The great tit HapMap project: a continental-scale analysis of genomic variation in a songbird

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Abstract

A major aim of evolutionary biology is to understand why patterns of genomic diversity vary among populations and species. Large-scale genomic studies of widespread species are useful for studying how the environment and demographic history shape patterns of genomic divergence, and with the continually decreasing cost of sequencing and genotyping, such studies are now becoming feasible. Here, we carry out one of the most geographically comprehensive surveys of genomic variation in a wild vertebrate to date; the great tit (*Parus major*) HapMap project. We screened *ca* 500,000 SNP markers across 647 individuals from 29 populations, spanning almost the entire geographic range of the European great tit subspecies. We found that genome-wide variation was consistent with a recent colonisation across Europe from a single refugium in South-East Europe, with bottlenecks and reduced genetic diversity in island populations. Differentiation across the genome was highly heterogeneous, with clear "islands of differentiation" even among populations with very low levels of genome-wide differentiation. Low local recombination rate in the genome was a strong predictor of high local genomic differentiation (F_{ST}), especially in island and peripheral mainland populations, suggesting that the interplay between genetic drift and recombination is a key driver of highly heterogeneous differentiation landscapes. We also detected genomic outlier regions that were confined to one or more peripheral great tit populations, most likely as a result of recent directional selection at the range edges of this species. Haplotype-based measures of selection were also related to recombination rate, albeit less strongly, and highlighted population-specific sweeps that likely resulted from positive selection. These regions under positive selection contained candidate genes associated with morphology, thermal adaptation and

colouration, providing promising avenues for future investigation. Our study highlights how comprehensive screens of genomic variation in wild organisms can provide unique insights into evolution.

Introduction

Since the first studies of allozyme variation in humans [1] and *Drosophila* [2,3], there has been great interest in explaining how evolutionary and ecological processes shape the patterns of genetic variation observed within and among natural populations. One focus of research and debate in this area has been on quantifying the roles of adaptive and neutral processes in explaining observed levels of genetic variation [4]. However, adaptation does not occur in isolation, but acts on genetic variation that is also shaped by mutation, recombination, gene flow, and genetic drift [5]. More recently there has been increased effort in understanding how these fundamental evolutionary forces operate in concert to generate and maintain the levels of genetic diversity commonly observed in natural populations [6,7].

The increasing feasibility of high-throughput sequencing and genotyping, alongside subsequent characterisation of genome-wide variation across large numbers of individuals, has revealed that at the genomic level, patterns of variation and divergence among natural populations and species are highly heterogeneous [8]. A key feature of these "genomic landscapes" of divergence that has received particular attention is the presence of so-called "islands of differentiation": outlier regions of the genome with high levels of divergence estimated from statistics such as F_{ST} or d_{xy} that consistently emerge at the same loci in different comparisons between populations or related species [8–11]. Initially these

139 regions were termed “islands of speciation”, and were thought to arise as a result of
140 reduced gene flow in genomic regions associated with reproductive isolation [8,12].
141 Subsequent research has revealed that highly heterogeneous patterns of genomic
142 divergence can occur even in the complete absence of gene flow, as a result of
143 recombination rate variation and linked selection [13,14]. In genomic regions of low
144 recombination, selection for beneficial mutations (positive selection), or against
145 deleterious mutations (background selection), will impact relatively large genomic regions
146 as a result of high levels of linkage disequilibrium (LD) among sites. Selection within these
147 regions reduces diversity within populations, and increases levels of differentiation among
148 them, resulting in “islands” of increased differentiation that persist over evolutionary time
149 [14,15]. Another, less well explored reason for which islands of divergence can arise is due
150 to the differential effects of genetic drift in response to variation in effective population size
151 across different genomic regions; something that may be particularly important in recently
152 colonised populations [16,17]. These circumstances promote fixation of haplotypes and
153 therefore result in either reduced or inflated local differentiation.

154 Comparing patterns of genomic differentiation among sets of populations or species at
155 different stages of the divergence/speciation continuum is a powerful way of disentangling
156 the forces that shape variation among populations. Martin et al. [18] showed that, across
157 multiple *Heliconius* butterfly populations and species, patterns of genomic variation were
158 shaped by a combination of gene flow and selection, particularly in genomic regions
159 harbouring genes involved in wing patterning. In contrast, Renaut et al. [10] showed that in
160 *Helianthus* sunflowers, genomic architecture was the main driver of genomic
161 differentiation across sets of populations. Similarly, recent research in birds has revealed

162 that differentiation landscapes are conserved across populations, species and even across
163 avian families, with the same islands of differentiation arising among populations of
164 distantly related species [19–21]. This latter pattern appears to have arisen, at least in part,
165 as a result of a highly conserved synteny and recombination landscape in birds [22], with
166 background selection in regions of low recombination producing recurrent islands of
167 differentiation [23].

168 It is now clear that the recombination landscape and linked selection are key drivers of
169 genomic variation within and among populations. However, we are only just beginning to
170 understand how this linked selection interacts with other evolutionary forces to shape
171 patterns of differentiation across natural populations and species [23–28]. A recent, large-
172 scale analysis of threespine sticklebacks (*Gasterosteus aculeatus*) showed that islands of
173 differentiation were more likely to arise in low recombination regions when gene flow
174 occurred between populations [29]. There is also a significant impact of divergence time; in
175 recently separated populations the differentiation landscape is most likely to reflect
176 selective sweeps. Then, as divergence accumulates, genomic architecture is expected to
177 play an increasingly important role in generating these genomic islands [23].

178 Widespread continental species are excellent models for studying how demography and
179 the environment shape genetic and phenotypic variation among populations, due to their
180 large effective population sizes and ecologically varied ranges. Insight into the evolutionary
181 history of such species can be gained if genetic variation is characterized across much of its
182 geographical range. Cross-population comparisons of genetic variation can then be utilised
183 to make inferences about phylogeography, levels of gene flow between populations and

184 how adaptation to different environmental and ecological conditions occurs [30]. The first
185 large-scale studies were performed in humans - i.e. the HapMap Projects [31–33] which
186 characterized human genetic variation on different continents, with a view to determining
187 the feasibility of association mapping studies. Similar studies have been conducted in
188 domesticated species and their wild ancestors [34–36], and in model organisms [37,38].
189 More recently, there is a growing appreciation that HapMap-type studies are useful for
190 studying signatures of selection and adaptation in natural populations of species with large
191 effective population sizes and high levels of gene flow [39–42].

192 The European great tit (*Parus major major*) is an excellent model for ecological and
193 evolutionary studies [43]. As is the case with several avian species which are amenable to
194 long-term study [44], a wealth of ecological data exists across multiple great tit populations
195 [45–47], enabling informed hypotheses about selection to be tested in this system.
196 Phyleogeographic research using mitochondrial DNA suggests that this species has recently
197 expanded across its European range, possibly from a single refugium in South-East Europe
198 [48]. Contemporary populations are characterised by large effective population sizes and
199 low levels of genetic differentiation [49,50]. However, these previous cross-population
200 molecular studies have relied on a modest number of microsatellite loci and mitochondrial
201 DNA, making the detection of genomic regions under selection impossible. The genome of
202 the great tit has recently been sequenced [51], and a high density panel of *ca* 500,000 SNP
203 markers has been developed [52]. A recent study of two European populations using this
204 marker panel suggests that rapid adaptation has occurred at the genomic and phenotypic
205 levels, with pronounced selection on morphology [53].

206 Here, we perform a HapMap study of 647 unrelated individuals across 29 populations (Fig.
207 1), to examine how genomic architecture, natural selection and population history have
208 shaped patterns of genomic variation across recently colonised European great tit
209 populations. Using a large SNP panel typed across all individuals, we first characterise
210 genome-wide patterns of variation within and among populations, in order to infer
211 population history. We then examine how variation is partitioned across the genome, and
212 test the hypothesis that highly divergent genomic regions have arisen in genomic regions of
213 low recombination [13,14]. Finally, we examine how genomic divergence accumulates
214 along the colonisation route of this species, with the aim of inferring how recent natural
215 selection and demography drive variation across the genome in the wild.

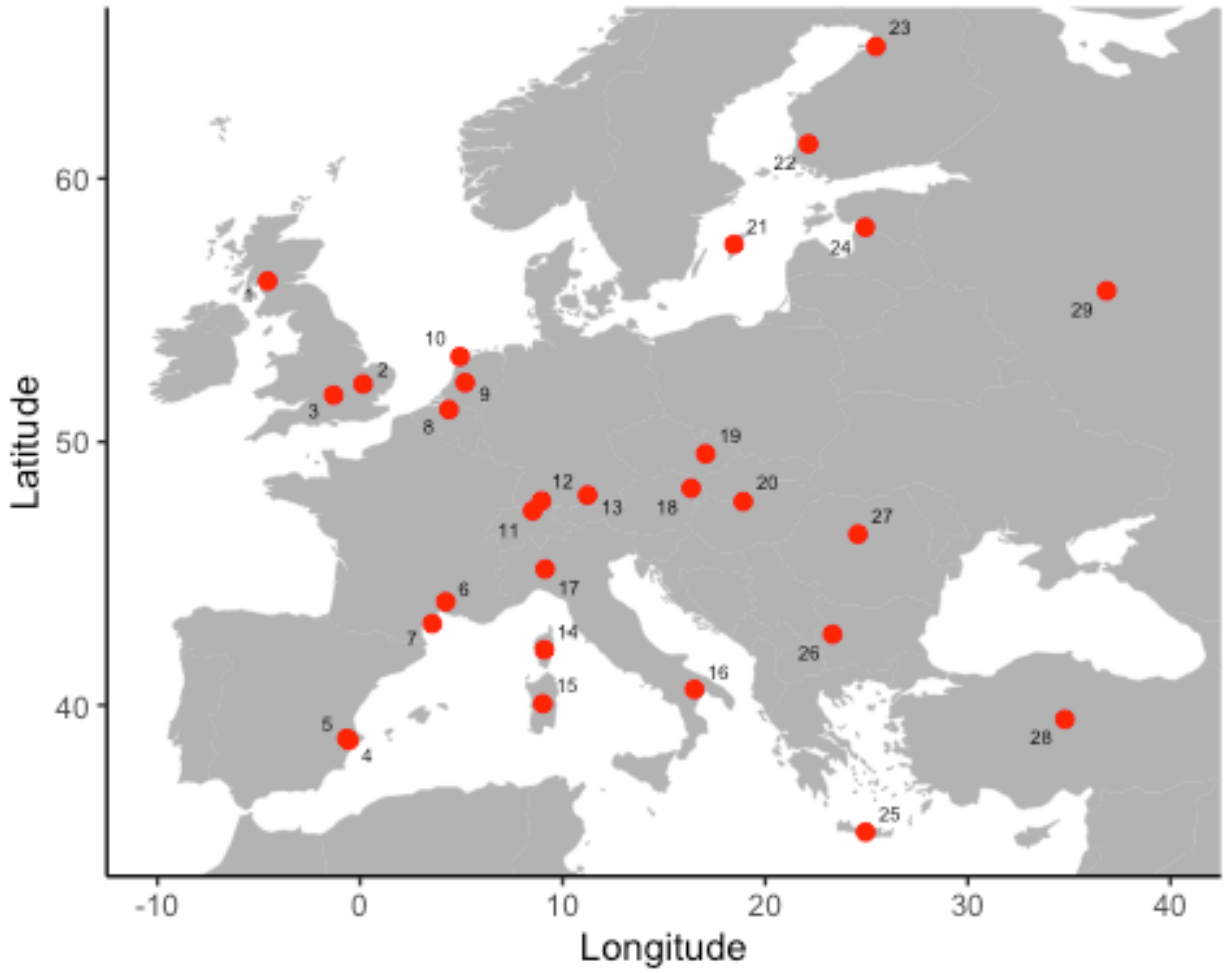

Figure 1 Sampling locations of great tit populations. Population names and sample sizes are given in Table S1, and numbers on the map correspond to the "code" column in Table S1.

Results and Discussion

Genetic diversity and population history

222 Sampling locations and sample sizes for each population are given in Table S1. Levels of
223 genetic diversity (π_{SNP}) were generally high, but we observed substantial differences
224 among populations (Fig. 2A). Similarly, LD declined rapidly with genomic distance in all
225 populations, reaching baseline levels within ~5kb in all populations, but also varied among
226 populations (Fig. S1). Highest levels of LD (and lowest levels of genetic diversity) were
227 observed in the Mediterranean island populations of Crete (Greece) and Sardinia (Italy),
228 with lowest levels of LD in central and western Europe (Fig. S1). This is consistent with
229 reduced effective population size in these island populations, either as result of the
230 colonisation process or more recent bottlenecks, along with low levels of subsequent gene
231 flow from the continent to the islands [54,55].

232 Mean genome-wide F_{ST} between all pairs of European great tit populations was 0.015, with
233 no significant pattern of isolation-by-distance (Mantel test; r = 0.13, p = 0.18; Fig. S2).
234 Instead, the highest levels of F_{ST} were found in comparisons involving the Mediterranean
235 island populations of Corsica (France), Sardinia and Crete (Fig. S2). Admixture analysis was
236 consistent with this pattern (Fig. S3); the K = 2 analysis assigned individuals in Sardinia
237 and Corsica to one genetic cluster, and the remaining populations to the second. Thus, it is
238 likely that much of the genetic structure between European great tit populations is a result
239 of genetic drift in these small island populations. Admixture analysis also revealed some
240 structure between (mainly peripheral) mainland and larger island populations. At K = 3
241 (the model that best fitted the great tit data; Fig. S4), Spain was separated from the rest of
242 mainland Europe. Increasing values of K resulted in the separation of populations in
243 Scotland (K = 4), Sardinia (from Corsica; K = 5), southern France (K = 6), Crete (K = 7) and
244 England (K = 8). The Admixture output at K = 8 is displayed in Fig. 2C as this gives the most

245 detailed picture of genetic structure among European great tit populations. Further
246 increases in K did not generate patterns of structure that corresponded to geographical
247 variation (Fig. S3), and were increasingly less well supported (Fig. S4). Thus, even with
248 hundreds of thousands of markers Admixture was unable to separate many of the
249 European populations, confirming that levels of divergence are extremely low [51]. PCA
250 largely corroborated the Admixture results, with PC1 separating Corsica and Sardinia from
251 the remaining populations, PC2 separating Spain, while PC3 and PC4 separated Scotland,
252 England, Corsica, Sardinia and Crete (Fig. S5).

253 Maximum likelihood analyses implemented in TreeMix showed that a model with no
254 migration explained 97.8% of variance in relatedness between populations [56]; increasing
255 the number of migration events substantially improved the percentage of relatedness
256 explained, up to 99.7% when 10 migration events were fitted (Fig. S6). In Figure 3 we
257 display the maximum likelihood tree with three migration events, after which the variance
258 in relatedness explained plateaued when more migration events were added (Fig. S6). The
259 tree was generally characterised by short branch lengths, with the exception of the island
260 populations of Sardinia and Crete, which were grouped with the population from mainland
261 Italy (Fig. 3). Thus, the TreeMix analysis is consistent with large populations and low
262 overall genomic divergence, with the exception of the Mediterranean island populations.
263 However, much (though not all) of the grouping that did occur among continental
264 populations made geographical sense, with populations from Finland and Estonia grouped
265 together, as were some populations from South-East Europe, and populations from
266 England and Scotland (Fig. 3). Interestingly, TreeMix grouped the Spanish and Corsican
267 populations, which is consistent with previous subspecies descriptions of European great

268 tits [57]. The three fitted migration edges all involved Sardinia, with migration from
269 eastern Europe to Sardinia, and from Sardinia to Corsica and Spain (Fig. 3).

270 We next tested the hypothesis that great tits colonised Europe from a single refugium in
271 South-East Europe. This scenario has been suggested before [48], but due to the low
272 number of genetic markers available there has been limited power with which to test this
273 hypothesis. Using our genome-wide panel of SNP markers, we compared genetic and
274 geographic distance between each population and the proposed refugial populations.
275 Because of the elevated structure in Corsica, Sardinia and Crete (Fig. S2), we excluded
276 comparisons involving these populations. We found that F_{ST} was significantly related to
277 distance from Turkey ($r = 0.81$, $p < 0.001$; Fig. 2B) and Romania/Bulgaria ($r = 0.44$, $p =$
278 0.001). The same relationship was not found for alternative potential refugial populations
279 [58] in Spain ($r = -0.09$, $p = 0.55$), or southern Italy ($r = 0.04$, $p = 0.77$). Our results
280 therefore lend empirical support to the hypothesis [48] that great tits colonised Europe
281 primarily from a single refugium in the south-east. Clearly, although our sampling was
282 extensive, it is not exhaustive, and more fine-scaled sampling in eastern Europe would be
283 required to determine the extact location and extent of refugial great tit populations.
284 Sampling in North Africa would also be useful to determine whether further refugia exist,
285 and to quantify the extent of admixture between European and African great tit
286 populations.

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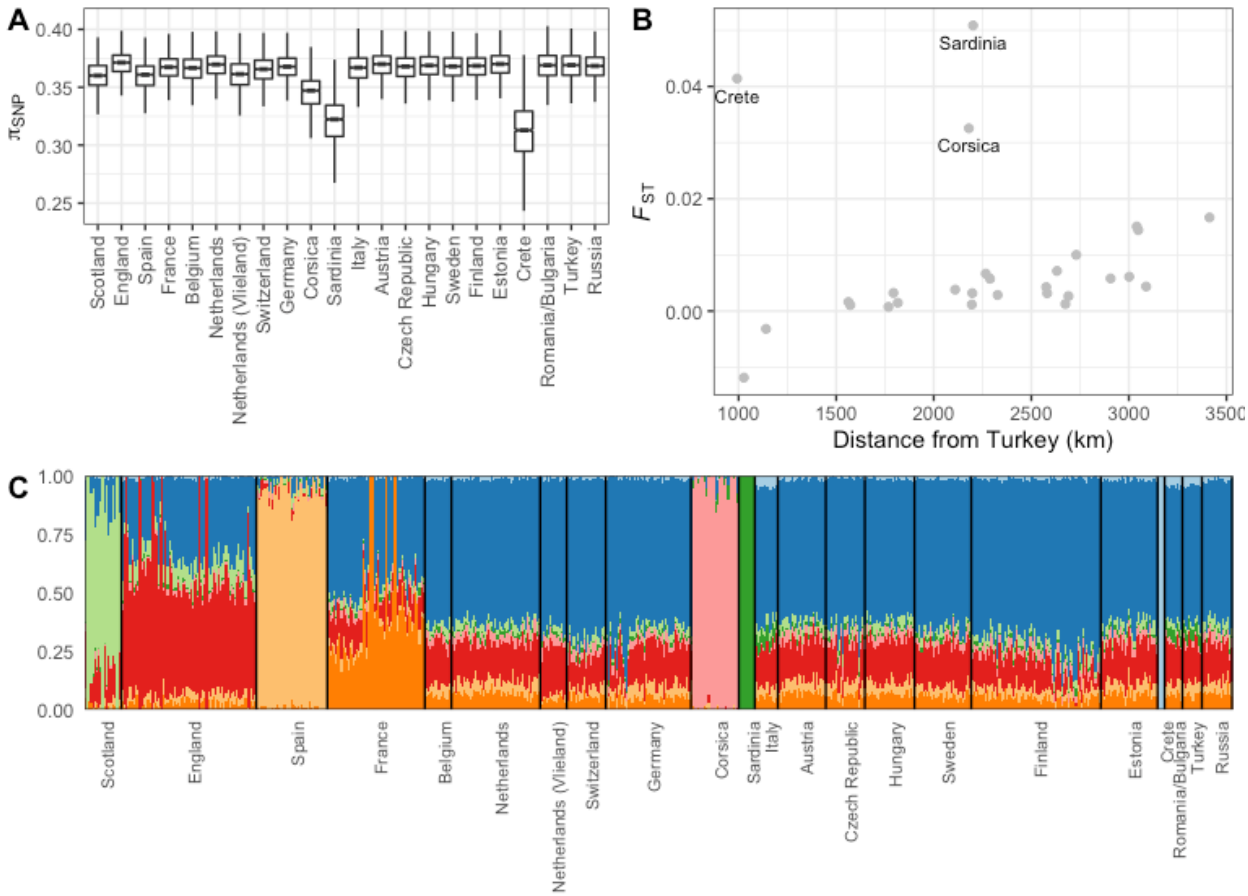

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289 **Figure 2** Genetic diversity and structure in European Great tit populations. **A** Nucleotide

290 diversity within each population. **B** Pairwise F_{ST} in relation to geographic distance from the

291 Turkey, only including comparisons involving Turkey. **C** Output from Admixture analysis at

292 K = 8. Population details can be found in Table S1.

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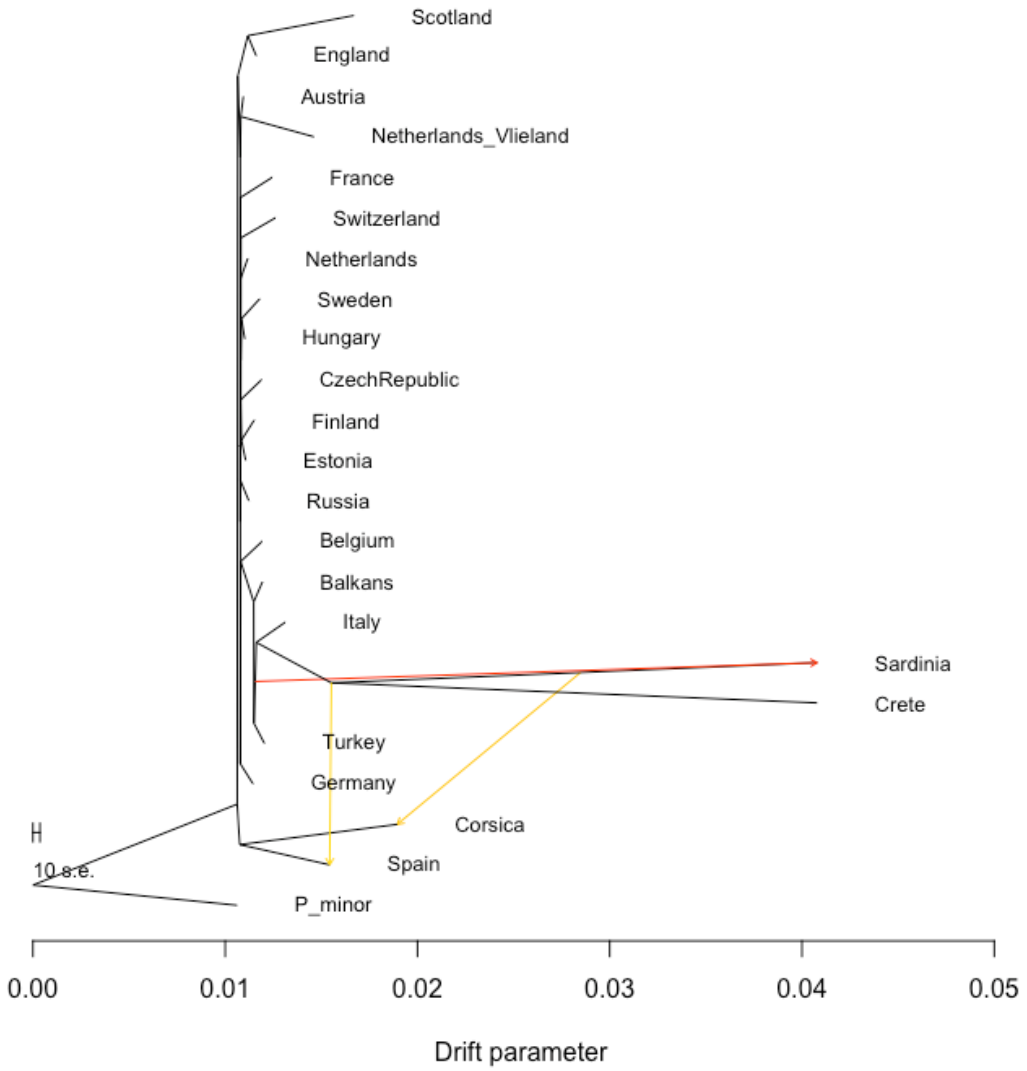

Figure 3 Maximum likelihood tree inferred by TreeMix, allowing three migration events. The two migration events (arrows) are coloured according to their weight (red = higher migration), and horizontal branch lengths are proportional to the amount of genetic drift that has occurred along the branch. A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

300

301 *Genomic landscapes of differentiation*

302 It is likely that many, and perhaps the majority, of wild populations are characterised by
303 highly heterogeneous patterns of differentiation across the genome [27]. This is the case in
304 European great tits - despite the extremely low average F_{ST}, we found genomic regions
305 with very high levels of genomic structure (maximum F_{ST} for 10kb and 500kb windows
306 was 0.98 and 0.07, respectively). To examine how landscapes of genomic divergence have
307 formed along the colonisation route of European great tits, we calculated windowed F_{ST} in
308 500kb bins between each population and the proposed refugial population in Turkey. We
309 found that F_{ST} varied markedly across the genome in all comparisons (Fig. 4; Fig. S7).
310 Outlier regions (windows with standardized F_{ST},hereafter zF_{ST}, > 10) were found in all
311 comparisons apart from Crete and Sardinia, in which overall levels of divergence were
312 highest, with some outlier regions found across multiple comparisons (Fig. 4). Our results
313 suggest, therefore, that genomic islands of differentiation can and do arise even among
314 recently separated populations.

315 Considering all populations, F_{ST} calculated in 500kb windows was strongly negatively
316 correlated with local variation in recombination rate (Spearman correlation, r = -0.50, p <
317 0.001). Although recombination rate and gene density were positively correlated, (r = 0.16,
318 p < 0.001), F_{ST} was only moderately correlated with gene density in 500kb windows (r = -
319 0.09, p < 0.001), and this correlation became weak when calculated in 10kb windows (r = -
320 0.01, p < 0.001). Thus it appears that the negative relationship between F_{ST} and
321 recombination rate is not driven by gene density. Examining how the relationship between
322 genomic differentiation and recombination varied among populations revealed that F_{ST}

323 was negatively related to recombination rate in almost all comparisons with Turkey (Fig.
324 5). The relationship between F_{ST} and recombination rate was generally weak, but in a
325 handful of populations this relationship was substantially stronger - most notably in the
326 island populations of Corsica, Sardinia and Crete, and in the peripheral mainland
327 populations of England, Scotland, Spain, Finland and France, which are the populations that
328 are likely most susceptible to drift (Fig. 5).

329 As a further examination of how allele frequency variation and haplotype structure may be
330 shaped by neutral and adaptive processes, we compared F_{ST} and recombination rate with
331 Rsb - a measure which aims to detect regions of the genome under positive selection by
332 comparing extended haplotype homozygosity profiles between populations, and is
333 expected to be less sensitive to variation in recombination rate because extended
334 haplotypes should be present in both populations when recombination frequency is lower
335 [59]. Specifically, we compared the distributions of these statistics in 500kb windows using
336 three populations in the Netherlands, Finland and Spain, with the aim of exploring how Rsb
337 varies with recombination rate and with F_{ST}. We found that, across the genome,
338 correlations between absolute Rsb and recombination rate were only slightly weaker
339 (Spearman rank r between -0.25 and -0.20) compared to those between F_{ST} and
340 recombination for the same pairwise comparisons (r between -0.36 and -0.20), and that the
341 regions of high F_{ST} in these comparisons tended to be in these same regions of low
342 recombination and high Rsb (Fig. S8). Notably, though, there were several cases where
343 windows with extreme Rsb values occurred in regions of high recombination, and most
344 high F_{ST} regions in high recombination regions were also Rsb outliers (Fig. S8). Below we

345 explore the possibility that such regions are strong candidates for recent adaptive
346 evolution.

347 Background selection is a key driver of genomic differentiation in birds [20,21], and other
348 organisms [60], but is not expected to play a major role in driving islands of differentiation
349 over the timescales (tens of thousands of years) relevant to this study [23,29]. On the other
350 hand, differential effects of drift operating across the genome could contribute
351 substantially to the highly heterogeneous patterns of differentiation observed in European
352 great tits. Our finding that the relationship between recombination rate and F_{ST} is strongest
353 in populations experiencing the greatest levels of drift (e.g. islands; Fig. 5) is consistent
354 with this - low recombining regions have reduced effective population size and increased
355 rates of lineage sorting due to drift. Drift has been suggested to be a driver of
356 heterogeneous genomic landscapes in other systems [16,17], and our study suggests that it
357 may play a key role in shaping genomic structure in widespread, continental species.

358 Outlier regions of very high differentiation (zF_{ST} > 10) mostly occurred in areas of the
359 genome with low recombination rates (Fig. 5) and the recombination rate of outlier regions
360 was (just) significantly lower than the genome-wide average (Wilcoxon test, P = 0.048). Of
361 the 11 outlier regions, nine were found in only one or two comparisons, while the other
362 two were found in 12 and 10 comparisons, respectively (Table S2). We hereafter refer to
363 outlier regions found in one or two comparisons as "population-specific" outlier regions,
364 and to those found in more than two comparisons as "shared" regions.

365 Regions of high differentiation that are not shared among populations could potentially
366 arise as a result of recent drift, but are also candidate regions for recent positive selection

367 [23]. Several lines of evidence suggest that this may be the case in European great tits.
368 Firstly, population-specific regions tended to occur in regions of higher recombination than
369 shared outliers, although low sample size prevents a formal statistical analysis of this.
370 Second, population-specific outliers tended to be found in the most peripheral European
371 great tit populations, with three found in Scotland, and two in England, Spain and Finland;
372 the remaining outlier regions were found in comparisons involving the Czech republic,
373 Russia, Vlieland (Netherlands) and Belgium (Table S2). Notably, these populations are not
374 the lowest in genetic diversity and therefore drift effects are not necessarily stronger in
375 these peripheral populations. Third, the Rsb tests, which indicate which population has
376 undergone a selective sweep, also tend to find evidence of positive selection in the more
377 peripheral population, as expected under an adaptive evolution scenario (Fig. S8). This
378 directionality cannot be assessed with F_{ST}, because it is a combined measure of allele
379 frequency differences. Of course, across the genome, both positive selection and linked
380 background selection are likely to be operating. Observational and experimental research
381 shows that adaptation at range edges is a key feature shaping divergence among recently
382 colonised and expanding populations [61–63]. Population-specific regions appeared less
383 likely to be in regions of low recombination than shared regions (Fig. 5), although the small
384 number of regions precluded testing this hypothesis formally. Thus, it is likely that genomic
385 architecture plays a key role in determining how both selection and drift have shaped
386 genomic variation across the recent evolutionary history of European great tits.

387 To further explore how selection may have shaped variation in F_{ST} outlier regions, we
388 estimated levels of nucleotide and haplotype diversity within these regions. Nucleotide
389 diversity (π_{SNP}) in outlier regions varied from 0.21 to 0.48, and diversity in these regions

390 was significantly lower than the genome-wide average (Wilcoxon test, P = 0.018; Fig. S9A).
391 However, there appeared to be little difference in nucleotide diversity between shared and
392 population-specific regions (Fig. S9A). Haplotype diversity varied substantially among
393 regions, with haplotype richness ranging from 72 to 1033. Both haplotype richness and
394 marker density in shared regions tended to be lower than those in population-specific
395 regions (Fig. S9B), consistent with lower recombination frequency. A detailed examination
396 of haplotype structure in one shared and one population-specific region is displayed in
397 Figure S10. The population-specific outlier region (to Finland, situated on chromosome 1A)
398 was characterised by a complex structure, with a single haplotype at high frequency in
399 Finland compared to other populations, indicating a population-specific selective sweep
400 and high background diversity (Fig. S10). In contrast, the shared region on chromosome 2
401 was much less complex, demonstrating high frequency haplotypes that are found across a
402 range of populations. Our data therefore suggest that examining patterns of haplotype
403 diversity in outlier regions may help to separate recent episodes of positive selection from
404 drift and background selection (Figs S9, S10).

405 Potential candidate genes found within shared and population-specific outlier regions are
406 displayed in Table S2. Perhaps most notable among these is *COL4A5*, a gene found to be
407 associated with bill length, and under selection between populations in England and the
408 Netherlands, in a recent great tit study [53]. Here we found that the *COL4A5* region is an F_{ST}
409 outlier in England and Scotland, but not in any other European populations (Table S2). UK
410 great tits have been described as a separate subspecies based on beak shape [64], and our
411 results here, combined with previous results, suggest that this divergence is the result of
412 recent natural selection in the UK [53]. Another notable candidate gene potentially

413 involved in beak morphology, and previously found to be under selection in UK great tits is
414 *BMPR1A*, which plays a key role in palate development [65] and in this study was found in
415 an outlier region in Scotland. Other candidate morphology and obesity genes in the
416 population-specific outlier regions in the UK included *PPP1CB*, which may play a role in
417 adipogenesis [66] and *GHITM*, which appears to have been subject to natural selection in
418 human pygmy populations [67]. Thus, morphological traits may frequently be involved in
419 adaptation in great tits.

420 In addition to morphological candidates in the UK, we found outlier regions specific to cold
421 populations in Scotland, Finland and Russia (Table S2), containing at least one candidate
422 gene for thermal stress (*CDKN1B*) [68]. Other genomic outlier regions contained potential
423 candidate genes associated with malaria infection (*MRPL33*) [69] and colour variation
424 (*SOX10*) [70]. This is thus far an exploratory analysis, and we are therefore reluctant to
425 speculate whether these candidate genes are genuine targets for natural selection, and
426 more reluctant still to speculate as to how selection might be driving variation at these
427 regions. Regardless, these candidates will provide useful starting points for future genomic
428 and ecological investigation.

429 HapMap style projects have been hugely informative in shaping our understanding of how
430 natural selection operates in humans and other model species [31,38]. This study is one of
431 the largest to date of genomic variation in a wild vertebrate, which has helped to reveal the
432 evolutionary history of great tits, and to identify candidate genes and traits that may have
433 been involved in adaptation during and/or after postglacial recolonisation. Further, this
434 work will form the foundation of many future analyses. Clearly, we have only touched on

435 haplotype-based methods to infer adaptation here, and this will be the subject of future
436 work. Environmental association approaches are also highly suited to detecting adaptation
437 in widespread continental species [71,72], and further work will test how variation in the
438 environment has shaped patterns of genomic variation in great tits [73]. This combination
439 of environmental and genomic data in species such as great tits, in which a wealth of
440 ecological and genomic resources are available, is likely to generate interesting insights
441 into the the genetic and phenotypic basis of natural selection.

442

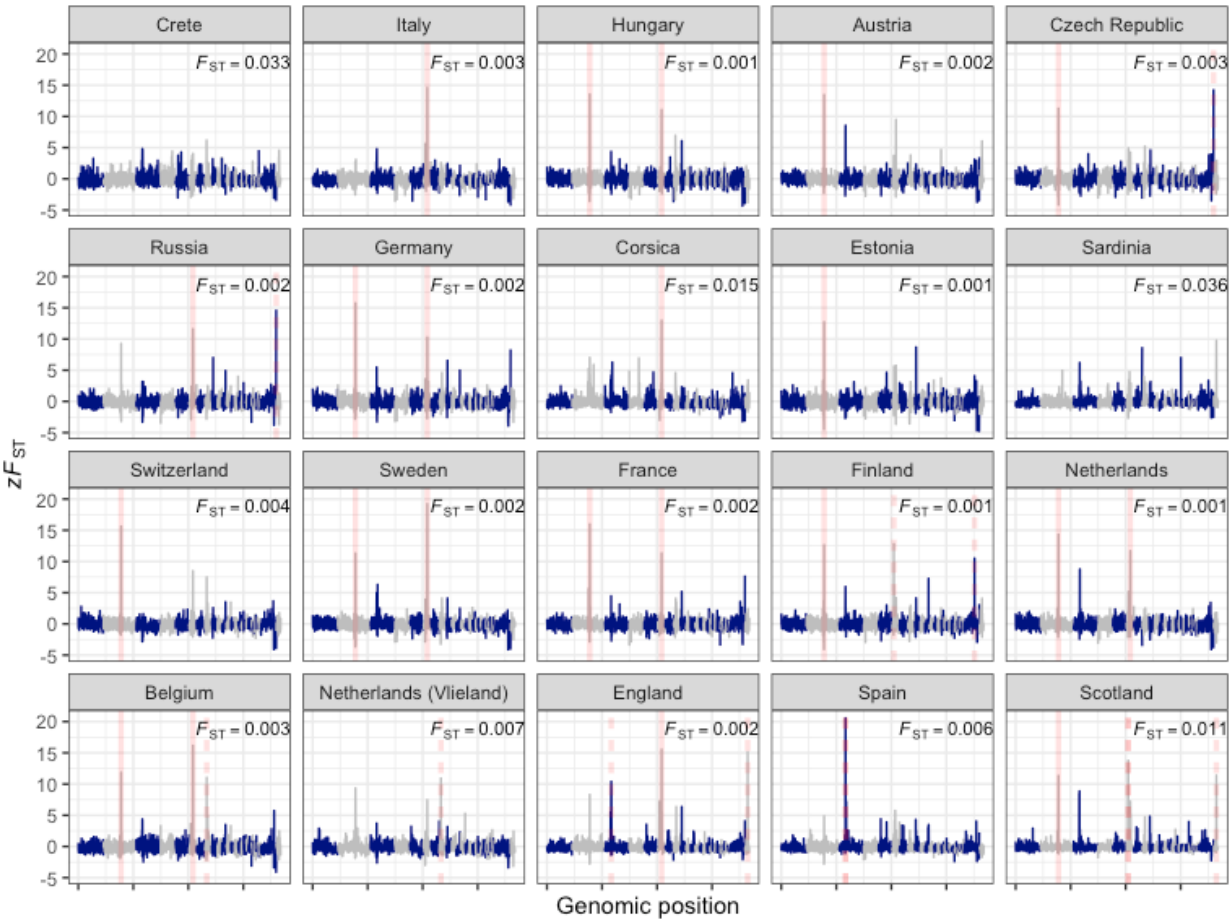

Figure 4 Landscapes of relative genomic differentiation in European great tit populations. zF_{ST} across the genome is averaged in 500kb windows, with each panel displaying a pairwise comparison with the proposed refugial population in Turkey. Red lines represent F_{ST} outliers (windows with mean F_{ST} values at least 10 standard deviations greater than the global mean for that comparison) shared across more than two comparisons (solid red lines), or specific to one or two comparisons (dashed red lines). Mean, untransformed F_{ST} values are given in the top-right of each panel, and are fully displayed in Fig. S7.

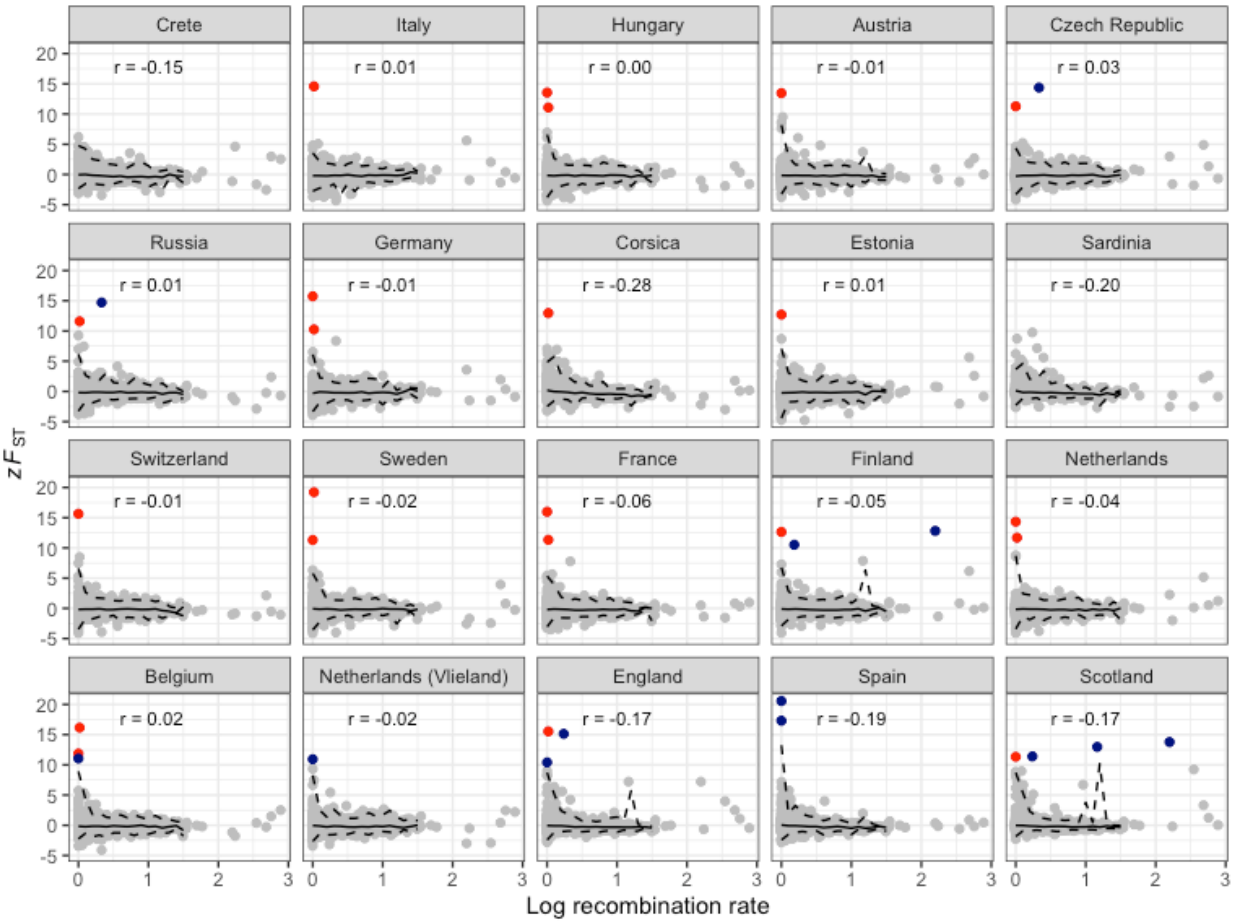

Figure 5 Genomic differentiation and recombination rate variation in European great tit populations. Each point is the mean of a 500kb window, with each panel displaying a pairwise comparison with the proposed refugial population in Turkey. Coloured points represent F_{ST} outliers (mean standardized F_{ST} values of zF_{ST} > 10) shared across more than two comparisons (red), or specific to one or two comparisons (dark blue). Solid and dotted lines represent median and 99% quantiles of F_{ST} windows from bins of 0.1 log cM/Mbp.

Materials and Methods

Sampling and molecular methods

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

Birds were trapped from nest boxes, or using mist nets, and ringed with a uniquely numbered aluminium ring. Blood was taken via brachial or tarsal venipuncture, and stored in either 1 ml Cell Lysis Solution (Gentra Puregene Kit, Qiagen, USA), Queen's buffer, or absolute ethanol. All samples were genotyped using a custom-made Affymetrix® great tit 650K SNP chip at Edinburgh Genomics (Edinburgh, United Kingdom), following the approaches outlined in [52]

Analyses

Unless stated otherwise, all population genetic statistics were calculated in PLINK version 1.9 [74], and downstream analysis and plotting was carried out in R version 3.3 [75]. For population genetic analyses, we used the filtering approaches outlined in [53]. Briefly, we randomly removed individuals from pairs with relatedness values > 0.4, and for demographic analyses we used a LD pruned dataset (based on VIF > 0.2), with SNPs

480 associated with an inversion on chromosome 1A [**???**] removed. After filtering, a total of
481 647 samples typed at 483888 SNPs were retained for analysis.

482 In each population, we estimated LD (R^2) for each pair of markers within 50kb on the same
483 chromosome, and compared this to physical distance between marker pairs. We calculated
484 observed heterozygosity for each SNP and population using a reduced SNP dataset, which
485 was pruned based on LD to remove all markers with $R^2 > 0.1$, then thinned with a
486 probability of retaining each variant of 0.25. We calculated genome-wide (mean) F_{ST}
487 between each pair of populations using the pruned and thinned dataset described above.
488 Pairwise F_{ST} was compared to geographic distance between populations using Mantel tests,
489 implemented in the Ecodist package in R [76]. We tested whether genetic structure was
490 related to distance from candidate refugial populations (in Romania/Bulgaria, Turkey,
491 Spain and Italy), using Pearson correlations. We also estimated population structure using
492 Admixture version 1.3, with default settings [77]. We varied values of K from one to ten; by
493 which point increasing values of K provided no informative information about population
494 structure (see results). Model support for each value of K was estimated by calculating 5-
495 fold cross-validation error. Finally, we visualised the evolutionary history among European
496 great tit populations by generating a maximum likelihood tree in TreeMix version 1.13
497 [56]. We rooted the tree using a sample of *P. minor* individuals sampled from Amur, Russia
498 [52]. We fitted models allowing for range of migration events (0-10), and used a window
499 size of 500 SNPs [56]. To assess model fit, we calculated the proportion of variance in
500 relatedness between populations explained by each model [56].

501 Recombination rates at each locus were estimated by comparing the location of SNPs on
502 the genome assembly (v1.1) with their location on the great tit linkage map [78]. Previous
503 linkage mapping, using a lower density SNP chip, was independently carried out in UK and
504 Netherlands great tit populations and the two maps were almost identical [78]. For the
505 purposes of this analysis, we used SNPs and marker intervals from the UK comprehensive
506 map. A total of 2706 SNPs were located on both the genome assembly and the linkage map.
507 Thus a positon in Mb and cM of each of these SNPs is known. All other SNPs on the HD chip
508 have a physical (Mb) position but no known linkage map position. The great tit genome
509 v1.1 is 1.02 Gb long, so the average physical interval between mapped SNPs is ~376Kb. The
510 linkage map position of each unmapped SNP was estimated by interpolation; by taking the
511 closest mapped SNP in either direction, and assuming a constant recombination rate in the
512 interval between those SNPs. For example, an unmapped SNP with physical position 1.4Mb,
513 flanked by mapped SNPs at 1.0Mb/0.5cM and 2.0Mb/1.0cM, would be estimated to be
514 located at 0.5 + (1.4-1.0)/(2.0-1.0) * (1.0-0.5) = 0.7cM. Having interpolated cM position of
515 every SNP, the local recombination rate was calculated as the cM interval spanned by the
516 nearest neighbouring SNPs, divided by the physical distance (bp) spanned by those same
517 neighbouring SNPs. In other words, for the ith SNP, the recombination rate is estimated as
518 the linkage distance between the i-1th and i+1th SNP, divided by the physical distance
519 between the i-1th and i+1th SNP. For downstream analyses, local recombination rates were
520 estimated by averaging across all SNPs in each 500kb window. We calculated gene density
521 in 10kb and 500kb windows using custom R scripts and the annotated great tit genome (v
522 1.1) [51].

523 We examined the genomic landscape of differentiation across European great tit
524 populations by calculating F_{ST} in 10kb and 500kb bins, using python scripts obtained from
525 Github (https://github.com/simonhmartin/genomics_general). We did not estimate d_{xy}, as
526 this parameter is difficult to estimate accurately from single SNP loci [14]. We also
527 calculated standardised F_{ST} (zF_{ST}) by mean-centring windowed values and dividing them
528 by the standard deviation among windows. We defined outlier regions as 500kb bins with
529 zF_{ST} values greater than ten. Finally, Rsb [59] was calculated for three pairwise
530 comparisons (in Spain, Finland and the Netherlands), using the R package Rehh [79], and
531 this averaged into 500kb windows. Unlike F_{ST}, the Rsb statistic gives an indication of which
532 population an adaptively important haplotype is under positive selection in, and is thought
533 to be less sensitive to local recombination rates [59]. Rsb estimation requires phased
534 genotype data, so phasing was performed using shapeIT2 v 2.r837 [80]. The –duohmm
535 argument was used to ensure that family information, where available, was used to
536 improve the accuracy of phasing. The –effective-size parameter was set at 500,000
537 reflecting the large effective population size of European great tit populations [51]. Local
538 recombination rates (measured in cM/Mbp) were used in the map files. Comparisons
539 between F_{ST}, Rsb, recombination rate and gene density were carried out using Spearman
540 rank correlation (as all statistics were highly skewed).

541 *Ethics Statement*

542 Great tit DNA samples were provided by researchers studying hole-nesting great tit
543 populations with permissions and licenses to study and sample birds granted by the
544 following organisations: Bundesministerium für Wissenschaft und Forschung (Ref. II/3b

Gentechnik und Tierversuche), Czech Republic Ministry of Education Youth and Sports (MSMT-56147/2012-310), Dutch legal entity Dier Experimenten Comissie (NIOO 14.11, NIOO 07.04), Dutch Central Authority for Scientific Procedures on Animals (AVD-801002017831), Estonian Ministry of Agriculture - Animal Procedures Committee (License 108), Ethical Committee of the Canton of Bern, Ethics Committee for animal experimentation of Languedoc Roussillon CEEA-LR (APAFIS#8608-2017012011062214), Ethics Committee of Lomonosov Moscow State University (Approval numbers 26ch, 37zh, 49zh, 59zh, 88zh), Federal Office for the Environment - Switzerland (License 234), Flemish Ministry - Agency for Nature and Forest, Generalitat Valenciana - Conselleria de Medi Ambient, Hellenic Ministry of Environment and Energy, Istituto Superiore per la Protezione e la Ricerca Ambientale - ISPRA (Law 157/1992 Art. 4 (1) and Art. 7 (5)), Mehmet Akif Ersoy University - Local Ethical Committee on Animal Experiments (93773921-9), Middle Danube Valley Inspectorate for Environmental Protection, Nature Conservation and Water Management (43355-1/2008), Regierung Oberbayern (55.2-1-54-2532-140-11), Regierungspräsidium Freiburg - Departments for Animal Welfare and Nature Conservation, Regional State Administrative Agency of Southern Finland (ESAVI/1650/04.10.03/2012, ESAVI-1804/04.10.03/2011), Romanian Academy of Sciences (Permit number 2257), Swedish Committee for Experiments on Animals (Licence number C 108/7), UK Home Office (License PPL 30/2409), University of Oxford - Department of Zoology Local Ethical Review Committee, University of Glasgow AWERB committee, Veterinary Office of the Canton of Zurich (License 195/2010)

Samples were imported to the UK under DEFRA general import license IMP/GEN/2011/01, according to regulations outlined by the UK Animal Health and Veterinary Laboratories Agency.

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Data Availability

All data and code to reproduce the results are available on Github:
https://github.com/lgs85/SpurginBosse_Hapmap

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