Insights into the genetic architecture of morphological traits in two passerine bird species

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Abstract

Knowledge about the underlying genetic architecture of phenotypic traits is needed to understand and predict their evolutionary dynamics. The number of causal loci, magnitude of their effects and location in the genome is however still largely unknown. Here we use genome-wide SNP data from two large-scale datasets on house sparrows and collared flycatchers to examine the genetic architecture of different morphological traits (tarsus length, wing length, body mass, bill depth, bill length, total and visible badge size and white wing patches). Genomic heritabilities were estimated using relatedness calculated from SNPs. The proportion of variance captured by the SNPs (SNP-based heritability) was lower in house sparrows compared to collared flycatchers, as expected given marker density (6,348 SNPs in house sparrows versus 38,689 SNPs in collared flycatchers). Indeed, after down-sampling to similar SNP density and sample size this estimate was no longer markedly different between species. Chromosome partitioning analyses demonstrated that the proportion of variance explained by each chromosome was significantly positively related to the chromosome size for some traits, and, generally, that larger chromosomes tended to explain proportionally more variation than smaller chromosomes. Finally, we found two genome-wide significant associations with very small effect sizes. One SNP on chromosome 20 was associated with bill length in house sparrows and explained 1.2% of phenotypic variation (V_P) and one SNP on chromosome 4 was associated with tarsus length in collared flycatchers (3% of V_P). Although we cannot exclude the possibility of undetected large-effect QTL, our results indicate a polygenic basis for morphological traits.

Introduction

Information about the genetic architecture of phenotypic traits is fundamental to our understanding of how these traits evolve. By revealing the number and effect size of the loci

47 controlling heritable traits we can improve predictions about trait evolution in natural populations
48 (Barton & Keightley 2002) and better understand the potential of populations to adapt to
49 environmental change. For example, the HMGA2 gene in Galápagos finches explains a substantial
50 portion of variation in beak morphology, and was associated with marked character displacement
51 during a severe, acute drought (Lamichhaney *et al*. 2016). While there has been an increasing
52 number of studies aiming at identifying genes underlying phenotypic variation in natural
53 populations (reviewed in Slate *et al*. 2010; Schielzeth & Husby 2014), the genetic architecture (i.e.
54 the number of genes, their effect sizes and location in the genome) of most morphological traits still
55 remains unknown.

56 A first step in understanding the genetic architecture is to establish if the trait is heritable,
57 something that traditionally has been done using quantitative genetic methods such as parent-
58 offspring regressions, sib analyses or the 'animal model'(Lynch & Walsh 1998). These models all
59 use the expected genetic relatedness among individuals to estimate heritability. However, advances
60 in high-density genotyping have made it possible to use genome-wide marker data to estimate
61 realized genetic relatedness between individuals and therefore the 'genomic heritability'
62 (Aulchenko *et al*. 2007; Yang *et al*. 2010; Zaitlen *et al*. 2013; Rönnegård *et al*. 2016). Genome-
63 wide marker data from a large number of individuals can also be used to estimate the proportion of
64 variation in the trait that is tagged by SNP arrays, the so called SNP-based heritability (Yang *et al*.
65 2010, 2011b). All these approaches have limitations. For example, pedigree-based heritability
66 estimates (h^2_{ped}) require information from known relatives and heritability values may be biased due
67 to shared environmental factors among relatives. At the same time, SNP-based estimates have been
68 less successful in capturing the full extent of known trait genetic variance. As a result, there is often
69 a gap between heritability estimated from pedigree approaches and heritability estimated obtained
70 by considering significant SNPs from genome-wide association studies (GWAS), which is referred
71 to as the "missing heritability" (Manolio *et al*. 2009).

The 'missing heritability' phenomenon is partly a result of very stringent criteria for determining that a SNP contributes significantly to the trait variance. All SNPs considered jointly explain a much higher proportion of the variance than individually significant SNPs considered jointly (Yang *et al*. 2010). However, this method requires only unrelated individuals to be used, substantially reducing sample size. To alleviate this, Zaitlen *et al*. (2013) developed a method to estimate both the proportion of trait variance explained by genotyped SNPs (SNP-based heritability – h^2_g) and the 'total narrow-sense heritability' (h^2_{gkin}), which is equivalent to the traditional pedigree based heritability (Zaitlen *et al*. 2013).

Given high enough marker density, kinship coefficients can also be estimated on a more regional scale instead of genome-wide. For example, Yang *et al*. (2011a) proposed partitioning genetic variance of traits onto chromosomes. This method can provide novel insights into the genetic architecture of traits because it is expected that under a polygenic model, chromosome size should scale positively with the amount of genetic variation explained by that chromosome. Chromosomes that contribute a disproportionate amount of variation given their size can therefore indicate the presence of large-effect loci on that chromosome or, alternatively, a cluster of loci of small effect (Schielzeth & Husby 2014).

Ultimately, we are interested in understanding how evolutionary forces act on complex traits. Genome-wide association methods have been extensively used in human and livestock studies to detect causal loci (e.g. Goddard & Hayes 2009; Yang *et al*. 2010) and the decreasing cost of genotyping many individuals at thousands of loci means that GWAS are increasingly applied in studies of non-model organisms (e.g. Johnston *et al*. 2014; Barson *et al*. 2015; Husby *et al*. 2015; Santure *et al*. 2015). Some of these studies have been successful in identifying large-effect loci (Johnston *et al*. 2014; Barson *et al*. 2015) while others have failed to identify genome wide significant variants (Santure *et al*. 2013). Even in cases where significant variants have been

96 detected, they only explain a relatively small proportion of the phenotypic variance (e.g. Bérénos *et*
97 *al*. 2015; Husby *et al*. 2015).

98 Traditionally GWAS do not utilize repeated measurements of the same individuals, but
99 many long-term ecological studies follow individuals throughout their lifetime and re-measure
100 phenotypic traits over ontogeny. This adds additional information that could be used in GWAS, and
101 Rönnegård *et al*. (2016) recently developed a method to incorporate such repeated measures in a
102 GWAS framework. Adding repeated measures can lead to increased power if there are large annual
103 variations in the expression of the trait or unbalanced records per individual. As many GWAS of
104 natural populations suffer from a lack of power as a result of low sample size (e.g. Kardos *et al*.
105 2016), incorporating repeated measures can therefore be a useful way to increase power to detect
106 QTLs (Rönnegård *et al*. 2016).

107 In this study we take advantage of genomic resources that have recently become available
108 for house sparrows (*Passer domesticus*; Hagen *et al*. 2013) and collared flycatchers (*Ficedula*
109 *albicollis*; Ellegren *et al*. 2012), two well studied model passerine species in evolutionary biology
110 and ecology (Anderson 2006; Qvarnström *et al*. 2010). Of relevance to the present study, Hagen *et*
111 *al*. (2013) designed a custom Illumina 10K SNP array for house sparrows and Kawakami *et al*.
112 (2014) a custom Illumina 50K SNP array for collared flycatchers. These arrays have an average
113 marker density of one SNP per 100,000 bp for house sparrows (Hagen *et al*. 2013) and one SNP per
114 22,000 bp for collared flycatchers. These genomic resources, together with the phenotypic data
115 collected offer the opportunity to examine the genetic architecture of phenotypic traits.

116 House sparrows and collared flycatchers group in different phylogenetic clades within
117 Passeriformes, Passeridae and Muscicapidae respectively, that diverged approximately 50 million
118 years ago (Jarvis *et al*. 2014). Comparing the genetic architecture of different phenotypic traits in
119 these two species gives the opportunity to identify patterns of genetic architecture of phenotypic

traits within passerines. Here we first aimed at estimating genomic heritabilities of morphological traits using both genome wide and chromosome specific approaches (see Table 1). Second, we used a recently developed method (Zaitlen *et al*. 2013) to estimate the proportion of genetic variance captured by the SNP arrays. To identify SNPs associated with the traits studied we carried out GWAS using both repeated phenotypic measures (GWAS rep) and mean phenotypic values (GWAS mean). Finally, we examined whether the genetic architecture is concordant across similar traits in the two species and across different approaches.

Methods

Study populations and phenotypic data

Phenotypic data from house sparrows were collected as part of a long-term individual based study on four islands in northern Norway: Aldra (66°25′N, 13°04′E), Hestmannøy (66°33′N, 12°50′E), Leka (65°06′N, 11°38′E) and Vega (65°40′N, 11°55′E) that has been running since 1993 (e.g. (Jensen *et al*. 2008). Five phenotypic characters were measured in adults of both sexes (Figure 1): tarsus length, wing length, body mass, bill depth and bill length. In addition, both total badge size and visible badge size (see Fig 1) were measured in adult males as there is evidence of different mechanisms for the expression of these two traits, and they may act as different signals (Veiga 1996). Total badge size was measured as the square root of the area covered by black feathers and feathers with black bases and gray tips on the throat and chest, while visible badge size was measured as the square root of the area covered by completely black feathers, i.e. excluding the feathers with gray tips (Jensen et al. 2008). Phenotypic measurements were corrected for fieldworker variation by adding the mean difference between T.H.R. measurement and a fieldworker measurement when this was significant ($p < 0.05$) as judged by a paired t-tests (see Kvalnes 2016). When using one value per individual ("mean phenotypic values"), any variation in

144 trait size due to age and season was accounted for by adjusting trait size to February-measures at the
145 age of one year. This was done by first fitting a general linear mixed effects model (using the lme4
146 package in R, Bates *et al*. 2015) for each trait and sex separately, with age, age^2 and month as
147 explanatory variables, and an individual random intercept and slope to separate out any between-
148 individual variation in the relationship with age. The predicted values from this model were used to
149 adjust each measurement of a trait through the life of an individual to its predicted value in
150 February at age one. Then, the mean of all adjusted measurements was used as an individual's mean
151 trait value (Kvalnes 2016). We used this adjusted measurement as the mean trait estimate in all of
152 the following analyses. The effects of sex, hatch year and hatch island were accounted for in the
153 models below (heritability estimation, chromosome partitioning and GWAS) when these factors
154 were significantly associated with the trait being analyzed (adjusted R^2 and p-values in Table S1).
155 For the repeated measurements, we did not adjust trait measurements for age and season prior to the
156 analyses, but accounted for the effects of sex, hatch year, hatch island, month and age of the
157 individual at the time of measurement directly in the GWAS (adjusted R^2 and p-values in Table S2).
158 Phenotypic data on collared flycatchers were collected from a nestbox population on the
159 Swedish island of Öland (57°10'N, 16°58E), which has been monitored since 2002 (Qvarnström *et*
160 *al*. 2010). Individuals were caught and ringed while breeding, or ringed as nestlings. For all adults,
161 tarsus length, body mass, wing length and the size of white wing patches were measured. The white
162 on the wing was measured using sliding calipers as the sum of the amount of white on primary
163 feathers (2 – 7). The effects of sex and study area were included in the models below (heritability
164 estimation, chromosome partitioning and GWAS). Sex was included as a fixed effect in the mean
165 models of body mass, wing length and white patches on the wings, while study area was included in
166 the model of body mass and white patches on the wings (adjusted R^2 and p-values in Tables S3,
167 S4). For repeated measures models, sex was included as a covariate for body mass, wing length and
168 white patches on the wings, and study area was included in models of tarsus, wing length and white

on the wings. A description of the phenotypic data and number of records available for the analyses of house sparrows and collared flycatchers is reported in Table 2.

Genotyping

For both species a small blood sample was taken from the brachial vein of each individual and stored in ethanol, Queens lysis buffer or FTA cards for subsequent DNA extraction. In total, we genotyped 1,898 house sparrows with a 10K SNP array (Hagen *et al*. 2013) and 825 adult collared flycatchers on a 50K SNP array (Kawakami *et al*. 2014). We excluded markers with a call rate less than 95%, minor allele frequency (MAF) of less than 0.01 and a *p*-value for rejection of Hardy-Weinberg equilibrium (HWE) of less than 0.001. We also excluded one of a pair of individuals where the identity by state (IBS) was greater than 0.9 (removing accidental duplicated samples e.g. due to pipetting the same sample twice and avoiding bias errors introduced by these overrepresented genotypes). For this quality control step we used the function *check.marker()* in GenABEL (Aulchenko *et al*. 2007). For house sparrows, the quality control for HWE was conducted independently for each population and markers that failed this test in all populations were excluded (i.e. when a marker was not at HWE in all populations it was excluded). After quality control 6,348 SNPs were available for analysis in 1,851 house sparrows and 38,689 SNPs for 825 collared flycatchers.

Genetic variance and heritability estimation

Three different software were used to estimate genetic variance and heritability (Table 1). We first estimated genomic heritability of the phenotypic traits using the R package RepeatABEL, using the function "*rGLS"* (Rönnegård *et al*. 2016), which allows the use of repeated measurements

of phenotypic traits when estimating genetic variance. We refer to the genomic heritability estimates from this approach as h^2_{kin} (rep). For comparison with other studies (Robinson *et al*. 2013; Santure *et al*. 2013) we also estimated heritability using the mean phenotype for each individual in the R package GenABEL, using the function "*polygenic*" (Aulchenko *et al*. 2007). We refer to this estimate as h^2_{kin} (mean). Finally, we used the software GCTA to estimate the genetic variance using mean phenotypic values. In addition, GCTA was used to estimate the proportion of variance tagged by the SNP arrays (see below). In each of these methods, when appropriate (Tables S1 and S2), we included various fixed effects. Ideally some of the fixed effects would be included as random effects (hatch year, hatch island) but this was not possible because not all software allow more than one or two random effects (which are typically the relatedness matrices).

In addition to estimating genome wide genetic variance we also used a recent method to estimate how much of the genetic variance was captured by the SNP arrays (h^2_g). Unlike the method by (Yang et al. 2010) which needs unrelated individuals, Zaitlen *et al*. (2013) use two genetic relationship matrices (GRMs) in a restricted maximum likelihood (REML) analysis to calculate both SNP-based heritability (h^2_g) and a pedigree equivalent heritability (h^2_{gkin}) using all individuals. This method has been implemented in the software GCTA (Yang *et al*. 2011a).

The variance explained by all autosomal SNPs was estimated using the mixed effects linear model $\mathbf{y} = \mathbf{X\beta} + \mathbf{g_G} + \mathbf{g_G^{un}} + \boldsymbol{\varepsilon}$, where $\mathbf{y}$ is a vector of phenotypes, $\boldsymbol{\beta}$ is a vector of fixed effects (e.g. sex, hatch island, hatch year) with its incidence matrix $\mathbf{X}$, $\mathbf{g_G}$ is a matrix of aggregate effects of all autosomal SNPs for all individuals, and $\mathbf{g_G^{un}}$ is a matrix of aggregate effects of all autosomal SNPs where unrelated individuals have off-diagonals that are < 0.05 set to 0 to distinguish them from related individuals. This model therefore uses mean phenotypic values and estimates additive genetic effects tagged by the genotyped SNPs ('SNP-based heritability' – h^2_g) and the pedigree equivalent heritability using information about genetic relationships of kin inferred from the marker data ('total narrow-sense heritability' – h^2_{gkin}). The estimated total narrow-sense

heritability (h^2_{gkin}) can therefore be compared to h^2_{kin} (mean) estimates from GenABEL (Zaitlen *et al*. 2013). Prediction errors due to imperfect LD were adjusted using the *--grm-adj 0* function when estimating genetic relationships (for similar approach see Bérénos *et al*. 2015).

As sample size and marker density differs between species (1,898 house sparrows genotyped on 6,348 SNPs versus 825 collared flycatchers genotyped on 38,689 SNPs), this makes it difficult to compare heritability estimates. We therefore randomly down sampled the number of SNPs (in the collared flycatcher) and number of individuals (in the house sparrows) across the dataset such that both sample size and marker density were the same in both species. Heritabilities were then estimated using the four approaches described above and in Table 1.

Partitioning of genetic variance between chromosomes

To partition genetic variance among chromosomes, we used the GCTA software (Yang *et al*. 2011a) to compute chromosome specific GRMs for the autosomes. The genetic variance attributable to each chromosome was estimated by fitting the GRMs of all chromosomes simultaneously in the model: $\mathbf{y} = \mathbf{X\beta} + \sum_{c=1}^{m} \mathbf{g_c} + \boldsymbol{\varepsilon}$, where $\mathbf{g_c}$ is a vector of genetic effects attributable to each chromosome with $(\mathbf{g_c}) = \mathbf{A_c} * \boldsymbol{\sigma}_c^2$ ($\mathbf{A_c}$ is the GRM from the SNPs on each chromosome and $\boldsymbol{\sigma}_c^2$ is the chromosome variance). The maximum number of chromosomes fitted differed between house sparrows (m= 29) and collared flycatchers (m=33). If the models did not converge (for all traits except body mass in house sparrows and for wing length in collared flycatchers), the chromosomes with the smallest number of SNP markers were iteratively excluded until the model converged. A maximum of 8 chromosomes were excluded for house sparrows and 10 chromosomes for collared flycatchers (Table S5 and S7).

To address convergence problems we first fitted separate models for each chromosome with the GRM of the focal chromosome and a GRM for all other chromosomes combined:

$\mathbf{y} = \mathbf{X\beta} + \sum_{c=1}^{m} \mathbf{g_{focal}} + \sum_{c=1}^{m} \mathbf{g_{rest}} \ \boldsymbol{\varepsilon}$, where $(\mathbf{g_{focal}}) = \mathbf{A}_{focal} * \boldsymbol{\sigma}^2_{focal}$, and A_{focal} the GRM of the focal chromosome. $(\mathbf{g_{rest}}) = \mathbf{A}_{rest} * \boldsymbol{\sigma}^2_{rest}$ estimate the variation explained by all other chromosomes but the focal chromosome. However, this did not solve the convergence issues. We therefore also tried to estimate a single GRM using marker data from all micro-chromosomes jointly. This should estimate the variance due to all micro-chromosomes together. Unfortunately, this also did not completely solve the problem, and we still had some traits where the models did not converge (Table S6). These convergence problems are likely because of the low number of markers on some chromosomes (the microchromosomes).

We estimated the proportion of variance explained by the Z chromosome in collared flycatchers compared to the proportion explained by all autosomes considered together (Table S8). Note that we do not have comparable information in house sparrows (markers on the Z chromosome were not included here because they have not been mapped to the genome and a linkage map for the Z chromosome is not available yet), and so we did not consider this further.

To estimate the relationship between chromosome size and the amount of variation it explained for each trait, we used linear regression models in R (R Core Team 2015). Chromosome sizes for both house sparrows and collared flycatchers were taken from the reference genome assemblies (house sparrows; NCBI accession number 17653, collared flycatchers; NCBI accession number 11872)

GWAS

Data on both house sparrows and collared flycatchers have been collected as part of long term individual based monitoring projects. This allowed us to take repeated measures of individuals and to take this nested data structure into account when testing for associations between SNPs and the phenotypic traits using the function "*rGLS*" in the R package RepeatABEL (Rönnegård *et al*. 2016). RepeatABEL allows a mixed model with both a repeated measures effect as well as relatedness between individuals to be included as random effects. For comparison, we also used individual mean measurements of phenotypic traits using the function "*grammar*" in the R package GenABEL (Aulchenko *et al*. 2007). Reported *p*-values are based on Wald tests and are corrected for population stratification (structure and relatedness) and the repeated sampling of the same individuals when using the repeated measures GWAS (Rönnegård *et al*. 2016). The genome-wide significance threshold was determined using a Bonferroni correction by dividing the significance value ($p = 0.05$) by the number of markers (Lander & Kruglyak 1995) resulting in $p = 7.80 \times 10^{-6}$ for house sparrows and $p = 1.29 \times 10^{-6}$ for collared flycatchers. This is a conservative *p*-value as it assumes that all markers are independent. We also report the additive genetic variance explained by each of the five SNPs with the smallest *p*-values, estimated as $V_{SNP} = 2pqa^2$, where *p* and *q* are the frequencies of the major and minor allele frequencies, respectively, and *a* is the additive SNP effect (Falconer & Mackay 1996). The heritability of each of these SNPs (h^2_{SNP}) was then estimated as V_{SNP}/V_P (where V_P is the phenotypic variance estimate obtained from the GWAS).

Results

Genomic heritability

Heritability estimated using repeated phenotypic measures (h^2_{kin} (rep)) ranged from 0.136 for total badge size to 0.415 for tarsus length in house sparrows, and from 0.149 for white wing patches to 0.289 for tarsus length in collared flycatchers (Table 2). In general, when using mean

285 phenotypic values heritability estimates (h^2_{kin} (mean)) tended to be higher for both species across all
286 traits, ranging from 0.228 for total badge size to 0.495 for bill length in house sparrows, and from
287 0.267 for white wing patches to 0.576 for tarsus length in collared flycatchers (Table 2).

288 When jointly estimating the SNP-based heritability (h^2_g) and total narrow-sense heritability
289 (h^2_{gkin}) using the Zaitlen *et al*. (2013) method we found that SNP-based heritability ranged from
290 zero for wing length to 0.185 for body mass in house sparrows, and from 0.080 for body mass to
291 0.538 for wing length in collared flycatchers. For most traits, the total narrow-sense heritability
292 estimates from the Zaitlen *et al*. (2013) method (Table 3) were similar to the h^2_{kin}(mean) values
293 from GenABEL (Table 2).

294 In general SNP-based heritabilities were higher for collared flycatchers compared to the
295 house sparrows. To examine this in more detail we thinned both data sets down to 825 individuals
296 and 6,348 SNPs. In house sparrows the reduction of sample size caused an inflation for many h^2
297 estimates and an increase in standard errors, whereas a reduction in marker density in collared
298 flycatchers had little effect (Tables 4 and 5).

299

300 *Chromosome partitioning*

301 We found a significant linear relationship between the proportion of variance explained by
302 each chromosome and chromosome size for tarsus length, body mass, bill length and visible badge
303 size in house sparrows (Figure 2), but not for wing length, bill depth and total badge size (Figure 2,
304 Table S5). The variance explained by each chromosome ranged from zero to 0.092 across all traits
305 (Table S5). Chromosome 2, which is the largest chromosome in house sparrows, did not explain
306 much of the variation for most traits, except for visible badge size. On the other hand, chromosome
307 1 (the second largest chromosome) explained a high proportion of the variance in most
308 morphological traits (tarsus length, wing length, bill depth, bill length and total badge) except for

body mass and visible badge size. Interestingly, a relatively small chromosome (14) explained a large proportion of the variation for wing length (Figure 2, Table S5).

Some models failed to convergence when including all chromosomes and estimating the variance using all micro-chromosomes together did not solve convergence issues (e.g. wing length). These values were similar to estimates when fitting the GRMs of all chromosomes simultaneously in the model (Tables S5 and S6). Fitting separate models with the GRM for a focal chromosome against the rest also did not solve convergence problems (see Methods). Some of the chromosome specific estimates should therefore be treated with caution.

For collared flycatchers, the relationship between the proportion of variance explained by each chromosome and chromosome size was significant for tarsus length (r=0.656), but not for wing length (r=0.269), body mass (r=0.144) or white wing patch (r=-0.041; Figure 3, Table S7). The proportion of variance explained by a single chromosome ranged from zero to 0.150 across all traits. As with house sparrows, chromosome 2 did not explain substantial variation in any trait, while chromosome 1 contributed substantially to both tarsus and wing length. Chromosome 4 also contributed substantially to tarsus length (Table S7), which reflects the presence of a significant marker for tarsus length on chromosome 4 (see below).

GWAS

After correcting for multiple testing, one SNP (11485) on chromosome 20 was significantly associated with bill length in house sparrows when using mean phenotypic values, and explained 2% of the phenotypic variation (Table S11, Figure S3). This SNP also had the lowest *p*-value when using repeated measures, although it was no longer significant after Bonferroni correction (Table S9, Figure S1). In general, each one of the top five SNPs (ranked by *p*-value) explained only a small proportion of the phenotypic variation and these values were similar between

the two approaches. The total amount of variation explained by the top five SNPs ranged from 3% for wing length to 5.8% for total badge using the repeated measures, and from 3.3% for wing length to 10.9% for total badge using the mean values (Tables S9 and S11). The ranking of the top five SNP associations was often similar between the two approaches, although they were not always shared (Tables S9 and S11).

As for house sparrows, the results from the two GWAS in collared flycatchers were also concordant. In neither approach did we find significant associations between SNP markers and any phenotypic traits with the exception of tarsus length (SNP N00199:174262 on chromosome 4); this SNP explained a small amount of the variation (3% using repeated measures and 4% using mean values). Across all the traits measured, allelic variation at the top five SNPs was responsible for between 3.3-11.4% of the phenotypic variation (Tables S9 and S12, Figure S2 and S4).

Discussion

Understanding the genetic architecture of traits in wild populations can better elucidate the mechanisms responsible for trait evolution, including the expected rate of evolutionary change (Barton & Keightley 2002). In this study, we used large-scale genotype data from custom SNP arrays from two passerine species to examine the genetic architecture of morphological traits. Using genomic data we demonstrate that these traits are heritable (Tables 2 and 3) and chromosome partitioning revealed that for many traits the proportion of variance explained by a chromosome scaled with its size, suggesting a polygenic basis (Figures 2 and 3). This interpretation was further supported by the GWAS that did not detect any large effect loci (Tables S9-S12). Overall, our results add further support for a polygenic basis in morphological, sexually selected and life-history traits as earlier documented for example in great tit (Santure *et al*. 2013, 2015), Soay sheep (Bérénos *et al*. 2015) and collared flycatcher (Husby *et al*. 2015; Kardos *et al*. 2016).

The different approaches used here to estimate heritability gave similar values to that seen in previous studies using pedigree approaches in both species (Gustafsson 1986; Jensen *et al*. 2003, 2008). As documented in a previous pedigree study (e.g. Åkesson *et al*. 2008), the use of repeated measures resulted in somewhat lower estimates of heritability, and this was also the case for genomic heritability in the GWAS context (Rönnegård *et al*. 2016). Our results support this finding (Table 2), which is a result of reduced residual variance in the mean trait models. Our estimates of heritability were generally lower than the average heritability estimates for morphological traits in wild systems (Postma 2014), which seems consistent with previous reports that genomic heritabilities tend to be lower than heritabilities estimated from pedigree based animal models (Zaitlen *et al*. 2013; de los Campos *et al*. 2015).

A relatively new measure is the SNP-based heritability, which estimates how much of the variation in a trait is tagged by the SNP array used after accounting for the variance explained by similarity between relatives. Studies in humans have demonstrated that the SNP-based heritability is generally lower than the pedigree heritability (Yang *et al*. 2010), suggesting that not all causal sites are tagged by the SNP arrays used. We used a recent approach developed by Zaitlen *et al*. (2013) to simultaneously estimate the SNP-based heritability (h^2_{g}) and the total narrow-sense heritability (h^2_{gkin}). In general, and as expected, the SNP-based heritability tended to be lower than the total narrow-sense heritability (Tables 3 and 5). This could be because of a relatively low density of SNPs, compared to relatively many related individuals. Thus, we might estimate a higher heritability by using genomic relatedness to assess resemblance between relatives than by assessing the phenotypic variance explained by tagged SNPs. The SNP-based heritability in house sparrows was generally lower than in collared flycatchers (zero to 0.185 versus 0.080 to 0.538 respectively), which was not unexpected given that marker density is higher for collared flycatchers than house sparrows. These differences in SNP-based heritability may also be the result of house sparrows being more related than collared flycatchers as a consequence of their life history characteristics.

We therefore thinned the collared flycatcher dataset to have 6,348 SNPs and the house sparrow dataset to have 835 individuals. Interestingly, both h^2_{gkin} and h^2(mean) showed there was an upward bias in the heritability estimates in house sparrows compared to the full data set (Tables 4 and 5). This indicates that there may be an inflation of heritability estimates and effect sizes at lower biological sample sizes. We did not see a similar effect in flycatchers, where we thinned the number of markers. This could be because the number of markers in the full data set was already relatively low, particularly for the LD structure typical in passerines (Kawakami *et al*. 2014; Kardos *et al*. 2016).

The Zaitlen *et al*. (2013) method has not yet, to our knowledge, been used in other studies of natural populations. Surprisingly, the proportion of the heritability explained by the SNPs in our study is similar or higher to that seen in humans (Yang *et al*. 2010). However, these datasets have substantial differences in terms of SNP density, sample sizes, level of relatedness between individuals and chromosome architecture. As we have demonstrated by thinning the sparrow data set to fewer individuals, there may be an inflation of the amount of phenotypic variation explained by kinship-based methods when fewer individuals are included in an analysis. Simulations are needed for a robust comparison and to understand the effects of these differences in the dataset when estimating SNP-based heritabilities.

For some traits, chromosome partitioning analyses demonstrated a significant positive association between the amount of variation explained by a chromosome and the size of that chromosome, as would be expected if the trait was polygenic (Figures 2 and 3). However, we did not find significant correlation between chromosome size and proportion of variance explained for wing length, bill depth and total badge in house sparrows, or wing length, body mass and white wing patches in collared flycatchers, although some larger chromosomes explained substantial amounts of the overall variation (Figures 2 and 3). Similar morphological traits have been identified as polygenic in other species; for example wing length, weight, tarsus length, clutch size and egg

407 weight in great tits (Robinson *et al*. 2013, Santure *et al*. 2015) and jaw size and body mass in Soay
408 sheep (Bérénos *et al*. 2015). Additionally, Schielzeth *et al*. (2012a) used a QTL linkage mapping
409 approach to find six genomic regions linked to variation in wing length in a captive population of
410 zebra finches. All putative regions showed similar effect sizes (3.9–8.3%) and together explained
411 only about half of the heritability in wing length. The many candidate genes within the QTL regions
412 further suggest a polygenic basis for wing length in zebra finches. In total, it seems that these
413 morphological traits in passerines could generally be polygenic.

414 One may still argue that our results are not totally consistent with previous findings.
415 However, it is important to keep in mind that the larger chromosomes tended to explain substantial
416 variance also in traits that did not show significant correlation between chromosome size and
417 proportion of variance explained (Tables S5-S7), as expected under a polygenic model. Moreover,
418 estimating relatedness on the micro-chromosomes is difficult because we have very few markers on
419 these, which makes estimation difficult (and potentially unreliable), as indicated by the problems
420 with model convergence. An additional consideration is that it is not clear that chromosomes that
421 contribute disproportionately to trait variation given their size should harbor large effect QTLs
422 because it is equally plausible that many small effect loci cluster on that chromosome. As pointed
423 out by Schielzeth & Husby (2014), such clustering of many loci of small effect on a single
424 chromosome is not uncommon and can involve association with biologically relevant pathways for
425 a specific trait. Some caution is therefore warranted when making predictions about the genetic
426 architecture of traits from regressions of chromosome size on proportion variance explained.
427 Finally, we did not find any significant single large effect-size markers for these traits on the
428 chromosomes that explained a disproportional part of the variance. Taken together, most evidence
429 therefore points in the direction of a polygenic basis also for these traits.

430 We only detected two SNP markers that met the genome-wide significant threshold: one
431 SNP on chromosome 20 for bill morphology in house sparrows that explained 1.9% of the

phenotypic variation and one SNP on chromosome 4 in the collared flycatcher for tarsus length explaining 3% of the variation. SNP 11485 on chromosome 20 associated with bill length in house sparrows might be related to a previously detected QTL on chromosome 20 for beak morphology in zebra finches (Knief *et al*. 2012). In the zebra finch this QTL was found to be located at 0.86–14.17 Mb and the position of the SNP in our study is 7.6 Mb. We are not aware of any previous studies on tarsus length that show an association in the region on chromosome 4 where the QTL for tarsus length in the collared flycatcher was located.

Another interesting finding in our study was the presence of two shared SNPs for different traits in house sparrows. SNP 15053 was among the top five SNPs associated with both total badge and visible badge size when using repeated values (Table S9), and SNP 11485 was among the top five SNPs associated with bill depth and bill length when using mean values (Table S11). Shared loci among traits will result in a genetic correlation between these traits (i.e., total badge vs. visible badge size and bill depth vs. bill length) and these are traits that have previously been found to be genetically correlated in this species (Jensen et al. 2008).

In summary, we genotyped a large number of individual house sparrows and collared flycatchers on custom genome-wide SNP arrays and examined the genetic architecture of a number of phenotypic traits. By estimating and using kinship matrices based on genome-wide SNP data we demonstrated that all traits showed substantial amount of genetic variance, in line with results from previous pedigree-based approaches. When applying a novel method to estimate the proportion of variance in the traits captured by the genotyped SNPs (SNP-based heritability, h^2_g), our estimates were somewhat larger than expected considering the sample size and number of SNPs used. The SNP-based heritability was lower than the total narrow-sense heritability in both species suggesting that not all causal sites are tagged by the SNP arrays used. Chromosome partitioning as well as GWAS showed several lines of evidence suggesting that the investigated traits are polygenic. This was indicated by a positive correlation between chromosome size and amount of variance explained

for most traits, a lack of any large effect QTLs, and the small amount of total variation explained by the top SNPs in the GWAS. Our results are in line with other recent studies showing a polygenic basis to phenotypic traits in natural populations.

Finally, one major conclusion to make from this work is that genomic techniques, even with low marker densities, can be useful to provide a better understanding of short-term evolutionary change of phenotypic traits in natural populations. We are currently transitioning to studies at the level of entire genomes but low-density SNP arrays can be very useful tools. In particular, these SNP arrays are a cost-efficient resource for addressing questions that require large sample sizes from natural populations.

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Data Accessibility

Genetic and phenotypic data will be deposited in Dryad. The deposit files contain data for each sample with the locality and genotypes for all individuals.

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601

602 Tables:

603

604 **Table 1** Information about the different approaches used for estimating heritabilities (abbreviation,
605 name, description with specific characteristics and underlying methodology and software used).
606 h^2_{kin}(mean) and h^2_{gkin} use the same data and statistical method and should give near identical
607 estimates (see Methods).

608

Abbreviation	Name	Description	Software	References
h^2_{kin} (rep)	Genomic heritability - using repeated measures	Genomic heritability estimated using genetic relatedness and using repeated measurements of phenotypic traits	R package RepeatABEL	Rönnegård *et al*. 2016
h^2_{kin} (mean)	Genomic heritability - using mean values	Genomic heritability estimated using genetic relatedness using the mean phenotype for each individual	R package GenABEL	Aulchenko *et al*. 2007
h^2_{g}	SNP-based heritability	Proportion of additive genetic effects captured by the genotyped SNPs using unrelated individuals	GCTA	Yang *et al*. 2011a; Zaitlen *et al*. 2013
h^2_{gkin}	Total narrow-sense heritability	Genomic heritability estimated using genetic relatedness inferred from marker data (mean phenotype for each individual)	GCTA	Yang *et al*. 2011a; Zaitlen *et al*. 2013

609

610 **Table 2** Descriptive information on number of individuals (N_i) and number of records (N_r) of each
611 trait with respective phenotypic mean and standard deviation (SD); heritability estimates with
612 respective standard errors (SE), total phenotypic variance (V_P) and total additive genetic variance
613 (V_A) for phenotypic traits of two passerines estimated using genomic heritabilities with repeated
614 measures (h^2_{kin} (rep)) and genomic heritabilities with mean values (h^2_{kin} (mean)). All estimates are
615 contingent on the fixed effects included in the analyses (see methods for the fixed effects included).

					Repeated measures				*Mean values*			
House sparrow	N_i	N_r	Mean	SD	h^2_{kin} (rep)	SE	V_P	V_A	h^2_{kin} (mean)	SE	V_P	V_A
Tarsus length	1443	3201	19.58	0.851	0.415	0.042	0.724	0.302	0.399	0.041	0.711	0.284
Wing length	1446	3210	79.92	2.032	0.388	0.037	4.865	1.888	0.481	0.040	3.927	1.889
Body mass	1448	3335	31.46	1.983	0.300	0.035	4.758	1.427	0.374	0.041	3.825	1.431
Bill depth	1442	3316	8.11	0.282	0.319	0.036	0.090	0.035	0.459	0.040	0.068	0.031
Bill length	1443	3314	13.74	0.542	0.390	0.037	0.340	0.108	0.495	0.039	0.253	0.125
Total badge size	721	1621	19.97	0.861	0.136	0.042	1.027	0.140	0.228	0.063	0.752	0.171
Visible badge size	720	1624	15.59	1.387	0.139	0.043	2.511	0.349	0.253	0.065	0.908	0.230
Collared flycatcher												
Tarsus length	798	1923	19.45	0.67	0.289	0.07	0.48	0.14	0.576	0.079	0.45	0.260
Wing length	800	1981	82.32	2.09	0.242	0.06	4.02	0.97	0.544	0.080	4.41	2.40
Body mass	794	1978	14.19	1.43	0.203	0.06	0.89	0.18	0.338	0.087	2.06	0.70
White wing patches	799	1974	32.93	16.87	0.149	0.05	195.6	29.2	0.267	0.083	284.6	75.97

616

617

Table 3 Descriptive information on heritability values with respective standard errors (SE), total phenotypic variance (V_P) and total additive genetic variance (V_A) for phenotypic traits of two passerines estimated using the Zaitlen *et al*. (2013) approach: SNP-based (h^2_g) and total narrow-sense heritability (h^2_{gkin}). Note that h^2_{gkin} is the sum of $h^2_g + h^2_{kin}$ (proportion of phenotypic variance not explained by the SNPs, not reported here).

		SNP-based heritability			*Total narrow-sense heritability*		
House sparrow	V_P	h^2_g	SE	V_A	h^2_{gkin}	SE	V_A
Tarsus length	0.700	0.052	0.078	0.037	0.399	0.073	0.279
Wing length[1]	2.408	0.000	0.051	0.000	0.114	0.065	0.275
Body mass	3.767	0.185	0.082	0.697	0.270	0.077	1.017
Bill depth	0.066	0.045	0.072	0.003	0.168	0.080	0.011
Bill length	0.240	0.119	0.081	0.028	0.147	0.074	0.035
Total badge	0.736	0.120	0.151	0.088	0.148	0.148	0.109
Visible badge	0.894	0.031	0.114	0.028	0.058	0.115	0.052
Collared flycatcher							
Tarsus length	0.464	0.45	0.17	0.209	0.651	0.080	0.302
Wing length	3.39	0.538	0.178	1.82	0.538	0.083	1.824
Body mass	0.649	0.080	0.181	0.052	0.310	0.082	0.201
White wing patches	141.19	0.083	0.162	11.70	0.185	0.094	26.12

[1] one variance component was constrained from the second iteration. When using the --reml-no-constrain option, the variance was negative.

Table 4 Descriptive information (for between species comparison) on number of individuals (N_i) and number of records (N_r) of each trait with respective phenotypic mean, standard deviation (SD) and total phenotypic variance (V_P); heritability estimates with respective standard errors (SE) and total additive genetic variance (V_A) for phenotypic traits of two passerines estimated using GWAS with repeated measures (h^2_{kin} (rep)) and GWAS with mean values (h^2_{kin} (mean)). These analyses use a thinned data set for both species such that marker density and sample size are identical (n= 825 individuals, n = 6,348 SNPs). Note that sample size for badge size traits in house sparrow are smaller (only present in males).

					Repeated measures				*Mean values*			
House sparrow	N_i	N_r	Mean	SD	h^2_{kin} (rep)	SE	V_P	V_A	h^2_{kin} (mean)	SE	V_P	V_A
Tarsus length	816	1560	19.63	0.84	0.484	0.066	0.720	0.348	0.459	0.065	0.711	0.330
Wing length	816	1564	80.18	2.25	0.376	0.057	3.962	1.490	0.505	0.064	3.927	2.001
Body mass	815	1612	31.61	2.21	0.287	0.056	4.198	1.205	0.410	0.066	3.825	1.721
Bill depth	816	1605	8.15	0.30	0.257	0.055	0.068	0.017	0.375	0.066	0.068	0.026
Bill length	816	1603	13.78	0.60	0.368	0.059	0.257	0.095	0.503	0.064	0.253	0.129
Total badge size	393	746	20.07	0.99	0.132	0.072	0.752	0.099	0.262	0.112	0.752	0.197
Visible badge size	390	747	15.88	1.56	0.099	0.066	0.952	0.094	0.099	0.097	0.908	0.094
Collared flycatcher												
Tarsus length	819	1923	19.45	0.67	0.284	0.06	0.48	0.14	0.466	0.07	0.45	0.20
Wing length	822	1981	82.32	2.09	0.233	0.06	4.02	1.60	0.397	0.07	4.41	1.75
Body mass	815	1978	14.19	1.43	0.203	0.05	0.89	0.26	0.290	0.08	2.06	0.60
White wing patches	820	1974	32.93	16.9	0.140	0.05	195.6	46.4	0.237	0.07	284.6	67.5

Table 5 Descriptive information (for between species comparison) on heritability values with respective standard errors (SE), total phenotypic variance (V_P) and total additive genetic variance (V_A) for phenotypic traits of two passerines estimated using the Zaitlen *et al*. (2013) approach: SNP-based (h^2_g) and total narrow-sense heritability (h^2_{gkin}). These analyses use a thinned data set for both species such that marker density and sample size are identical (n= 825 individuals, n = 6,348 SNPs). Note that sample size for badge size traits in house sparrow are smaller (only present in males).

		SNP-based heritability			*Total narrow-sense heritability*		
House sparrow	V_P	h^2_g	SE	V_A	h^2_{gkin}	SE	V_A
Tarsus length[1]	0.722	0.000	0.136	0.000	0.412	0.140	0.297
Wing length[2]	2.320	0.000	0.149	0.000	0.282	0.144	0.654
Body mass	4.051	0.358	0.163	1.451	0.358	0.145	1.450
Bill depth	0.065	0.342	0.165	0.022	0.342	0.145	0.022
Bill length	0.245	0.103	0.141	0.025	0.205	0.128	0.050
Total badge	0.763	0.149	0.311	0.114	0.257	0.305	0.196
Visible badge	0.970	0.000	0.314	0.000	0.258	0.291	0.250
Collared flycatcher							
Tarsus length	0.465	0.232	0.117	0.108	0.620	0.083	0.288
Wing length	3.38	0.417	0.122	1.411	0.529	0.083	1.791
Body mass	0.649	0.163	0.126	0.106	0.307	0.081	0.199
White wing patches	141.22	0.048	0.126	6.77	0.186	0.093	26.27

[1] one variance component was constrained from the second iteration. [2] one variance component was constrained from the first iteration.

Figures:

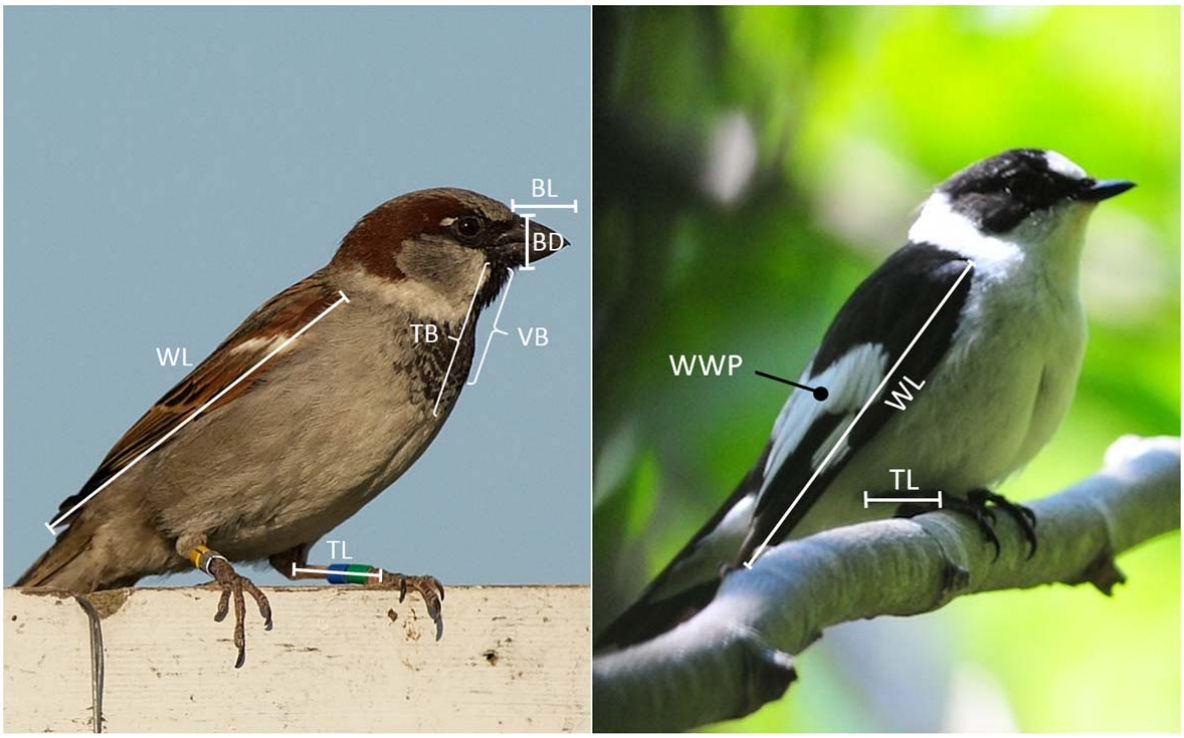


Figure 1 Schematics of phenotypic measurements in house sparrows (BL: bill length, BD: bill depth, TB: total badge, VB: visible badge, WL: wing length and TL: tarsus length) and collared flycatchers (TL: tarsus length, WL: wing length, WWP: white wing patches). Photos by H. Jensen (male house sparrow) and A. Husby (male collared flycatcher).

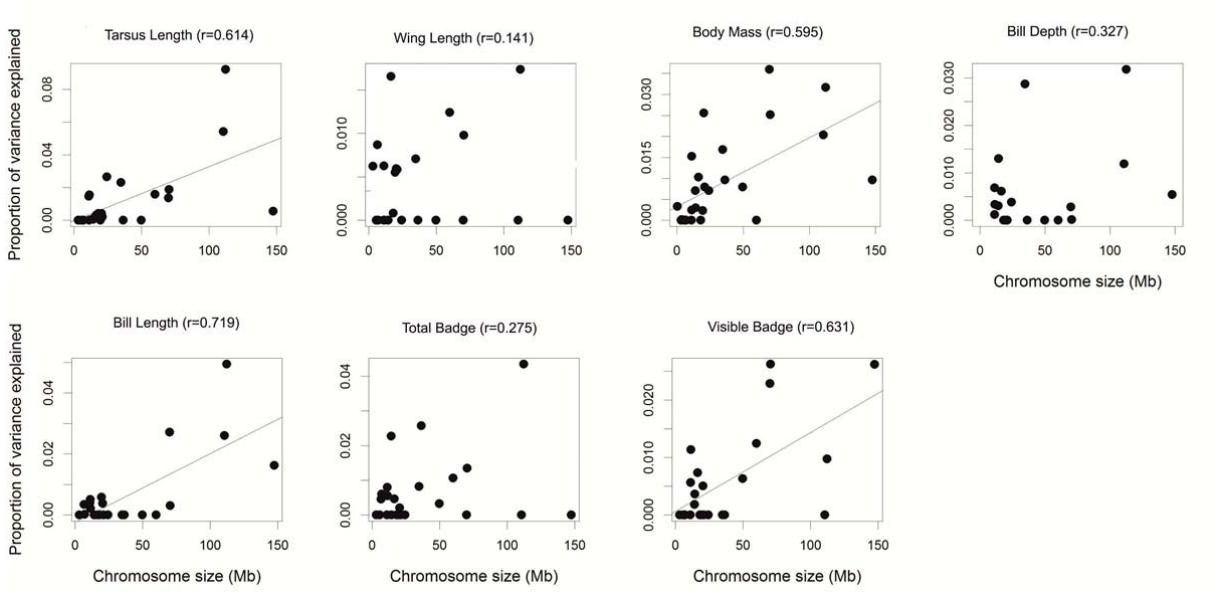
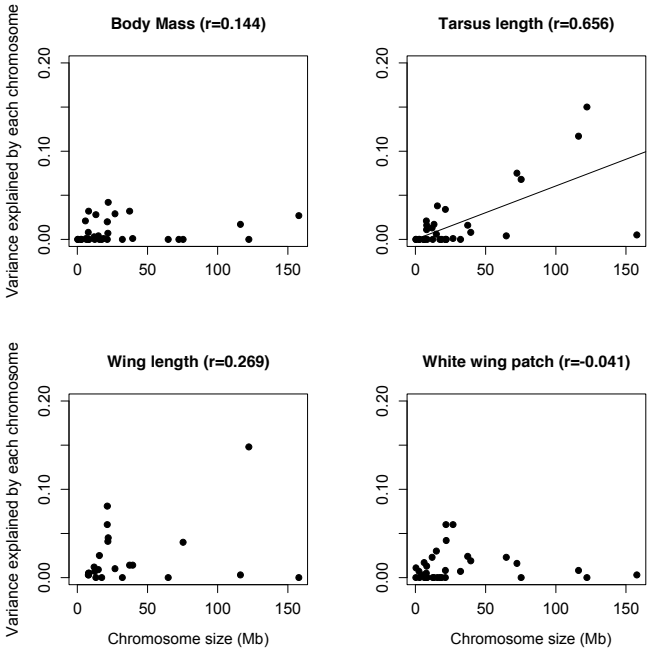
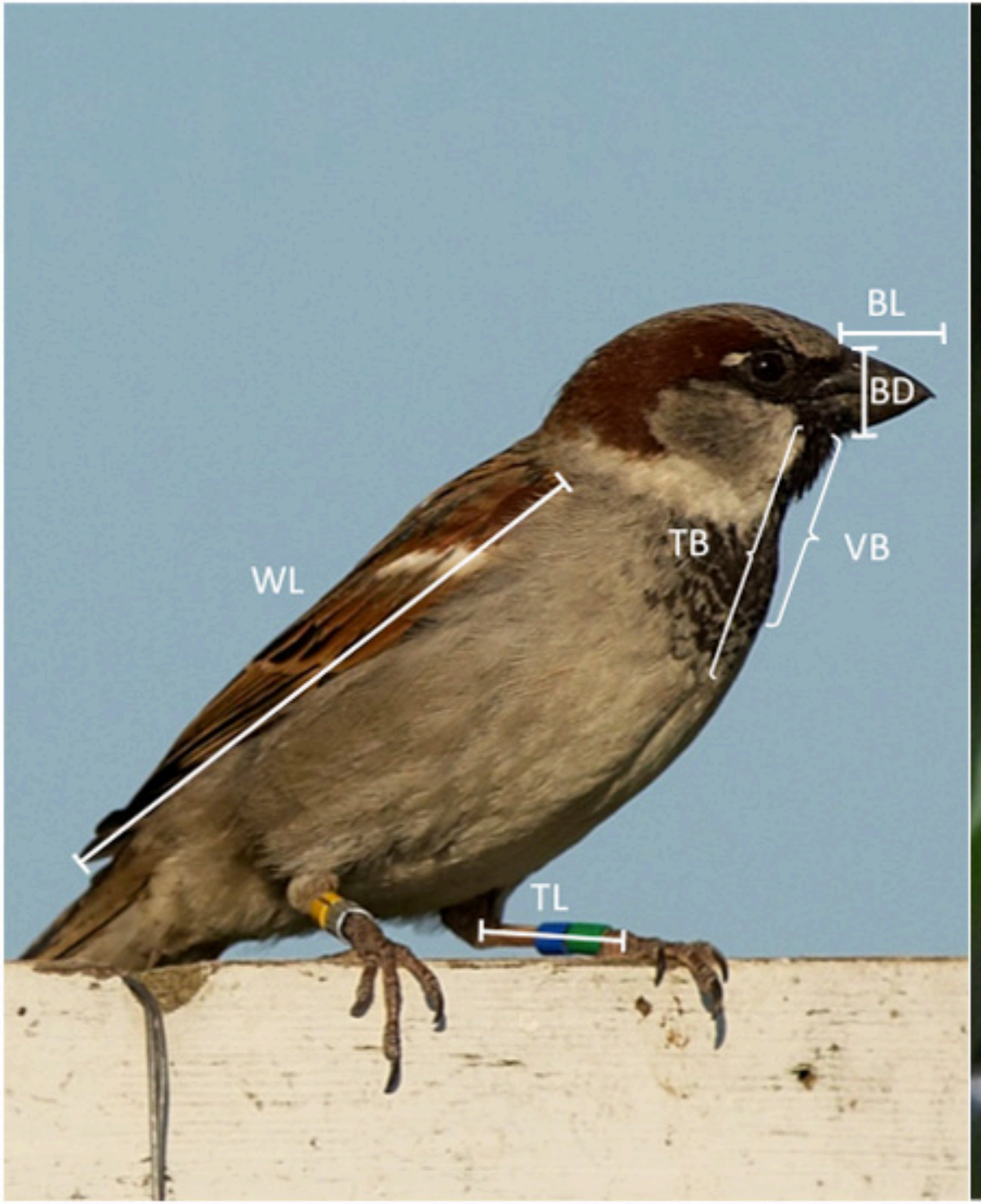


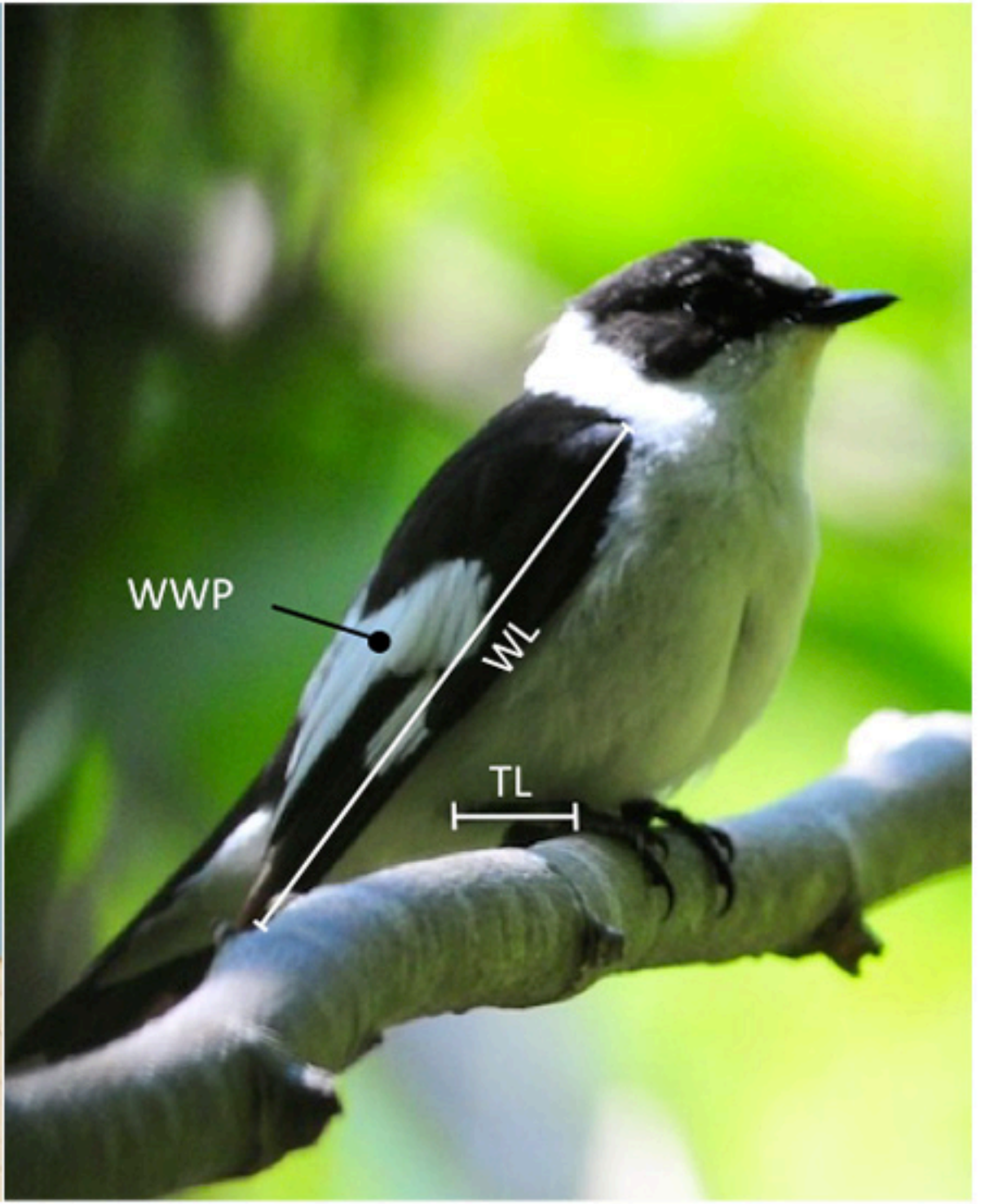
Figure 2 Scatterplot of the relationship between chromosome size (Mb) and the variance explained by each chromosome for seven phenotypic traits of house sparrows (Pearson correlation: $p < 0.05$ for tarsus length, body mass, bill length and visible badge; $p > 0.05$ for wing length, bill depth and total badge).

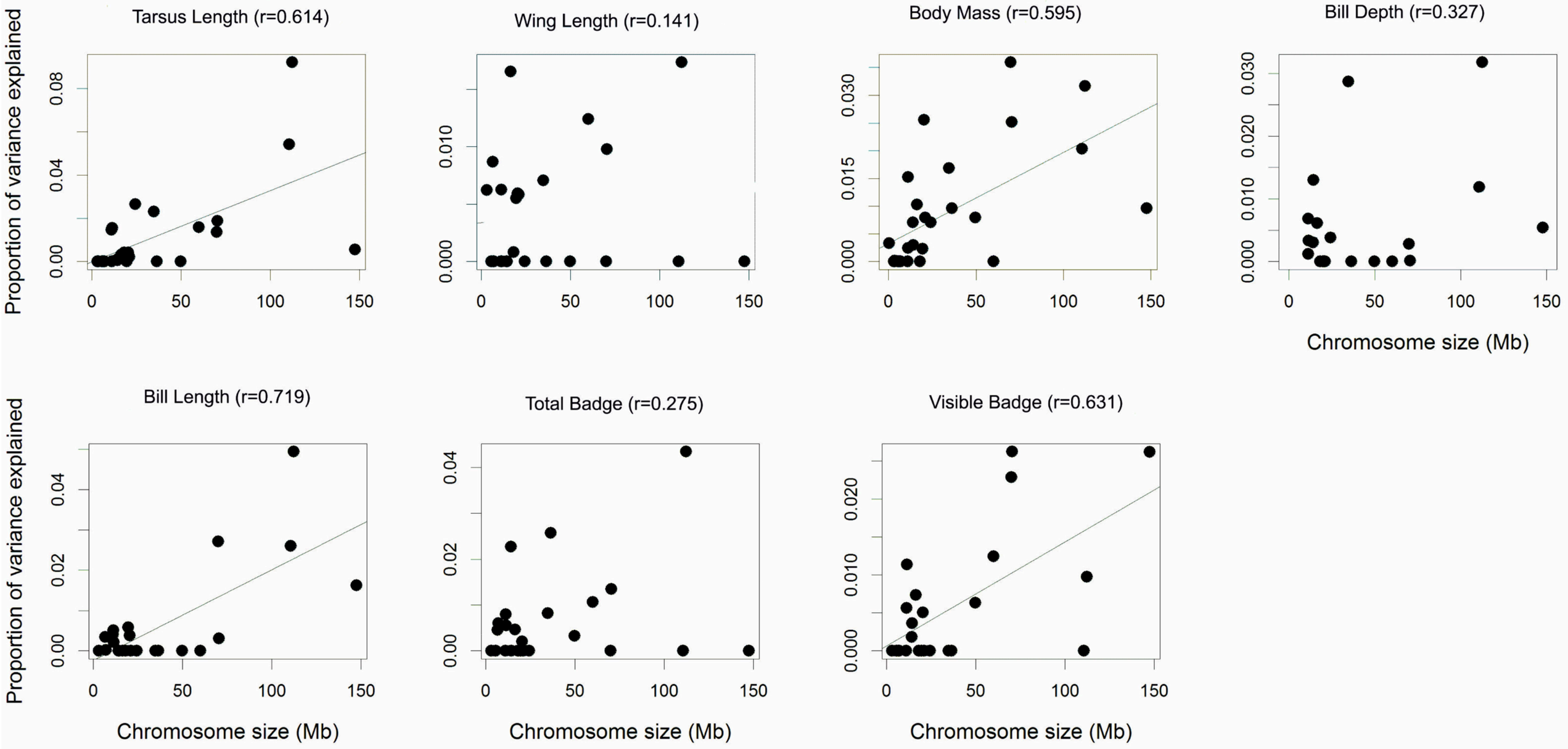


662

663 **Figure 3** Scatterplot of the relationship between chromosome size (Mb) and the variance explained
664 by each chromosome for tarsus length ($p < 0.01$), wing length ($p = 0.215$), mass ($p = 0.431$) and
665 white wing patches ($p = 0.824$) for collared flycatchers.


BL
BD
TB
VB
WL
TL


WWP
WL
TL


Tarsus Length (r=0.614)
Wing Length (r=0.141)
Body Mass (r=0.595)
Bill Depth (r=0.327)
Bill Length (r=0.719)
Total Badge (r=0.275)
Visible Badge (r=0.631)
Proportion of variance explained
Chromosome size (Mb)
0.00
0.04
0.08
0.000
0.010
0.015
0.020
0.030
0.02
0
50
100
150

Body Mass (r=0.144)

Tarsus length (r=0.656)

Wing length (r=0.269)

White wing patch (r=-0.041)

Variance explained by each chromosome

Chromosome size (Mb)

0.00

0.10

0.20

0

50

100

150