

1 The great tit HapMap project: a continental-scale analysis of ge- 2 nomic variation in a songbird

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85 **Abstract**

86 A major aim of evolutionary biology is to understand why patterns of genomic variation vary among
87 populations and species. Large-scale genomic studies of widespread species are useful for studying how
88 the environment and demographic history shape patterns of genomic divergence, and with the continually
89 decreasing cost of sequencing, such studies are now becoming feasible. Here, we carry out one of the most
90 comprehensive surveys of genomic variation in a wild vertebrate to date; the great tit (*Parus major*) HapMap
91 project. We screened *ca* 500,000 SNP markers across 647 individuals from 29 populations, spanning almost
92 the entire geographic range of the European great tit subspecies. We found that genome-wide variation was
93 consistent with a recent colonisation across Europe from a single refugium in the Balkans and/or Turkey, with
94 bottlenecks and reduced genetic diversity in island populations. Differentiation across the genome was highly
95 heterogeneous, with clear “islands of differentiation” even among populations which are ostensibly panmictic.
96 These islands of differentiation were consistently found in regions of low recombination, suggesting that
97 background selection can rapidly promote population differentiation among even the most recently colonised
98 populations. We also detected genomic outlier regions that were unique to peripheral great tit populations,
99 most likely as a result of recent directional selection at the range edges of this species. These “unique”
100 outlier regions contained candidate genes for morphology, thermal adaptation and colouration, supporting
101 previous research in this species, and providing avenues for future investigation. Our study suggests that
102 comprehensive screens of genomic variation in wild organisms can provide unique insights into evolution.

103 **Author summary**

104 Studying patterns of genetic variation is a useful way of determining why populations and species differ in
105 nature. Genetic variation is shaped by natural selection, but also by the present and past size of populations,
106 the amount of migration, and by features of the genome, such as variation in recombination rate, of the
107 organism being studied. Teasing apart the effects of these different processes on genomic diversity is difficult,
108 but one way that this can be achieved is by studying genomic variation across the entire range of a species.
109 We performed a continental-scale analysis of genetic variation in the great tit - a widespread songbird that
110 has been the focus of extensive ecological research. We first used genomic data to reconstruct the historical
111 colonisation of great tits across Europe, and showed that during the last ice age, this species was likely
112 restricted to a single region in Eastern Europe, from which they spread across the continent. We then
113 studied how patterns of variation differ along the genome, and show that recombination rate is a key driver
114 of variation among all populations. Importantly, by comparing many populations we were able to identify

115 genes that have been subject to natural selection in specific geographical regions. We found that natural
116 selection appeared to be strongest in populations on the edges of the great tit's range acting on traits such as
117 morphology, stress response and colouration. Large-scale genetic analyses such as ours are therefore useful
118 approaches for understanding how evolution operates in the wild.

119 Introduction

120 Since the first studies of allozyme variation in humans [1] and *Drosophila* [2,3], there has been great interest in
121 explaining how evolutionary and ecological processes shape the patterns of genetic variation observed within
122 and among natural populations. One focus of research and debate in this area has been on quantifying the roles
123 of adaptive and neutral processes in explaining observed levels of genetic variation [4]. However, adaptation
124 does not occur in isolation, but acts on genetic variation that is also shaped by mutation, recombination, gene
125 flow, and genetic drift. More recently there has been increased effort in understanding how these fundamental
126 evolutionary forces operate in concert to generate and maintain the levels of genetic diversity commonly
127 observed in natural populations [5,6].

128 The increasing feasibility of high-throughput sequencing and subsequent characterisation of genome-wide
129 variation across large numbers of individuals has revealed that at the genetic level, patterns of variation
130 and divergence among natural populations and species are highly heterogeneous [7]. A key feature of these
131 “genomic landscapes” of divergence that has received particular attention is the presence of so-called “islands
132 of differentiation”: outlier regions of the genome with high levels of divergence estimated from statistics
133 such as F_{ST} or d_{xy} [7–10]. Initially these regions were termed “islands of speciation”, and were thought
134 to arise as a result of reduced gene flow in genomic regions associated with reproductive isolation [7,11].
135 Subsequent research has revealed that highly heterogeneous patterns of genomic divergence can occur even in
136 the complete absence of gene flow, as a result of recombination rate variation and linked selection [12,13].
137 In genomic regions of low recombination, selection for beneficial mutations (positive selection), or against
138 deleterious mutations (background selection), will impact relatively large genomic regions as a result of high
139 levels of linkage disequilibrium (LD) among sites. Selection within these regions reduces diversity within
140 populations, and increases differentiation among them, resulting in “islands” of increased differentiation that
141 persist over evolutionary time [13,14].

142 Comparing patterns of genomic differentiation among sets of populations or species at different stages of the
143 divergence/speciation continuum is a powerful way of disentangling the forces that shape variation among
144 populations. Martin et al. [15] showed that, across multiple *Heliconius* butterfly populations and species,

145 patterns of genomic variation were shaped by a combination of gene flow and selection, particularly in
146 genomic regions harbouring genes involved in wing patterning. In contrast, Renaut et al. [9] showed that
147 in *Helianthus* sunflowers, genomic architecture was the main driver of genomic differentiation across sets of
148 populations. Similarly, recent research in birds has revealed that differentiation landscapes are conserved
149 across populations, species and even across avian families, with the same islands of differentiation arising
150 among populations of distantly related species [16–18]. This latter pattern appears to have arisen, at least in
151 part, as a result of a highly conserved recombination landscape in birds, with background selection in regions
152 of low recombination producing recurrent islands of differentiation [19].

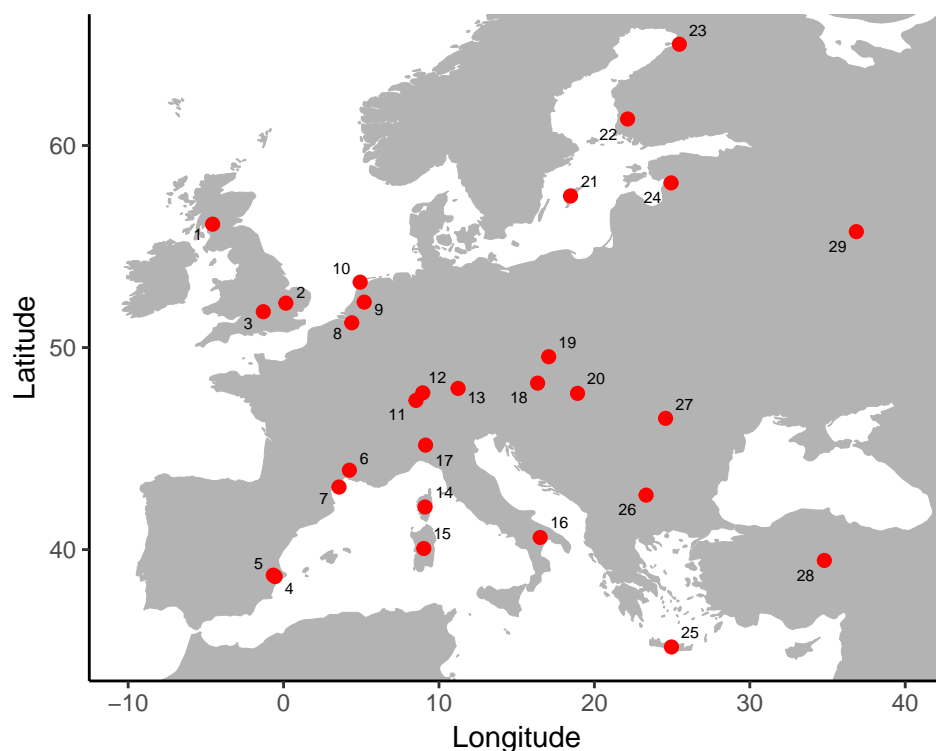
153 It is now clear that the recombination landscape and linked selection are key drivers of genomic variation within
154 and among populations. However, we are only just beginning to understand how this linked selection interacts
155 with other evolutionary forces to shape patterns of differentiation across natural populations and species
156 [19–23]. A recent, large-scale analysis of threespine sticklebacks (*Gasterosteus aculeatus*) showed that islands
157 of differentiation were more likely to arise in low recombination regions when gene flow occurred between
158 populations [24]. There is also a significant impact of divergence time; in recently separated populations the
159 differentiation landscape is most likely to reflect selective sweeps. Then, as divergence accumulates, genomic
160 architecture is expected to play an increasingly important role in generating these genomic islands [19].

161 Widespread continental species are excellent models for studying how demography and the environment
162 shape genetic and phenotypic variation among populations, due to their large effective population sizes and
163 ecologically varied ranges. Insight into the evolutionary history of such species can be gained if genetic
164 variation is characterized across much of its geographical range. Cross-population comparisons of genetic
165 variation can then be used to make inferences about phylogeography, levels of gene flow between populations
166 and how adaptation to different environmental and ecological conditions occurs. Whole-genome resequencing
167 and customized SNP genotyping arrays have made studies that characterize all, or much, of the genome
168 tractable. The first large-scale studies were performed in humans - i.e. the HapMap Projects [25–27] which
169 characterized human genetic variation on different continents, with a view to determining the feasibility
170 of association mapping studies. Similar studies have been conducted in domesticated species and their
171 wild ancestors [28–30], and in model organisms [31,32]. More recently, there is a growing appreciation that
172 HapMap-type studies are useful for studying signatures of selection and adaptation in natural populations of
173 species with large effective population sizes and high levels of gene flow [33–36].

174 The European great tit (*Parus major major*) is an excellent model for ecological and evolutionary studies [37].
175 A wealth of ecological data exists across multiple great tit populations [38–40], enabling informed hypotheses
176 about selection to be tested in this system. Phylogeographic research using mitochondrial DNA suggests that

177 this species has recently expanded across its European range, possibly from a single refugium in the Balkans
178 [41]. Contemporary populations are characterised by large effective population sizes and high levels of gene
179 flow among populations, resulting in low levels of genetic differentiation [42,43]. However, these previous
180 cross-population molecular studies have relied on a modest number of microsatellite loci and mitochondrial
181 DNA, making the detection of genomic regions under selection impossible. The genome of the great tit has
182 recently been sequenced [44], and a high density panel of SNP markers has been developed [45]. A recent
183 study of two European populations using this marker panel suggests that rapid adaptation has occurred at
184 the genomic and phenotypic levels, with pronounced selection on morphology [46].

185 Here, we perform a HapMap study of 647 unrelated individuals across 29 populations (Fig. 1), to examine
186 how genomic architecture, natural selection and population history have shaped patterns of genomic variation
187 across recently colonised European great tit populations. Using a large SNP panel typed across all individuals,
188 we first characterise genome-wide patterns of variation within and among populations, in order to infer
189 population history. We then examine how variation is partitioned across the genome, and test the hypothesis
190 that highly divergent genomic regions have arisen in genomic regions of low recombination [12,13]. Finally,
191 we examine how genomic divergence accumulates along the colonisation route of this species, with the aim of
192 inferring how recent natural selection and background selection drive variation across the genome in the wild.



193

194 **Figure 1** Sampling locations of great tit populations. Population names and sample sizes are given in Table
195 S1, and numbers on the map correspond to the “code” column in Table S1.

196 Results and Discussion

197 Genetic diversity and population history

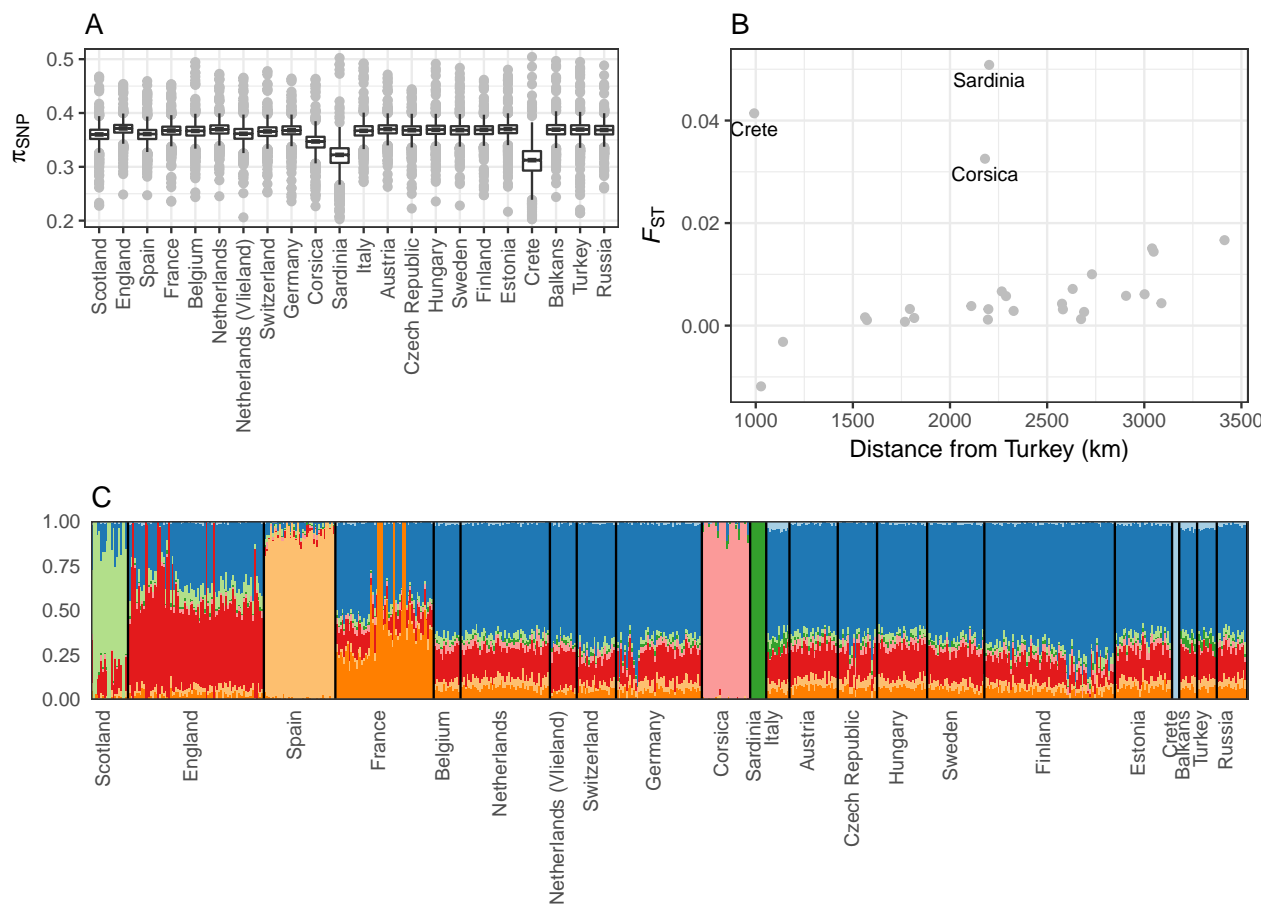
198 Sampling locations and sample sizes for each population are given in Table S1. Levels of genetic diversity
199 (π_{SNP}) were generally high, but we observed substantial differences among populations (Fig. 2A). Similarly,
200 LD declined rapidly with genomic distance in all populations, reaching baseline levels within ~5kb in all
201 populations, but also varying among populations (Fig. S1). Highest levels of LD (and lowest levels of
202 genetic diversity) were observed in the Mediterranean island populations of Crete (Greece) and Sardinia
203 (Italy), with lowest levels of LD in central and western Europe (Fig. S1). This is consistent with reduced
204 effective population size in these island populations, either as result of the colonisation process or more recent
205 bottlenecks, along with low levels of subsequent gene flow from the continent to the islands [47,48].

206 Genome-wide F_{ST} between European great tit populations was 0.007, with no significant pattern of isolation-
207 by-distance (Mantel test; $r = 0.13$, $p = 0.18$; Fig. S2). Instead, the highest levels of F_{ST} were found in
208 comparisons involving the Mediterranean island populations of Corsica (France), Sardinia and Crete (Fig.
209 S2). Admixture analysis was consistent with this pattern (Fig. S3); the $K = 2$ analysis assigned individuals
210 in Sardinia and Corsica to one genetic cluster, and the remaining populations to the second. Thus, it is
211 likely that much of the genetic structure between European great tit populations is a result of genetic drift in
212 these small island populations. Admixture analysis also revealed some structure between (mainly peripheral)
213 mainland and larger island populations. At $K = 3$ (the model that best fitted the great tit data; Fig. S4),
214 Spain was separated from the rest of mainland Europe. Increasing values of K resulted in the separation of
215 populations in Scotland ($K = 4$), Sardinia (from Corsica; $K = 5$), southern France ($K = 6$), Crete ($K =$
216 7) and England ($K = 8$). The Admixture output at $K = 8$ is displayed in Fig. 2C as this gives the most
217 detailed picture of genetic structure among European great tit populations. Further increases in K did not
218 generate patterns of structure that corresponded to geographical variation (Fig. S3), and were increasingly
219 less well supported (Fig. S4). Thus, even with hundreds of thousands of markers Admixture was unable to
220 separate many of the European populations, confirming that levels of divergence are extremely low. PCA
221 largely corroborated the Admixture results, with PC1 separating Corsica and Sardinia from the remaining
222 populations, PC2 separating Spain, while PC3 and PC4 separated Scotland, England, Corsica, Sardinia and
223 Crete (Fig. S5).

224 Maximum likelihood analyses implemented in TreeMix showed that a model with no migration explained 97.8%
225 of variance in relatedness between populations [49]; increasing the number of migration events substantially
226 improved the percentage of relatedness explained, up to 99.7% when 10 migration events were fitted (Fig.

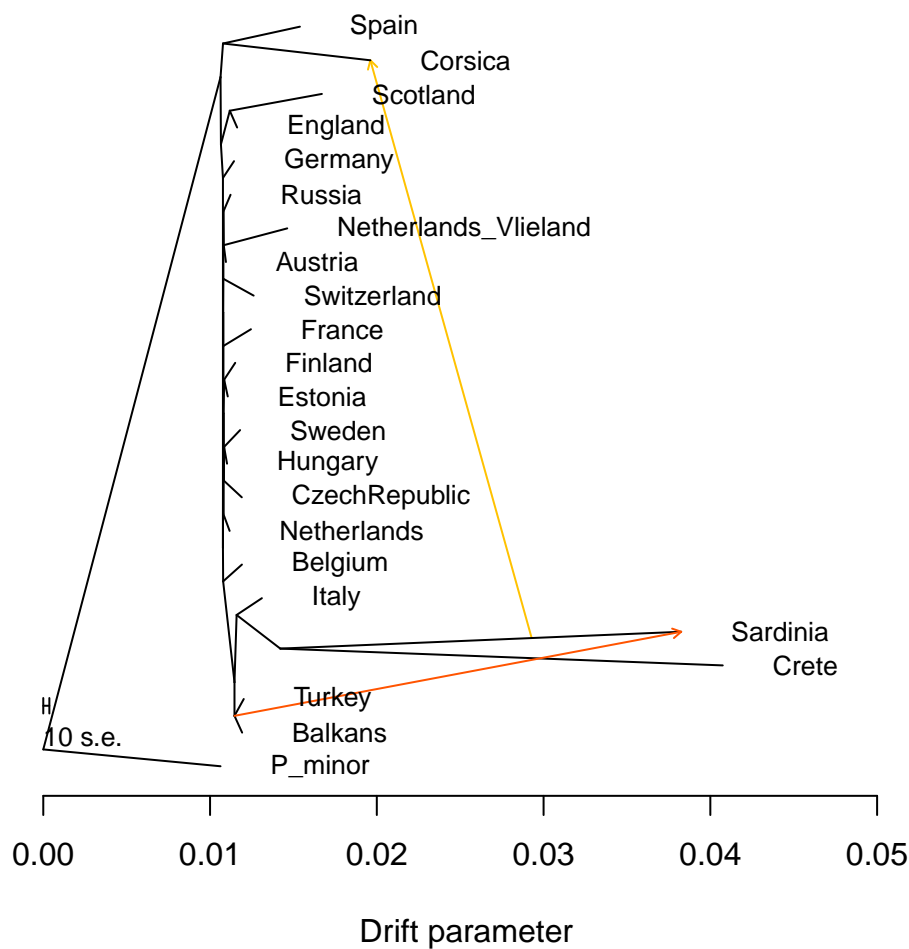
227 S6). In Figure 3 we display the maximum likelihood tree with two migration events, after which the variance
228 in relatedness explained plateaued when more migration events were added (Fig. S6). The tree was generally
229 characterised by short branch lengths, with the exception of the island populations of Sardinia and Crete,
230 which were grouped with the population from mainland Italy (Fig. 3). Thus, the TreeMix analysis is
231 consistent with the pattern of low overall genomic divergence, with the exception of the Mediterranean island
232 populations. However, much (though not all) of the grouping that did occur among continental populations
233 made geographical sense, with populations from Finland and Estonia grouped together, as were populations
234 from Turkey and the Balkans, and populations from England and Scotland (Fig. 3). Interestingly, TreeMix
235 grouped the Spanish and Corsican populations, which is consistent with previous subspecies descriptions of
236 European great tits [50]. The two fitted migration edges both involved Sardinia, with migration from eastern
237 Europe to Sardinia, and from Sardinia to Corsica (Fig. 3).

238 We next tested the hypothesis that great tits colonised Europe from a single refugium in Turkey and the
239 Balkans. This scenario has been suggested before [41], but due to the low number of genetic markers available
240 there has been limited power with which to test this hypothesis. Using our genome-wide panel of SNP markers,
241 we compared genetic and geographic distance between each population and the proposed refugial populations.
242 Because of the elevated structure in Corsica, Sardinia and Crete (Fig. S2), we excluded comparisons involving
243 these populations. We found that F_{ST} was significantly related to distance from Turkey ($r = 0.81$, $p <$
244 0.001 ; Fig. 2B) and the Balkans ($r = 0.44$, $p = 0.001$). The same relationship was not found for alternative
245 potential refugial populations in Spain ($r = -0.09$, $p = 0.55$), or southern Italy ($r = 0.04$, $p = 0.77$). Our
246 results therefore lend empirical support to the hypothesis [41] that great tits colonised Europe from a single
247 refugium in the south-east. Clearly, although our sampling was extensive, it is not exhaustive, and more
248 fine-scaled sampling in eastern Europe would be required to determine the exact location and extent of
249 refugial great tit populations. Sampling in North Africa would also be useful to determine whether further
250 refugia exist, and to quantify the extent of admixture between European and African great tit populations.



251

252 **Figure 2** Genetic diversity and structure in European Great tit populations. **A** Nucleotide diversity
 253 within each population. **B** Pairwise F_{ST} in relation to geographic distance from the Turkey, only including
 254 comparisons involving Turkey. **C** Output from Admixture analysis at $K = 8$. Population details can be
 255 found in Table S1.



256

257 **Figure 3** Maximum likelihood tree inferred by TreeMix, allowing two migration events. The two migration
258 events (arrows) are coloured according to their weight (red = higher migration), and horizontal branch lengths
259 are proportional to the amount of genetic drift that has occurred along the branch. A population of the great
260 tit's sister species, *Parus minor*, was used as an outgroup. Population details are given in Table S1.

261 Genomic landscapes of differentiation

262 It is likely that many, and perhaps the majority, of wild populations are characterised by highly heterogeneous
263 patterns of differentiation across the genome [23]. To examine how landscapes of genomic divergence have
264 formed along the colonisation route of European great tits, we calculated windowed F_{ST} in 500kb bins between
265 each population and the proposed refugial population in Turkey. We found that F_{ST} varied markedly across
266 the genome in all comparisons (Fig. 4; Fig. S7). Outlier regions (windows with standardized F_{ST} , hereafter
267 zF_{ST} , > 10) were found in all comparisons apart from Crete and Sardinia, in which overall levels of divergence
268 were highest (Fig. 4). Our results suggest, therefore, that genomic islands of differentiation can and do arise
269 even among populations that could be considered panmictic.

270 Genomic differentiation was negatively related to recombination rate in almost all comparisons with Turkey
271 (Fig. 5). The relationship between F_{ST} and recombination rate was generally weak, with correlation
272 coefficients < 0.1 . In a handful of populations this relationship was substantially stronger - most notably
273 in the island populations of Corsica, Sardinia and Crete, and in England, Scotland, Spain, Finland and
274 France (Fig. 5). Outlier regions of very high differentiation ($zF_{ST} > 10$) almost exclusively occurred in
275 relatively low recombining regions, and accordingly, the recombination rate of outlier regions was lower
276 than the genome-wide average, albeit not significantly (Wilcoxon test, $P = 0.079$). Nonetheless, there is a
277 suggestion that linked selection in regions of low recombination may play a key role in driving patterns of
278 genomic differentiation even in the very earliest stages of population separation.

279 Of the 11 outlier regions, nine were found in only one or two comparisons, while the other two were found
280 in 12 and 10 comparisons, respectively (Table S2). We hereafter refer to outlier regions found in one or
281 two comparisons as “unique” outlier regions, and to those found in more than two comparisons as “shared”
282 regions. It appears that European great tits have colonised from a single refugial population, and as such truly
283 independent comparisons are not available. Therefore an outlier region shared among multiple populations
284 could represent either selection in the ancestral population, or background selection. Given the pervasiveness
285 of background selection in birds [17,18], and other organisms [51], and the overall negative relationship
286 between F_{ST} and recombination rate in this study (Fig. 5), it is likely that this background selection is the
287 driver of the shared outlier regions in great tits. Thus, it appears that background selection can generate
288 islands of differentiation in the very earliest stages of population separation. This is not necessarily what we
289 would expect - it is often assumed that peaks of high differentiation in recently separated populations are
290 the product of selective sweeps, and that only after time do correlated patterns of genomic divergence arise
291 [19,24]. Further research into genomic landscapes of differentiation among widespread continental species
292 will help us better understand on the role of background selection in shaping genomic divergence over short

293 evolutionary timescales.

294 Regions of high differentiation that are not shared among populations are more likely to be the result
295 of recent positive selection [19]. We found that unique outlier regions tended to be found in the most
296 peripheral European great tit populations, with three found in Scotland, two in England, Spain and Finland;
297 the remaining outlier regions were found in comparisons involving the Czech republic, Russia, Vlieland
298 (Netherlands) and Belgium (Table S2). Observational and experimental research shows that adaptation at
299 range edges is a key feature shaping divergence among recently colonised and expanding populations [52–54].
300 There appeared to be no difference in the recombination rate between shared and unique outlier regions (Fig.
301 5), although the small number of regions precluded testing this hypothesis formally. Thus, it is likely that
302 genomic architecture plays a key role in determining how both positive and background selection have shaped
303 genomic variation across the recent evolutionary history of European great tits.

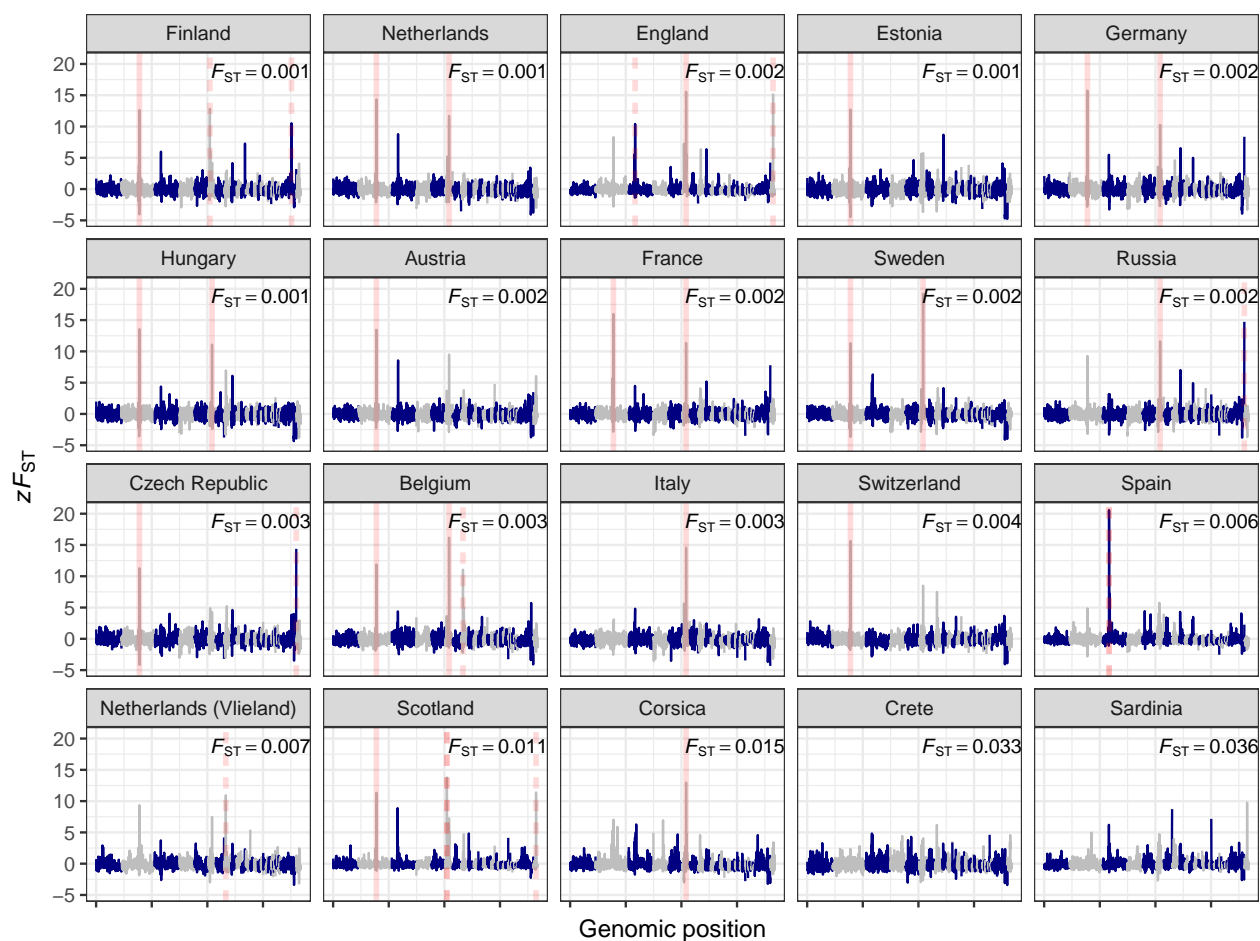
304 Genes found within shared and unique outlier regions are displayed in Table S2. Perhaps most notable among
305 these is *COL4A5*, a gene found to be associated with bill length, and under selection between populations in
306 England and the Netherlands, in a recent great tit study [46]. Here we found that the *COL4A5* region is
307 an F_{ST} outlier in England and Scotland, but not in any other European populations (Table S2). UK great
308 tits have been described as a separate subspecies based on beak shape [55], and our results here, combined
309 with previous results, suggest that this divergence is the result of recent natural selection in the UK [46].
310 Another notable candidate gene potentially involved in beak morphology, and previously found to be under
311 selection in UK great tits is *BMPRI1A*, which plays a key role in palate development [56] and in this study
312 was found in an outlier region in Scotland. Other candidate morphology and obesity genes in the unique
313 outlier regions in the UK included *PPP1CB*, which may play a role in adipogenesis [57] and *GHITM*, which
314 appears to have been subject to natural selection in human pygmy populations [58]. Thus, morphological
315 traits may frequently be involved in adaptation in great tits.

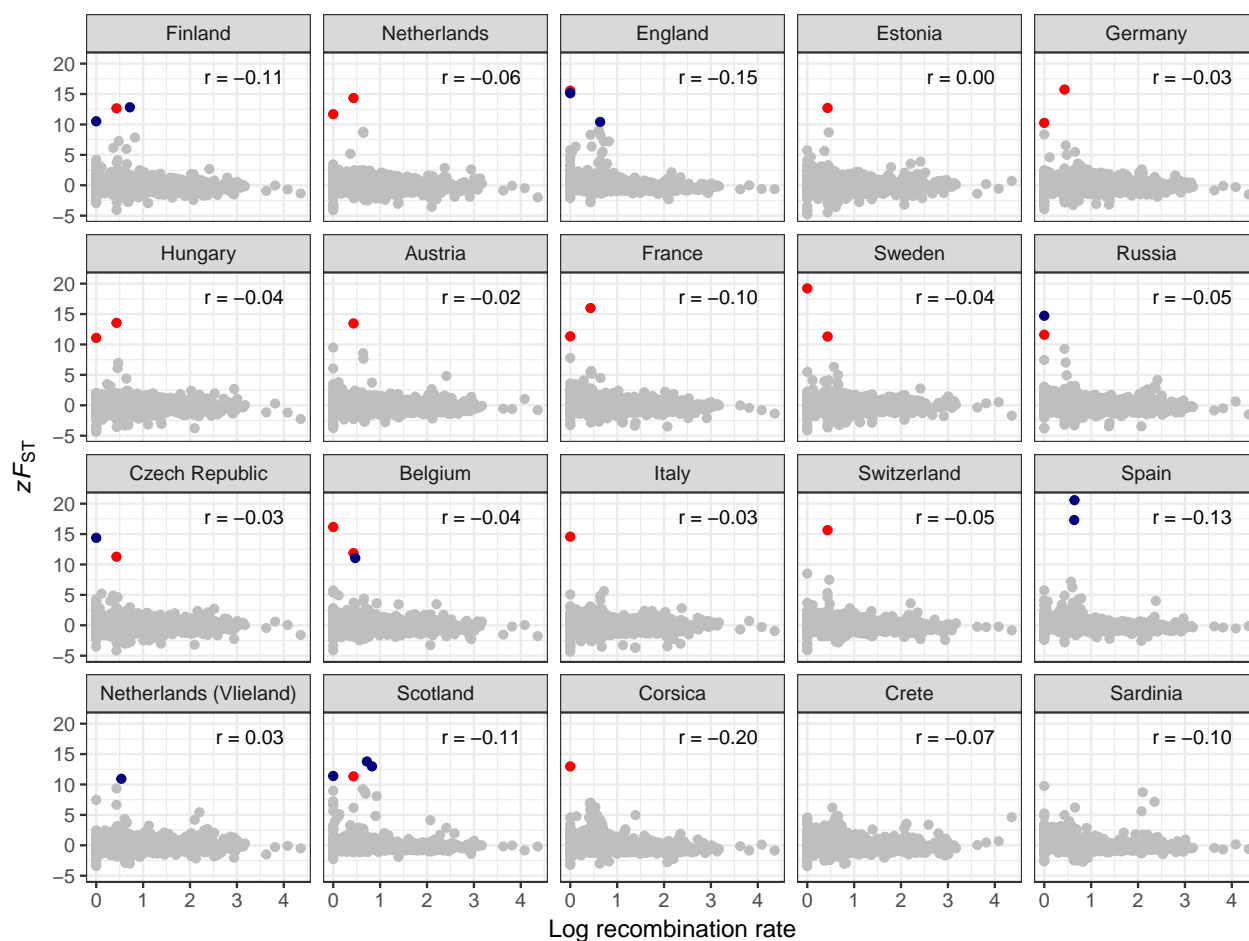
316 In addition to morphological candidates in the UK, we found outlier regions unique to cold populations in
317 Scotland, Finland and Russia (Table S2), containing at least one candidate gene for thermal stress (*CDKN1B*)
318 [59]. Other genomic outlier regions contained potential candidate genes for malaria infection (*MRPL33*) [60]
319 and colour variation (*SOX10*) [61]. This is thus far an exploratory analysis, and we are therefore reluctant to
320 speculate whether these candidate genes are genuine targets for natural selection, and more reluctant still to
321 speculate as to how selection might be driving variation at these regions. Regardless, these candidates will
322 provide useful starting points for future genomic and ecological investigation.

323 To further explore how selection may have shaped variation in F_{ST} outlier regions, we estimated levels

324 of nucleotide and haplotype diversity within these regions. Nucleotide diversity (π_{SNP}) in outlier regions
325 varied from 0.21 to 0.48, and diversity in these regions was significantly lower than the genome-wide average
326 (Wilcoxon test, $P = 0.018$; Fig. S8A). However, there appeared to be no difference in nucleotide diversity
327 between shared and unique regions (Fig. S8A). Haplotype diversity varied substantially among regions, with
328 haplotype richness ranging from 72 to 1033. Both haplotype richness and marker density in shared regions
329 tended to be lower than those in unique regions (Fig. S8B). A detailed examination of haplotype structure in
330 one shared and one unique region is displayed in Figure S9. The unique outlier region (to Finland, situated
331 on chromosome 1A) was characterised by a complex structure, with a single haplotype at high frequency
332 in Finland compared to other populations, indicating a population-specific selective sweep (Fig. S9A,C).
333 In contrast, the shared region on chromosome 2 was much less complex, demonstrating higher haplotype
334 frequencies across a range of populations. Our data therefore suggest that examining patterns of haplotype
335 diversity in outlier regions may help to separate recent episodes of positive selection from the gradual process
336 of background selection (Figs S8, S9).

337 HapMap style projects have been hugely informative in shaping our understanding of how natural selection
338 operates in humans and other model species [25,32]. This study is one of the largest to date of genomic
339 variation in a wild vertebrate, which has helped to reveal the evolutionary history of great tits, and to
340 identify candidate genes and traits that may have been involved in adaptation during and/or after postglacial
341 recolonisation. Further, this work will form the foundation of many future analyses. Clearly, we have only
342 touched on haplotype-based methods to infer adaptation here, and this will be the subject of future work.
343 Environmental association approaches are also highly suited to detecting adaptation in widespread continental
344 species [62,63], and further work will test how variation in the abiotic environment has shaped patterns of
345 genomic variation in great tits. This combination of environmental and genomic data in species such as great
346 tits, in which a wealth of ecological and genomic resources are available, is likely to generate interesting
347 insights into the the genetic and phenotypic basis of natural selection.





355

356 **Figure 5** Genomic differentiation and recombination rate variation in European great tit populations. Each
 357 point is the mean of a 500kb window, with each panel displaying a pairwise comparison with the proposed
 358 refugial population in Turkey. Coloured points represent F_{ST} outliers (mean standardized F_{ST} values of
 359 $zF_{ST} > 10$) shared across more than two comparisons (red), or unique to one or two comparisons (dark blue).

360 **Materials and Methods**

361 **Sampling and molecular methods**

362 Samples were collected from 29 populations from 22 regions across Europe (Fig. 1; Table S1). Samples were
363 pooled into regions either based on geographical proximity (e.g. Cambridge and Wytham woods), or based
364 on sample size (e.g. Romania and Bulgaria). An exploratory analysis considering all sampled populations
365 separately yielded virtually identical results to those shown here, and in no cases did we observe substructure
366 within pooled populations in our Admixture analyses (Fig. S3).

367 Birds were trapped from nest boxes, or using mist nets, and ringed with a uniquely numbered aluminium
368 ring. Blood was taken via brachial or tarsal venipuncture, and stored in either 1 ml Cell Lysis Solution
369 (Gentra Puregene Kit, Qiagen, USA), Queen's buffer, or absolute ethanol. All samples were genotyped using
370 a custom-made Affymetrix® great tit 650K SNP chip at Edinburgh Genomics (Edinburgh, United Kingdom),
371 following the approaches outlined in [45], and the filtering approaches outlined in [46]. After filtering, a total
372 of 647 samples typed at 483888 SNPs were retained for analysis.

373 **Analyses**

374 Unless stated otherwise, all population genetic statistics were calculated in PLINK version 1.9 [64], and
375 downstream analysis and plotting was carried out in R version 3.3 [65]. In each population, we estimated LD
376 (R^2) for each pair of markers within 50kb on the same chromosome, and compared this to physical distance
377 between marker pairs. We calculated observed heterozygosity for each SNP and population using a reduced
378 SNP dataset, which was pruned based on LD to remove all markers with $R^2 > 0.1$, then thinned with a
379 probability of retaining each variant of 0.25.

380 We calculated genome-wide (mean) F_{ST} between each pair of populations using the pruned and thinned
381 dataset described above. Pairwise F_{ST} was compared to geographic distance between populations using
382 Mantel tests, implemented in the Ecodist package in R [66]. We tested whether genetic structure was related
383 to distance from candidate refugial populations (in the Balkans, Turkey, Spain and Italy), using Pearson
384 correlations. We also estimated population structure using Admixture version 1.3, with default settings
385 [67]. We varied values of K from one to ten; by which point increasing values of K provided no informative
386 information about population structure (see results). Model support for each value of K was estimated by
387 calculating 5-fold cross-validation error. Finally, we visualised the evolutionary history among European
388 great tit populations by generating a maximum likelihood tree in TreeMix version 1.13 [49]. We rooted the
389 tree using a sample of *P. minor* individuals sampled from Amur, Russia. We fitted models allowing for range

390 of migration events (0-10), and used a window size of 500 SNPs [49]. To assess model fit, we calculated the
391 proportion of variance in relatedness between populations explained by each model [49].

392 We examined the genomic landscape of differentiation across European great tit populations by calculating
393 F_{ST} in 500kb bins, using python scripts obtained from Github ([https://github.com/simonhmartin/genomics_](https://github.com/simonhmartin/genomics_general)
394 [general](https://github.com/simonhmartin/genomics_general)). We did not estimate d_{xy} , as this parameter is difficult to estimate accurately from single SNP loci
395 [13]. We also calculated standardised F_{ST} (zF_{ST}) by mean-centring windowed values and dividing them by
396 the standard deviation among windows. We defined outlier regions as 500kb bins with zF_{ST} values greater
397 than ten. We tested whether the landscape of genomic differentiation was related to recombination rate
398 variation using a recombination map previously developed for the great tit using a 10K SNP chip [68], from
399 which we estimated recombination rates using third-order polynomials [69].

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