Title: On the timing of bill length evolution in British great tits: A comment on Bosse et al. 2017.

Running title: Evolution of bill length in great tits

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Abstract:

Bosse et al. (Science - 2017) investigated the genomic architecture and spatio-temporal variation in the bill length of the Great tit *Parus major*. They report a polygenic control of bill length and their analysis also suggests that UK birds have evolved longer bills than birds on mainland-Europe, potentially recently caused by the use of bird feeders. In contrast, using an alternative statistical approach, our analysis suggests a recent decrease in bill length in UK birds, even though they do have longer bills than great tits on mainland-Europe. This raises questions about the evolutionary mechanisms underlying increase on the long-term, but recent shrink, of bill length in UK great tits.

Main Text:

Avian bill morphology has played a prominent role in empirical studies of evolution and natural selection (*1*, *2*) and is notoriously plastic, being shaped by its use (3, 4). Bosse et al. investigated the genomic architecture of bill length in the Great tit *Parus major*, using a tremendous amount of data from two long-term research programs (5). Applying modern analyses of both population and quantitative genomics on 2322 birds genotyped at 500k single nucleotide polymorphisms, the authors provide insight into the genomic origins of variation in bill length. In line with quantitative genetics theory, the authors showed that i) the proportion of variance in bill length explained by each chromosome was scaled to its size, ii) about 1% of the SNPs contributed significantly to bill length variation, and iii) none of the detected SNPs reached genome wide significance in genome wide association tests (GWAS) with bill length. Moreover, the study provides evidence for larger bills in the UK compared to mainland-Europe, with genomic predictions based on the polygenic variation linked to bill length accurately predicting bill length differences. These last results suggest that bill length could have evolved *via* divergent selection between both geographic regions. This study could become a textbook case study of the genetic architecture of an illustrious polygenic trait in a wild population, uncovered by combining ambitious long-term field monitoring with extensive genomic analyses.

Media attention has, however, focused on a different aspect of the story, as illustrated by The Guardian's headline "British birds evolve bigger beaks to use garden feeders" (*6*). This massively communicated strong assertion was based on what was rather a speculation by the authors, based on a compelling collection of connected arguments. Among these arguments, Bosse et al. reported that COL4A5-C, an allele of the SNP with the highest (yet non-significant at the genome wide level) association with bill length, was at higher frequency in the UK than on mainland-Europe, was under selection, and was associated to 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 (5)) and increased from 1982 to 2007 in the UK (Figure 4B in (5)).

Here, we argue that the last of these arguments was not supported by the data. Specifically, we propose that bill length did not increase but instead decreased among the birds recently measured in the UK, although great tits do have longer bills in the UK than on mainland-Europe. Intrigued by the distribution of the annual mean bill-length data, especially by the striking change from 1986 to 1987 (Figure 4b in (3)) and apparent departures from the linear model, we re-analyzed the temporal dataset. As expected, breakpoint computations (*coin* R-package) proposed that the best cutpoint was between 1986 and 1987 (maxT = 7.41, p-value < 2.2E-16). Measures from the five years preceding 1987 differed from subsequent years (t-test: t = -7.28, df = 674.53, p-value = 9.1E-13). The origin of this cutpoint is important to investigate, and could have arisen due to abiotic, biotic or methodological factors. Removing the five measures prior to 1987, bill length significantly decreased from 1987 to 2007 (Figure 1A, Linear model taking into account or not tarsus length, 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 half of the record. Strikingly, tarsus length also decreased significantly from 1987 to 2007, but also from 1982 to 2007 (Figure 1B, F = 6.61, p-value = 0.01 & F = 24.07, p-value = 9.9E-07, respectively). Correspondingly, the bill length / tarsus length ratio did not change significantly from 1987 to 2007 (Figure 1C, F = 1.51, p-value = 0.28), indicating no change in bill length, when scaled to tarsus length, over this period. Finally, it may be important to note that differences in bill length across Europe are much greater than recent bill length changes in the UK. These results overall contradict the supposition by Bosse et al.'s of a recent increase of bill length in British great tits, as well as the supposed recent role of feeders.

The recent shortening of both bill and tarsus length could result from an evolutionary process, yet it is at present not possible to decipher whether bill length or tarsus length or both, if any, were targeted by selection. Indeed, both traits are commonly phenotypically and genetically

positively correlated in passerines (7), hence should often evolve together. This may be coherent with the authors' findings that the most significantly enriched GO-terms, implicating osteoblasts and palate development, were identified among the genes found clearly under selection in the UK but not significantly associated with bill length at the genome wide level. Alternatively, this recent shortening of bill and tarsus could have been driven, not by rapid evolution, but by a plastic response to environmental change. On the longer term, the observed bill length variation across Europe could be the result of evolution on a longer time scale than the period covered by phenotypic analyses in Bosse et al.. Hence, a halfway explanation between these two scenarios, partly compatible with the conclusions of Bosse et al., would involve a long-term genetic evolution towards longer bill (and tarsus length?) in the UK, combined with a recent plastic decrease of both bill and tarsus length (8).

Opportunely, this context offers exciting avenues of research to unravel the determinants of both recent and long-term variation in bill length. Recent bill length variation could be unraveled by combining trait plasticity and feeders usage in a multi-trait genome wide association tests, which could be inspired from a pioneering study on great tit breeding phenology (9). A formal selection analysis relating great tit bill length to over-winter survival when birds are most likely to benefit from food provided by people, would be needed to elucidate the nature of the evolutionary forces behind bill length variation. Approximating the age of genetic mutations (10, 11) linked to bill length, and other genetically correlated traits, would provide insights on the long-term evolutionary mechanisms underlying bill length variation. Finally, a comparative study of the genetic variation associated with bill length across the species distribution would also be highly valuable given the differences at this trait across countries (Figure 2).

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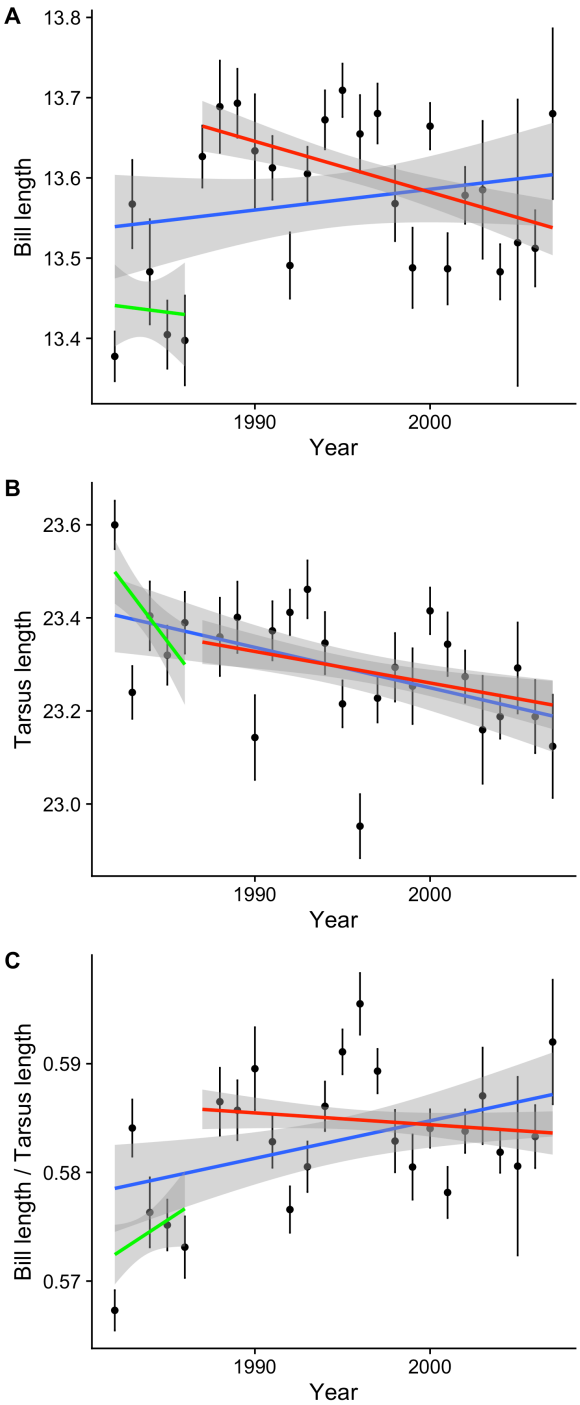

Figure 1. Decrease of mean bill length (A) and tarsus length (B) but conservation of the allometry between both traits (C), from 1987 to 2007 (in red) in Wytham great tits. Regressions from 1982 to 2007 presented by Bosse et al. are shown in blue. Regressions from 1982 to 1986 are shown in green.

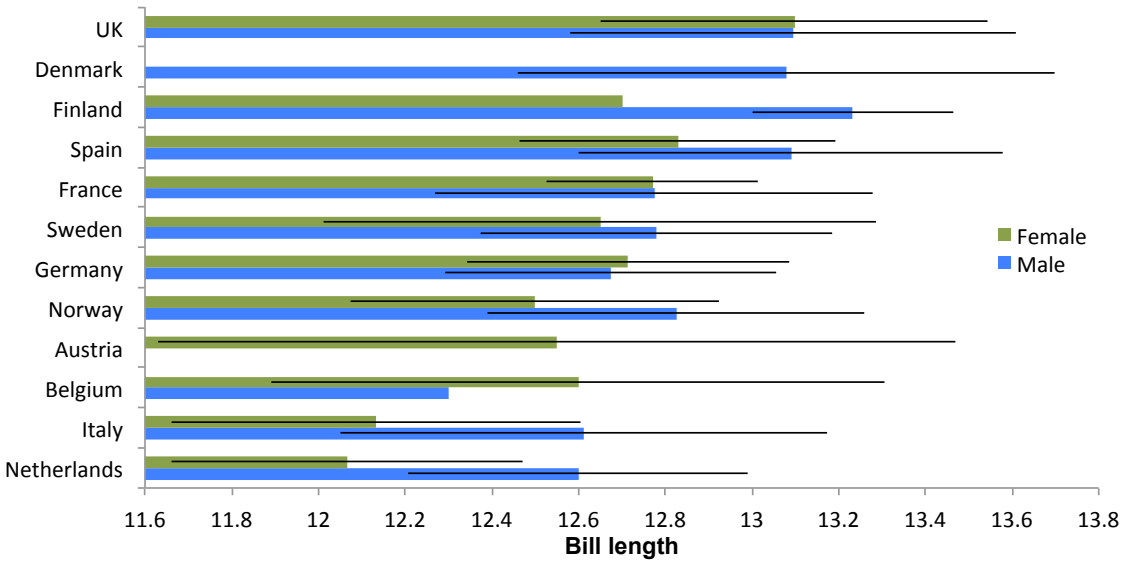

Figure 2. Variations in bill length across European countries for both male (in blue) and female (in green) great tit specimens from museums. Error bars indicate standard deviations.