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1 **Title: Recent natural selection causes adaptive evolution of an avian polygenic trait**

2

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18 **One Sentence Summary:** We identify genomic regions that have evolved under selection, and that
19 explain variation in bill length and fitness in great tits.

20

21 **Abstract:** We use extensive data from a long-term study of great tits (*Parus major*) in the UK and
22 Netherlands to better understand how genetic signatures of selection translate into variation in fitness
23 and phenotypes. We found that genomic regions under differential selection contained candidate genes
24 for bill morphology, and used genetic architecture analyses to confirm that these genes, especially the
25 collagen gene COL4A5, explained variation in bill length. COL4A5 variation was associated with
26 reproductive success which, combined with spatiotemporal patterns of bill length, suggested ongoing
27 selection for longer bills in the UK. Finally, bill length and COL4A5 variation were associated with usage
28 of feeders, suggesting that longer bills may have evolved in the UK as a response to supplementary
29 feeding.

Main Text:

To demonstrate evolutionary adaptation in wild populations we must identify phenotypes under selection, understand the genetic basis of those phenotypes along with effects on fitness, and identify potential drivers of selection. The best-known demonstrations of genes underlying evolution by natural selection usually involve strong selection ('hard sweeps') on genetic variants, that may be recently derived, with a major effect on variation in preselected phenotypes (1–3). However, most quantitative phenotypes are polygenic (4) and for these traits selection is likely to act on many pre-existing genetic variants of small effect (5). Detecting so-called polygenic selection is challenging because selection acts on multiple loci simultaneously and selection coefficients are likely to be small (6). Most attempts to detect polygenic selection have focused on gene sets, rather than individual loci (e.g. (7)). Furthermore, even if population genomics analyses identify genes under selection, these analyses are rarely combined with detailed ecological and behavioral data (8–10), and as a result linking all three components of the genotype-phenotype-fitness continuum remains a challenge. In this study we combine fine-scale ecological and genomic data to study adaptive evolution in the great tit (*Parus major*), a widespread and abundant passerine bird and well-known ecological model system (11) with excellent genomic resources (12). To do so, we analyzed genomic variation within and among three long-term study populations from the UK (Wytham, $n = 949$) and the Netherlands (Oosterhout, $n = 254$ and Veluwe, $n = 1812$; Fig. 1A).

After filtering (see methods), our dataset comprised 2322 great tits typed at 485,122 SNPs. Levels of genetic diversity were high and linkage disequilibrium (LD) decayed rapidly within all three sample sites (fig. S1). Admixture and principal component analyses (PCA) both suggest that genetic structure is low (Fig. 1, B and C). These findings demonstrate a large effective population size and confirm high levels of gene flow in the species (12, 13), making the long-term study populations well suited to studying evolutionary adaptation.

55 To identify loci under divergent selection between the UK and Dutch populations, we ran a genome-
56 wide association study using the first eigenvector from the PCA as a ‘phenotype’ (EigenGWAS (14)).
57 We identified highly significant outlier regions of the genome likely to be under divergent selection (fig.
58 2A, S2), which were supported by F_{ST} analyses (fig. S3). The majority of these outlier regions contained
59 candidate genes (e.g. COL4A5, SIX2, TRPS1, NELL1) involved in skeletal development and
60 morphogenesis (Fig. 2, A to C, table S1 and external database S1). Genes associated with the ontology
61 term “palate development” (GO:0060021; genes ALX4, BMPR1A, SATB2, INHBA, GLI3) were more
62 significantly overrepresented than any other GO term (Fig. 2C; Bonferroni-corrected $p = 2.9 \times 10^{-5}$;
63 external database S1). The strongest single-marker signal was found at the LRRIQ1 gene (table S1,
64 external database S1), where there was evidence of selection in Wytham, but not Veluwe (fig. S4).
65 LRRIQ1 is one of four genes located in the 240kb region associated with beak shape in Darwin’s finches
66 – arguably the best-known example of a trait undergoing adaptive evolution in the wild (15). Another
67 EigenGWAS peak contained VPS13B, a gene also associated with bill morphology in the Darwin’s finch
68 study, and with facial dysmorphism in humans (16).

69

70 Our genetic analyses therefore suggested bill morphology as a key trait involved in differentiation
71 between UK and Dutch great tit populations. Previously UK great tit populations have been characterized
72 as a different subspecies (*P. major newtoni*) compared to the rest of mainland Europe based on bill length,
73 but this classification is disputed (17) and it is unknown whether any bill length differences are adaptive
74 in this species. We examined the genetic architecture of bill length in the UK population, using two
75 complementary approaches. First, we fitted all SNPs simultaneously in a mixture model analysis (18),
76 and estimated that 3009 (95% credible interval 512-7163), or 0.8%, of the SNPs contributed to bill length
77 variation, suggesting that bill length is highly polygenic. Collectively these SNPs explained ~31% of the
78 phenotypic variation. The proportion of variance in bill length explained by each chromosome scaled
79 with its size, which is also consistent with a polygenic architecture (4) (fig. S5). Second, and consistent

80 with the mixture model analysis, we found multiple nominally significant SNPs in a GWAS on bill length
81 in Wytham, but even the most significant ($p = 1.6 \times 10^{-6}$) was not genome-wide significant after
82 accounting for multiple testing, perhaps as a consequence of small effect size and modest sample size.
83 Nonetheless, the SNPs were associated with bill length variation independently of overall body size
84 (Table S2). Using a sliding window approach, we found that the most significant GWAS regions largely
85 overlapped with the most significant regions in the EigenGWAS and F_{ST} analyses (Fig. 2, A and B, fig.
86 S3), suggesting that genes involved in bill length have been under divergent selection between
87 populations. We extracted SNPs from the most significant EigenGWAS peaks, calculated the summed
88 effect of those SNPs on bill length, and compared this against a null distribution generated by randomly
89 resampling the same number of SNPs and regions from across the genome. The regions under selection
90 explained a small amount of variation (0.54%) in bill length in the UK population, but this is more than
91 expected by chance ($p = 0.004$; fig. S6). Moreover, genomic prediction analysis using just the SNPs from
92 the EigenGWAS peaks showed that UK birds had breeding values for longer bills than birds from the
93 Netherlands (fig. S7), confirming that inter-population differences in bill length is at least partially
94 attributable to the loci that have been under recent selection.

95

96 The three genomic regions most notably associated with bill length variation and under likely divergent
97 selection (Fig. 2, A and B) all contained genes with annotations that make them candidates for
98 involvement in bill length. SOX6 is a transcription factor, and PTHrP a member of the parathyroid
99 hormone family; both are essential for bone development (19, 20). COL4A5 is a type IV collagen gene
100 best known for its association with Alport's syndrome in humans (21), that has also been identified as a
101 candidate for craniofacial disorders (22). The ~400kb region of chromosome 4A containing the COL4A5
102 gene was the region most notably associated with bill length (4 of the 24 most significant SNPs in the
103 GWAS were in COL4A5; Table S2), and belongs to the top three regions under strongest divergent
104 selection between birds from the UK and Netherlands (Fig. 2, A and B). A closer inspection of the

individual SNPs within SOX6 and PTHrP reveals numerous SNPs that are nominally significantly associated with bill length, but none as strongly as the COL4A5 SNPs; thus we focus on the COL4A5 locus hereafter. Patterns of genetic variation at COL4A5 reveal a clear signature of recent selection for longer bills in the UK. First, the allele at the SNP that is most significantly associated with increased bill length (hereafter 'COL4A5-C'; Fig. 3D), is at higher frequency in the UK (0.54, bootstrap 95% confidence intervals = 0.52-0.56) compared to the two Dutch populations (Veluwe: 0.28, CI = 0.27-0.29; Oosterhout: 0.26, CI = 0.23-0.29). Second, extended haplotype homozygosity tests confirm that the haplotype carrying the COL4A5-C allele extends further than alternative haplotypes within Wytham (Fig. 3, A to C). The COL4A5-C haplotype is longer and more abundant in Wytham compared to Veluwe, and LD at this locus is much higher in Wytham, suggesting selection is UK-specific (fig. S8). Third, SNP data from 15 European populations, including 3 UK populations, shows that the COL4A5-C allele is at a higher frequency across the UK than across Europe (LGS et al. In Prep), consistent with selection on this gene in the UK.

To further elucidate how natural selection has shaped variation in bill length across the two populations, we tested how variation at the COL4A5 locus was related to annual reproductive success. We found differences in the relationship between COL4A5 genotype and the number of chicks fledged between the two populations (zero-inflated Poisson GLMM, interaction between genotype and population: $n = 3076$ breeding attempts from 1790 birds, estimate = -0.40 ± 0.17 , $p = 0.016$, Fig. 3E). The interaction was significant because the associations between genotype and bill length in the two populations were in opposite directions; in the UK, the number of copies of the 'long-billed' COL4A5-C allele was positively associated with fledgling production ($n = 868$ breeding attempts from 516 birds, estimate = 0.23 ± 0.11 , $p = 0.046$, Fig. 3E; fig. S9), whereas in the Dutch birds COL4A5-C was negatively, but not significantly, associated with fewer fledglings ($n = 2208$ breeding attempts from 1274 birds, estimate = -0.16 ± 0.10 ,

129 $p = 0.093$). The relationship between fledgling production and COL4A5 genotype did not arise because
130 long-billed genotype birds were more likely to produce offspring (binomial GLMM: $n = 3076$ breeding
131 attempts from 1790 birds, estimate = -0.20 ± 0.17 , $p = 0.91$); rather, when we only considered
132 “successful” breeding attempts in which at least one fledgling was produced, long-billed genotype birds
133 produced more fledglings (Poisson GLMM: $n = 2690$ breeding attempts from 1612 birds, estimate =
134 0.058 ± 0.024 , $p = 0.018$). Thus, we suggest that the COL4A5 allele associated with longer bills confers
135 a fitness advantage in the UK population.

136

137 To better understand the evolutionary consequences of selection for longer bills in the UK population,
138 we examined spatiotemporal variation in bill length. In museum samples from the UK and mainland
139 Europe, the UK individuals had considerably longer bills ($n = 291$, estimate = 0.40 ± 0.06 mm, $p = 5.2$
140 $\times 10^{-12}$, $R^2 = 0.16$, Fig. 4A), in accordance with a previous study (17). Using a 26-year dataset from live
141 birds in Wytham, we found that bill length has increased significantly over recent years (1982-2007; $n =$
142 2489, estimate = 0.004 ± 0.001 mm per year, $p = 0.0038$, R^2 of year effect = 0.004, Fig. 4B, table S3;
143 with tarsus length fitted as a covariate, the significant temporal increase in bill length remained
144 significant - $n = 2485$, estimate = 0.005 ± 0.001 mm per year, $p = 0.0001$, R^2 of year effect = 0.003). This
145 effect, though weak in terms of the variance explained, is not due to stochastic variation among years
146 (randomization test, $P = 0.02$, Supplementary Materials), and is equivalent to an evolutionary rate of
147 change of 0.0154 Haldanes; in a large review of phenotypic change in wild animal populations this rate
148 was exceeded in just 641 of 2420 estimates (23).

149

150 Selection on bill-length has been documented multiple times in birds, and is typically associated with
151 variation in food availability (24). No differences in the natural diet of great tits between the UK and
152 mainland Europe are known. In contrast, bird feeding by the public has been widespread in the UK since

153 the 19th Century; it is estimated it occurs in over 50% of gardens (25) and that the UK's expenditure on
154 bird seed is twice that spent in the whole of mainland Europe (26). Great tits are particularly good at
155 exploiting bird feeders (27), and therefore we investigated whether supplementary feeding could have
156 been a driver of selection on bill length in UK great tits, similar to that proposed in UK blackcap (*Sylvia*
157 *atricapilla*) populations (28). Radio Frequency Identification (RFID) bird feeders throughout Wytham
158 recorded RFID-tagged great tit utilization of supplementary food over the course of three winters (29).
159 We found that COL4A5-C homozygotes displayed a higher propensity to use the feeders compared to
160 heterozygotes or short-billed homozygotes ($n = 444$, estimate = -0.17 ± 0.08 , $p = 0.03$, Fig. 3F). There
161 was some variation in the extent of this effect across winter seasons (Fig. S10), and the strength and
162 consistency of this effect, along with the mechanisms behind it, requires further investigation.
163 Encouragingly, however, a follow-up analysis using a more recent dataset gathered from high-resolution
164 RFID feeders (but on un-genotyped birds) showed a positive relationship between feeding propensity
165 and bill length ($n = 1806$ observations of 183 birds, estimate = 0.15 ± 0.05 , $p = 0.004$, Fig. S11).
166
167 Together, our results provide a detailed example of natural selection in a wild animal. Starting with a
168 bottom-up analysis of genomic data, and no-preselected phenotypes, we have demonstrated polygenic
169 adaptation by providing associations between loci that have responded to selection, fitness variation,
170 phenotypic variation, microevolutionary change and a possible driver of selection. Combining large-
171 scale genomic and ecological data in natural populations will significantly enhance our understanding of
172 both the mechanistic basis and evolutionary consequences of natural selection.

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276 samples. M.A.M.G., K vO., M.E.V. and J.S. coordinated collection of SNP data. M.B., L.G.S, V.N.L.
277 and J.S. cleaned and QC checked SNP data. M.B., L.G.S and J.S. wrote the manuscript with input from
278 all other authors. The data described in the paper are archived on Dryad with accession number XXX.

279 **Supplementary Materials**

280 Materials and Methods

281 Supplementary Text

282 Tables S1 – S3

283 Fig S1 – S9

284 Caption for database S1

285 References (30–44)

286

287 **Fig. 1. Population structure of Western European great tits.** (A) Worldwide distribution of *P. major*
288 and sampling locations in Wytham (▲) Oosterhout (■) and Veluwe (●). (B) Principal component
289 analysis of genotype data. (C) ADMIXTURE plot with $K=3$, which is both the most likely number of
290 clusters and the number of geographically distinct sampling sites. Levels of genetic structure are low
291 (F_{ST} Veluwe-Wytham = 0.006, and F_{ST} Veluwe-Oosterhout = 0.003).

292

293 **Fig. 2. Differentiation and regions under selection across two great tit populations.** (A) Upper panel:
294 EigenGWAS on PC1 across all autosomes, averaged over 200kb sliding windows. Genes surrounding or
295 covering peaks are indicated. Gene names highlighted in bold green belong to the most significant GO-
296 term 'palate development'. Lower panel: GWAS for bill length in the UK population, averaged over
297 200kb sliding windows. Color-highlighted regions indicate peaks found in both the GWAS and
298 EigenGWAS analyses. (B) EigenGWAS p-values in relation to bill length GWAS p-values averaged
299 over 200kb windows. Color-highlighted points correspond with the highlighted regions in (A). (C) Gene
300 Ontology network of genes in or surrounding the EigenGWAS peaks. Size of circles indicates
301 significance and line thickness indicates proportion of shared genes.

302

303 **Fig. 3. COL4A5 locus on chromosome 4A.** (A) 2Mb zoom of EigenGWAS (green triangles) and GWAS
304 (black circles) p-values at the COL4A5 region (highlighted blue in Fig. 2A). Red horizontal bars indicate
305 gene locations (B and C) Bifurcation diagram for haplotypes in Wytham, starting from the two alleles at
306 the most significant GWAS SNP. Note the extended haplotype at the COL4A5-C-allele in (C), relative
307 to the shorter haplotypes at the COL4A5-T allele in (B), consistent with a recent selective sweep around
308 the COL4A5-C allele in the UK. (D) Bill length and COL4A5 genotype; the C allele is associated with
309 longer bills ($R^2 = 0.035$). (E) The COL4A5-C allele is associated with greater annual fledgling production
310 in the UK population ($R^2 = 0.015$). (F) COL4A5-C allele birds display greater winter feeding site activity
311 – the y axis is \log_{10} transformed cumulative activity records ($R^2 = 0.01$). Lines and shaded areas in d-f
312 are fitted values and 95% confidence limits from general(ized) linear models (full data are plotted in Figs
313 S8 and S9).

314

315 **Fig. 4. Spatiotemporal variation in bill length.** (A) Bill lengths of museum samples from the UK and
316 mainland Europe.(B) Temporal variation in bill length in the Wytham population plotting annual
317 means with standard error from 1982-2007. Line and (narrow) shaded area in b are fitted values and
318 95% confidence limits from a linear regression ($R^2 = 0.004$); note different scales on axes in A and B.



Supplementary Materials for

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Materials and Methods
Supplementary Text
Figs. S1 to S11
Tables S1 to S3
Caption for database S1

Other Supplementary Materials for this manuscript includes the following:

Database S1 as zipped archives: Markers used and results from gene ontology analyses

Materials and Methods

Sampling

Sample sites: Samples were collected from three distinct forest areas in Western Europe (Fig. 1A): Wytham (UK); Oosterhout (Netherlands) and Veluwe (Netherlands). All three sample locations are long-term study sites for great tit research.

Sampling: Blood was collected from a total of 949 specimens in Wytham, 254 in Oosterhout and 2058 in Veluwe. Blood samples were stored in either 1 ml Cell Lysis Solution (Gentra Puregene Kit, Qiagen, USA) or Queen's buffer. DNA was extracted from these samples by using the FavorPrep 96-Well Genomic DNA Extraction Kit (Favorgen Biotech corp.). DNA quality and DNA concentration were measured on a Nanodrop 2000 (Thermo Scientific).

Genotyping and filtering

Great tits were genotyped using a custom made Affymetrix® great tit 650K SNP chip at Edinburgh Genomics (Edinburgh, United Kingdom).

Netherlands birds: A total of 2066 female great tits were genotyped and passed quality control. SNP calling was done following the Affymetrix® best practices steps in Axiom® Genotyping Solution Data Analysis Guide by using the Affymetrix® Genotyping console 4.2.0.26. Eight individuals with dish quality control value of <0.82 were discarded. SNP quality control was done by using Affymetrix Power Tools software package 1.16.1 and the functions `Ps_Metrics` and `Ps_Classification`. The recommended SNP group consisted of 505,604 SNPs while 105 366 SNPs were discarded because their call rate was below the threshold (<0.97), because they were “off-target” variants or because they belonged to the “other” group of suboptimal SNPs. In addition to the SNPs that did not pass the `Ps_Metrics` and `Ps_Classification` steps, an additional 388 SNPs were removed because they were duplicates or the genomic position was missing. Altogether 505,216 SNPs passed initial quality control.

UK birds: SNP calling was performed using the Affymetrix Axiom Analysis Suite 1.1.0.616, the successor of the Genotyping Console described above. The same quality control thresholds were used as for the Netherlands birds; samples with dish QC < 0.82 or call rates <0.95 were discarded, as were SNPs with call rates <0.97 . A total of 1,846 samples typed at 498,036 SNPs were retained for analysis. 996 of the samples, which included replicates for error checking, were from Wytham Woods – the remainder were from other populations that are not the focus of this study. Replicated error samples suggested a per SNP genotyping error rate of 0.004 (among samples with call rates >0.98 , the error rate was 0.002).

After this initial filtering, the two datasets were merged. Only SNPs that passed filtering steps in both populations were selected for further analysis. This final step resulted in 485,122 SNPs that passed our quality control steps in both populations.

Genetic diversity analyses

Pairwise relatedness between individuals and linkage disequilibrium for all SNP pairs up to 200kb apart within populations were calculated per population in PLINK v1.90b3x (30). Based on a visual inspection of the distribution of relatedness values, we removed individuals from pairs with relatedness > 0.4 for all further analyses, leaving us with 2322 birds from the three sample sites. Pairwise F_{ST} between the three populations was calculated in PLINK. The filtered dataset was LD-pruned in PLINK (Variance Inflation Factor > 2) which resulted in 375,846 SNPs. Principal component analysis was performed on the filtered and pruned dataset using the GCTA package (31). A small percentage of birds from all three populations displayed atypical clustering based on SNPs on chromosome 1A, possibly representing a large inversion (data not shown). Therefore, chromosome 1A, as well as chromosome Z and the small linkage groups were excluded from PCA analysis. Admixture analysis was performed on the filtered and pruned data using the software package ADMIXTURE v1.23 (32) with K ranging from 2 to 5.

Selection analyses

The populations were screened for between-population signatures of selection with two distinct measures. The EigenGWAS package (14) was used to apply a GWAS framework to the first two eigenvectors of the principal component analysis. The main rationale for using EigenGWAS over an F_{ST} outlier locus test, is that it is more flexible. There is no need to predefine populations (although clearly we can do so here), and the analysis accounts for population stratification (e.g. due to the presence of relatives). The EigenGWAS is quick and easy to implement, and the results are conceptually comparable to a standard GWAS (i.e. where markers are used to identify genomic regions that explain phenotypic variation). Chromosome 1A was excluded from the PCA but included in the EigenGWAS, due to the potential inversion (see above). As a comparison to the EigenGWAS test, we also calculated single-marker pairwise F_{ST} using PLINK, with predefined clusters according to sampling sites. F_{ST} and EigenGWAS results were almost identical (fig. S2) We used Pearson's product-moment correlation in R(33) to test for correlation between the EigenGWAS corrected p-value and F_{ST} between Veluwe and Wytham.

We screened the populations for more recent selective sweeps at the COL4A5 locus with an extended haplotype homozygosity (EHH) test (34). First, the full dataset was phased with Shapeit v2 (35) with inclusion of pedigree information. After filtering for related individuals ($IBD > 0.4$), EHH was generated for each SNP in both populations, identifying long and frequent haplotypes as implemented in the R package rehh (36). We screened for population-specific extended haplotypes with Rsb, a statistic that compares EHH between populations to detect between-population selection (37). At the most significant GWAS marker at the COL4A5 locus the p-value was generated from the normal cumulative density function for genome-wide Rsb values. Starting from the core marker with highest GWAS p-value, a bifurcation diagram was created for both alternative alleles using rehh.

Gene ontology analyses

Regions under selection were tested for an overrepresentation of genes belonging to specific gene ontology terms. Candidate genes for all EigenGWAS peaks containing markers with p -value $<10^{-9}$ were extracted if they were 1) overlapping the most significant marker; 2) surrounding the peaks if no overlapping gene was present. Since the peaks are relatively narrow, mostly only one or two genes overlapped the peaks (see Additional Data table S1). Candidate genes were extracted from the great tit reference annotation (NCBI Parus major Annotation Release 100).

Functional relatedness of Gene Ontology (GO) terms was performed using the Cytoscape plugin ClueGO 2.2.4 (38). ClueGO constructs and compares networks of functionally related GO terms with kappa statistics. A two-sided hypergeometric test (enrichment/depletion) was applied with GO term fusion, network specificity was set to 'medium' and false discovery correction was carried out using the Bonferroni step down method. We used both human (8.3.2016) and chicken gene ontologies (9.3.2016) for comparison. With human gene ontologies we detected 16 functional groups of GO terms (Supplementary Data). These groups were mainly involved in functions concerning palate development, positive regulation of osteoblast differentiation and mesoderm formation. When using the chicken orthologues the results were comparable, but with more significant GO groups (26 groups) and with higher P values (Supplementary Data). This is because the chicken genes were not as well GO-annotated as the human genes.

Genetic architecture of bill length

To understand the genetic architecture of bill length we used two fundamentally different approaches. First, a genome-wide association study (GWAS) was performed to test for associations between SNP genotypes and the focal trait, fitting one SNP at a time. However, a GWAS on a dataset of this magnitude is unlikely to detect genome-wide significant loci, unless there are major effect loci affecting the trait. Second, to further understand the architecture of bill length we ran analyses that fitted all SNPs in one model; hereafter we call this the 'BayesR' analysis after the method (39) and software (18) used to perform the analysis. BayesR can simultaneously estimate effect sizes of individual SNPs, making it possible to estimate a trait's heritability, partition variation across the genome, and perform genomic prediction. There were several rationales for performing this analysis. First, we could investigate whether bill length is a polygenic trait. Second, we could estimate the effects of all SNPs on bill length in one model, and then use these estimated effects to ask whether regions under selection disproportionately contribute to bill length variation. Third, we could perform genomic prediction to test the extent to which the differences in bill length between the UK and Netherlands populations are caused by the SNPs under selection.

GWAS analysis

A GWAS was conducted on bill length in using a dataset of 150 measurements from 89 Wytham birds, using the GenABEL package (40). Due to repeated measures, a mixed effects model was first fitted to extract individual random effects for bill length. Random

effects in the model included individual identity, year of birth and age category (used to distinguish recent fledglings, adult birds of different ages and birds of unknown age). Sex was fitted as a fixed effect. The GenABEL functions `polygenic` and `grammar` were used to run a GWAS that uses genomewide realized relatedness to control for population structure caused by the presence of related individuals (41). To test whether the effect was caused by correlated traits, we ran the same analysis on bill length with bill depth and tarsus lengths for the same birds included as covariates in the model.

BayesR analysis

The BayesR analysis was performed using default parameters - SNP effects were assumed to be drawn from a mixture of 4 normal distributions, with SNPs having a variance of 0, 0.0001, 0.001 or 0.01 of the genetic variation. MCMC chains were run for 50,000 samples, with a 20,000 sample burn-in, followed by every 10th sample being used. This gave a total of 3000 used samples. For each sample the number of SNPs in the non-zero effect size distributions were counted. The mean and 95% confidence intervals for the number of SNPs contributing to trait variance was determined from the 3000 samples. SNP effect sizes (β) were reported in terms of the phenotypic change caused by an allelic substitution from one allele to another. The proportion of trait variation explained by each SNP was then estimated as $V_{\text{SNP}} = 2 * \beta^2 * p * (1-p)$ where p is the frequency of the minor allele. BayesR also returns an estimate of trait heritability and the number of typed SNPs that contribute to trait variation (or, more likely, tag causal variants because they are in LD with them).

If a trait is polygenic, then the proportion of variance explained by each chromosome should scale with chromosome size (4). We tested this by estimating the proportion of variance explained by each chromosome. This was done by summing, across chromosomes, the effect sizes of all SNPs from the non-zero distributions, and estimating the proportion of additive genetic variance explained by each chromosome. The process was performed for each of the 3000 samples, from the MCMC chain.

Do SNPs under selection explain bill length variation?

The GO term analysis suggested that the EigenGWAS SNPs under selection ('candidate SNPs') should affect craniofacial traits, and in particular bill length. To test for an overlap between the most significant EigenGWAS regions and peaks in the GWAS on bill length, we used a sliding window approach, averaging the signal from all markers within 200kb windows sliding in steps of 50kb along the genome. The rationale for this is that due to low LD between sites, allele frequency differences and SNPs imperfectly tagging sites under selection, single markers in a significant region do not necessarily result in a high signal for both EigenGWAS and GWAS statistics, even when the underlying region is the same. Sliding window based approaches are therefore more powerful for identifying regions that overlap between the EigenGWAS and GWAS.

We also tested whether regions under selection (i.e. those with low EigenGWAS p-values) disproportionately contributed to bill length variation using a randomization test on the

BayesR estimates of SNP effect sizes. We first defined which SNPs were included in the EigenGWAS peaks. For all eigenGWAS peaks (peaks where $P < 10^{-9}$), the core SNPs with the lowest EigenGWAS p-values were extracted (17 regions in total, see Table S1). Starting from these core SNPs, flanking markers were included until 10 consecutive markers did not include 1 marker with an EigenGWAS signal within the top 1% of most significant EigenGWAS p-values. This way, 16 genomic candidate regions were extracted with a total number of 1530 SNPs. The randomization test sampled the same number of SNPs, in the same number of regions, at randomly chosen positions in the genome, and then summed the effects of those SNPs on bill length variation. By sampling the genome 1000 times, we were able to generate a null distribution for the amount of bill length variation explained by 1530 SNPs. We tested our observed data from the eigenGWAS candidates against this null distribution.

Genomic prediction

We predicted that the SNPs that were under selection in the eigenGWAS analysis would cause the UK birds to have longer bills than NL birds. Genomic prediction uses estimates of SNP effects in one population (a ‘training’ population), to then predict genomic estimated breeding values (GEBVs) in a second population that has been genotyped at the same SNPs (the ‘test’ population). Note that the phenotypes in the ‘test’ population are not used to estimate breeding values in that population. Therefore, inter-population differences in GEBVs should be attributable to genetic differences between the two populations. Genomic prediction analyses were performed using the `—predict` command in BayesR. The test was done reciprocally, using the eigenGWAS candidate SNP. First, SNP effects were estimated in 87 UK birds and used to predict genomic estimated breeding values (GEBVs) in 194 genotyped Netherlands birds (fig. S6). Next, the SNP effects were estimated from the 194 Netherlands birds and these estimates were used to predict GEBVs in the 87 UK birds. The sample sizes are small, making it hard to reliably predict variation in breeding values within a population, but because inter-population variation is typically greater than intra-population variation, the analysis should be capable of detecting population differences. Comparisons between the GEBVs of each population were performed by two sample t-tests with Welch’s correction for unequal variances.

Spatiotemporal trends in bill length

We investigated spatiotemporal variation in bill length, using both 291 museum specimens from across Europe and temporal data available from Wytham. The museum specimens and Wytham data were each measured by a single measurer (KM and AGG, respectively), following a standardized methodology (42). Using the museum specimens, we tested for a difference in bill length between UK and mainland European samples using a general linear model, with bill length as the response variable, and population ID, year of collection, age and sex as explanatory variables.

To test for temporal trends in the UK population, we used a large number of bill length measurements taken from live birds from the Wytham population between 1982 and 2007. The data presented in the main text were collected on 2489 birds measured in May or June

when aged 1 year old. A small number of birds were measured more than once, and for these individuals a mean measurement was taken. Sex and Year of Birth were fitted as main effects in a linear model. One bird had an exceptionally long bill, but was retained in the model. The results are robust to its exclusion. We confirmed that temporal changes in bill length were not due to trends in overall body size, by rerunning the models with tarsus length, an indicator of overall body size, fitted as a covariate. We measured the rate of change in bill length in Haldanes, using the framework described in Hendry et al (23). The difference in bill length between the start and end of the time series was divided by the product of the standard deviation of bill length and the number of generations that had elapsed during the time series. Thus, the rate of change is measured in standard deviations per generation. Bill length was natural log-transformed prior to estimation. Generation length was assumed to be 1.81 years, following Bouwhuis et al. (43). The difference in bill length between the UK and NL populations is approximately 1.27 standard deviations.

A larger dataset contained 9980 records collected on 5145 birds. Modelling this data was more complex as birds were of different ages and measurements were taken at different times of year - bill length is known to vary seasonally (39). Therefore, a linear mixed effects model implemented in MCMCglmm (44) was fitted. Fixed effects were year of birth (mean-centered) and sex, while random effects included ID, month of measurement, age category at measurement, and whether or not the bird was an immigrant.

To check that our observed change in bill length over time (see results) could not come about due to stochastic, yet highly significant, year-to-year variation, instead of a temporal trend, we performed a simple simulation. We randomly re-assigned cohort years, while keeping the same individuals “together” in cohorts. Using such an approach, we expect high levels of year-to-year variation, but this variation should be random with respect to variation over time. We generated 500 randomised datasets in this way, and performed a linear model of bill length against (randomised) birth year in each dataset.

COL4A5 and reproductive success

We tested how variation at COL4A5 was related to fitness in the UK using long-term data from the Wytham population. We used variation at the SNP identified as being most significantly associated with bill length using GWAS as numerical explanatory term (with individuals coded as having 0, 1 or 2 copies of COL4A5-T, the ‘short billed’ allele). We tested how variation at COL4A5 was related to reproductive success, using generalized linear mixed models implemented in the R package glmmADMB. We used a zero inflated Poisson model, with the number of fledglings produced in an individual year as a response. COL4A5 genotype, age and sex were fitted as explanatory terms and year and individual ID were fitted as random effects. We also ran separate GLMMs to test whether the effect of genotype on fledgling production was due to the production of fledglings versus no fledglings (binomial error structure, using all observations), or the number of offspring produced (Poisson error structure), when excluding observations where no offspring were produced.

Visits to supplementary feeding sites in Wytham

We tested how variation at COL4A5 was related to the propensity for individuals to use supplementary food resources. Since 2007, all captured great tits have been fitted with Radio Frequency Identification (RFID) tags for studies of social behavior (29). These tags allow the automated recording of their visits to bird feeders fitted with RFID antennae and filled with sunflower seeds (hereafter ‘RFID feeders’). We used data over three winters (2007-2010), for which we had reasonable sample sizes of genotyped birds ($N = 167$ for 2007-2008, 142 for 2008-2009 and 135 for 2009-2010).

The RFID feeding locations comprised of a stratified grid of 67 locations throughout Wytham Woods, but whether or not an RFID feeder was present at each location at any given time depended on the temporal feeding regime. Through these winters, 16 of the 67 locations contained RFID feeders at any one time. In winters beginning 2007 and 2008, RFID feeders were rotated every 4 days in a structured random design so that each of the eight similarly sized sections of Wytham contained two feeders. In 2009, rotations took place on a 7-day basis. In this way, each of the 67 grid locations contained an RFID feeder twice a month in the winters beginning in 2007 and 2008, and once a month (but for a longer period) in 2009. The RFID feeders utilized over these periods scanned for RFID tags 16 times per second, and observations showed that >99% of visits by RFID-tagged birds to the feeder were successfully recorded. During the winters 2007-2010, the RFID feeders automatically binned all records of the same bird into 15s time intervals i.e. for each minute, only one record of each bird would be stored in the time intervals of 0-14s, 15-29s, 30-44s and 45-59s of that minute. More recently (2012 onwards), higher resolution RFID feeders have been deployed which store up to two records by each bird each second. This high resolution data has allowed fine-scale estimates of actual seed consumption to be determined (29). However, upon binning the high resolution data into 15s time bins, we found that the raw number of records from this procedure correlates strongly with the actual estimated number of seeds consumed ($r = 0.98$). Therefore, bird feeder activity recorded in this way is likely to be an ecologically relevant measure of supplementary food usage.

From the RFID feeder records, we then calculated three measures of individual activity on bird feeders for each of the winter seasons (2007-08, 2008-09, 2009-10) separately. First, we calculated the mean number of records each bird showed per day it was recorded. Second, we calculated the number of days each bird was recorded utilizing the feeders. Finally, we summed the total number of records for each bird over the winter season. We then ran GLMMs with a poisson error distribution, using each of these measures as the response variable. Genotype was modeled as a continuous variable (0 = CC, 1 = CT and 2 = TT) to reduce the degrees of freedom. Sex, month (ordinal from start of winter, with a quadratic term fitted) and season were also included as fixed effects, individual ID was fitted as a random effect, and an observation-level random effect was fitted to the GLMMs to account for overdispersion.

After the genotyping study period had been completed, we also collected data on un-genotyped birds’ bill length from April 2016. This resulted in 384 beak measurements of

341 great tits, taken by the same individual (KM). This allowed us to directly test, using an entirely separate dataset, whether bill length was related to activity at bird feeders. In January-March of 2016 and 2017, as part of a wider study of great tit behaviour at Wytham (29), RFID- feeders in fixed locations (as described above) were open every weekend, scanning for RFID-tags from pre-dawn until post-dusk. Although the data set provides less information on feeding and seed consumption over the entire winter (due to shorter time span and fewer days of recording), the modern RFID-feeders provide high-resolution data (two records per second rather than 15s bins) that allows fine-scale assessment of activity upon the feeders. Therefore, GLMMs with a poisson error distribution were fitted in exactly the same way as the feeder/genotype models above, but with fine-scale daily feeding activity as the response variable, and bill length replacing genotype as a covariate.

Supplementary Figures

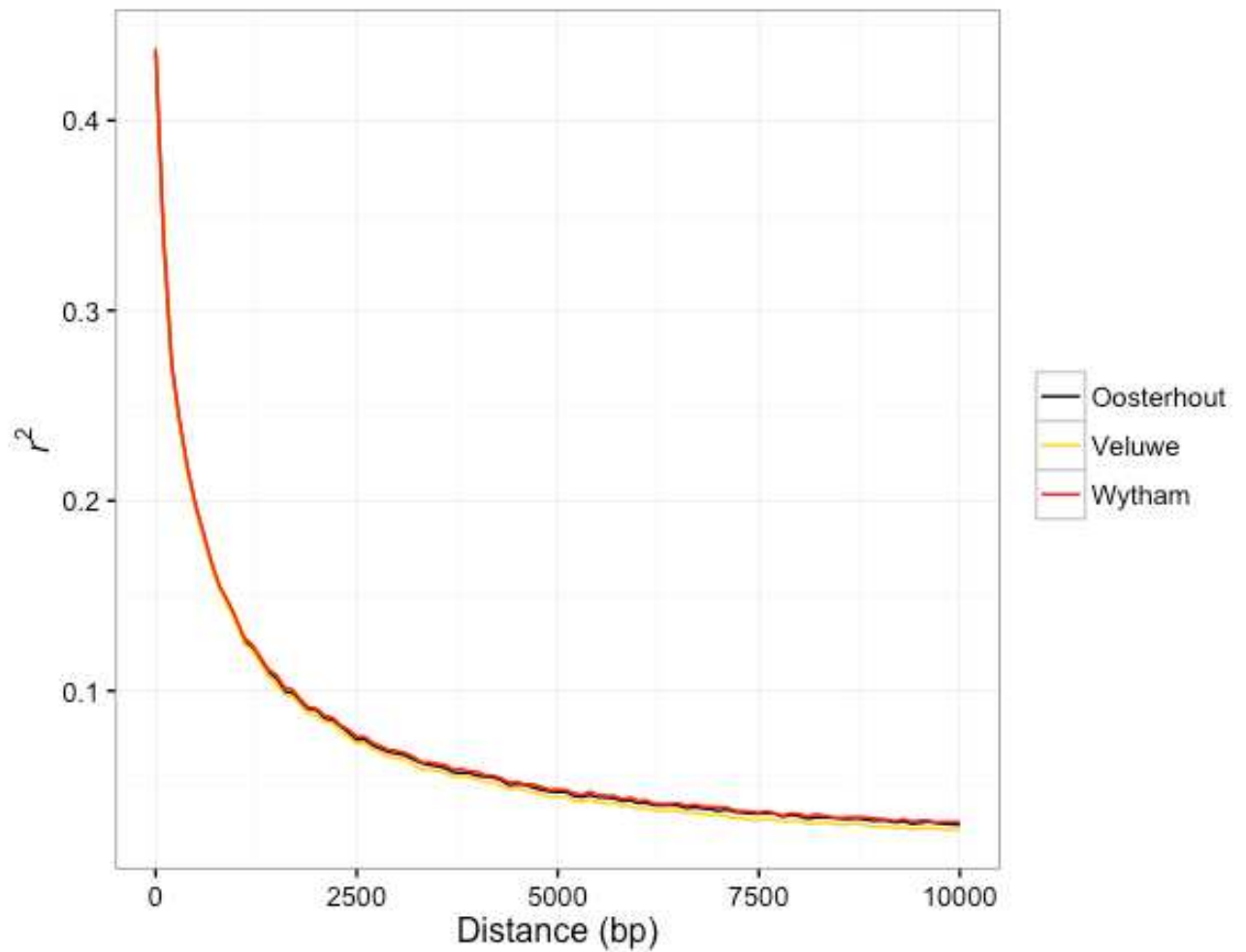


Fig. S1. Linkage disequilibrium (LD) decay in the three great tit populations. Distance between markers in base-pair is displayed along the x-axis and r^2 between markers on the y-axis. Lines represent mean values between all pairs of markers in 100bp distance bins. LD drops rapidly in all populations along the first ~2000bp.

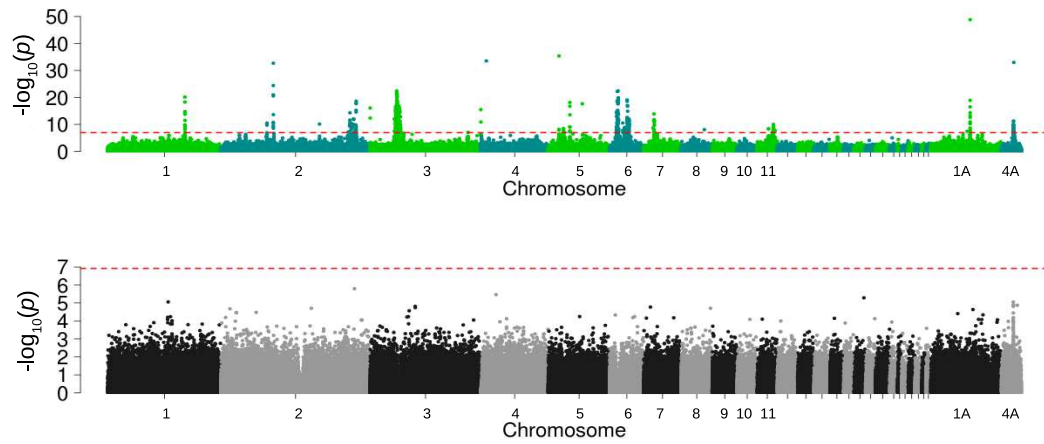


Fig. S2. Single marker ($-\log_{10}$) p-value for EigenGWAS on PC1 (green and blue dots, above) and GWAS on bill length (gray tones, below). Genomewide significance thresholds were generated by performing Bonferroni correction on the effective number of independent tests, estimated with the Genetic Type 1 Error Calculator (downloadable at <http://grass.cgs.hku.hk/gec/>). The EigenGWAS peak on Chromosome 1A is over the LRRIQ1 and ALX1 loci (see fig. S4).

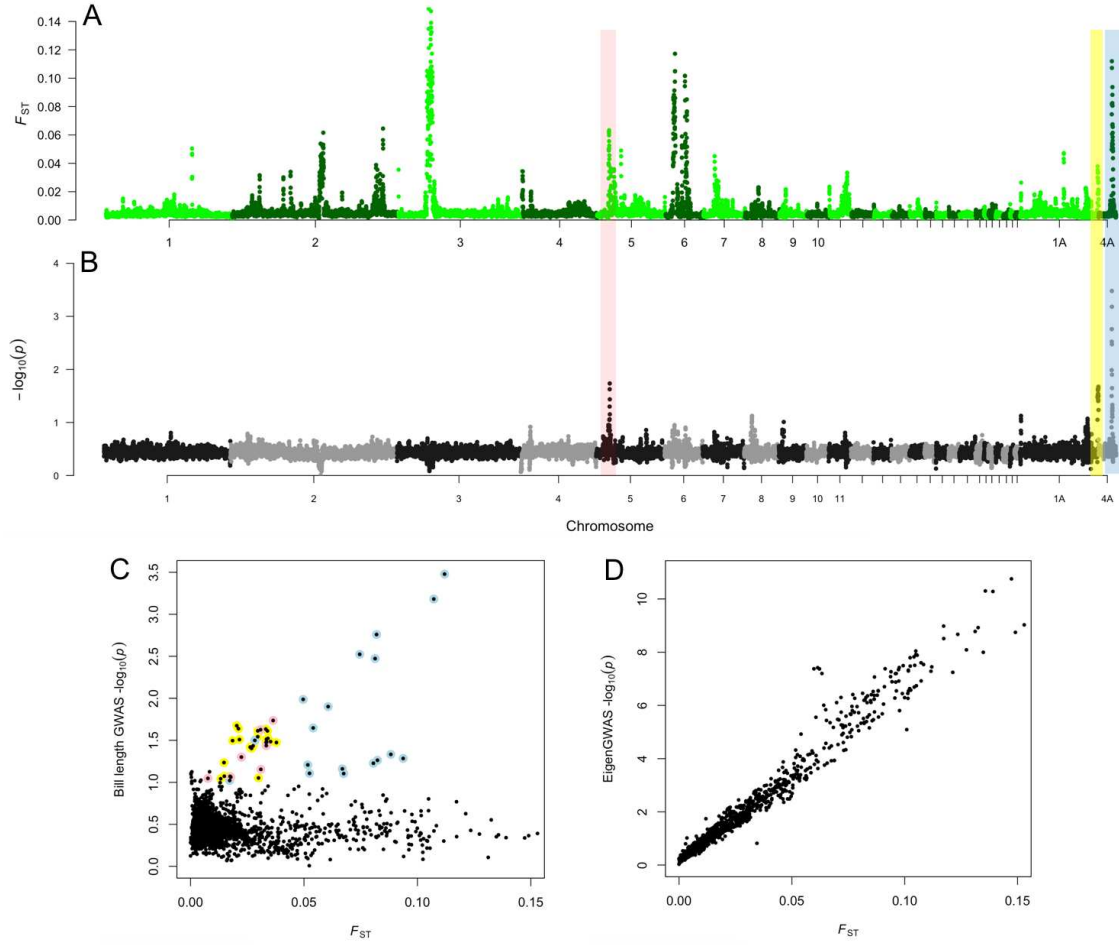


Fig. S3. F_{ST} and EigenGWAS analyses reveal identical patterns of divergent selection, including at genes associated with bill length. **A** 200kb sliding window F_{ST} and **B** 200kb sliding window p values from a GWAS of bill length. Shaded regions correspond to the same shaded regions in figure 2 in the main text. **C** F_{ST} values and GWAS p values are correlated, with three shaded regions showing high levels of structure and associations with bill length. **D** The reason the effects are identical is that F_{ST} and EigenGWAS (PC1) p-values are highly correlated ($r = 0.98$, $P < 10^{-16}$).

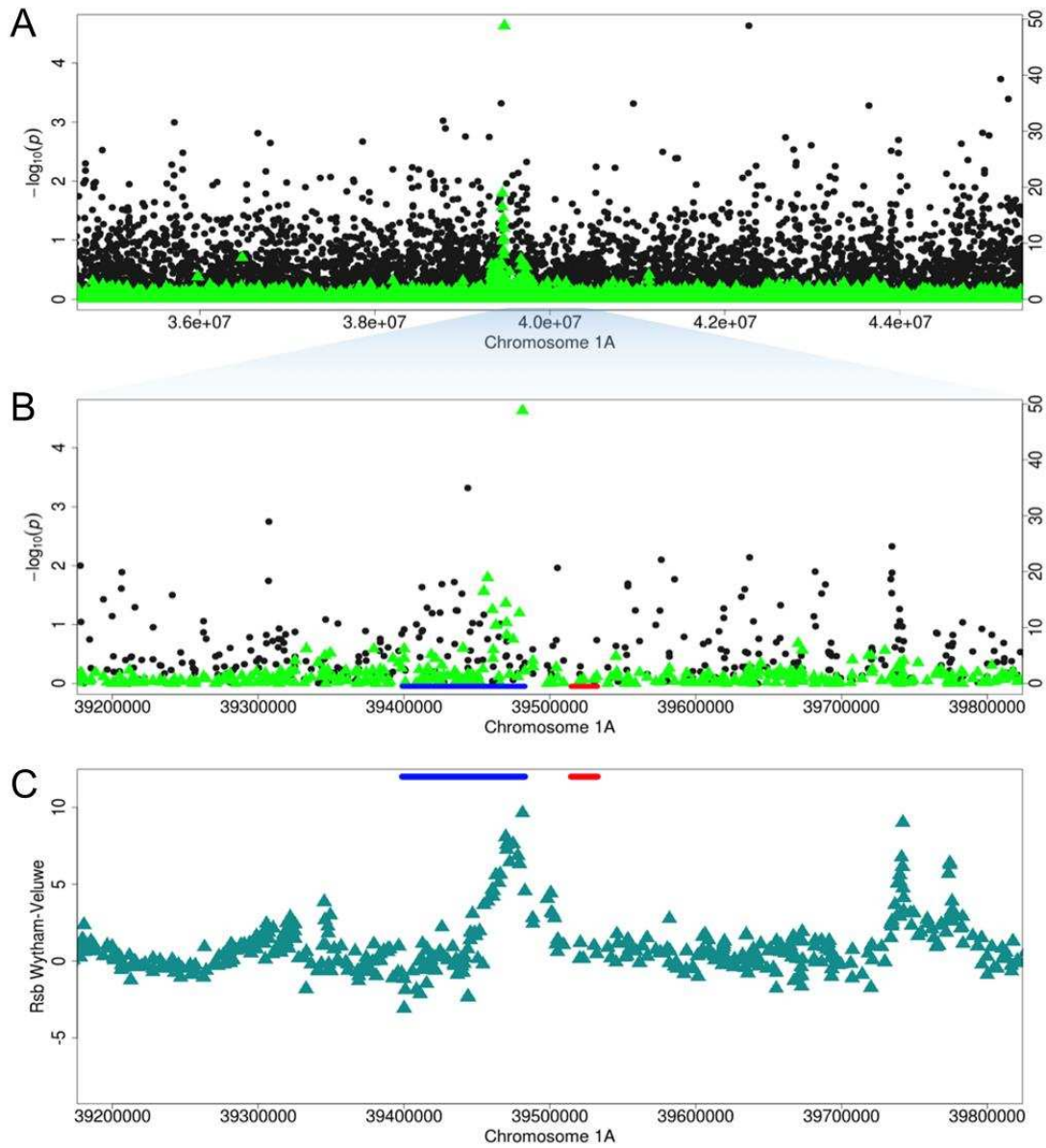


Fig. S4. (A and B) Zoom of the LRRIQ1 and ALX1 region on Chromosome 1A. Black dots represent bill length GWAS ($-\log_{10}$) p-values (left y-axis) and bright green triangles represent EigenGWAS ($-\log_{10}$) p-values (right y-axis). (C) R_{sb} across the same region. Values higher than zero indicate selection for longer haplotypes at higher frequency in Wytham compared to Veluwe, while values below zero indicate selection in Veluwe. The region under selection in Wytham covers LRRIQ1 (blue lines) but not ALX1 (red lines).

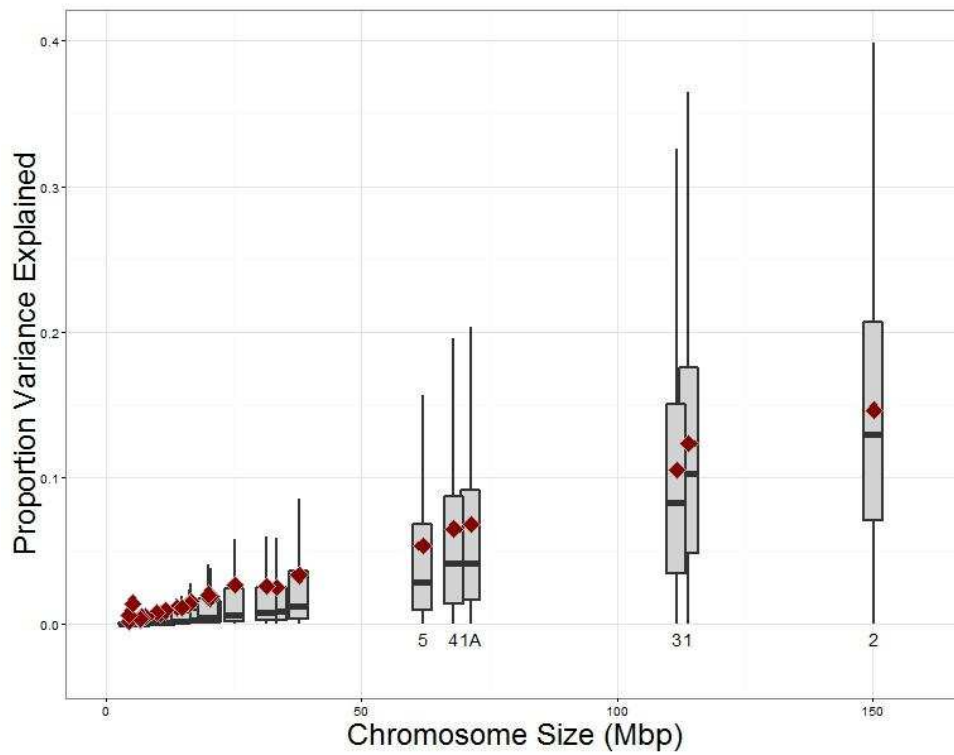


Fig. S5. The proportion of additive genetic variance of bill length explained by a chromosome in relation to chromosome size. Estimates are obtained from 3000 samples of the MCMC chain. Mean values for each chromosome are overlaid onto the boxplots (red diamonds). Only the largest 6 chromosomes are labelled. In 85.2% of samples, chromosome length was significantly associated with the proportion of variance explained by the chromosome. The proportion of variance explained by chromosome size across the 3000 samples had a mean = 0.40 (SE = 0.005), and increased with chromosome size. Therefore, the data are consistent with bill length being explained by a large number of loci, of small effect, spread approximately evenly across the genome.

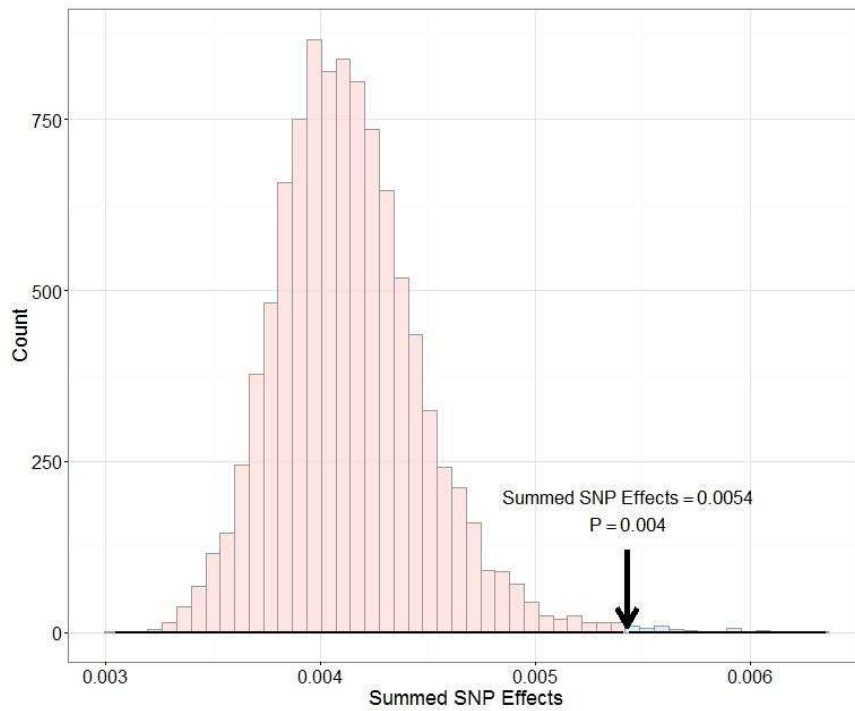


Fig. S6. Randomization test for summed SNP effect on bill length. Distribution of summed SNP effects across 1000 randomized datasets in Wytham. The arrow indicates the observed summed SNP effects, expressed as a proportion of additive genetic variance explained ($\sim 0.54\%$), for the EigenGWAS candidate SNPs. The p value ($p = 0.004$) represents the proportion of simulated datasets in which the summed SNP effects are larger than the observed value. Therefore, even though bill length is highly polygenic, we were able to detect an elevated contribution of our candidate regions under selection to bill length variation.

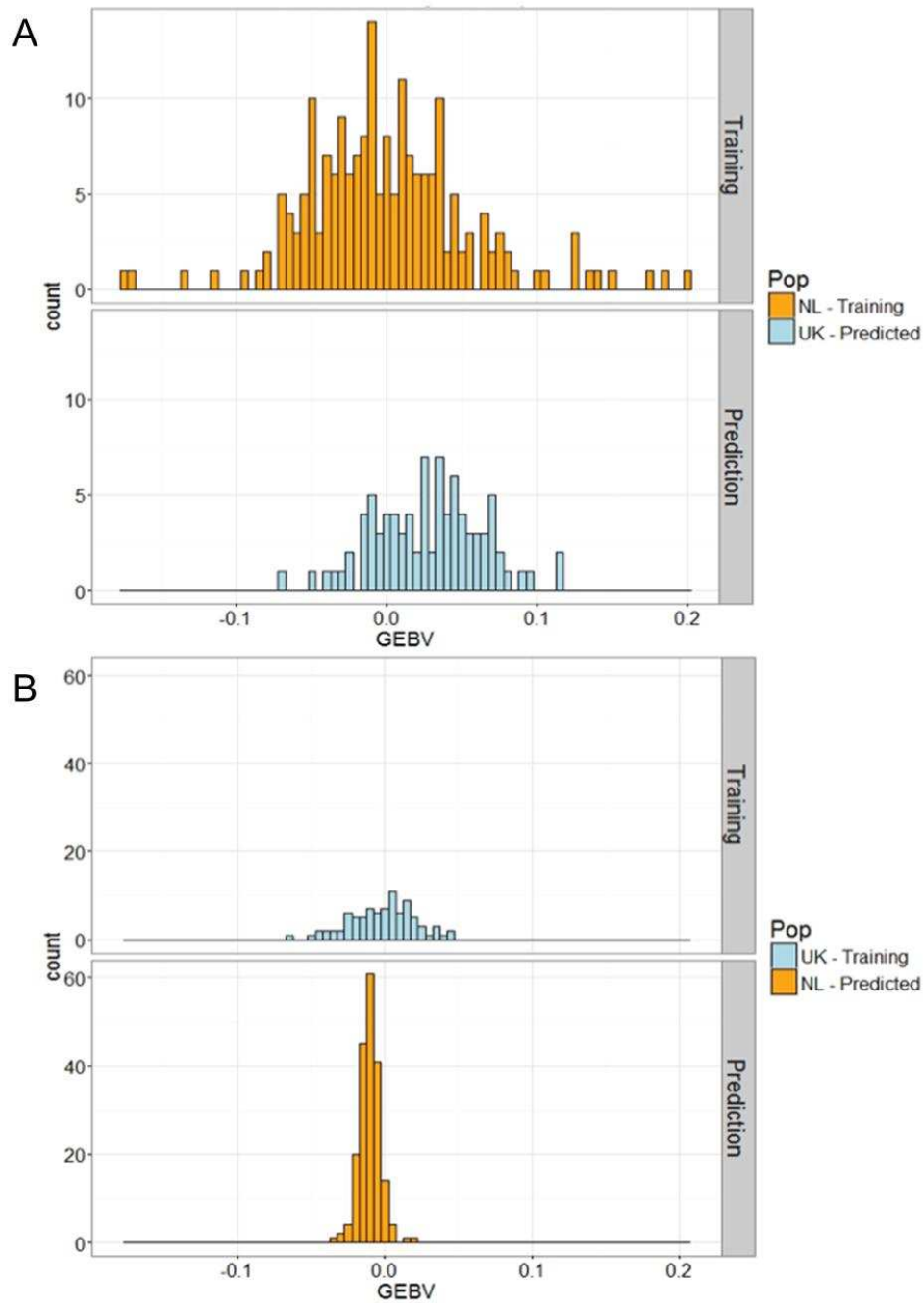


Fig. S7. Genomic estimated breeding values (GEBVs) for bill length. **(A)** GEBVs for Wytham using candidate SNP effect sizes, predicted from Veluwe as a training population. Wytham GEBVs are greater than Veluwe GEBVs ($t = 4.897$, d.f. = 246.14, $p = 1.8 \times 10^{-6}$) **(B)** GEBVs for Veluwe using candidate SNPs effect sizes, predicted from Wytham as a training population are shorter than Wytham GEBVs ($t = 3.592$, d.f. = 94.04, $p = 0.0005$). In both panels the training populations are zero-centered.

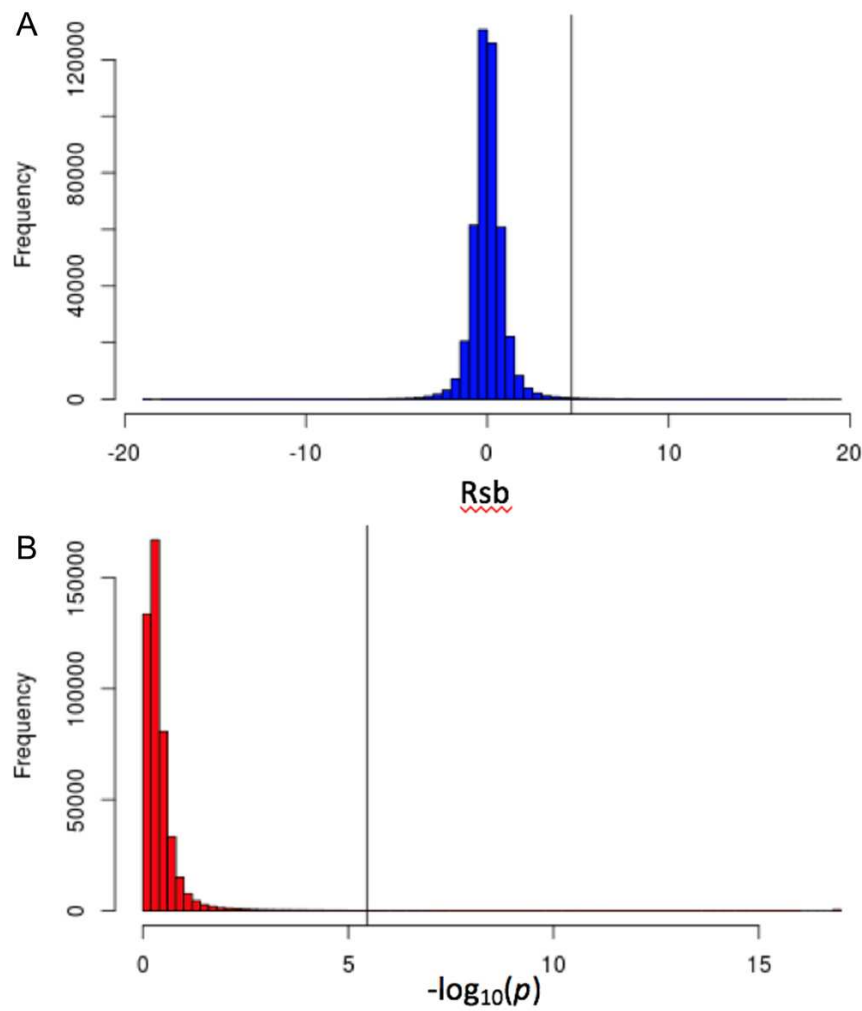


Fig. S8. Distribution of the Rsb statistic (A) and p-values (B) for all SNPs. Rsb values greater than zero represent selection for longer and more frequent haplotypes in Wytham, while negative values represent selection in Veluwe. Horizontal lines indicate the Rsb and p-value for the most significant GWAS marker at COL4A5, highlighting that the region surrounding this marker is under strong selection in Wytham.

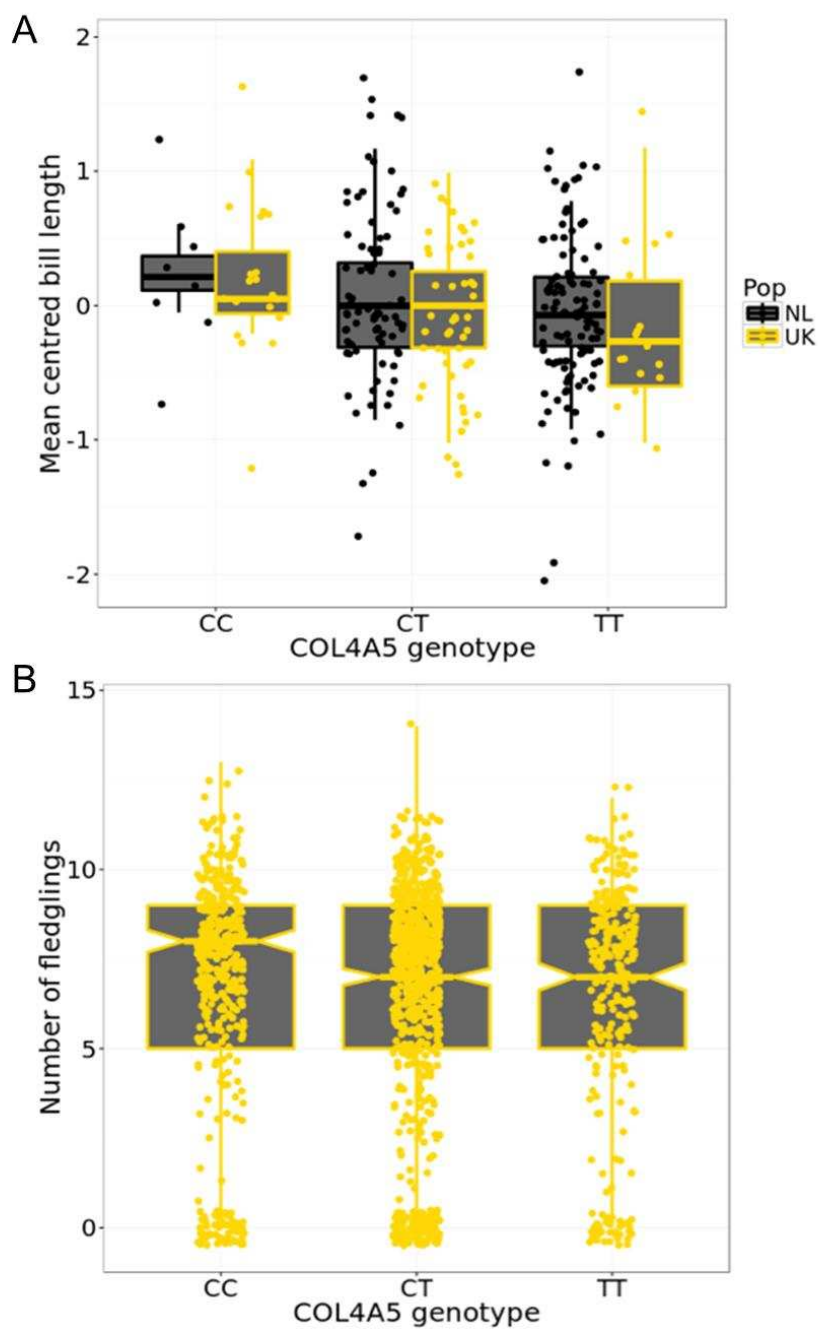


Fig. S9. (A) Mean-centered bill length in relation to genotype in the UK and Dutch populations. (B) Number of fledglings per year in the UK population in relation to genotype at the most significant bill-length GWAS SNP at COL4A5.

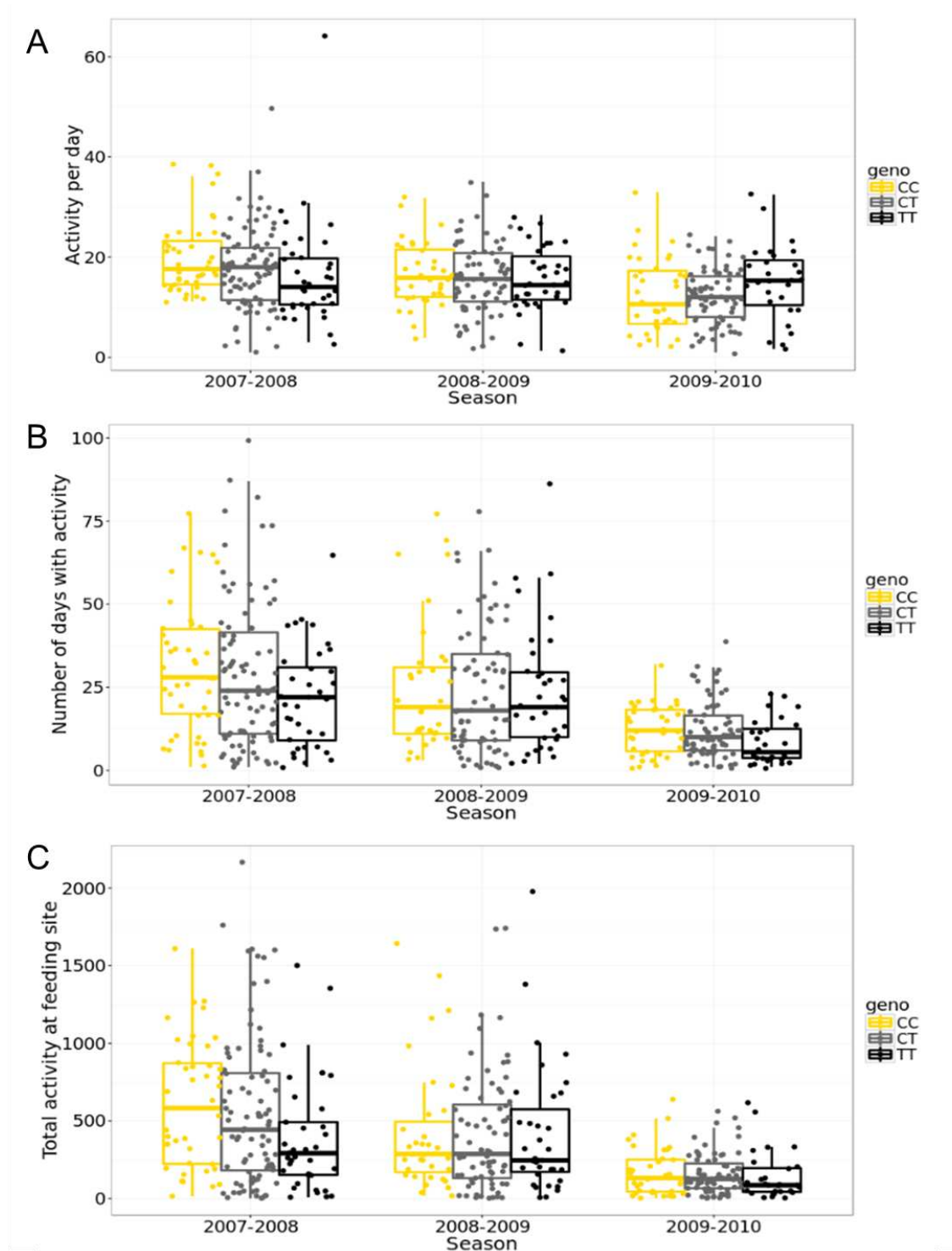


Fig. S10. Activity at feeding stations and COL4A5 genotype. (A). Genotype and mean number of activity records per day for each bird across three seasons ($n = 444$, estimate = -0.37 , $p = 0.45$). (B) Genotype and number of days the birds visited the feeding site across the season. CC birds visited the feeder more often than CT or TT birds ($n = 444$, estimate = -0.12 , $p = 0.03$). (C). Genotype and total number of activity records for each bird over the

season. CC birds consume more seeds across the winter than CT or TT birds ($n = 444$, estimate = -0.16, $p = 0.04$) We found no evidence for an interaction between genotype and season for any of the three models (linear models with genotype x season interaction fitted, all $p > 0.2$).

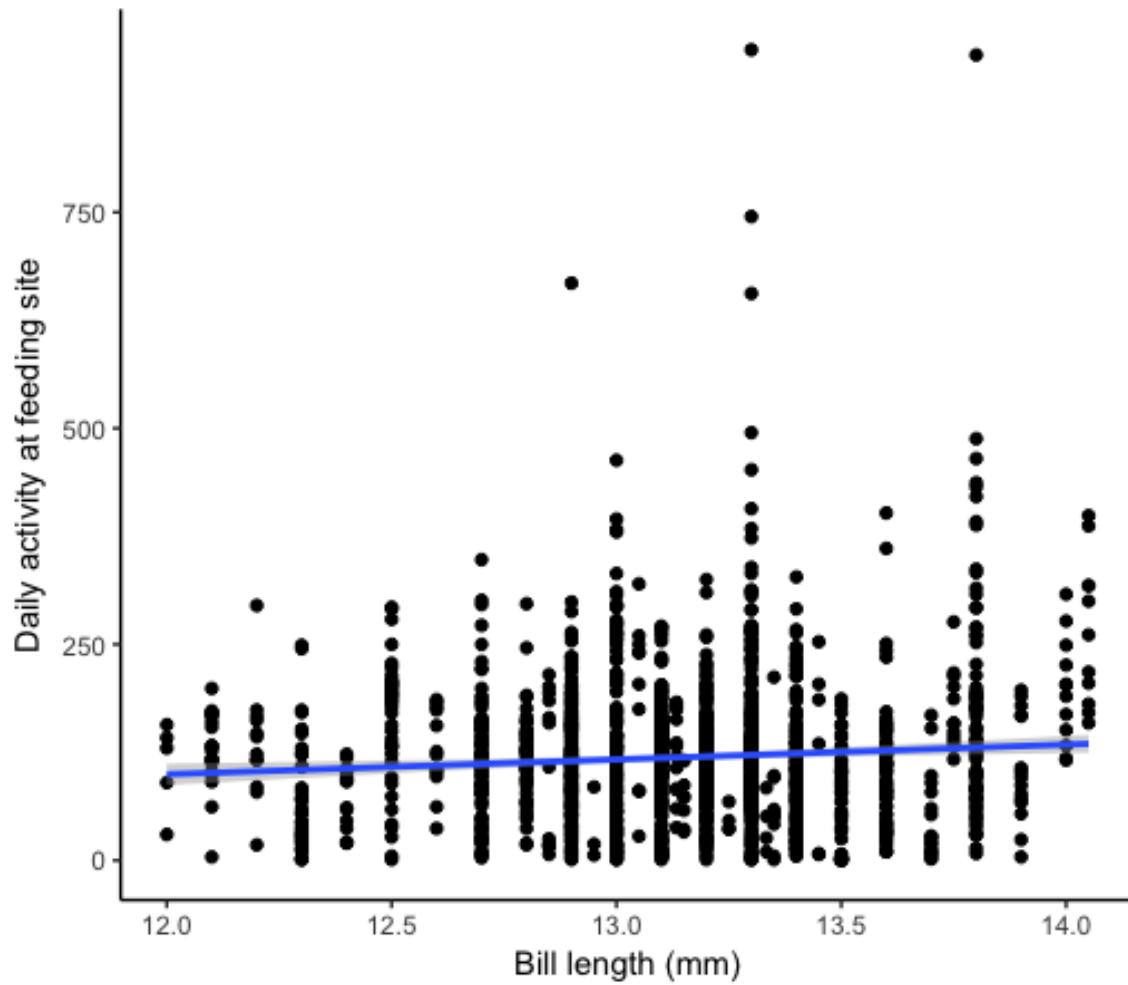


Fig. S11. Activity at feeding stations (number of activity records per day; see methods) and bill length. For bill length, we used the measurement taken at the nearest capture to the day of the feeding record.

Table S1. List of most significant markers in EigenGWAS peaks. Regions included in the genomic prediction and BayesR analysis surrounding the top markers are indicated, and candidate genes for those regions are listed. Start and Stop refer to the locations of the entire EigenGWAS peaks, not gene locations. Numbers in brackets indicate total number of genes in the region.

SNP	CHR	BP	Freq	Pgc	Start	Stop	Candidate
AX-100016177	1	77,696,913	0.216	7.45E-21	77,663,865	77,762,204	HEPHL1
AX-100056392	2	52,689,192	0.132	2.08E-33	52,658,347	52,748,564	GLI3
AX-100827868	2	129,918,616	0.209	4.88E-15	129,804,789	129,938,703	VPS13B
AX-100550602	2	136,196,505	0.205	2.47E-19	136,026,079	136,211,161	TRPS1
AX-100957595	3	25,770,657	0.252	5.91E-17	25,579,744	26,000,833	SRBD1/ SIX2/ SIX3
AX-100923788	3	26,810,200	0.19	2.23E-22	26,722,719	28,252,952	SOCS5 (10)
AX-100516656	3	29,160,891	0.368	1.84E-17	28,442,822	31,002,208	DAAM2 (39)
AX-100959055	5	10,662,578	0.122	3.90E-36	10,449,814	11,190,618	SLC17A6/ ANO5/ LOC107206397/ NELL1
AX-100690978	5	21,629,221	0.297	7.36E-19	21,567,485	21,675,638	ALX4 CD82
AX-100474351	5	34,236,075	0.229	2.33E-18	34,222,212	34,237,466	LOC107205269/ LOC107205369
AX-100471694	6	7,561,954	0.218	6.29E-23	6,927,592	8,874,881	LDB3/BMPRI1A (19)
AX-100402843	6	8,342,760	0.218	3.52E-23	6,927,592	8,874,881	CDHR1/NRG3 (19)
AX-100289034	6	17,406,899	0.223	8.44E-20	17,383,685	18,150,016	SHD24B (6)
AX-100326794	7	10,164,383	0.15	1.35E-14	10,143,016	10,241,782	SATB2/ LOC107207327
AX-100350351	11	16,307,406	0.282	9.72E-11	16,245,749	16,358,113	VAT1L/ ADAMTS18
AX-100642371	1A	39,481,264	0.205	1.49E-49	39,454,093	39,504,418	LRRIQ1
AX-100712161	4A	12,433,942	0.062	1.04E-33	11,810,428	13,263,572	COL4A5 (27)

Table S2. List of most significant markers in the GWAS for bill length. The P value is corrected using a lambda inflation factor. The Last three columns report the results with bill depth and tarsus length fitted as covariates. Effect size and SE are the effect of an allelic substitution in mm. Where SNPs are within genes, the gene name is reported. SNPs significant at 5×10^{-5} are reported.

SNP	CHR	Position	P	Effect	SE	Gene	P	Effect	SE
Without covariates							Tarsus length & bill depth as covariates		
AX-100161487	2	134,506,393	1.60E-06	0.361	0.075		3.84E-07	0.382	0.075
AX-100162335	4	15,291,962	3.40E-06	0.546	0.118		4.56E-07	0.587	0.116
AX-100219258	18	9,840,163	5.20E-06	0.441	0.097	<i>MYOCD</i>	4.04E-06	0.441	0.096
AX-100772359	1	60,802,871	8.70E-06	-0.392	0.088	<i>LRCH1</i>	3.02E-05	-0.375	0.090
AX-100866146	4A	11,968,430	8.90E-06	-0.387	0.087	<i>COL4A5</i>	1.91E-05	-0.375	0.088
AX-100415796	4A	15,974,127	1.33E-05	-0.884	0.203	<i>CMC4</i>	2.25E-05	-0.866	0.204
AX-100790037	4A	11,948,539	1.35E-05	0.376	0.086	<i>COL4A5</i>	3.28E-05	0.361	0.087
AX-100763101	3	45,380,348	1.55E-05	-0.343	0.079		1.11E-05	-0.348	0.079
AX-100983338	4A	11,971,129	1.55E-05	-0.374	0.086	<i>COL4A5</i>	2.88E-05	-0.363	0.087
AX-100427980	7	6,552,913	1.68E-05	-0.541	0.126		1.28E-05	-0.550	0.126
AX-100121530	3	45,463,659	1.81E-05	-0.834	0.194		2.91E-05	-0.809	0.194
AX-100317140	8	29,545,515	1.97E-05	-0.330	0.077	<i>CACHD1</i>	5.92E-05	-0.312	0.078
AX-100344380	2	91,033,979	1.97E-05	0.475	0.111	<i>ERP44</i>	4.21E-05	0.455	0.111
AX-100551558	2	9,029,184	2.10E-05	-0.488	0.115	<i>PTPRN2</i>	2.45E-05	-0.484	0.115
AX-100268275	1A	42,273,688	2.32E-05	-0.565	0.134	<i>EEA1</i>	4.04E-06	-0.616	0.134
AX-100043541	3	39,378,229	2.71E-05	-0.400	0.095		2.56E-05	-0.401	0.095
AX-100395747	2	35,488,576	3.34E-05	0.363	0.088	<i>TBC1D5</i>	1.33E-05	0.381	0.088
AX-100354346	2	15,476,538	3.47E-05	1.136	0.274	<i>MPP7</i>	1.49E-05	1.198	0.277
AX-100510816	2	15,509,848	3.47E-05	1.136	0.274	<i>MPP7</i>	1.49E-05	1.198	0.277
AX-100855556	2	15,493,743	3.47E-05	1.136	0.274	<i>MPP7</i>	1.49E-05	1.198	0.277
AX-100537714	4A	11,971,623	3.59E-05	0.343	0.083	<i>COL4A5</i>	7.24E-05	0.332	0.084
AX-100928290	1A	26,968,602	3.89E-05	-0.352	0.086	<i>CNTN1</i>	4.17E-05	-0.349	0.085
AX-100605138	1A	52,409,448	4.56E-05	-0.470	0.115	<i>TXNRD1</i>	1.54E-04	-0.444	0.116
AX-100277824	6	5,560,627	4.68E-05	-0.682	0.168	<i>CCDC6</i>	4.11E-05	-0.685	0.167

Table S3: Summary of general linear mixed model of temporal trends in bill length in Wytham dataset (9980 measurements taken on 5145 birds between 1976 and 2010). Repeatability of bill length is 0.62 (95% CI = 0.60-0.64).

Random Effects

Term	Variance*	95% CI	Δ DIC
ID	0.1256	0.1194 - 0.1341	5924.13
Month measured	0.0136	0.0065 - 0.0532	912.97
Age Category	0.0007	0.0001 - 0.0096	27.72
Resident / Immigrant	0.0001	0.0000 - 0.3033	6.67
Residual	0.0750	0.0721- 0.0782	

*Posterior mode

Fixed Effects

	Posterior mode	95% credible interval	pMCMC
Intercept	13.42 mm	13.24 - 13.64	< 0.001
Birth year	0.0020 mm p.a	0.0007 - 0.0037	0.004
Sex - male	-0.041 mm	-0.063 - -0.018	0.002

As with the restricted dataset (1 year-old birds) a temporal trend for increasing bill lengths was significant (increase = 0.0020mm p.a., 95% credible interval = 0.0007-0.0037 mm p.a., pMCMC < 0.001)

Additional Data table S1 (separate file) Markers used and results from gene ontology analyses