bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Title: On the importance of time scales when studying adaptive evolution

Running title: Time scale of bill length evolution

Authors: Charles PERRIER[1] & Anne CHARMANTIER[1]

Affiliations: [1]CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valery Montpellier, EPHE 1919, route de Mende - 34293 Montpellier cedex 05 – France

Corresponding authors: Charles.perrier@cefe.cnrs.fr

Abstract:

Long-term field studies coupled with quantitative genomics offer a powerful means to understand the genetic bases underlying quantitative traits and their evolutionary changes. However, analyzing and interpreting the time scales at which adaptive evolution occurs is challenging. First, while evolution is predictable in the short term, with strikingly rapid phenotypic changes in data series, it remains unpredictable in the long term. Second, while the temporal dynamics of some loci with large effect on phenotypic variation and fitness have been characterized, this task can be complicated in cases of highly polygenic trait architecture implicating numerous small effect size loci, or when statistical tests are sensitive to the heterogeneity of some key characteristics of the genome, like recombination rate variations. After introducing these aforementioned challenges, we discuss a recent investigation of the genomic architecture and spatio-temporal variation in great tit bill length, which was related to the recent use of bird feeders. We discuss how this case study illustrates the importance of considering different temporal scales and evolutionary mechanisms both while analyzing trait temporal trends and when searching for and interpreting the signals of putative genomic footprints of selection. More generally this commentary discusses interesting challenges for unraveling the time scale at which adaptive traits evolve and their genomic bases.

Key words: Adaptation, Phenotypic variation, Long-term data series, Genome scans, Recombination

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Impact summary:

An important goal in evolutionary biology is to understand how individual traits evolve, leading to fascinating variations in time and space. Long-term field studies have been crucial in trying to understand the timing, extent, and ecological determinants of such trait variation in wild populations. In this context, recent genomic tools can be used to look for the genetic bases underlying such trait variation and can provide clues on the nature and timing of their evolution. However, the analysis and the interpretation of the time scales at which evolution occurs remain challenging. First, analyzing long-term data series can be tricky; short-term changes are highly predictable whereas long-term evolution is much less predictable. A second difficult task is to study the architecture of complex quantitative traits and to decipher the timing and roles of the several genomic mechanisms involved in their evolution. This commentary introduces these challenges and discusses a recent investigation of the nature and timing of ecological and genomic factors responsible for variation in great tit bill length. Overall, we raise cautionary warnings regarding several conceptual and technical features and limitations when coupling analyses of long-term and genomic data to study trait evolution in wild populations.

Main Text:

Longitudinal field studies have brought invaluable insight for the understanding of evolutionary processes (Clutton-Brock & Sheldon 2010). Long-term studies notably allowed characterization of the temporality of trait variation and the strength and directionality of natural selection underlying such variation. Some of these long-term examinations of key phenotypic traits detected strikingly fast phenotypic change, driven by rapid ecological changes (Grant & Grant 2006). In contrast, many long-term studies failed to reveal micro-evolutionary change and response to selection (Merila *et al.* 2001; Pujol *et al.* 2018). While examining longitudinal data looking for both

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

long-term trends as well as short-term fluctuations has the potential to shed light on evolutionary trajectories in natural populations, our ability to understand and more notably predict evolution remains limited outside the laboratory. Quantitative genetic models were initially developed with the aim of predicting evolutionary change, based on estimates of selection and additive genetic variation (Falconer 1960). Their predictive power worked efficiently for the genetic improvement of complex traits in many animal and plant breeding programs. Yet when spatio-temporal ecological heterogeneity is involved, evolution in the wild remains largely unpredictable (Pemberton 2010; Pujol et al. 2018).

Coupling such long-term studies with genomic tools is a powerful way to improve our understanding of the genetic bases underlying evolutionary changes in response to environmental variation. Rapid and recent monogenic adaptations based on de-novo mutations are often used as examples, for instance the rise of the melanic morph of the peppered moth *Biston betularia* following the industrial revolution (van't Hof et al. 2016). Similarly, there are famous examples of rapid parallel monogenic or oligogenic adaptation based on long lasting standing genetic variation segregating in heterogeneous environments, for example coloration in deer mice *Peromyscus* and armor plates in stickleback *Gasterosteus aculeatus* (Barret & Hoekstra 2011; Nelson & Cresko 2018). However, these genomic analyses are facing several challenges when it comes to making inferences about polygenic adaptation and quantitative trait evolution. First, loci effect sizes are often small, requiring thousands, if not millions, of both SNPs and individuals for genome wide association studies (GWAS) to reveal significant effects. Such an investigation requires technical commitments (Wellenreuther & Hansson 2016; Gienapp et al. 2017a), but also a conceptual shift towards suppressing our desire to discover large effect alleles that are, in theory, rarely responsible for quantitative variation (Rockman 2011). Second, genome characteristics and especially variation in recombination rate along the genome can cause variation in the extent of background selection (defined as the loss of genetic diversity at a neutral locus due to negative selection against linked

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

deleterious alleles) (Charlesworth et al. 1993; Charlesworth 2012; Nordborg et al. 2009) that can confound or bias detections of positive selection and hence our comprehension of the timing and nature of evolutionary trajectories based on genomic data (Roesti et al. 2012; Burri et al. 2015; Berner & Roesti 2017; Burri 2017; Comeron 2017; Delmore et al. 2018). Nevertheless, several studies have begun to decipher the polygenic mechanisms of rapid evolution of quantitative traits.

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 bill length was predictable as a rapid response to strong selection, it remained unpredictable on a slightly longer microevolutionary scale. Although the question of predictability of evolution across time scales remains challenging, even in the genomic era (Nosil et al 2018), genomic tools did provide insights on the evolution of bill size in the context of the rapid diversification of Darwin's finches. A handful of genes were found to be significantly associated with bill size and shape in the medium ground finch *Geospiza fortis* (Lamichhaney et al. 2016), among which a major locus has been shown to influence bill dimensions in the Darwin finches' entire radiation (Lamichhaney et al. 2015). These genomic analyses hence cracked the genomic architecture of this trait variation at both small and large microevolutionary scale, with the predominant control of a few large effect loci.

In a recent study, Bosse et al. (2017) investigated the genetic architecture of bill length in the Great tit *Parus major*, using a tremendous amount of data from long-term research programs in

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Wytham woods in the UK and in the Netherlands (NL). Applying modern analyses of both population and quantitative genomics using 500k single nucleotide polymorphisms, the authors provide insight into the signatures of divergent selection in the studied populations and the genomic architecture of variation in bill length. In line with the quantitative nature of variation in bill length and with quantitative genetics theory (Lynch & Walsh 1998), the authors showed that the genetic architecture of bill length was highly polygenic. Specifically, the authors showed, using a mixture analysis fitting all the SNPs simultaneously, that 3009 SNPs explained collectively 31% of bill length phenotypic variation. None of these SNPs reached genome wide significance in the GWAS with bill length, revealing small effect sizes of individual variants. In accordance with recent quantitative genomic findings notably for other traits in great tits (*eg* Robinson et al. 2013), the proportion of variance in bill length explained by each chromosome amazingly scaled with its size, demonstrating that the many SNPs additively explaining bill length are distributed throughout the genome. The polygenic analysis also predicted the difference in bill length observed between the UK and the NL, further illustrating the polygenic nature of this trait variation. This evidence for a polygenic control of bill length with no large effect SNPs is hence very different from the previous example on Darwin finches' bill with large effect loci. Nevertheless, Bosse et al. (2017) also showed that variation at a single gene, col4A5, was associated with bill length. Bosse et al. (2017) then discussed whether an extended use of feeders, that are more abundant in the UK, might have driven the evolution of larger bills in the UK compared to the NL. Among the arguments pointing to feeders as drivers of longer bill lengths in UK great tits, Bosse et al. (2017) reported that col4A5 was associated with bill length in the UK but not in the NL, was highly differentiated between the UK and the NL, and was associated with greater reproductive success and higher activity at feeding sites in the UK. In addition, they reported that bills were longer in the UK compared to mainland Europe (Figure 4A in Bosse et al. 2017) and increased from 1982 to 2007 in the UK (Figure 4B in Bosse et al. 2017).

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

We argue in this comment that the speculation on the role of feeder on bill length evolution is not well supported by these arguments. Based on both phenotypic and genomic data, we propose instead that differences in bill length between the UK and the NL might have been evolving on a longer time scale than the contemporary use of feeders. First, an examination of bill length monitoring shows puzzling temporal and geographic patterns that may not incriminate the use of feeders. Second, the genomic patterns found at the region containing col4A5 might be compatible with the combined effect of variation in recombination rate and background selection, hence questioning the putative recent role of feeders as agents of positive selection.

The bill length trend inferred from the long-term monitoring is highly dependent on the time scale considered. Inspired by the readings of papers such as the Grant & Grant (2006) study 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 rapid 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 subsequent years (t-test: t = -7.28, df = 674.53, p-value = 9.1E-13). Although a linear regression through the entire period, from 1982 to 2007, as implemented by Bosse et al., yielded a positive slope, removing the five measures prior to 1987, bill length significantly decreased from 1987 to 2007 (Figure 1A, Linear model from 1987 to 2007 either taking tarsus length into account, or not, F = 7.13, p-value = 8.2E-4; F = 13.2, p-value: 2.9E-4, respectively). A LOESS model confirms the decrease in bill length during the second part of the record. More generally, slopes of linear models linking bill length to time, using all of the possible combinations of 10 to 25 consecutive years (simply removing 1 to 16 years at the beginning, or the end, or both, of the data-series), were often positive when including one or more data points collected between 1982 and 1987, while negative slopes were often observed when excluding these years (Figure 2), further

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

148 illustrating that both time periods yield opposite patterns. Furthermore, tarsus length also decreased
149 significantly both over the 1987-2007 and the 1982-2007 periods (Figure 1B, F = 6.61, p-value = 0.01
150 & F = 24.07, p-value = 9.9E-07, respectively). Correspondingly, the bill length / tarsus length ratio did
151 not change significantly from 1987 to 2007 (Figure 1C, F = 1.51, p-value = 0.28), indicating no change
152 in bill length, when scaled to tarsus length, over this period. The origin of this cutpoint is important
153 to investigate, since it could constitute an evidence for a response, either genetic or plastic, of both
154 bill and tarsus lengths to a rapid abiotic or biotic change or to a methodological change. Yet, it seems
155 at present difficult to decipher whether bill length or tarsus length or both, if any, were targeted by
156 selection. Indeed, both traits are commonly phenotypically and genetically positively correlated in
157 passerines (Teplitsky et al. 2014; Poissant et al. 2016), hence should often evolve together. Overall,
158 these results very likely rule out the possibility of a contemporary (1982-2007) positive effect of
159 feeders on bill length.

160 One could nevertheless argue that bill length could have increased at a longer time scale
161 than the 1982-2007 period, but still recent enough to incriminate the use of feeders. Figure 3
162 however shows the absence of a clear pattern of bill length increase in museum specimens collected
163 in the UK. Although more data are needed to confirm the pattern, it suggests that larger bill length in
164 the UK seems to have evolved over a longer time period than the one during which feeders have
165 been used. Moreover, bill length across Europe does not display a clear dichotomy between the UK
166 and mainland Europe but rather smooth spatial variations (Figure 4), with an ANOVA showing a
167 significant effect of country (p = 3.75e-07) but not of sex (p = 0.345), with UK birds indeed having
168 longer bills compared to three other countries (France, Italy, and the NL). This spatial variation also
169 challenges the suggestion by Bosse et al. (2017) of a recent increase in bill length in British great tits
170 caused by feeders.

171 We then question whether the evolution of the genomic region containing the gene col4A5,
172 which was the corner stone linking bill length, activity at feeders, reproductive success and divergent

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

173 selection, could have been influenced by recombination rate variation and background selection,
174 rather than recent positive selection due to feeders. While the decay of LD is very fast in the great tit
175 genome (marginal after 2kb, as shown in Figure S1 in Bosse et al. 2017, and in Figure 5A here (see
176 supplementary material 1 for method details)), col4A5 was found in a large (>1Mb) genomic region
177 with high long distance (20 to 200Kb) LD in the UK (Figure 5B). The F_{ST} between the UK and the NL
178 was high along this region (Figure 5D here; figure S3A in Bosse et al. 2017). The eigenGWAS in this
179 region was significant using both populations simultaneously for the entire set of SNPs (upper panel
180 figure S2 in Bosse et al. 2017) and for chromosome 4A only (Figure 5E). As argued by Bosse et al.
181 (2017), such results are compatible with recent strong positive selection in the UK over this large
182 stretch of DNA on chromosome 4A. However, this large stretch of high LD around col4A5 was not
183 only found in the UK but also in the NL (Figure 5C). The eigenGWAS in this region was also significant
184 for both populations separately (Figure 5F & G). Furthermore, this large region was previously
185 identified by Laine et al. (2016) as showing a signature of selective sweeps and reduced nucleotide
186 diversity at the scale of the entire species distribution and not only in the UK (Sixth chromosome in
187 Figure 2 in Laine et al. 2016). This same region was also identified as showing elevated differentiation
188 in several lineages and populations of *Ficedula* flycatchers (Ellegren et al. 2012; Figure 1C in Burri et
189 al. 2015). Therefore, given the existence of this large genomic region with reduced variation,
190 increased LD, and increased divergence at several spatial scales in great tits, and in flycatchers, it
191 seems unlikely that the mechanism shaping this region has been acting only in the UK, recently, and
192 implying mainly positive section. Burri et al. (2015) determined that the high differentiation and high
193 LD at this region, shared across flycatcher lineages, was due to the effect of linked selection
194 combined with low recombination and issued a crucial warning: “scans are likely to identify
195 recombination-mediated elevations of differentiation not necessarily attributable to selective
196 sweeps”. Accordingly, we propose that low recombination (potentially reflecting pericentromeric
197 regions) and background selection in the region containing col4A5 in great tits could have resulted in

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

198 locally reduced genetic diversity, increased differentiation and increased LD that could have
199 altogether mimicked signatures of recent positive selection.

200 To illustrate the general challenge raised here in differentiating recombination rate variation
201 combined with background selection from positive selection, we present a very simple simulation of
202 a 20Mb chromosome (hence comparable to the length of the chromosome 4A) containing a region
203 of 1.6Mb with greatly reduced recombination (comparable to the length of the col4A5 haplotypes),
204 with random occurrence of neutral and deleterious mutations but no beneficial ones (see
205 supplementary material 2 for methods details). We show that for an average LD decay comparable
206 to what was found in great tits (Figure 6A), long distance LD (Figure 6B), eigenGWAS (Figure 6C),
207 integrated extended haplotype homozygosity (Figure 6D) and nucleotide diversity (Figure 6E) all
208 show striking deviations in the recombination coldspot compared to the rest of the chromosome.
209 Therefore, this simple simulation illustrates that such a pattern as that observed by Bosse et al.
210 (2017) is compatible with the combined action of large-scale variation in recombination and
211 background selection.

212 Neglecting the effects of variation in recombination and LD along the genome might have
213 not only resulted in false positive footprints of selection in coldspots of recombination but also in
214 false negatives in regions with a higher recombination rate (Berner & Roesti 2017). The large sliding
215 window used in Bosse et al. (sliding over 200kb while the average LD is highly reduced after 2Kb)
216 potentially worsened this problem by capturing principally elevated differentiation in large regions
217 with increased LD possibly resulting from extended lower recombination due to structural variations.
218 It may have also diluted narrow (*ie* narrower than the sliding window) peaks of elevated
219 differentiation in more common regions with lower LD outside of recombination coldspots.
220 Unfortunately, this probably relatively common caveat holds regardless of the nature of selection (*ie*
221 positive or purifying) and is a purely mathematical problem of mismatch between the sliding window
222 length and the extent of LD variation along the genome. It typically occurs when the unit of the

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

223 sliding window is the base pair instead of the centimorgan. In fact, such large sliding windows
224 relative to the LD decay should be used as neutral local baselines to ascertain the effects of variation
225 in LD and recombination along the genome, with local outliers detected when comparing local
226 residuals to such baselines (Roesti 2012; Burri 2017).

227 All these considerations suggest that, although the pattern of increased divergence at
228 col4A5 is apparently compatible with strong positive selection, as suggested by Bosse et al (2017),
229 the combined role of background selection, strong recombination rate variation and invariably large
230 averaging window while long distance LD is variable, should be more comprehensively tested.
231 Correspondingly, including these factors may also uncover more variants under stronger positive
232 selection located outside of the few low recombination regions. In this context, the causality of the
233 associations between col4A5 and bill length, activity at feeders and reproductive success in the UK,
234 requires further clarifications.

235 This case study offers exciting avenues of research to unravel the determinants of both
236 recent and long-term as well as spatial variations in quantitative traits in the Great tit but also in
237 other emblematic species displaying quantitative trait variations. Spatial trait variation could be
238 unraveled in multi-trait GWAS using polygenic frameworks, inspired by a pioneering study on great
239 tit breeding phenology (Gienapp et al. 2017b). Additionally, a formal selection analysis relating bill
240 length to over-winter survival when birds are most likely to benefit from food provided by people, is
241 required to elucidate the nature of the evolutionary forces behind bill length variation. Finally,
242 including the effect of variation in recombination rate, background selection and LD along the
243 genome to draw local neutral envelopes of genomic differentiation and modulate the local width of
244 sliding windows, will likely help identify further candidate loci (Roesti et al. 2012; 2013; Burri 2017;
245 Comeron 2017; Delmore et al. 2018). Genomic analyses performed in other populations across
246 Europe could also help determine the timing and the nature of selection, by taking into account the
247 temporal dynamics of the differentiation landscape (Burri 2017). We wish to conclude by

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

248 emphasizing the importance of integrating, or at least recognizing, the widespread and sometimes
249 strong variation in recombination rate along genomes, which can in some circumstances distort our
250 understanding of evolutionary processes based on genomic investigations (Roesti et al. 2012; Burri et
251 al. 2015; Berner & Roesti 2017; Burri 2017; Comeron 2017; Delmore et al. 2018).

252

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Acknowledgements

We thank several colleagues from the Institute of Evolutionary Sciences of Montpellier (ISEM) and from the Center for Functional and Evolutionary Ecology (CEFE), especially Paul Jay, Tim Janicke, Nicolas Bierne, Pierre-Alexandre Gagnaire, François Bonhomme, and Patrice David, for discussions regarding the impact of recombination rate variation on the landscape of diversity and differentiation. We thank Caroline Rose for comments on the English syntax. We thank the associate editor, three anonymous reviewers and JC Senar for constructive comments on previous versions of the manuscript. Both authors are presently funded by a European Research Council Starting grant – *Selection in Heterogeneous Environment* (ERC-2013-StG-337365-SHE). The authors declare no conflict of interest.

References and Notes

Barrett RD & Hoekstra HE (2011). Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics*, *12*, 767.

Berner D, Roesti M (2017) Genomics of adaptive divergence with chromosome-scale heterogeneity in crossover rate. *Molecular Ecology*, **26**, 6351–6369.

Boag PT, Grant PR (1981) Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galapagos. *Science*, **214**, 82–85.

Bosse M, Spurgin LG, Laine VN *et al.* (2017) Recent natural selection causes adaptive evolution of an avian polygenic trait. *Science (New York, N.Y.)*, **358**, 365–368.

Burri R (2017) Interpreting differentiation landscapes in the light of long-term linked selection. *Evolution Letters*, **1**, 118–131.

Burri R, Nater A, Kawakami T *et al.* (2015) Linked selection and recombination rate variation drive the evolution of the genomic landscape of differentiation across the speciation continuum of Ficedulaflycatchers. *Genome Research*, **25**, 1656–1665.

Charlesworth B. (2013). Background selection 20 years on: the Wilhelmine E. Key 2012 invitational lecture. *Journal of Heredity*, ***104***(2), 161-171.

Charlesworth B., Morgan, M. T., & Charlesworth, D. (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics*, ***134***(4), 1289-1303.

Clutton-Brock T, Sheldon BC (2010) Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology & Evolution*, **25**, 562–573.

Comeron JM (2017) Background selection as null hypothesis in population genomics: insights and challenges from Drosophila studies. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **372**, 20160471–13.

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Delmore KE, Lugo Ramos JS, Van Doren BM *et al.* (2018) Comparative analysis examining patterns of genomic differentiation across multiple episodes of population divergence in birds. *Evolution Letters*, **2**, 76–87.

Ellegren H, Smeds L, Burri R *et al.* (2012) The genomic landscape of species divergence in Ficedula flycatchers. *Nature*, **491**, 756–760.

Falconer, D. S. (1960). *Introduction to quantitative genetics*. Oliver And Boyd; Edinburgh; London.

Gienapp P, Fior S, Guillaume F *et al.* (2017a) Genomic Quantitative Genetics to Study Evolution in the Wild. *Trends in Ecology & Evolution*, 1–12.

Gienapp P, Laine VN, Mateman AC, Van Oers K, Visser ME (2017b) Environment-Dependent Genotype-Phenotype Associations in Avian Breeding Time. *Frontiers in Genetics*, **8**, 1392–9.

Grant BR, Grant PR (1993) Evolution of Darwin's Finches Caused by a Rare Climatic Event. *Proceedings of the Royal Society B-Biological Sciences*, **251**, 111–117.

Grant PR (1999) *Ecology and evolution of Darwin's finches*. Princeton University Press.

Grant PR, Grant BR (2002) Unpredictable Evolution in a 30-Year Study of Darwin's Finches. *Science*, **296**, 707–711.

Grant PR, Grant BR (2006) Evolution of character displacement in Darwin's finches. *Science (New York, N.Y.)*, **313**, 224–226.

Haller BC & Messer PW (2017) SLiM 2: Flexible, interactive forward genetic simulations. *Molecular Biology and Evolution 34*, 230–240.

Lack D (1947) *Darwin's finches*. Cambridge University Press, London.

Laine VN, Gossmann TI, Schachtschneider KM *et al.* (2016) Evolutionary signals of selection on cognition from the great tit genome and methylome. *Nature Communications*, **7**, 1–9.

Lamichhaney S, Berglund J, Almén MS *et al.* (2015) Evolution of Darwin's finches and their beaks revealed by genome sequencing. 1–16.

Lamichhaney S, Han F, Berglund J, Wang C, 2016 A beak size locus in Darwin's finches facilitated character displacement during a drought. *science.sciencemag.org*

Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Associates Incorporated.

Merila J, Sheldon BC, Kruuk LE (2001) Explaining stasis: microevolutionary studies in natural populations. *Genetica*, **112-113**, 199–222.

Nelson TC & Cresko WA (2018). Ancient genomic variation underlies repeated ecological adaptation in young stickleback populations. *Evolution Letters*, *2*, 9-21.

Nordborg M, Charlesworth B, & Charlesworth D (1996). The effect of recombination on background selection. *Genetics Research*, *67*, 159-174.

Poissant J, Morrissey MB, Gosler AG, Slate J, Sheldon BC (2016) Multivariate selection and intersexual genetic constraints in a wild bird population. *Journal of Evolutionary Biology*, **29**, 2022–2035.

Pemberton JM (2010) Evolution of quantitative traits in the wild: mind the ecology. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *365*, 2431-2438.

Pujol B, Blanchet S, Charmantier A *et al.* (2018) The Missing Response to Selection in the Wild. *Trends in Ecology & Evolution*, 1–10.

Rockman MV (2011) The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution*, **66**, 1–17.

Roesti M, Hendry AP, Salzburger W, Berner D (2012) Genome divergence during evolutionary diversification as revealed in replicate lake–stream stickleback population pairs. *Molecular Ecology*, **21**, 2852–2862.

Roesti M, Moser D, Berner D (2013) Recombination in the threespine stickleback genome-patterns and consequences. *Molecular Ecology*, **22**, 3014–3027.

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Teplitsky C, Tarka M, Møller AP *et al.* (2014) Assessing Multivariate Constraints to Evolution across Ten Long-Term Avian Studies. **9**, e90444–15.

van't Hof AE, Campagne P, Rigden DJ, Yung CJ, Lingley J, Quail MA [...] & Saccheri IJ (2016). The industrial melanism mutation in British peppered moths is a transposable element. *Nature*, *534*, 102.

Wellenreuther M, Hansson B (2016) Detecting Polygenic Evolution: Problems, Pitfalls, and Promises. *Trends in Genetics*, **32**, 155–164.

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

344 **Figures legends**

345 Figure 1. Decrease of mean bill length (A) and tarsus length (B) but conservation of the allometry
346 between both traits (C), from 1987 to 2007 (red lines) in Wytham great tits. Regressions from
347 1982 to 2007 as in the original paper by Bosse et al. are shown by the blue lines. Dots and bars
348 illustrate means and standard errors, respectively, for each year for each variable. Shaded areas
349 represent 95% standard error around regressions lines.

350

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

354

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

357

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

361

362 Figure 5. Genomic re-analysis of the chromosome 4A using the data from Bosse et al (2017). A)
363 Smoothed linkage disequilibrium (LD) decay with genomic distance in UK and NL (pooled) great
364 tits; Smoothed long distance (between SNPs distant from 20 to 200Kb) LD variation in UK (B) and
365 NL (C); D) Single-marker F_{ST} between UK and NL; Single-marker significance of eigenGWAS for

bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

366 chromosome A4 in the entire sample (E) and only UK (F) and NL (G). The grey area indicates the
367 region in which col4A5 is located.

368

369 Figure 6. Genomic analysis of a 20Mb chromosome evolving in a single population, with a 1.6Mb
370 region of reduced recombination (indicated by a grey area), and random occurrence of neutral
371 and deleterious mutations and absence of beneficial mutations, simulated in SLiM. A) Smoothed
372 LD decay with genomic distance, B) Smoothed long distance (between sites distant from 20 to
373 200Kb) LD variation, C) Single-marker and smoothed significativity of eigenGWAS, D) Single-
374 marker and smoothed integrated Extended Haplotype Homozygosity (iES), E) 200kb and
375 smoothed nucleotide diversity.

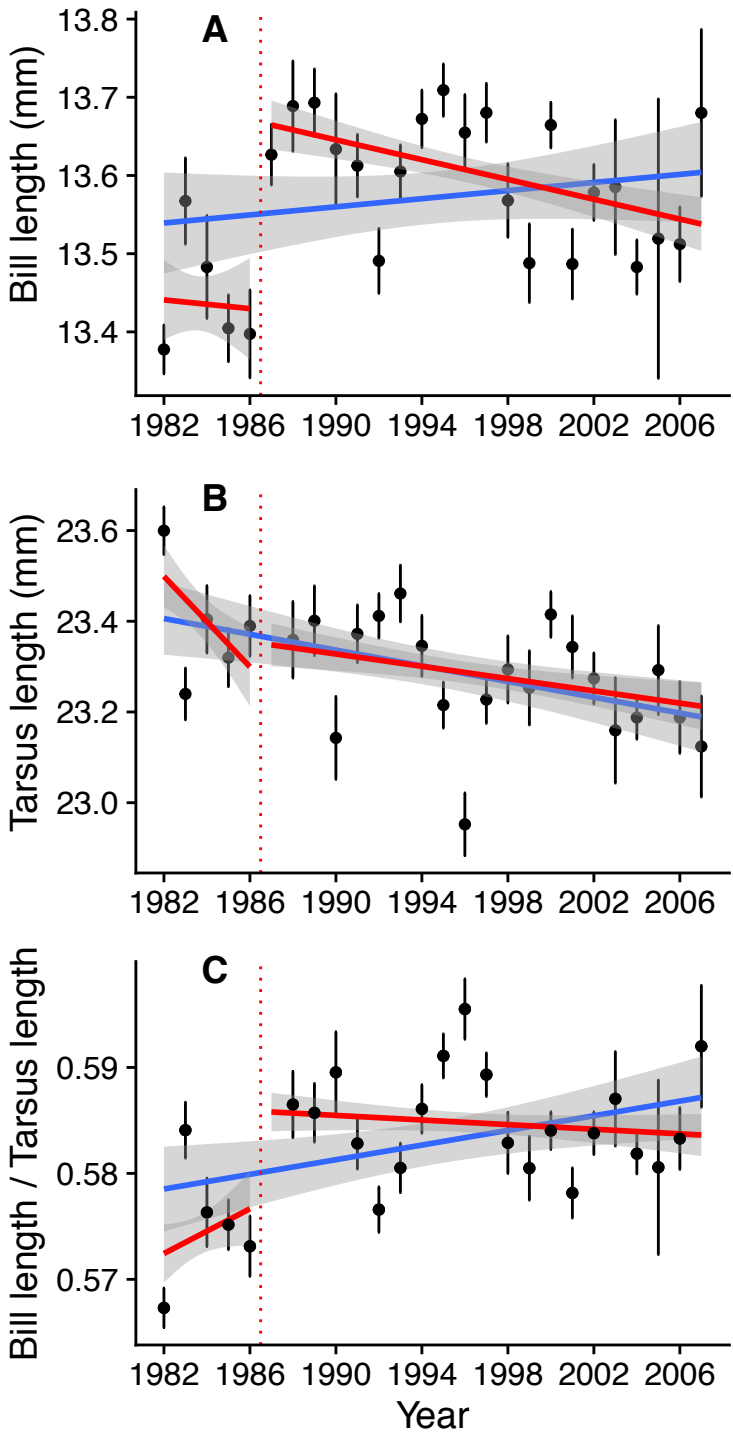
376

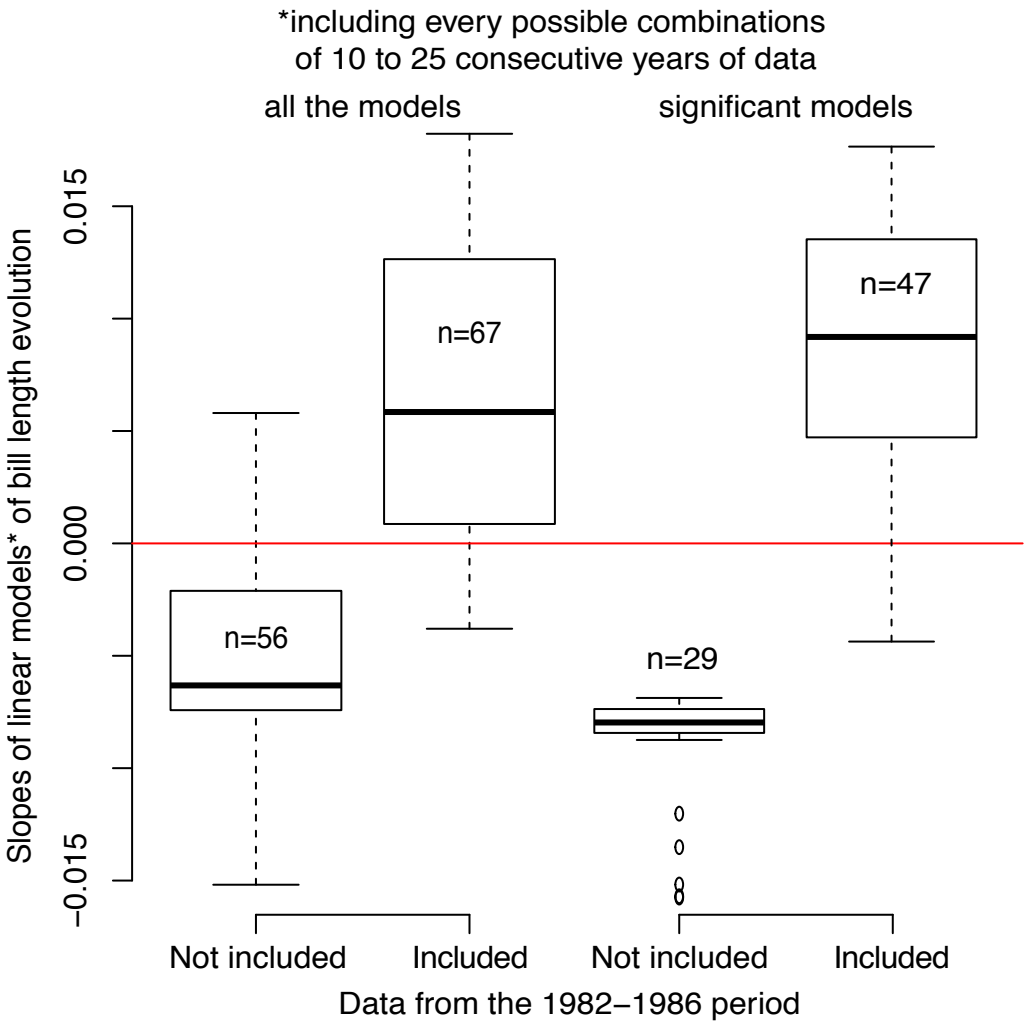
377 Supplementary material 1. Reanalysis of the COL4A5 region. We downloaded the SNP dataset
378 from Bosse et al. 2017 and used vcftools (Danecek et al. 2011) to estimate LD across the
379 genome for the entire dataset. We then estimated LD for both populations separately along the
380 chromosome 4A. To represent long distance LD, we kept LD values between each pairs of
381 markers distant from 20kb to 200kb. We smoothed these statistics using the loess function
382 native to R. We estimated F_{ST} using Plink (Purcell et al. 2007). We performed eigenGWAS tests
383 (Chen et al. 2017) in the UK, the NL and both. EigenGWAS were performed in chromosome 4A
384 only and not on the entire genome, in order to be more comparable with the simulation outputs
385 on one chromosome. Results from whole genome eigenGWAS can be found in figure S2 in Bosse
386 et al. (2017).

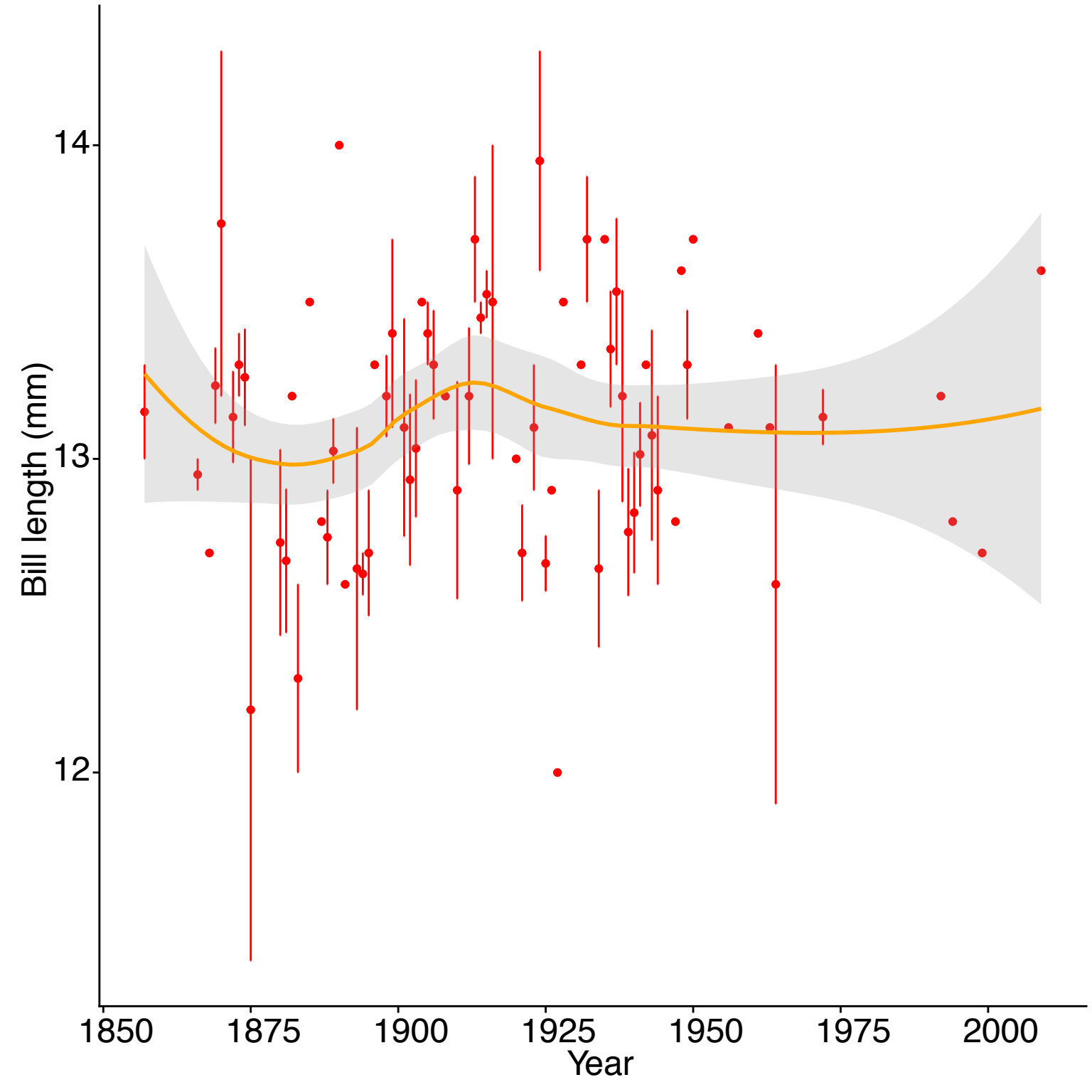
387

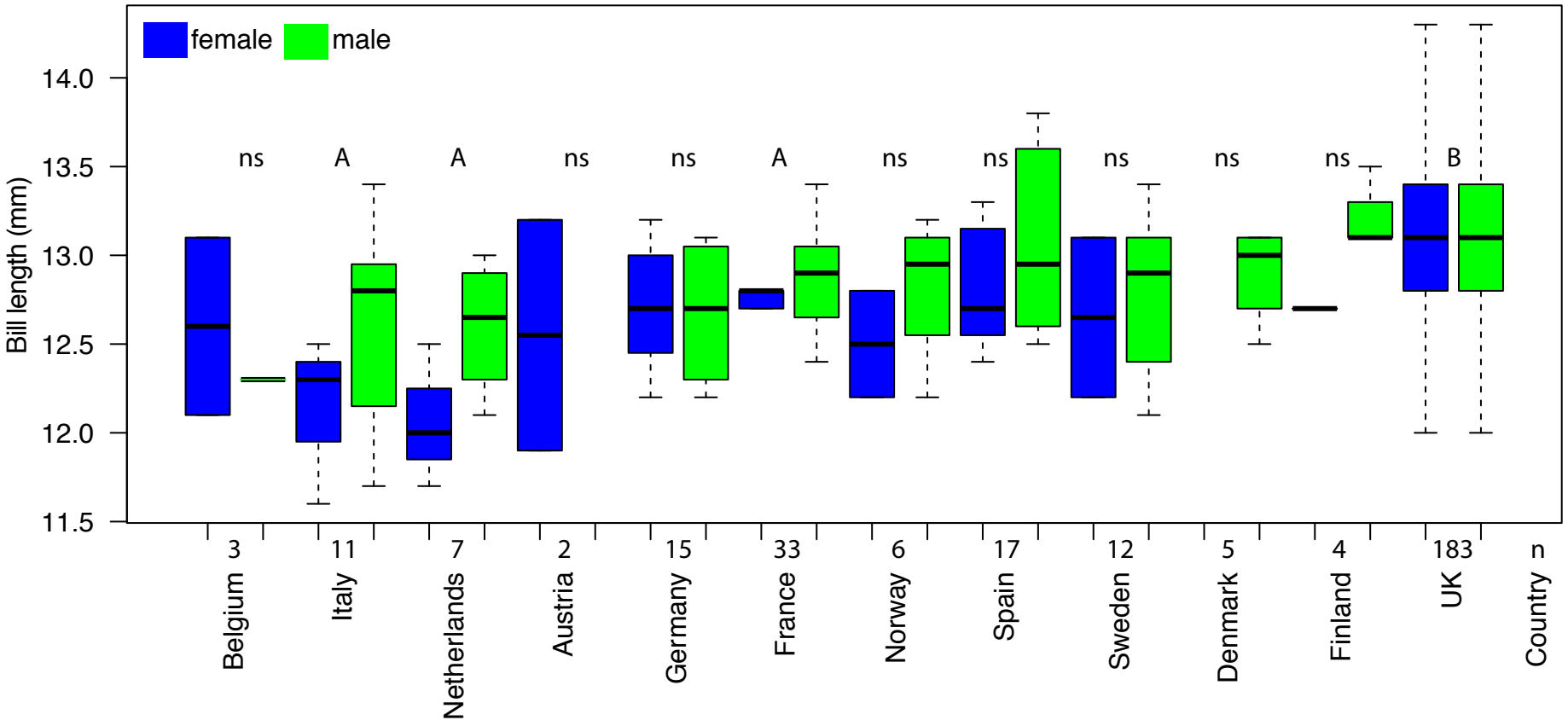
bioRxiv preprint doi: https://doi.org/10.1101/269175; this version posted October 4, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

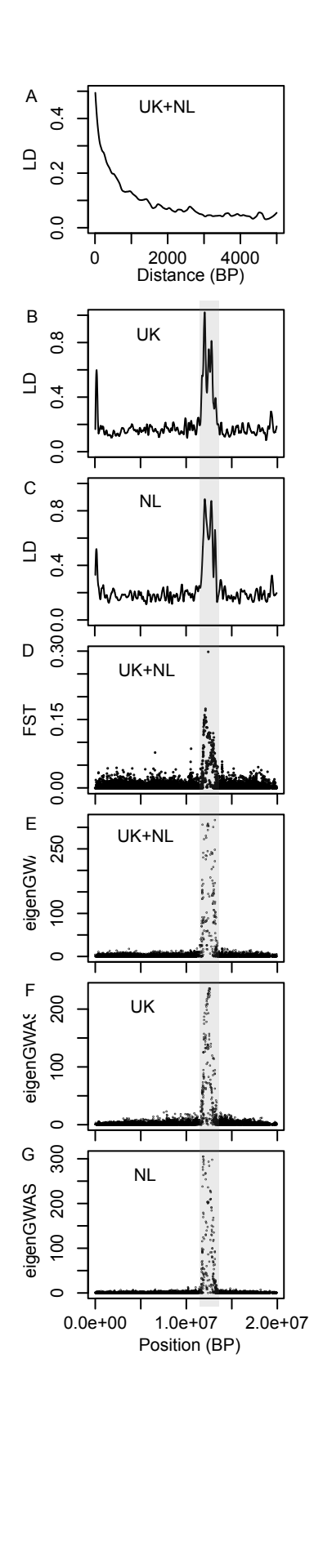
Supplementary material 2. Simulation of a chromosome similar in size to the chromosome 4A in great tit, and containing a region of reduced recombination of approximately the same size as the COL4A5 region. We used SLiM (Haller et al. 2017) to simulate a very simple scenario of reduced recombination in a 1.6Mb region within a 20Mb long chromosome. Recombination rate was equal to 1e-5 from 0 to 11.5Kb and from 13.1Kb to 20Mb. Recombination rate was equal to 1e-100 11.5Kb to 13.1Kb. Population size was equal to 1000. Simulation lasted 2000 generations. Mutation rate was 1e-7. We implemented 3 types of mutations: i) neutral, 50% of the mutations, ii) slightly deleterious (fitness effect of -0.1), 25% of the mutations, iii) severely deleterious (fitness effect of -0.9), 25% of the mutations. We exported a VCF dataset containing about 8500 SNPs from a randomly chosen simulation. We used vcftools to estimate LD across the chromosomes and nucleotide diversity (PI) over 200 kb windows. To represent long distance LD, we kept LD values between each pairs of markers distant from 20kb to 200kb. We performed eigenGWAS tests (Chen et al. 2017). We estimated iES (Sabesti et al. 2007) using the R package rehh (Gautier et al. 2016). We smoothed statistics using the loess function native to R.

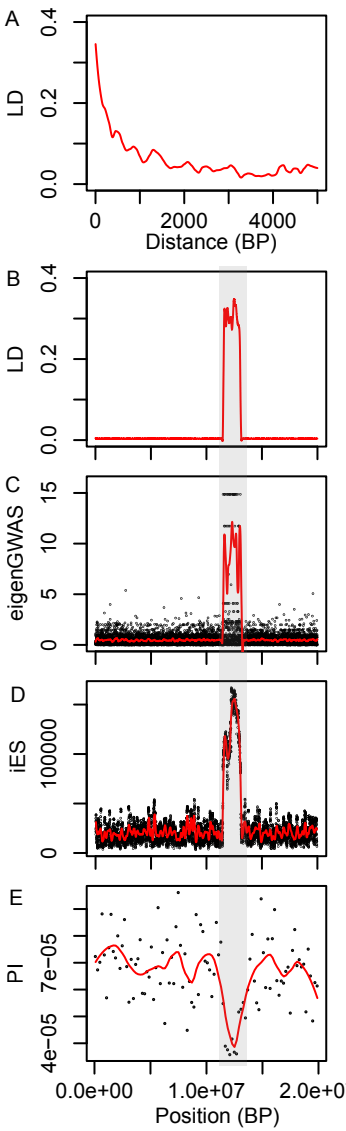

A
Bill length (mm)
13.8
13.7
13.6
13.5
13.4
1982
1986
1990
1994
1998
2002
2006
B
Tarsus length (mm)
23.6
23.4
23.2
23.0
C
Bill length / Tarsus length
0.59
0.58
0.57
Year


*including every possible combinations
of 10 to 25 consecutive years of data
all the models
significant models
Slopes of linear models* of bill length evolution
0.015
0.000
−0.015
n=67
n=56
n=47
n=29
Not included
Included
Not included
Included
Data from the 1982−1986 period


Bill length (mm)
14
13
12
1850
1875
1900
1925
1950
1975
2000
Year


female
male
Bill length (mm)
14.0
13.5
13.0
12.5
12.0
11.5
ns
A
A
ns
ns
A
ns
ns
ns
ns
ns
B
3
11
7
2
15
33
6
17
12
5
4
183
n
Belgium
Italy
Netherlands
Austria
Germany
France
Norway
Spain
Sweden
Denmark
Finland
UK
Country


A
UK+NL
LD
0.0
0.2
0.4
0
2000
4000
Distance (BP)
B
UK
LD
0.0
0.4
0.8
C
NL
LD
0.0
0.4
0.8
D
UK+NL
FST
0.00
0.15
0.30
E
UK+NL
eigenGW
0
100
250
F
UK
eigenGWAS
0
100
200
G
NL
eigenGWAS
0
100
200
300
0.0e+00
1.0e+07
2.0e+07
Position (BP)


A
LD
0.0
0.2
0.4
0
2000
4000
Distance (BP)
B
LD
0.0
0.2
0.4
C
eigenGWAS
0
5
10
15
D
iES
0
100000
E
PI
4e−05
7e−05
0.0e+00
1.0e+07
2.0e+07
Position (BP)