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

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

131 The gene COL4A5 was the corner stone linking bill length and feeders, via the association of
132 COL4A5 with bill length, activity at feeders, reproductive success, and divergent selection. However,
133 the association of COL4A5 was non-significant at the genome wide level. The authors acknowledged
134 that this locus probably has a small effect on the focal trait, which is in line with the demonstrated
135 polygenic control of bill length discussed above. The authors also propose that the small sample size
136 could limit the power of the analysis. Indeed, only 89 out of the initial 2322 individuals were used for
137 this GWAS analysis of quantitative variation of bill length. Furthermore, bill length had a relatively
138 low repeatability of 0.62, estimated over several thousands of individuals. Moreover, contrary to
139 Wytham, in Dutch birds the 200kb genomic regions surrounding COL4A5 did not yield significant p-
140 values in the GWAS with bill length, although the focal SNP was polymorphic in both popualtions
141 (data not shown). Collectively, although COL4A5 genomic region was linked to bird fitness and
142 displayed high Fst between Wytham and Netherlands, COL4A5 might only have a minor contribution
143 to the entire additive genetic variance of bill length.

144 SNPs in COL4A5 nevertheless showed elevated differentiation between Wytham and
145 Netherlands, which could be due to positive selection. However, COL4A5 was found in a relatively
146 large genomic region with very low SNP density (Figure 5), likely due to low recombination, which
147 might coincide with the centromere of the chromosome 4A. Importantly, although the authors did
148 not discuss this key fact, nearly all of the regions identified as highly differentiated (Figure 1A in
149 Bosse et al 2017) seem to coincide with regions of low SNP density (Supplementary figure 1), most

150 likely corresponding to low recombination regions hence with centromeres (Nordborg *et al.* 2009;
151 Limborg *et al.* 2015). Consistently, most of these regions were also identified by Laine et al. (2016) as
152 putative selective sweeps (Figure 2 in Laine et al. 2016), with reduced diversity and increased
153 divergence over large DNA regions, at the geographic scale of the species entire distribution,
154 suggesting a common origin of such large-scale patterns of differentiation among populations.
155 Specifically, the chromosome 4A region containing COL4A5 (Figure 1C in (Burri *et al.* 2015)) has
156 already been identified as showing elevated differentiation in several lineages and populations of
157 *Ficedula* flycatchers (Ellegren *et al.* 2012; Burri *et al.* 2015). Burri et al. (2015) determined that such
158 high differentiation shared across lineages was due to a reduction in effective population size of this
159 genomic region in the ancestral population, due to the effect of linked selection in interaction with
160 low recombination, since it displayed lower absolute divergence (dxy) and larger linkage
161 disequilibrium than the genomic background. Burri et al. concluded on a crucial warning: "scans are
162 likely to identify recombination-mediated elevations of differentiation not necessarily attributable to
163 selective sweeps". More broadly, variation in recombination rate and background selection have
164 received increasing attention since they are likely to shape larges-scale patterns of diversity and
165 differentiation along the genome and it has therefore been demonstrated that they should be
166 considered thoroughly when looking for footprints of divergent selection (Roesti *et al.* 2012; Berner
167 & Roesti 2017; Burri 2017; Comeron 2017; Delmore *et al.* 2018). Hence, the principal peaks of high
168 genetic differentiation identified in Bosse et al. 2017, matching the main areas of reduced diversity
169 displaying signatures of selective sweeps identified in Laine et al. 2016, may not necessarily illustrate
170 relatively recent divergent selection, but instead ancestral reduction of effective population size due
171 to low recombination, background selection and drift. Symmetrically to the risk of identifying false
172 positive loci in coldspots of recombination, it is also expected that neglecting the effects of variation
173 in recombination results in false negative loci localized outside of recombination coldspots (Berner &
174 Roesti 2017). Accordingly, both studies could have also missed candidate loci outside of these

175 recombination coldspots. Unfortunately, the large averaging sliding window (200kb) used in Bosse et
176 al. relatively to the average short distance linkage disequilibrium (r2 = 0.1 at 2kb) might have
177 worsened the problems raised above, by capturing even more principally large-scale areas of
178 recombination-elevated-differentiation. Correspondingly, this large window much likely increased
179 the risk of missing narrow peaks of elevated differentiation actually tagging candidate genes
180 targeted by relatively recent positive selection, and especially so outside of recombination coldspots.
181 In fact, such a large sliding window probably rather represents a neutral local baseline (Roesti 2012;
182 Burri 2017) that could have been implemented to detect local outlier markers showing locally
183 elevated divergence (e.g. supplementary figure 2). These caveats challenge the results and
184 conclusions of Bosse et al, questioning the potential role of divergent selection versus large-scale
185 variation in recombination and background selection in shaping the few large regions with elevated
186 differentiation. These considerations cast further doubt on the potential contemporary role of
187 feeders on bill length evolution.

188 Opportunely, this context and the available data offer exciting avenues of research to
189 unravel the determinants of both recent and long-term variation in bill length as well as adaptive
190 divergence between great tit populations across Europe. Spatial variation in bill length could be
191 unraveled in a multi-trait genome wide association test, using a polygenic framework, which could
192 be inspired from a pioneering study on great tit breeding phenology (Gienapp *et al.* 2017b). Besides,
193 a formal selection analysis relating great tit bill length to over-winter survival when birds are most
194 likely to benefit from food provided by people, is required, to elucidate the nature of the
195 evolutionary forces behind bill length variation. Finally, taking into account the effect of variation in
196 recombination rate and background selection along the genome to draw local neutral envelops of
197 genomic differentiation will likely help to validate or invalidate the potential footprints of divergent
198 selection identified almost only in coldspots of recombination, and to identify other footprints
199 outside of these regions (Roesti *et al.* 2012; 2013; Burri 2017; Comeron 2017; Delmore *et al.* 2018).

Providentially, the genomic analyses performed in other populations across Europe could also help determining whether elevated population differentiation was driven by population-specific relatively recent positive selection or by ancestral variation due to physical feature of the species genome (e.g. Figure Box 3 in Burri 2017).

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Figures

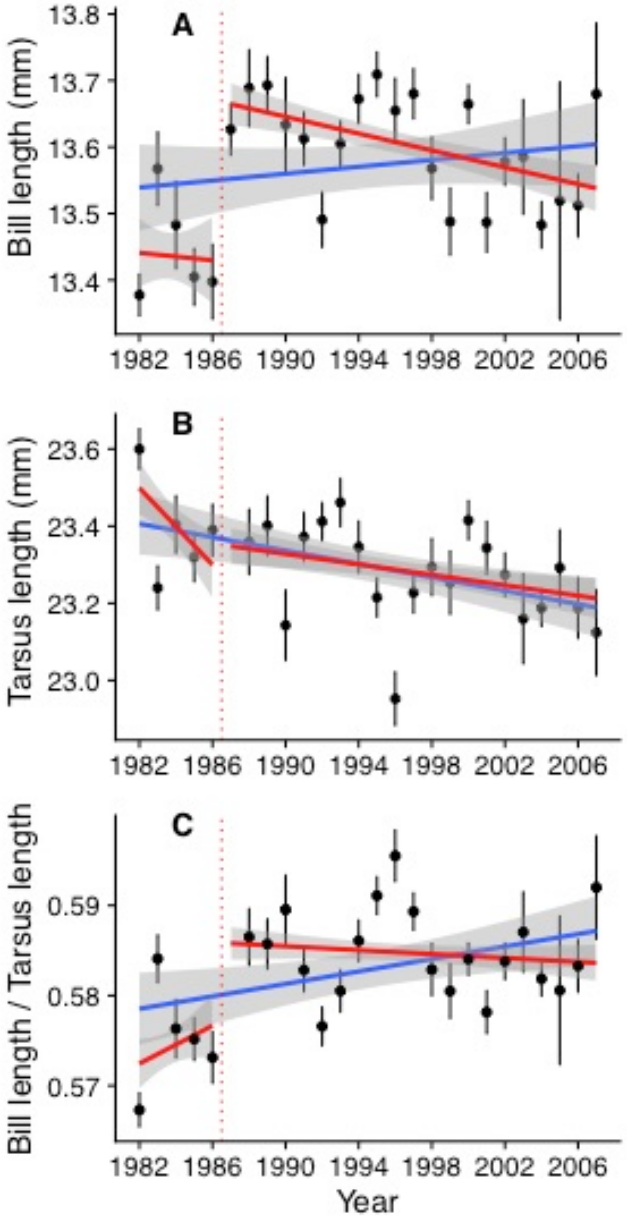


Figure 1. Decrease of mean bill length (A) and tarsus length (B) but conservation of the allometry between both traits (C), from 1987 to 2007 (in red) in Wytham great tits. Regressions from 1982 to 2007 presented by Bosse et al. are shown in blue.

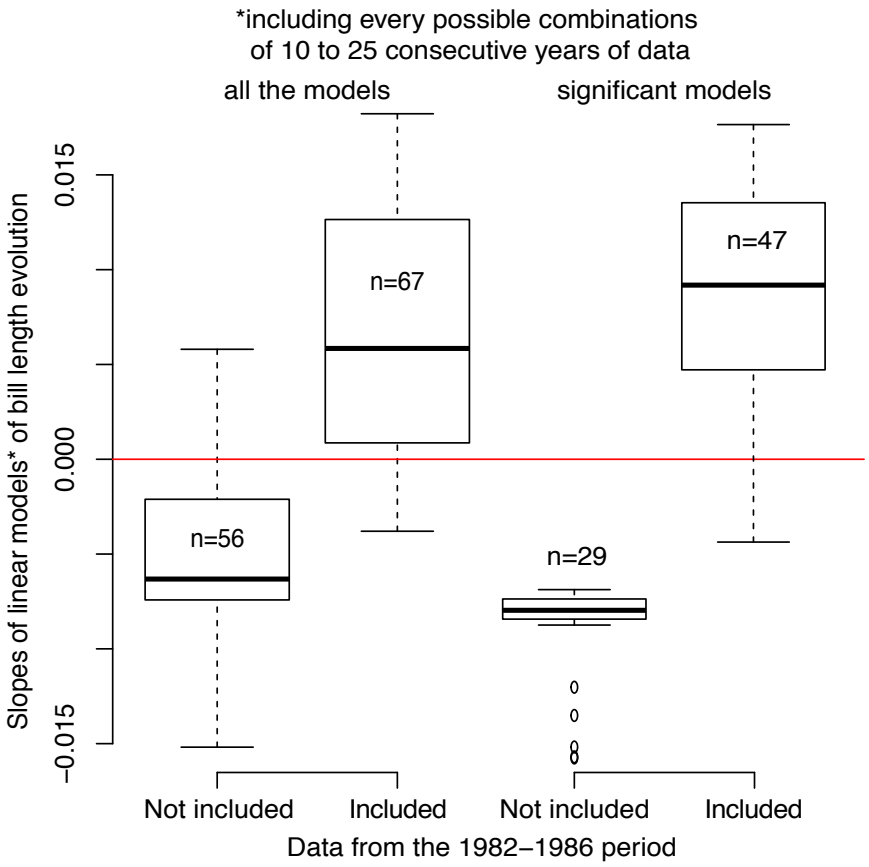


Figure 2. Slopes of linear models of bill length temporal evolution, including every possible combination of 10 to 25 consecutive years of data, with or without one to several years of data from 1982 to 1986.

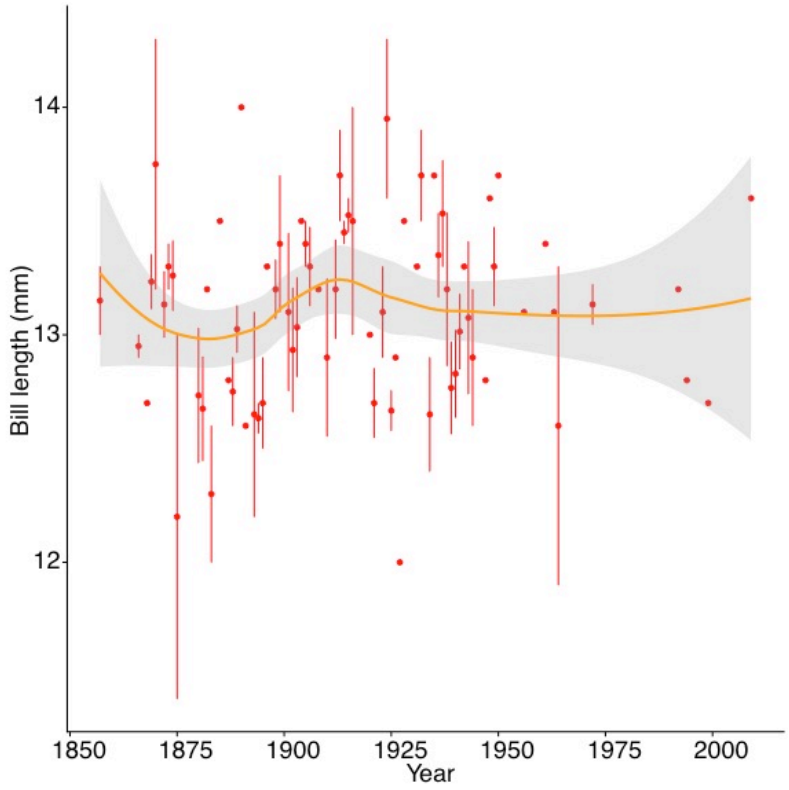
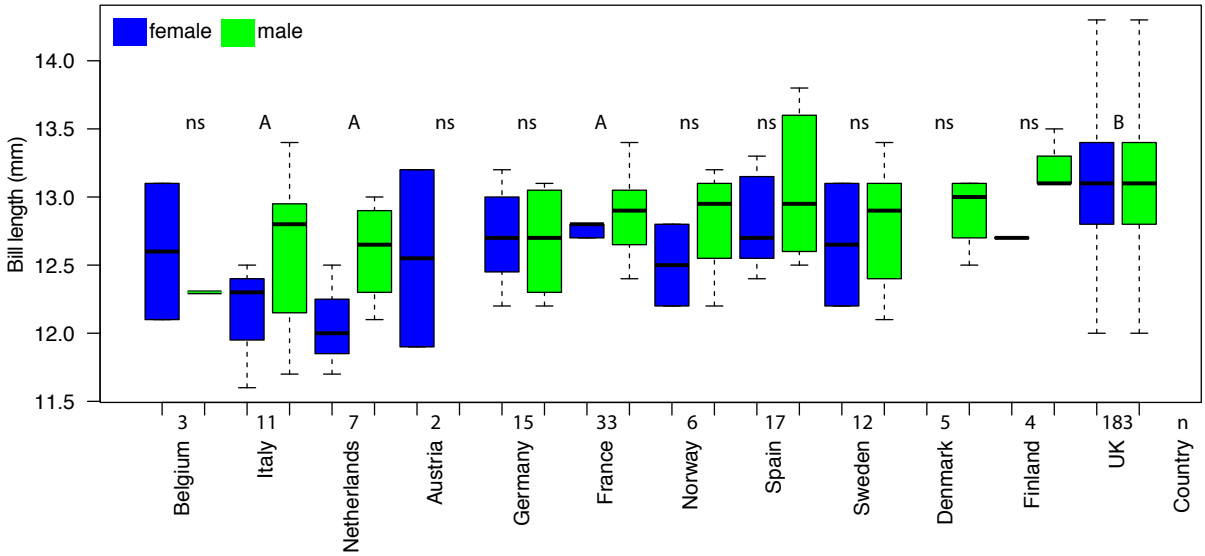


Figure 3. Variation of mean bill length in UK from 1850 to 2007, based on museum records used in Bosse et al. 2017.



290

291 Figure 4. Variation in bill length across European countries for both female (in blue) and male (in

292 green) great tit specimens from museums. Letters A and B illustrate significant differences

293 between sites and 'ns' refers to non-significant differences (Tukey HSD).

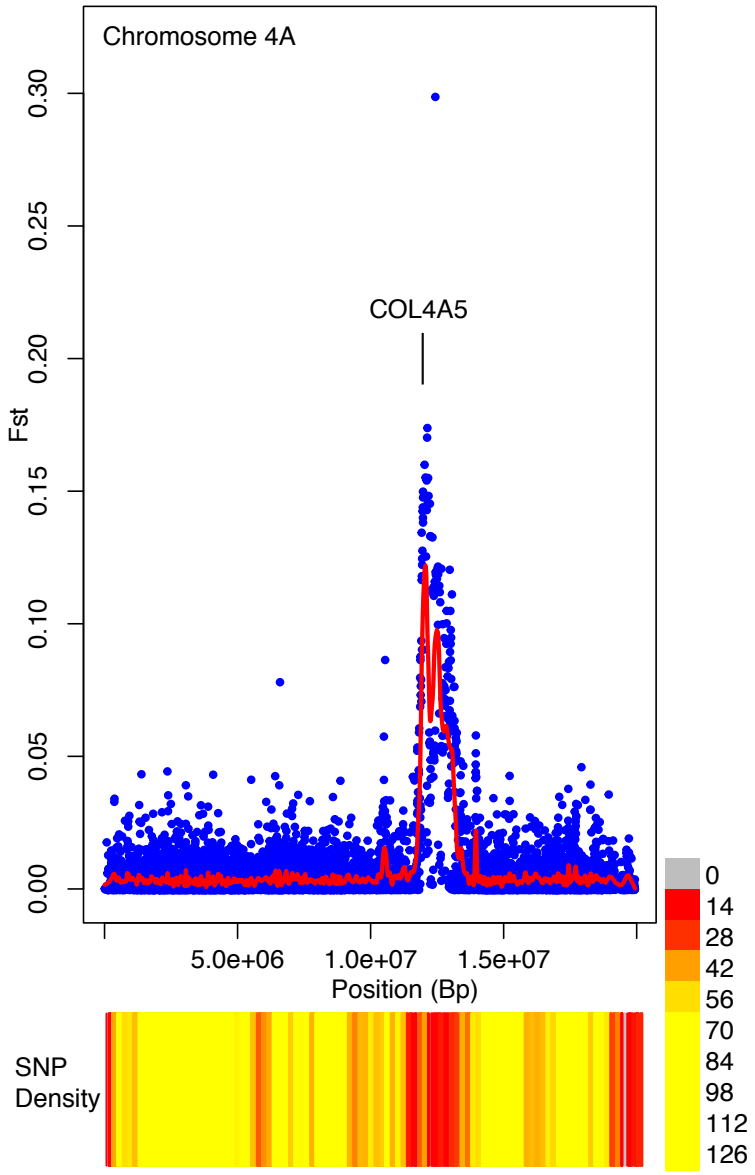
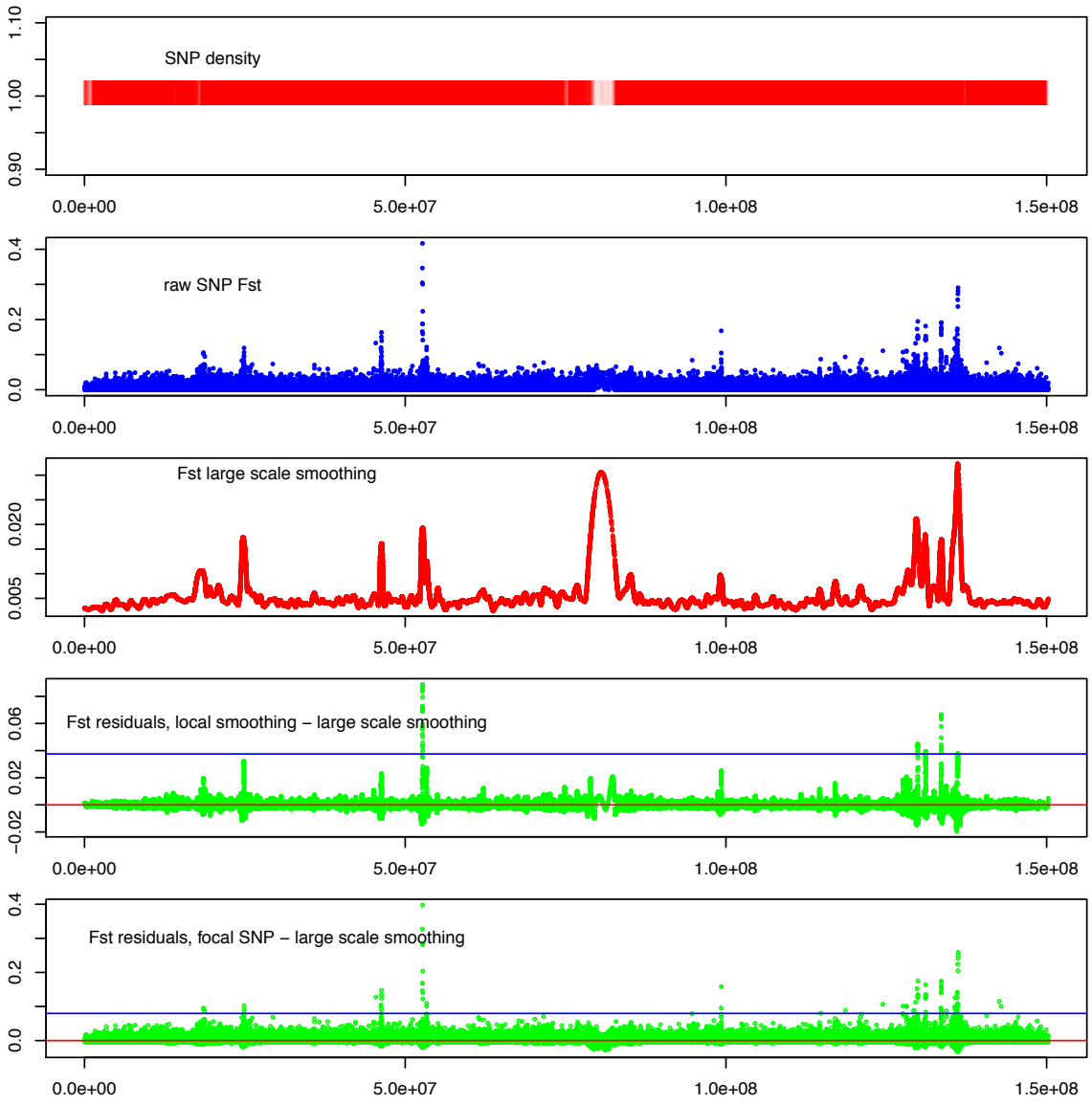


Figure 5. Fst at each SNP from chromosome 4A (blue dots), sliding window (red), and SNP density over 200 Kb regions.

Supplementary figure 1: 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 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

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

308 Fst outliers.

309