the IBD segments shared among pairs of individuals
338 inferred with *IBDseq* as described in the previous section. Subsequently we have classified
339 the shared genetic fragments as "short" (between 2 and 6 cM) and "long" (more than 6 cM),

340 and performed two independent MAPS runs for each length class to assess convergence.
341 Estonian territory was modeled as having a total of 200 demes. Each run had 5 million
342 iterations thinned every 10,000 and preceded by a burn-in of 2 million discarded cycles. The
343 obtained migration surfaces were subsequently plotted using the plotmaps R package¹⁵. We
344 repeated the whole procedure after removing samples belonging to clusters from Figure 2
345 with mean sharing above 60 cM to assess their effect on MAPS results.

346 *IBD-based fineSTRUCTURE (IBD/FS)*

347 In order to exploit patterns of genetic similarity between samples that arose very recently
348 and get insight into fine genetic structure of the Estonian population, we used total genetic
349 length of IBD segments longer than 2 cM as a measure of genetic similarity between pairs of
350 individuals. We refer to this measure as “IBD-sharing”. Next, to obtain natural genetic
351 grouping of the samples we used a matrix of IBD-sharing as input for fineSTRUCTURE
352 v2.0.7¹⁴. Although our approach is different from the original
353 CHROMOPAINTER/fineSTRUCTURE method¹⁴, it is very similar in its idea to the approach
354 used in ³, and, put loosely, treats each cM shared between a pair of individuals as a
355 CHROMOPAINTER chunk copied by the recipient from the donor. The fineSTRUCTURE
356 algorithm already has an inbuilt method of compensating for the fact that the units used to
357 measure similarity/relatedness between samples (either chunks in the classical approach or
358 cM in our approach) don’t represent fully independent pieces of information by transforming
359 the raw value into an effective one by applying a *c*-factor. The *c*-factor was calculated using
360 the *fs combine* command with the -C option applied to matrices of IBD-sharing for each
361 individual chromosome. For more details see Supplementary Information SI1:2.1 and
362 SI1:2.2. When running fineSTRUCTURE for both R50+ and the entire dataset the first
363 2,000,000 MCMC iterations were removed as burn-in and subsequently MCMC was run for
364 additional 2,000,000 MCMC iterations sampling every 10,000th run. When building the tree
365 we used the approach described in Leslie et al., 2015¹ and corresponding to the “1” value of
366 the -T option in fineSTRUCTURE v2, which, put informally, maximizes the concordance

367between samples' final cluster assignment and its' assignment in individual MCMC runs. To
368validate this approach we applied it to the simulated data used in Lawson et al., 2012¹⁴, and
369calculated the same measure of correlation between real and inferred cluster assignment of
370the samples for different number of chromosomal regions used to detect IBD segments
371(SI1:2.2).

372We applied this approach to the R50+ dataset (468 samples) and the entire dataset (2,305
373samples). In both cases fineSTRUCTURE was run twice to assess convergence (SI1:2.3,
374Tables SI2-2.3-I and SI2-2.3-II). In the case of the R50+ dataset to reduce the number of
375clusters revealed by the fineSTRUCTURE algorithm we have hierarchically joined together
376clusters with short terminal branches by cutting the tree at such a level so as to avoid having
377clusters consisting of less than 5 samples. In the case of the entire dataset clusters referred
378to throughout the text were obtained by cutting the tree at a level chosen after visual
379inspection (SI1:2.3).

380*Fst calculations*

381Fst between Estonian clusters was calculated using smartpca from the EIGENSOFT
382package v7.2.0⁴¹ after LD-pruning ($r^2 > 0.4$, windows of 1,000 SNPs) and removing sites with
383MAF < 0.05 and missing rate > 0.1 .

384Per-site Weir and Cockerham³² Fst estimator between SE and nonSE subsets was
385calculated using vcfTools⁴⁰ after filtering sites for LD, MAF and missing rate the same way as
386described above.

387*Geographic data visualization*

388Geographic coordinates of the corresponding birth town/parish were assigned to each
389sample with birth place information available (2,168 out of 2,305 samples). For MAPS these
390coordinates were used directly. When plotting IBD/FS and NNLS results for the R50+ panel,
391coordinates of the samples were changed manually to avoid over-plotting. When plotting
392samples from the entire dataset jittering was used for the same purpose. Shp objects used
393to plot maps of Estonia with parish and county borders were retrieved from the Estonian

394Land Board website (Administrative and settlement units, 2018.11.01,
395[https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division-](https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division-396p312.html)
396p312.html). Geographic data were visualized in R⁴⁴ with the aid of the following packages:
397sp^{45,46}, sf⁴⁷, rgdal⁴⁸, rgeos⁴⁹ and ggplot2⁵⁰.

398*IBDNe*

399In order to reconstruct recent Ne dynamics we used *IBDNe* version 07May18.6a¹⁹ with
400default settings (npairs=0, nits=1000, nboots=80, trimcm=0.2). *IBDNe* was applied to sets of
401no less than 100 individuals. In all cases IBD segments used as input for *IBDNe* were
402detected with *IBDseq*¹³. Recombination map in PLINK format used to convert physical
403distances to the genetic ones was taken from
404http://bochet.gcc.biostat.washington.edu/beagle/genetic_maps/plink.GRCh37.map.zip.

405To get independent evidence of regional differences in Ne dynamics we applied *IBDNe* to
406samples from the People of the British Isles¹ dataset grouped by the region of origin of
407individuals' grandparents. The following regions were used: Scotland, Wales and North-East,
408North-West, South-East and South-West England. For the list of counties comprising these
409regions see Table S2:4.3-I.

410*Genetic simulations*

411To simulate genetic data under various demographic scenarios to test the behavior of *IBDNe*
412we used mspms which is an ms-compatible version of msprime⁵¹. Commands used for
413simulation are provided in the Supplementary Information S1:4.1.

414*Estimating actual census size based on Ne*

415Several lines of evidence, based both on theoretical reasoning⁵² and empirical
416comparisons¹⁹ suggest that in industrial human societies census size (Nc) is roughly 3 fold
417the Ne assuming a panmictic and isolated population. However, application of this coefficient
418is limited to populations with specific reproductive characteristics, for example 1:1 male to
419female ratio and Poisson distribution of number of offspring among individuals capable of

420reproducing. We therefore adapted a more general approach from²² which incorporates the
 421inbreeding coefficient (F_{is}), relative fraction of males (m) and excess in variance of
 422reproductive success compared to the Poisson distribution (DV):

$$423 \quad N_{b(t)} = \frac{(1+F_{is})}{4} \times \left(\frac{1}{(1-m) \times m} + DV \right) \times N_{e(t)}$$

424 [1]

425Formula [1] yields the N_b , the number of breeding individuals (individuals capable of
 426reproducing) at time t under the assumption of absence of gene flow and population
 427structure, non-overlapping generations and equal variance of number of offspring between
 428sexes. It is dependent on parameters such as m , F_{is} and DV that cannot be reliably
 429estimated for each time bin. We therefore explored a range of plausible scenarios described
 430by different values of m , F_{is} and DV based on the following assumptions: i) F_{is} calculated on
 431chromosome 1 for contemporary human populations from the 1000 genomes dataset⁵³ as
 432well as for Estonians ranges from -0.016 to 0.004 (S1:4.4), leading to the conclusion that for
 433most human populations the term $(1+F_{is})$ can be safely approximated to 1; ii) m , the relative
 434fraction of reproducing males, must be comprised between 0.1 and 0.9, considering further
 435polarizations of this parameter as implausible for our species; iii) DV , the difference between
 436the expected and the observed variance in number of offspring per adult can be estimated to
 437range between -1 and 3. The latter estimate was obtained by taking Poisson distributions
 438constrained between 0 and 10 (considering 10 as the maximum number of surviving children
 439per adult) with an average between 1 and 5, and by empirically inflating the most extreme
 440bins (0 or 10 children per adult) 5-fold. Such an exercise yields DVs ranging between -0.2
 441and 2.5, which we conservatively rounded to -1 and 3, respectively. This range is also
 442consistent with data from contemporary Estonians, available from the Estonian Biobank, and
 443showing a DV of -0.76 based on 7,863 females born between 1900 and 1955 and in the age
 444of menopause at the time of enrolment. When plugged together into formula [1], these
 445estimates yield a minimum of 0.75 (with $m=0.5$ and $DV=-1$) and a maximum of 3.53 (with
 446 $m=0.1$ or 0.9 and $DV=3$). To provide a single point estimate of N_c we rewrite formula [1] as

$$447 \quad N_{b(t)} = 1.63 \times N_{e(t)}$$

448[2]

449 using a geometric mean between 0.75 and 3.5 and thus making our estimate slightly more
450 than 2-fold away from the provided range boundaries. Note, that although there are
451 indications that in some human populations DV can be higher than 3^{54} , such cases can be
452 considered to be at the very extreme of human reproductive behaviour spectrum as even
453 hypothetical “super-male” populations would have a sex-average DV of 1.8 given m equals
454 to 0.5^{55} . Hence we suggest our approach to be applicable to many human populations
455 provided that immigration and population structure can be properly accounted for. In
456 addition, the range of DV can be changed to study populations with extreme inequality in
457 reproductive success.

458 The value estimated using [2] corresponds to the number of individuals in reproductive age.
459 It can be converted into total census size (N_c) of a human population at a given time point by
460 dividing it by the estimated fraction of breeding individuals, which we here assume to be
461 roughly 0.33. This is supported by actual data on the Estonian population from the “Statistics
462 Estonia” database (<http://andmebaas.stat.ee/Index.aspx?lang=en#>) showing that the fraction
463 of people between 20 and 40 years old was between 0.33 to 0.38 during the period between
464 1970 and 2018. Incorporating this idea into [2] results in equation [3].

$$465 \quad N_{c(t)} = 4.89 \times N_{e(t)}$$

466[3]

467 which we used to obtain the curve in Figure 6B. Sources of historical estimates of Estonian
468 population size used in that figure are provided in S4.2-II.

469 When using N_e as a proxy for actual population size one should keep in mind the potential
470 effect of gene flow between populations. For example under a stepping stone model with
471 constant population size and migration N_e estimated using samples from one deme is
472 expected to increase when going back in time as more and more ancestors of sampled
473 individuals would represent other demes⁵⁶. In other words, coalescent-based N_e estimates

474 reflect the number of ancestors of a sampled population, which may have lived in any
475 location in space, rather than strictly the number of individuals in a given area at a given time
476 point.

477 *Singleton density score (SDS) selection scan*

478 As SDS²⁰ does not handle missing data, imputed genomes of 2,301 unrelated individuals
479 (four PCA outliers removed) were used. SDS²⁰ analysis was applied to three datasets
480 separately, namely, the entire dataset and its two subsets, Estonia SE and Estonia nonSE.
481 The latter two were defined based on the IBD/FS results (Figure 5): SE (individuals with
482 South-East Estonian ancestry belonging to clusters eSE_1-eSE_5) and nonSE (individuals
483 coming from the other parts of the country and belonging to other clusters). We did not apply
484 SDS to finer subclusters of the dataset due to sample size issues. The number of individual
485 genomes used in the analysis has a direct impact on the number of SNPs analyzed, the
486 power to detect selection at any given SNP and the length of the terminal branches of the
487 coalescent tree and hence the timing of the selection events that can be inferred²⁰. Since in
488 this study we are focusing on recent selection signals and differences between
489 subpopulations that show only limited differentiation, we aimed at using samples of at least
490 1000 individuals. As the dataset needs to be homogeneous in terms of number of singletons
491 per individual, this value was calculated with vcfTools 0.1.14⁴⁰ independently for each of the
492 datasets and individuals with extreme values (below 5th or above 95th quantiles) were
493 removed. Final datasets included 2,076, 927 and 1,132 samples for the entire dataset, SE
494 and nonSE subsets respectively. Predicted functional effect of the test SNPs was assessed
495 using Combined Annotation-Dependent Depletion tool (CADD)²⁹. In addition, two alternative
496 enrichment tests were performed to see whether candidate SNPs are enriched in a certain
497 category of genes^{30,31} or in certain GWAS catalogue categories
498 (<http://www.ebi.ac.uk/gwas/home>;⁵⁷). Candidate SNPs, as well as SNPs in linkage
499 disequilibrium with those ($R^2 > 0.5$), were checked for known e-QTL effects using the

500eQTLGen Consortium⁵⁸ (<http://www.eqtlgen.org/>) database. Details of SNP annotation and
501enrichment analyses are specified in S1:5.2.

502DATA AVAILABILITY

503The sequencing data are available on demand. The procedure of applying for the access to
504the data can be found under the following link:
505<https://www.geenivaramu.ee/en/biobank.ee/data-access>.

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642 complex traits using blood eQTL metaanalysis. *bioRxiv* 2018; : 447367.

643 **Author Contribution**

644VP, LP, AM and MM designed the study. LS, TE, RM, LP and AM conducted sample
645management and provided access to data. MS provided historical data. VP, FM, AK, GH, FJ,
646RF, DM, MK, AA, DJL and LP analyzed the data. VP, FM, AK, GH, FJ, LS, RF, DM, MS, UV,
647MK, CT, MMo, LM, AA, DJL, TE, RM, LP, AM, MM contributed to the interpretation of results.
648VP, FM, AK, GH, FJ, RF, DM, MS, CT, DJL, LP, MM wrote the manuscript.

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666advice.

667**Competing interests**

668Authors declare no competing interests.

669**Figures**

670Figure 1. Principal components analysis of 2,305 Estonian samples in the context of West
671Eurasian populations. Estonian samples were projected onto PC space defined by European
672samples (Methods, SI1:1.1, SI1:1.2, Table SI2:1.1-I, Table SI2:1.2-I). Outlined labelled dots
673correspond to medians of European populations or Estonian counties while non-outlined
674dots represent individual samples. Estonian samples are shown in colour corresponding to
675the geographic region of origin: NW (North-West) included Harjumaa (Ha), Läänemaa (Lä),
676Raplamaa (Ra), Järvamaa (Jä), Hiiumaa (Hi) and Saaremaa (Sa) counties; NE (North-East)
677includes Lääne-Virumaa (LV), Ida-Virumaa (IV) and Jõgevamaa (Jõ) counties; SW (South-
678West) includes Pärnumaa (Pä) and Viljandimaa (Vi); SE (South-East) includes Valgamaa
679(Va), Tartumaa (Ta), Põlvamaa (Põ) and Võrumaa (Võ). NA are individuals with no birth

680place information available. Individual non-Estonian samples are shown in grey. Medians of
681non-Estonian populations are coloured according to geographic regions as shown in the
682legend. Russians (N) and Russians (C/S) refers to Russians North and Central/South (Table
683SI2:1.2-I). Inset in bottom left corner shows a map of Estonian counties. This map was
684created in R (<https://www.R-project.org/>)⁴⁴ using an shp object of the Administrative and
685settlement units provided by the Estonian Land Board, 2018.11.01
686(<https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division->
687p312.html). See Methods for more details.

688Figure 2. Genetic clustering of R50+ samples based on pairwise sharing of IBD segments. a,
689Hierarchical relationships (tree) and the average total length of IBD segments shared
690between cluster members (heatmap) as inferred by fineSTRUCTURE. The length of the tree
691branches does not reflect any relationship between the clusters. Clusters are named to
692reflect their geographic distribution (E - “East”, NW - “North-West”, NE - “North-East”, SW -
693“South-West”, SE - “South-East”). Numbers in grey next to cluster names refer to the sample
694size of each cluster. b, Geographic distribution of inferred genetic clusters. Each symbol on
695the Estonian map corresponds to one individual from the R50+ subset. See SI1:2.3 for
696details. This map was created in R (<https://www.R-project.org/>)⁴⁴ using an shp object of the
697Administrative and settlement units provided by the Estonian Land Board, 2018.11.01
698(<https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division->
699p312.html). See Methods for more details.

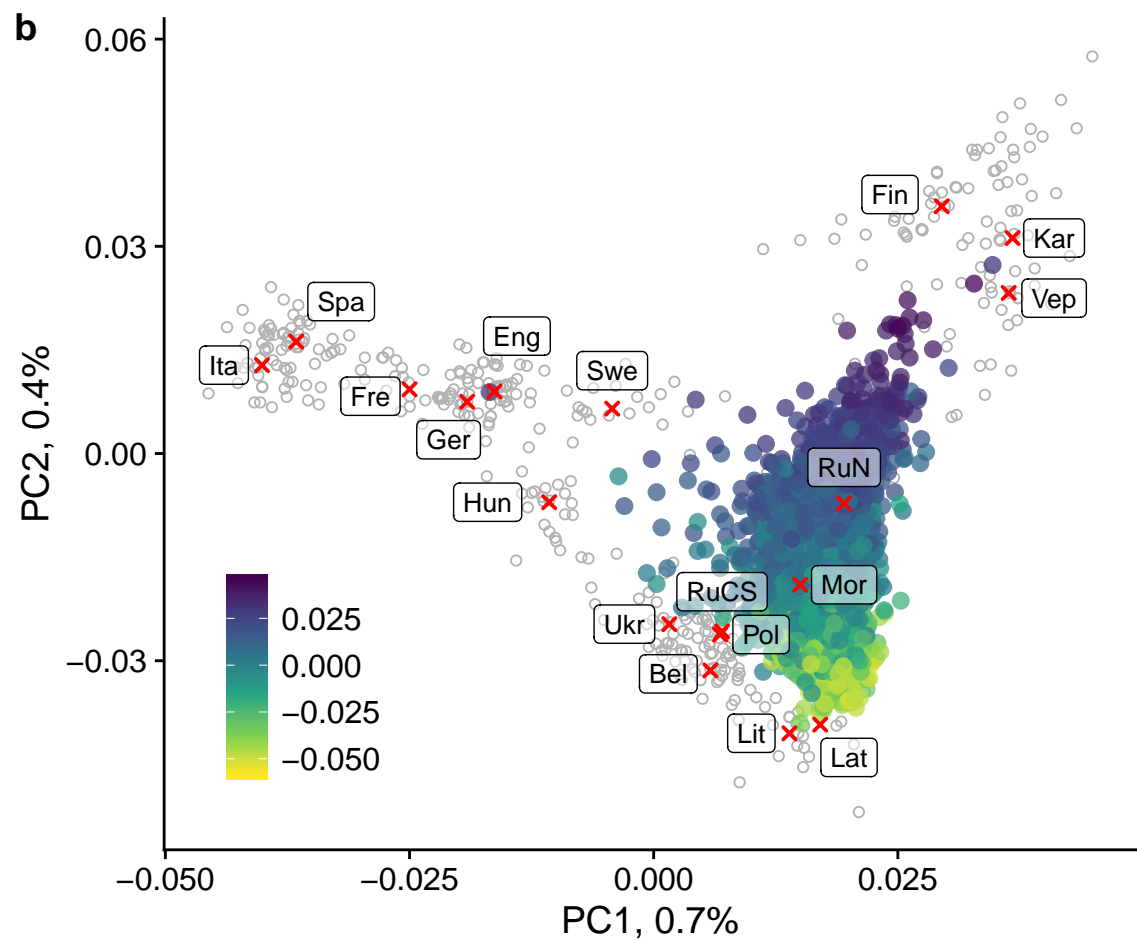
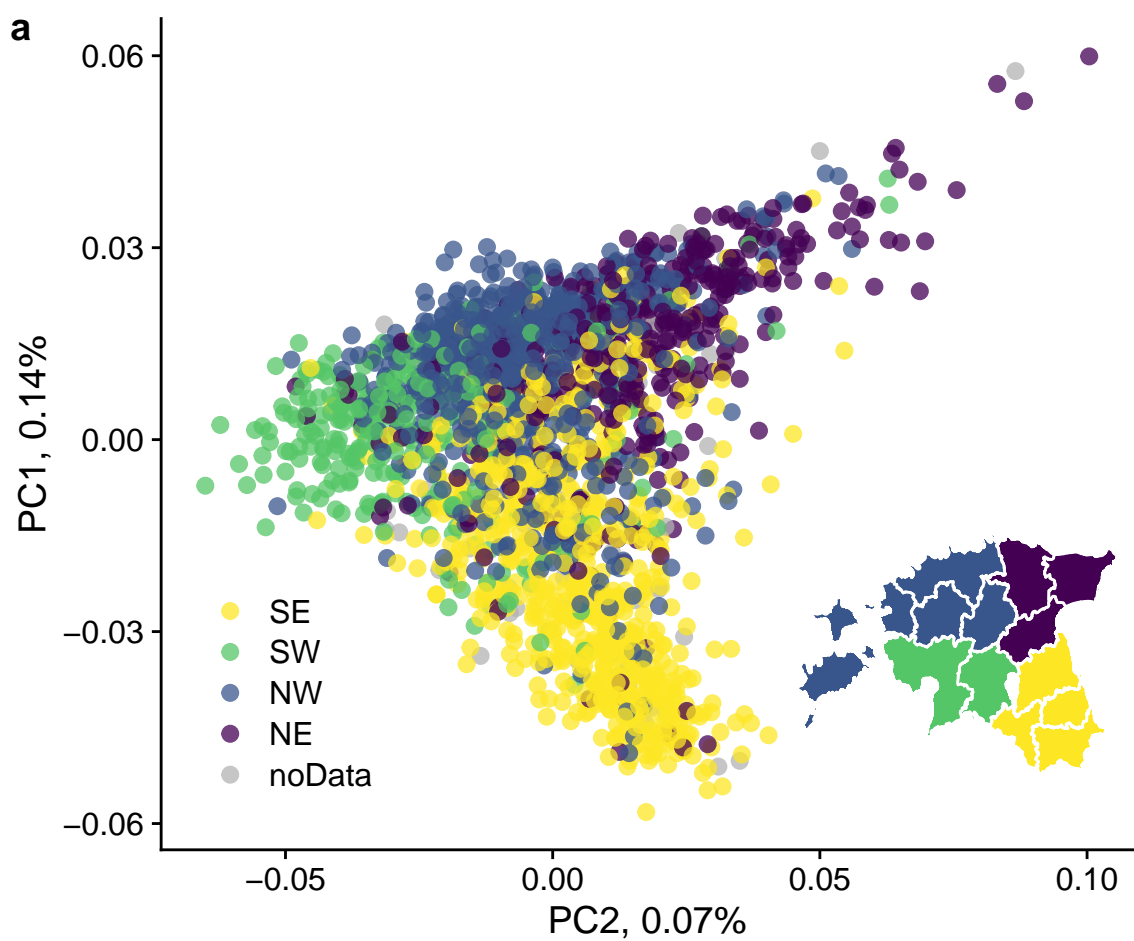
700Figure 3. Homozygosity-by-descent in the R50+ dataset. Boxplots show distribution of per-
701genome total length of Homozygosity-By-Descent (HBD) tracts within clusters shown in
702Figure 2. HBD tracts were detected using IBDseq13. The boxes show 25th, 50th and 75th
703quantiles, while the whiskers show values within 1.5 times the inter-quantile range (IQR) of
70425th and 75th quantiles. Individual dots show outliers (values out of the range shown by the
705whiskers).

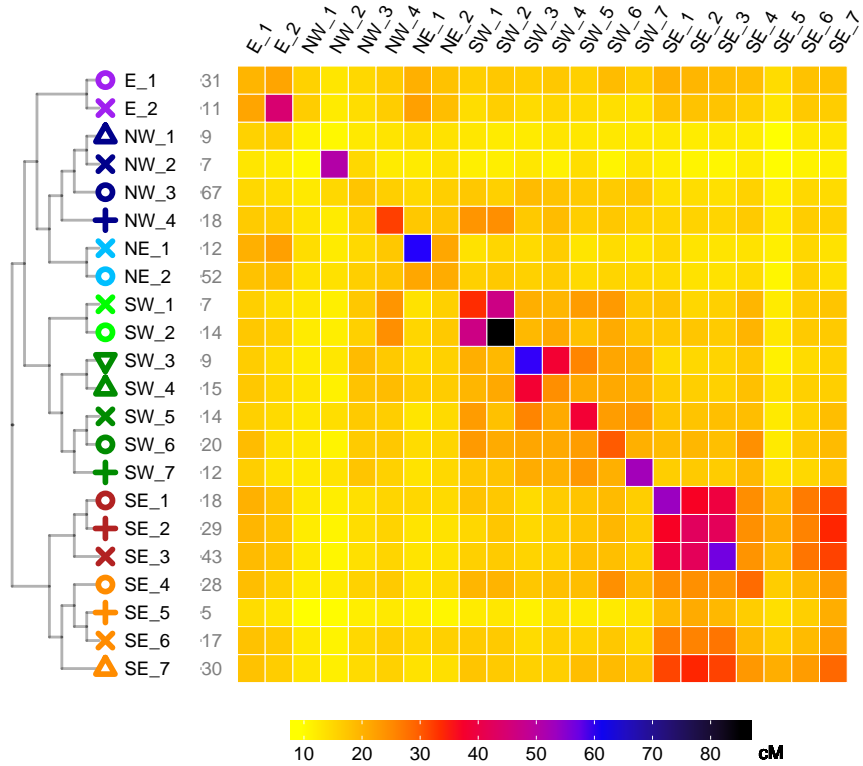
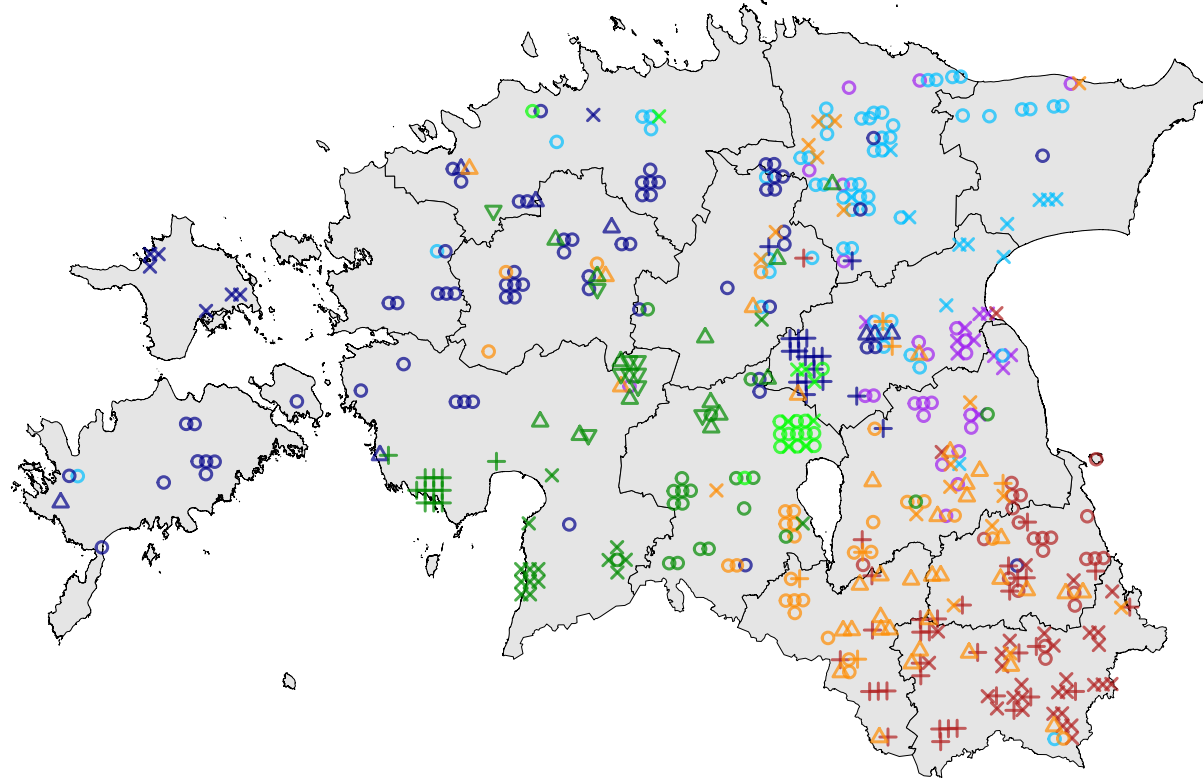
706 Figure 4. Relative proportions of “Baltic”, “Slavic”, Finnish and Swedish ancestry in the R50+
707 subset. Modelled relative ancestral proportions of «Balts» (Latvians and Lithuanians) (a),
708 «Slavs» (Belarusians, Poles, Russians, Ukrainians) (b), Finns (c), and Swedes (d) attributed
709 by applying non-negative least squares approach (NNLS) to
710 CHROMOPAINTER/fineSTRUCTURE (CP/FS) results are shown. For details on source
711 group composition, as well as for results for other groups see SI1:3.1. The colour of each
712 parish reflects mean values of samples coming from this parish. Parishes with no samples in
713 the R50+ dataset are filled with grey. The scale is the same for all four panels. See SI1:3.1
714 for more details. These maps were created in R (<https://www.R-project.org/>)⁴⁴ using an shp
715 object of the Administrative and settlement units provided by the Estonian Land Board,
716 2018.11.01 ([https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-](https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division-p312.html)
717 [Division-p312.html](https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division-p312.html)). See Methods for more details.

718 Figure 5. Genetic clustering of the entire Estonian dataset (2,305 samples). Samples were
719 clustered using the fineSTRUCTURE clustering algorithm based on pairwise total genetic
720 length of IBD segments as described in Methods. Obtained clusters were pulled together
721 based on their position on the tree resulting in 12 higher order clusters shown here (SI1:2.3).
722 A: Hierarchical relationships (tree) and average total length of IBD segments shared
723 between clusters (heatmap). The length of the tree branches does not reflect any
724 relationship between the clusters. Numbers in grey next to cluster names show the number
725 of samples in each cluster. B: Geography of inferred clusters. Each dot within the contour of
726 Estonia corresponds to one individual, while waffle plots show samples for 15 major
727 Estonian towns with each dot corresponding to 5 individuals. This map was created in R
728 (<https://www.R-project.org/>)⁴⁴ using an shp object of the Administrative and settlement units
729 provided by the Estonian Land Board, 2018.11.01
730 ([https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division-](https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division-p312.html)
731 [p312.html](https://geoportaal.maaamet.ee/eng/Spatial-Data/Administrative-and-Settlement-Division-p312.html)). See Methods for more details.

732 Figure 6. Estonian effective population size dynamics. a, Effective population size estimates
733 obtained by applying IBDNe19 to the entire dataset and to 4 clusters from Figure 5: eNW_1,
734 eNE, eSW_2 and eSE_5. b, Comparison of historical and genetic estimates of Estonian
735 population size. Historical estimates combine census data and reconstructions based on
736 written or archaeological sources (S1:4.2-II). Genetic estimates are derived from IBDNe
737 results, for which Est1527 subset was used (SI1:4.4-II) and refer to the broader population
738 that contributed over time to the genomes of contemporary Estonians. When converting time
739 points of the IBDNe curve into actual years we used the same logic as in the original
740 publication19 and set generation 0 to correspond to the year when individuals in our sample
741 had a mean age of 25 (1988). Generation time of 29 years was assumed. For year 1200 the
742 minimum and maximum estimates are provided. In panel a shaded areas show 95%
743 confidence intervals. In panel b shaded area corresponds to the range between the
744 minimum and maximum genetic estimates of N_c (Methods), while the light blue line shows
745 the geometric mean between the two. In both panels on the y axis, “k” stands for
746 “thousands” and “M” for millions.

747 Figure 7. Genome-wide plots of positive standardized SDS scores for the entire dataset (a)
748 as well as SE (b) and nonSE (c) subsets. Conditional suggestive (blue) and genome-wide
749 (red) significance lines are drawn. Gene names are highlighted for intragenic variants with –
750 $\log_{10}(p) > 5$. Datasets are described in the text and Supplementary Information SI1:5.1.

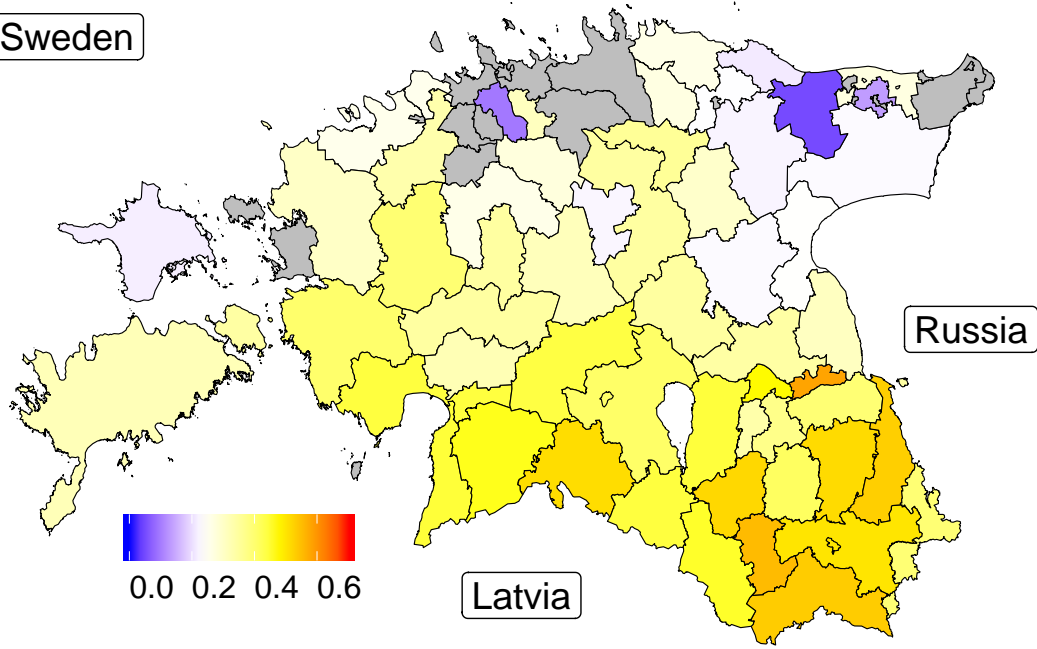


a**b**

Balts

Finland

Sweden



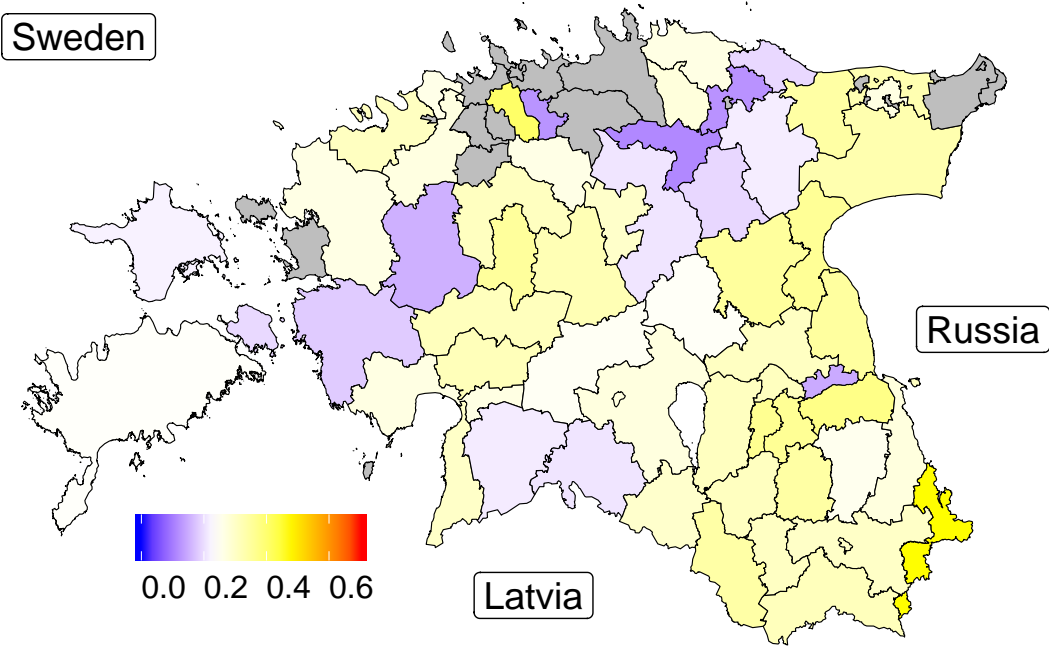
Russia

Latvia

Slavs

Finland

Sweden



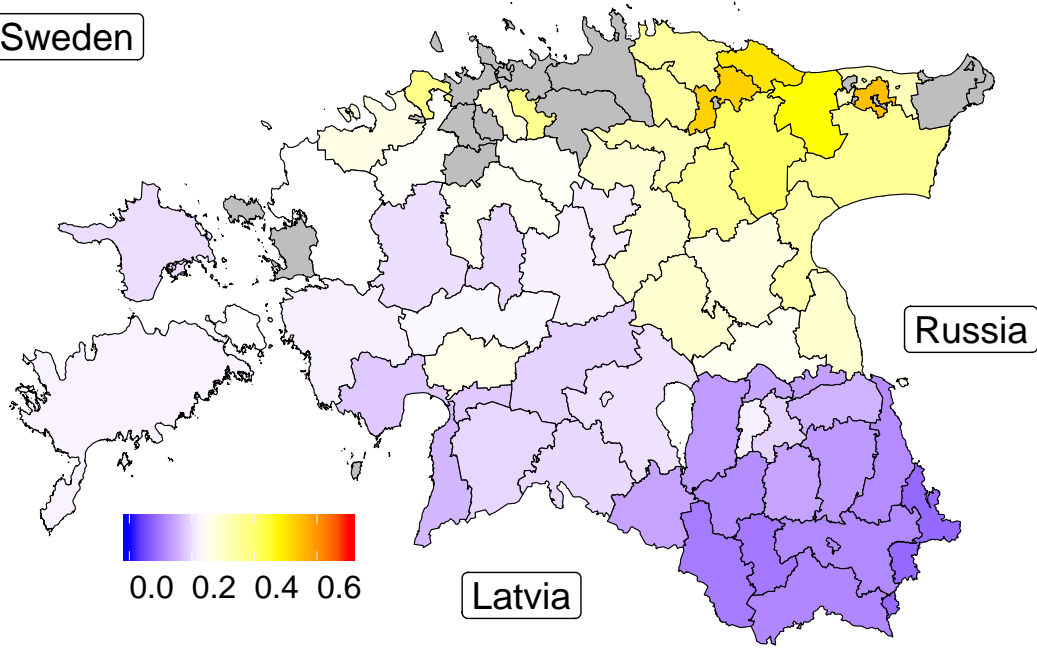
Russia

Latvia

Finns

Finland

Sweden



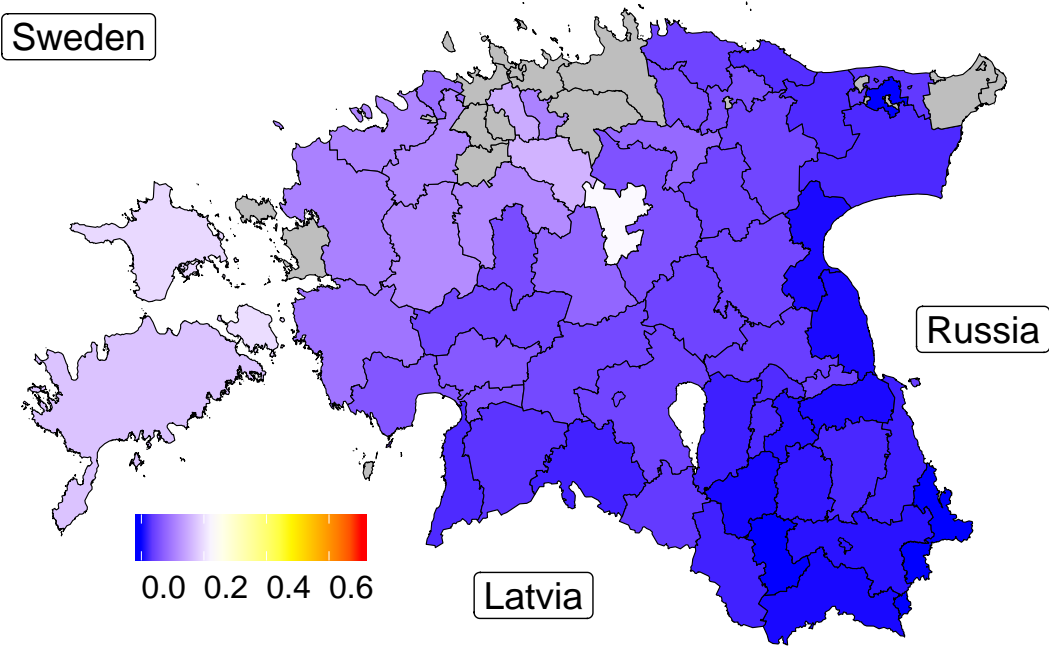
Russia

Latvia

Swedes

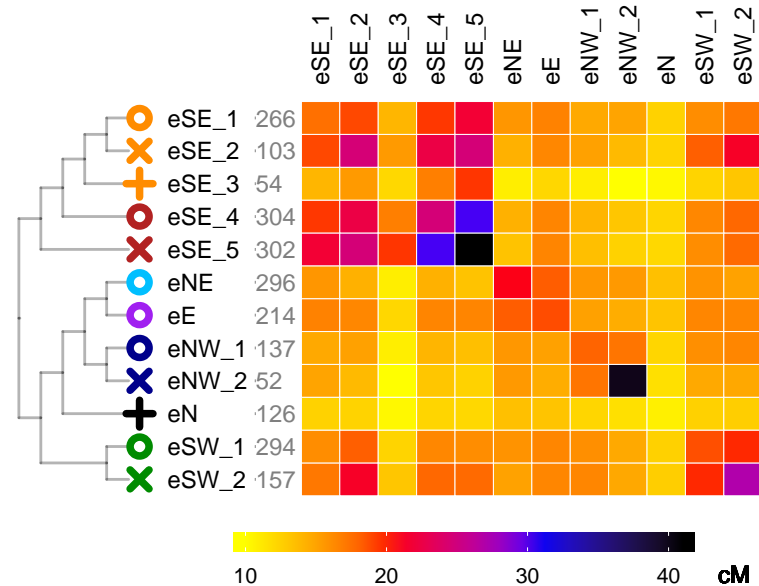
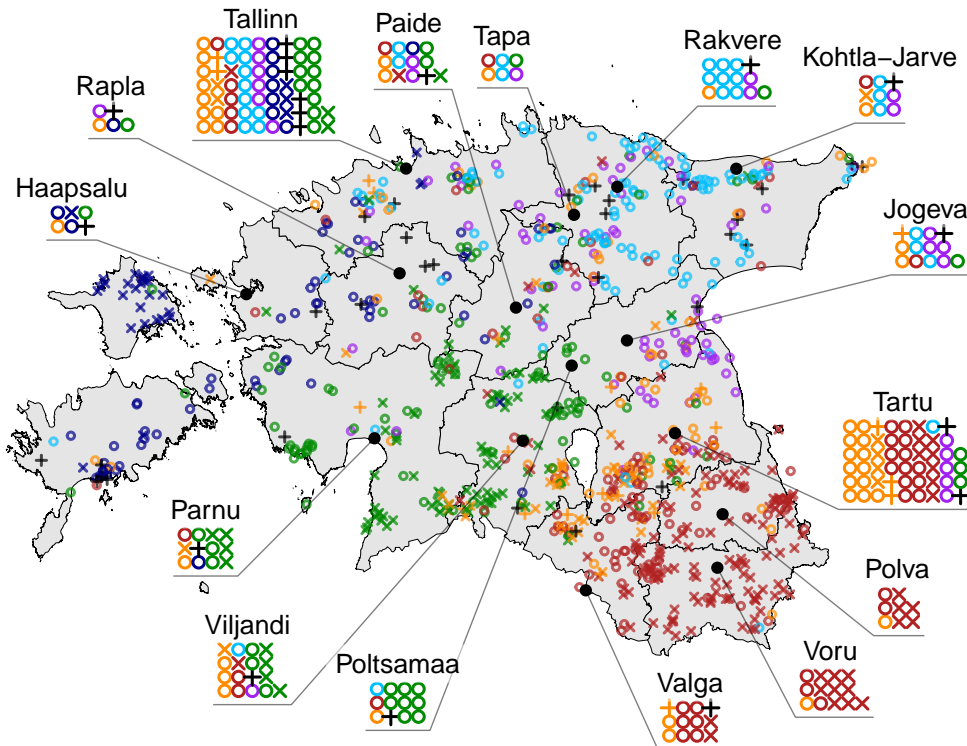
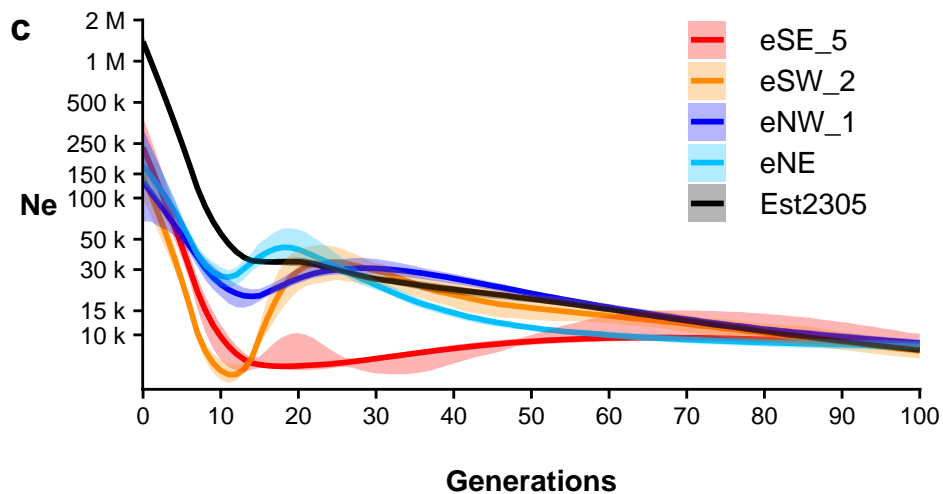
Finland

Sweden



Russia

Latvia

a**b****c****d**