Title: On the importance of scales when studying adaptive evolution

Running title: Time scale of bill length evolution

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Abstract:

Long-term individual field studies coupled with quantitative genomics offer a powerful means to understand the genetic bases underlying quantitative traits and their evolutionary changes. A recent investigation on the genomic architecture and spatio-temporal variation in great tit bill length reports on a polygenic control of this trait, and suggests that UK great tits have evolved longer bills than other birds in Europe, possibly because of their relatively recent use of bird feeders. We discuss how this case study reveals the importance of considering different temporal scales, since an alternative analysis leads to drastically different conclusions, with a significant decrease in bill length over the last twenty years and an absence of clear trend over 150 years, most likely disqualifying the feeder use as driver of an apparently old bill length variation. This study also illustrates the cautionary steps that are required when interpreting the genomic architecture of quantitative trait evolution. Especially, large coldspots of recombination most likely boosted large peaks of genetic differentiation that should be considered carefully when searching for footprints of selection. This study overall raises a stimulating challenge to unravel the environmental determinants and genomic bases underlying short-term and long-term quantitative variation in bill length in great tits both in UK and across Europe.

Main Text:

Longitudinal field studies have brought invaluable insight for the understanding of evolutionary processes (Clutton-Brock & Sheldon 2010). Long-term studies notably allowed characterizing the temporality of traits' variations and the strength and directionality of natural selection underlying such variations. Some of these long-term examinations of key phenotypic traits detected strikingly fast phenotypic variations, driven by prompt ecological changes (Grant & Grant 2006). In contrast, many long-term studies failed to reveal micro-evolutionary changes and response to selection (Merila *et al.* 2001; Pujol *et al.* 2018). Hence, examining longitudinal data looking for both long-term trends and embedded prompt anomalies has the potential to shed light on traits' evolution and evolutionary trajectories in natural populations. Coupling long-term studies with recent genomic developments is presently the most powerful way to understand the genetic bases underlying evolutionary changes.

Avian bill morphology has played a prominent role in empirical studies of evolution and natural selection (Lack 1947; Grant 1999), perhaps because the size and shape of bills show large variations across and within bird species, and are shaped by strong selective forces since they directly determine foraging efficiency on various food sources. For instance, the emblematic study of Darwin's finches on the Galapagos island of Daphne Mayor aimed at capturing evolutionary changes in bill size (Boag & Grant 1981; Grant & Grant 1993). From 1977 to 1978, bill size increased markedly after a severe drought in 1977. This analysis clearly demonstrated that extreme climatic events such as an El niño event are strong drivers of bill size evolution in this species. After 30 years of perspective, however, Grant & Grant (2002) concluded that while evolution of beak length was predictable as a rapid response to strong selection, it remained unpredictable on a slightly longer microevolutionary scale. Using recent genomic tools, a handful of genes were found significantly associated to bill size and shape in the medium ground finch *Geospiza fortis* (Lamichhaney et al. 2016), among which the strongest candidate gene has also been shown influencing bill dimensions in

50 the Darwin finches' entire radiation (Lamichhaney *et al.* 2015). These genomic analyses hence
51 cracked the oligogenic nature of this trait variation at both small and large microevolutionary scale.

52 In a recent study, Bosse et al. (2017) investigated the genetic architecture of bill length in
53 the Great tit *Parus major*, using a tremendous amount of data from long-term research programs in
54 Wytham woods and in Netherlands. Applying modern analyses of both population and quantitative
55 genomics using 500k single nucleotide polymorphisms, the authors provide insight into the
56 signatures of divergent selection in the studied populations and the genomic architecture of
57 variation in bill length. In line with the quantitative nature of variation in bill length and with
58 quantitative genetics theory (Lynch & Walsh 1998), the authors showed that the genetic architecture
59 of bill length was highly polygenic. Specifically, the authors showed, using a mixture analysis fitting
60 all the SNPs simultaneously, that 3009, or 0.8% of the SNPs, contributed significantly to bill length
61 variation, explaining collectively 31% of the phenotypic variation. Interestingly, none of these SNPs
62 reached genome wide significance in genome wide association tests (GWAS) with bill length,
63 revealing small effect sizes of individual variants. Accordingly with recent quantitative genomic
64 findings, the proportion of variance in bill length explained by each chromosome amazingly scaled
65 with its size, showing that the many SNPs additively explaining bill length are distributed throughout
66 the genome but not clustered in a single chromosome. The polygenic analysis also predicted the
67 difference in bill length observed between Wytham and Netherlands, further illustrating the
68 polygenic nature of this trait variation. This evidence for a polygenic control of bill length, with no
69 major effect SNP, is hence very different from previous examples of oligogenic architecture revealed
70 for example for Darwin finches' beak. This study might become a textbook case study of the
71 polygenic architecture of an illustrious quantitative trait in wild populations, uncovered by combining
72 ambitious long-term field monitoring with extensive genomic analyses in a polygenic framework.
73 Although such a polygenic investigation required these technical commitments and recent advances
74 (Wellenreuther & Hansson 2016; Gienapp *et al.* 2017a), it also requires a conceptual revolution

consisting in silencing our hope to find large effect alleles that are theoretically rarely responsible for quantitative variation (Rockman 2011).

Bosse et al. (2017) however concluded on a different interpretation of their data, proposing that variation at one gene was associated with use of feeders and that this might have driven the evolution of larger bills in UK compared to Netherlands. Among the arguments pointing to feeders as drivers of longer bill lengths in UK great tits, Bosse et al. (2017) reported that COL4A5, the locus with the highest association with bill length in Wytham, was also highly differentiated between Wytham and Netherlands, and was associated with greater reproductive success and higher activity at feeding sites in Wytham. In addition, they reported that bills were longer in UK compared to mainland Europe (Figure 4A in Bosse et al. 2017) and increased from 1982 to 2007 in UK (Figure 4B in Bosse et al. 2017). This gene was located in one of several large genomic regions strikingly highly differentiated between Wytham and Netherlands, overall containing genes that could make them good candidates for bill morphology. We argue in this comment that this speculation on the role of feeder on bill length evolution is not well supported by these arguments. We propose instead that differences in bill length between Wytham and Netherlands might have been evolving on the longer term than during the contemporary use of feeders and that the role of COL4A5, as well as other highly differentiated regions, might be tested more comprehensively.

Bill length temporal variation might not be contemporary to the use of feeders. Indeed, we show that the bill length trend is highly dependent on the time scale considered. Inspired by the readings of papers such as the one of Grant & Grant (2006) relating the effects of rare and extreme events on the evolution of phenotypic traits, we carefully inspected the evolution of bill length during the studied period, looking for particularly prompt changes. We reanalyzed the data using a breakpoint computations method (coin R-package) aiming at localizing such striking change. The best cutpoint was found between 1986 and 1987 (maxT = 7.41, p-value < 2.2E-16), which corresponds to a conspicuous change in bill length. Measures from the five years preceding 1987 differed from

100 subsequent years (t-test: t = -7.28, df = 674.53, p-value = 9.1E-13). Although a linear regression
101 though the entire period, from 1982 to 2007, as implemented by Bosse et al, yielded a positive slope,
102 removing the five measures prior to 1987, bill length significantly decreased from 1987 to 2007
103 (Figure 1A, Linear model from 1987 to 2007 taking into account or not tarsus length, F = 7.13, p-
104 value = 8.2E-4; F = 13.2, p-value: 2.9E-4, respectively). A LOESS model confirms the decrease in bill
105 length during the second part of the record. More generally, slopes of linear models linking bill
106 length to time, using several combinations of 10 to 25 consecutive years, were often positive when
107 including one or more data points collected between 1982 and 1987, and negative slopes were often
108 observed when excluding these years (Figure 2), further illustrating that both time periods yield
109 opposite patterns. Besides, tarsus length also decreased significantly both over the 1987-2007 and
110 the 1982-2007 periods (Figure 1B, F = 6.61, p-value = 0.01 & F = 24.07, p-value = 9.9E-07,
111 respectively). Correspondingly, the bill length / tarsus length ratio did not change significantly from
112 1987 to 2007 (Figure 1C, F = 1.51, p-value = 0.28), indicating no change in bill length, when scaled to
113 tarsus length, over this period. The origin of this cutpoint is important to investigate, since it could
114 constitute an evidence for a response, either genetic or plastic, of both bill and tarsus lengths to a
115 prompt abiotic or biotic factor or to a methodological change. Yet, it seems at present difficult to
116 decipher whether bill length or tarsus length or both, if any, were targeted by selection. Indeed, both
117 traits are commonly phenotypically and genetically positively correlated in passerines (Teplitsky *et*
118 *al.* 2014; Poissant *et al.* 2016), hence should often evolve together. Overall, these results much likely
119 rule out the possibility of a contemporary (1982-2007) positive effect of feeders on bill length.

120 One could nevertheless argue that bill length could have increased at a longer time scale
121 than the period 1982-2007, but still recent enough to incriminate the use of feeders. Figure 3
122 however shows the absence of a clear pattern of bill length increase in museum specimens collected
123 in UK. Although more data might be needed to confirm the pattern, it suggests that larger bill length
124 in the UK seems to have evolved on a larger time period than the one during which feeders have

been used. Moreover, bill length across Europe is not displaying a clear dichotomy between UK and mainland Europe but rather smooth spatial variations (Figure 4) with an ANOVA showing a significant effect of country ($p = 3.75e-07$) but not of sex ($p = 0.345$), with indeed UK birds having longer bills compared to three other countries (France, Italy, and Netherlands). This spatial variation challenges, once again, the supposition by Bosse et al.'s (2017) of a recent increase of bill length in British great tits that would have been caused by feeders.

The gene COL4A5 was the corner stone linking bill length and feeders, via the association of COL4A5 with bill length, activity at feeders, reproductive success, and divergent selection. However, the association of COL4A5 was non-significant at the genome wide level. The authors acknowledged that this locus probably has a small effect on the focal trait, which is in line with the demonstrated polygenic control of bill length discussed above. The authors also propose that the small sample size could limit the power of the analysis. Indeed, only 89 out of the initial 2322 individuals were used for this GWAS analysis of quantitative variation of bill length. Furthermore, bill length had a relatively low repeatability of 0.62, estimated over several thousands of individuals. Performing a GWAS on Dutch birds, we found that SNP statistically associated (without correction for multiple testing) to bill length variation were essentially different from the ones identified in Wytham (Supplementary figure 1). The GWAS with bill length in Dutch birds yielded larger p-values for the SNPs in COL4A5 and the entire genomic region surrounding COL4A5, than for Wytham birds (Figure 5A&B). Collectively, although COL4A5 genomic region was linked to bird fitness and displayed high Fst between Wytham and Netherlands, COL4A5 might only have a minor contribution to the entire additive genetic variance of bill length, as first proposed by the authors, and only in Wytham and not in Netherlands. These considerations therefore cast further doubt on the potential contemporary role of feeders on bill length evolution. More generally, the results of the GWAS performed here might be to interpret with caution since they identified only small effect variants, or that sample size was too small, and did not yield reproducible results between both populations. Alternatively, the lack of reproducibility

150 of the GWAS between both populations could originate from evolutionary processes independent
151 from the evolution of the focal trait.

152 SNPs in COL4A5 showed elevated bill length association in UK and genetic differentiation
153 between Wytham and Netherlands (Figure 5C). Two similar patterns were observed elsewhere along
154 the genome (in chromosome 5 and 1A). These coincident elevated associations and differentiations
155 were reasonably interpreted by Bosse et al. (2017) as an indication that selection was probably
156 acting on bill length. This is in line with previous interpretations of similar signals in human height in
157 which polygenic scores for height showed differentiation among populations, hence potentially
158 revealing polygenic response to selection for height (eg Turchin et al. 2012). However, Berg et al.
159 (2018) very recently discussed the effect of the variation along the genome of recombination rate
160 together with background selection, on LD score for height, genetic differentiation among
161 populations, and the correlation of both. Berg et al. (2018) showed that loci association scores were
162 negatively correlated to recombination rate, with the highest association scores found among
163 regions with almost null local recombination rate (S17 in Berg et al. 2018). Moreover, they show that
164 background selection explains much of the relationship between association score and allele
165 frequency differentiation. Overall, this reanalysis demonstrated that recombination rate variation,
166 combined with background selection, vastly explains coincidence of particularly high phenotype-
167 genotype association and allele differentiation between populations. In the context of the great tit, is
168 it possible that variation in recombination rate, in combination with background selection, could
169 have influenced the GWAS results and the genetic differentiation between populations, leading to
170 the observed coincidence of a few genomic regions showing elevated recombination and
171 differentiation? Specifically, is it possible that locally reduced recombination over relatively large
172 genomic regions, combined with background selection, could have contributed to elevated genetic
173 differentiation between populations concomitantly to increased association with bill length?

174 COL4A5 was found in a relatively large genomic region with very low SNP density (Figure
175 5D), likely due to low recombination, which might coincide with the centromere of the chromosome
176 4A. Importantly, although the authors did not discuss this key fact, nearly all of the regions identified
177 as highly differentiated (Figure 1A in Bosse et al. 2017) seem to coincide with large and regions of
178 low SNP density (Figure 6), most likely corresponding to low recombination regions, hence with
179 centromeres for most of them (Nordborg *et al.* 2009; Limborg *et al.* 2015). Consistently, most of
180 these regions were also identified by Laine et al. (2016) as putative selective sweeps (Figure 2 in
181 Laine et al. 2016), with reduced diversity and increased divergence over large DNA regions, at the
182 geographic scale of the species entire distribution, suggesting a similar evolutionary process
183 implicated in such large-scale patterns of differentiation among populations. Specifically, the
184 chromosome 4A region containing COL4A5 (Figure 1C in (Burri *et al.* 2015)) has already been
185 identified as showing elevated differentiation in several lineages and populations of *Ficedula*
186 flycatchers (Ellegren *et al.* 2012; Burri *et al.* 2015). Burri et al. (2015) determined that such high
187 differentiation shared across lineages was due to a reduction in effective population size of this
188 genomic region in the ancestral population, due to the effect of linked selection in interaction with
189 low recombination, since it displayed lower absolute divergence (dxy) and larger linkage
190 disequilibrium than the genomic background. Burri et al. concluded on a crucial warning: "scans are
191 likely to identify recombination-mediated elevations of differentiation not necessarily attributable to
192 selective sweeps". More broadly, variation in recombination rate and background selection have
193 received increasing attention since they are likely to shape larges-scale patterns of diversity and
194 differentiation along the genome and it has therefore been demonstrated that they should be
195 considered thoroughly when looking for footprints of divergent selection (Roesti *et al.* 2012; Berner
196 & Roesti 2017; Burri 2017; Comeron 2017; Delmore *et al.* 2018). Hence, the principal peaks of high
197 genetic differentiation identified in Bosse et al. 2017, matching the main areas of reduced diversity
198 displaying signatures of selective sweeps identified in Laine et al. 2016, may not necessarily illustrate

199 relatively recent divergent selection, but instead ancestral reduction of effective population size due
200 to low recombination, background selection and drift. Symmetrically to the risk of identifying false
201 positive loci in coldspots of recombination, it is also expected that neglecting the effects of variation
202 in recombination results in false negative loci localized outside of recombination coldspots (Berner &
203 Roesti 2017). Accordingly, both studies could have also missed candidate loci outside of these
204 recombination coldspots. Unfortunately, the large averaging sliding window (200kb) used in Bosse et
205 al. relatively to the average short distance linkage disequilibrium (r2 = 0.1 at 2kb) might have
206 worsened the problems raised above, by capturing even more principally large-scale areas of
207 recombination-elevated-differentiation. Correspondingly, this large window much likely increased
208 the risk of missing narrow peaks of elevated differentiation actually tagging candidate genes
209 targeted by relatively recent positive selection, and especially so outside of recombination coldspots.
210 In fact, such a large sliding window probably rather represents a neutral local baseline (Roesti 2012;
211 Burri 2017) that could have been implemented to detect local outlier markers showing locally
212 elevated divergence (e.g. supplementary figure 2). These caveats question the potential role of
213 divergent selection versus large-scale variation in recombination and background selection in
214 shaping the few large regions with elevated differentiation. Concerning the three main large
215 genomic regions (chromosomes 5,1A, 4A) showing elevated association with bill length in Wytham,
216 the fact that they also show elevated differentiation between populations, weak association in
217 Netherlands, and had particularly low SNP density, questions the role of local recombination drop
218 and background selection in generating these patterns (Berg et al. 2018).

219 Opportunely, this context and the available data offer exciting avenues of research to
220 unravel the determinants of both recent and long-term variation in bill length as well as adaptive
221 divergence between great tit populations across Europe. Spatial variation in bill length could be
222 unraveled in a multi-trait genome wide association test, using a polygenic framework, which could
223 be inspired from a pioneering study on great tit breeding phenology (Gienapp *et al.* 2017b). Besides,

224 a formal selection analysis relating great tit bill length to over-winter survival when birds are most
225 likely to benefit from food provided by people, is required, to elucidate the nature of the
226 evolutionary forces behind bill length variation. Taking into account the effect of variation in
227 recombination rate and background selection along the genome to draw local neutral envelops of
228 genomic differentiation will likely help to validate or invalidate the potential footprints of divergent
229 selection identified almost only in coldspots of recombination, and to identify other footprints
230 outside of these regions (Roesti *et al.* 2012; 2013; Burri 2017; Comeron 2017; Delmore *et al.* 2018).
231 Providentially, the genomic analyses performed in other populations across Europe could also help
232 determining whether elevated population differentiation was driven by population-specific relatively
233 recent positive selection or by ancestral variation due to physical feature of the species genome (e.g.
234 Figure Box 3 in Burri 2017). Lastly, taking into account the joint effect of recombination and
235 background selection simultaneously on phenotype-genotype associations and genetic
236 differentiation between populations would be important (Berg et al. 2018).

237

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246 **References and Notes**

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Figures

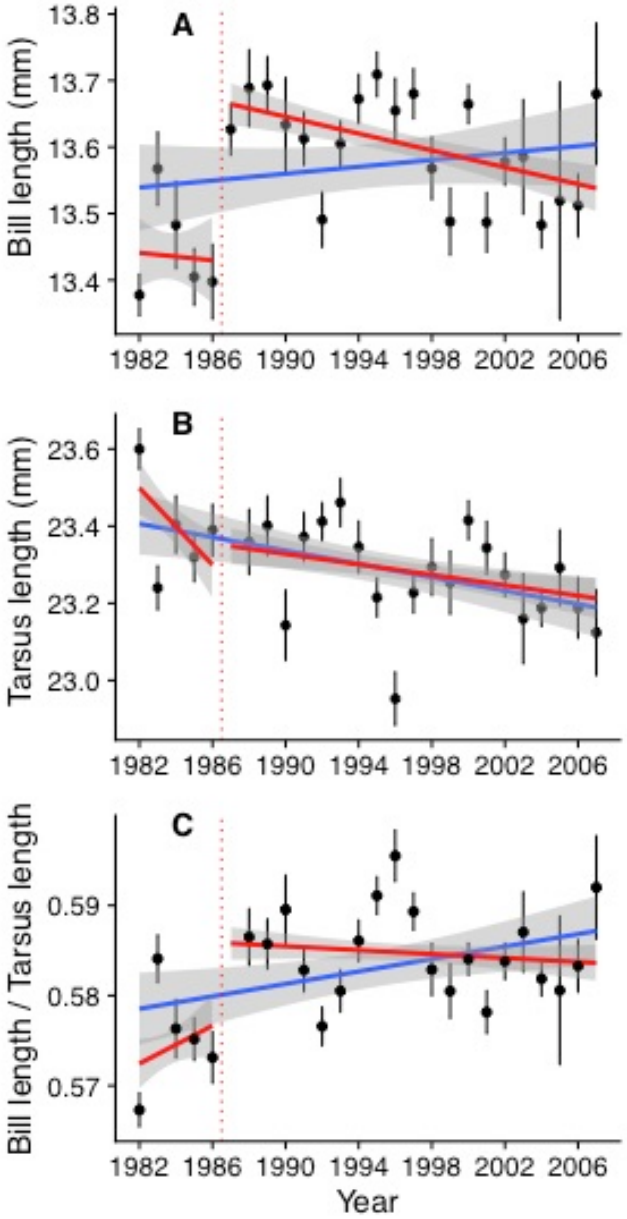
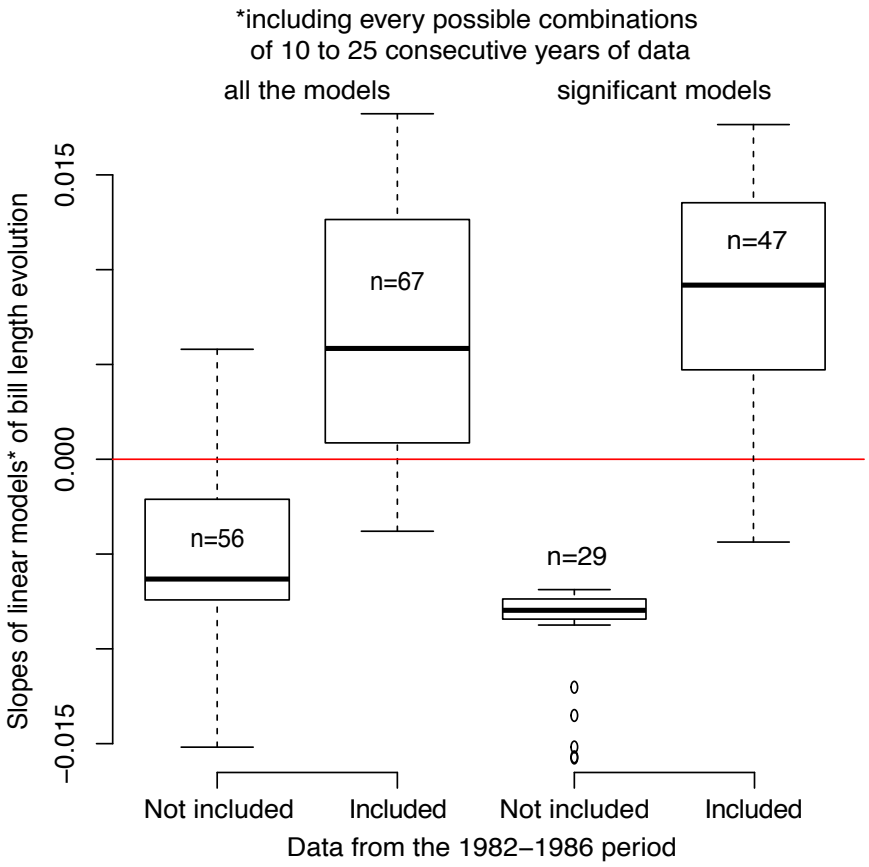


Figure 1. Decrease of mean bill length (A) and tarsus length (B) but conservation of the allometry between both traits (C), from 1987 to 2007 (in red) in Wytham great tits. Regressions from 1982 to 2007 presented by Bosse et al. are shown in blue.



321

322 Figure 2. Slopes of linear models of bill length temporal evolution, including every possible

323 combination of 10 to 25 consecutive years of data, with or without one to several years of data

324 from 1982 to 1986.

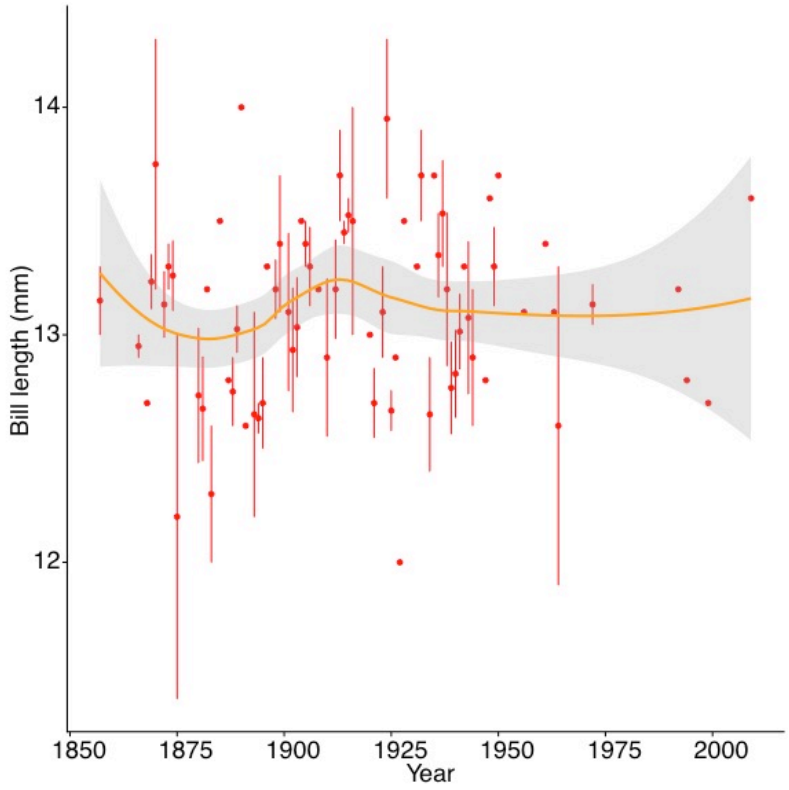


Figure 3. Variation of mean bill length in UK from 1850 to 2007, based on museum records used in Bosse et al. 2017.

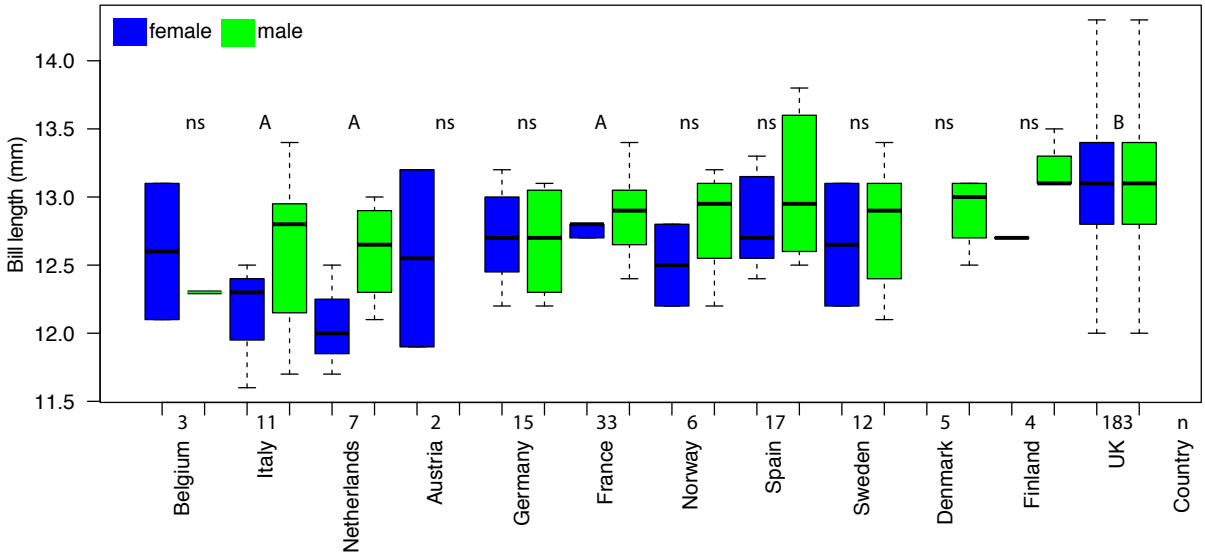


Figure 4. Variation in bill length across European countries for both female (in blue) and male (in green) great tit specimens from museums. Letters A and B illustrate significant differences between sites and 'ns' refers to non-significant differences (Tukey HSD).

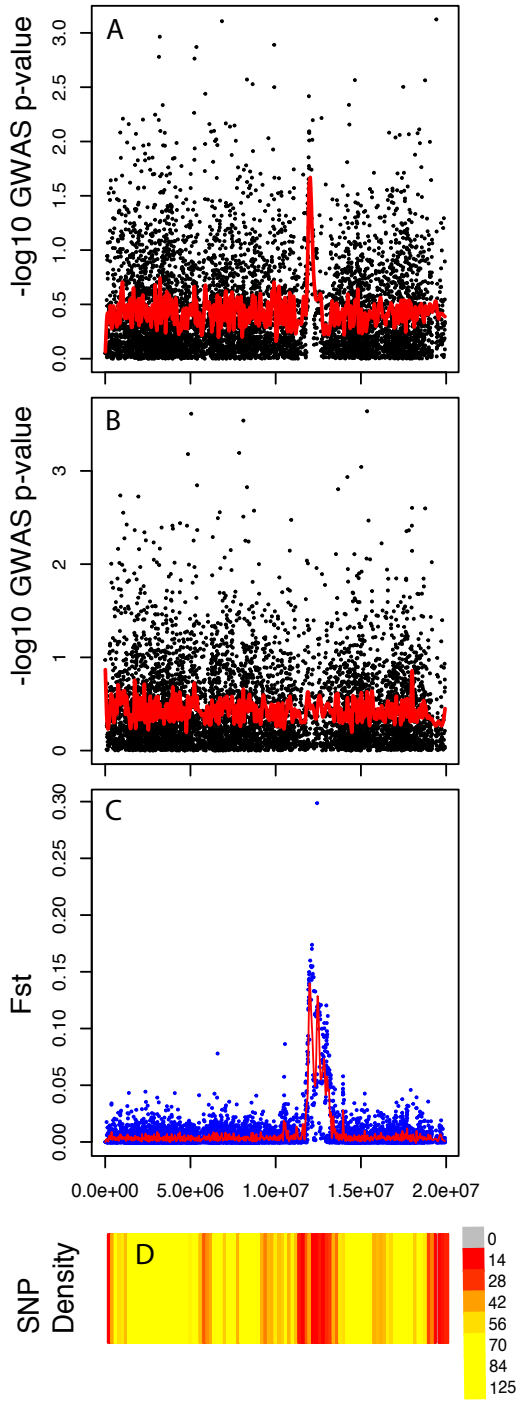
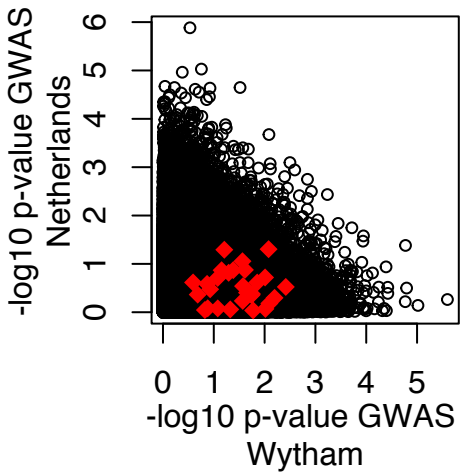
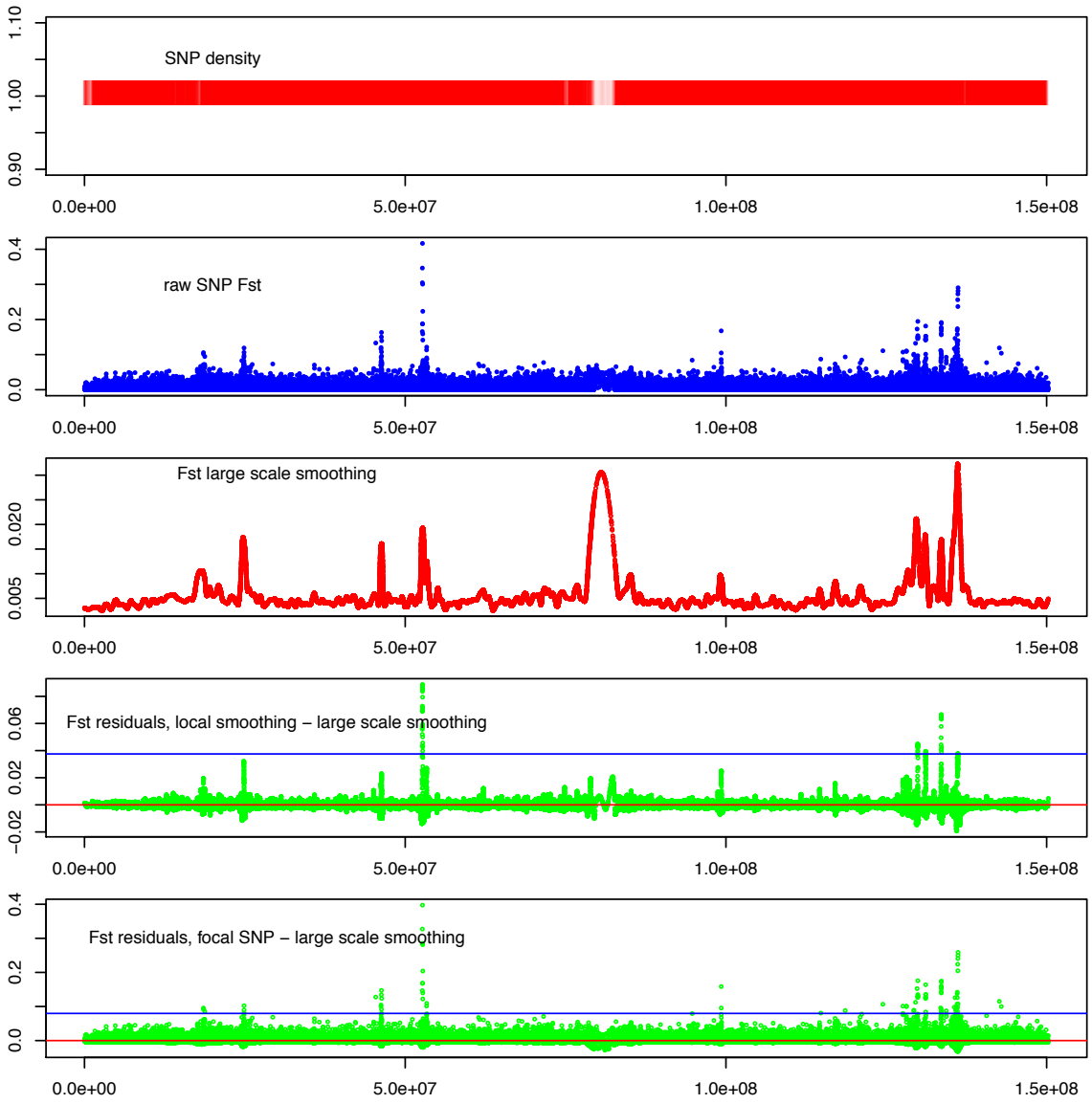


Figure 5. GWAS result with bill length for chromosome 4A in Wytham (A) and in Netherlands (B); Fst between Wytham and Netherlands on chromosome 4A (C); SNP density over 200 Kb regions (D).

Figure 6: Number of SNPs per 200 Kb window in chromosomes 2, 3, 5, 6, 1A & 4A, in which main Fst and eigenGWAS peaks have been identified.



Supplementary figure 1: Results of the GWAS with bill length for Wytham and Netherlands. Red diamonds correspond to the SNP in COL4A5.



Supplementary figure 2: Illustration on chromosome 2, of the combination of variation in recombination rate and large scale Fst smoothing, on the discovery of loci with elevated Fst between Wytham and Netherlands. SNP density, raw Fst per SNP, large scale Fst smoothing, local Fst residuals from the large-scale smoothing, SNP Fst residuals from the large-scale smoothing. The centromere regions result in a locally elevated peak of divergence that is not found anymore when calculating the residuals between local or SNP Fst and the large scale

350 smoothing. In contrast, peaks in the middle of both chromosome arms likely correspond to true

351 Fst outliers.

352