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**Heritability estimates from genome wide relatedness matrices in wild populations:
application to a passerine, using a small sample size**

Running head: GRM in Corsican blue tits

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partitioning; phenotype; blue tit; SNP; *Cyanistes caeruleus*

Abstract

Genomic developments have empowered the investigation of heritability in wild populations directly from genome wide relatedness matrices (GRM). Such GRM based approaches can in particular be used to improve or substitute approaches based on social pedigree (PED-social). However, measuring heritability from GRM in the wild has not been widely applied yet, especially using small samples and in non-model species. Here, we estimated heritability for four quantitative traits (tarsus length, wing length, bill length and body mass), using PED-social, a pedigree corrected by genetic data (PED-corrected) and a GRM from a small sample ($n = 494$) of blue tits from natural populations in Corsica genotyped at nearly 50,000 filtered SNPs derived from RAD-seq. We also measured genetic correlations among traits and we performed chromosome partitioning. Heritability estimates were slightly higher when using GRM compared to PED-social, and PED-corrected yielded intermediate values, suggesting a minor underestimation of heritability in PED-social due to incorrect pedigree links, including extra-pair paternity, and to lower information content than the GRM. Genetic correlations among traits were similar between PED-social and GRM but credible intervals were very large in both cases, suggesting a lack of power for this small dataset. Although a positive linear relationship was found between the number of genes per chromosome and the chromosome heritability for tarsus length, chromosome partitioning similarly showed a lack of power for the three other traits. We discuss the usefulness and limitations of the quantitative genetic inferences based on genomic data in small samples from wild populations.

Introduction

Estimating additive genetic variance and heritability of quantitative traits is a major aim in evolutionary biology because they are crucial components of all evolutionary models. Further, fine-scale dissection of the genomic basis of quantitative traits in the wild, using methods such as genome wide association and linkage mapping, requires validation that the traits being mapped are actually heritable. New sequencing technologies have empowered the estimation of genomic relatedness matrices (GRM) between individuals (Visscher *et al.* 2008; Huisman 2017; Gienapp *et al.* 2017), feasibly without any social pedigree (PED-social) recorded in the field. Since in a quantitative framework, common SNPs additively explain a large part of genetic additive variance, it is possible to use such realized relatedness estimated from genomic data to measure traits' heritability in wild populations (Yang *et al.* 2010). Furthermore, by aligning markers against a reference genome, heritability can then be partitioned among chromosomes (or at other genomic scales) enabling testing quantitative models of increasing heritability with the number of genes per chromosomes (Yang *et al.* 2011b), or alternatively, identifying chromosomes displaying higher genetic variance than expected. GRM estimates and chromosome partitioning, first applied on human data (Yang *et al.* 2010; 2011b), were then applied in well-known free-ranging animal populations (Great tits, *Parus major*, Robinson *et al.* 2013; Soay sheep, *Ovis aries*, Bérénos *et al.* 2015) for which thousands of individuals were genotyped at thousands of loci. There have been fewer empirical applications of these methods to smaller samples (but see Wenzel *et al.* 2015; Silva *et al.* 2017), although they have great potential in the current context of genomic tools

being increasingly applied to natural populations (Gienapp *et al.* 2017) that often rely on relatively small samples.

Three important limitations for estimating GRM and GRM based heritability are the number of SNPs, the number of individuals and the sampling variance (Visscher & Goddard 2015). The limitation from the number (as well as diversity and quality) of markers and their ability to resolve relatedness essentially vanished when we entered the genomic era (Csillery 2006; Stanton-Geddes *et al.* 2013; Gienapp *et al.* 2017). The sample size of individuals genotyped and phenotyped, however, remains an issue. For a given dataset, the number of individuals included will influence both error and median values of heritability estimates, as tested in two studies (Stanton-Geddes *et al.* 2013; Visscher & Goddard 2015). Nevertheless, reasonable heritability estimates can be reached using 150 to 200 individuals, as showed in a study on barrelclover (*Medicago trunculata*, Stanton-Geddes *et al.* 2013). However, different species, demographic contexts, and sampling strategies will likely perform differently for a similar number of individuals, number of markers and traits' architecture. Visscher & Goddard (2015) showed that for designs that use genetic markers to estimate relatedness among randomly sampled individuals from a population (which may be a common situation for non-model species in natural populations), the error in heritability estimation is inversely proportional to the squared sample size and is proportional to the effective population size. Therefore, heritability estimates based from genetic marker relatedness in extremely large populations will likely require thousands of samples (e.g. marine pelagic fish (Gagnaire & Gaggiotti 2016)), but also thousands of loci (given the small linkage disequilibrium among loci). Nevertheless, for populations of smaller size, a sampling

strategy aiming at capturing variance in realized relatedness offers possibilities to obtain robust heritability estimates bounded by reasonable standard errors.

While genomics offers promising potential to obtain quantitative genetic parameters for virtually any species or population (Gienapp et al. 2017), it has only been used in a few studies to-date and more applications in smaller samples and non-model species are needed. The first studies to estimate genomic heritability and partition variation across the genome elsewhere than in humans were, understandably, focusing on already well-known populations and species. In particular, analysis of 2644 individuals at 7203 SNPs from a long term population study of great tits was the first application of quantitative genomics to a wild population (Robinson *et al.* 2013). The Soay sheep (*Ovis aries*) also benefited from such a genomic quantitative genetic study, with 5805 individuals genotyped at 37,037 autosomal SNPs (Bérénos *et al.* 2014). This study revealed that most of the additive genetic (co)variances in sheep body size traits were captured by half of the SNPs. These pioneering studies enabled the verification of the power of quantitative genomics on known populations with long-term pedigrees and characterized quantitative genetic parameters (Edwards 2013). Other authors also estimated heritability from GRM in datasets with much less individuals, e.g. 200 barrelclover (*Medicago trunculata*) individuals genotyped at more than 5 million SNPs. Wenzel *et al.* (2015) estimated genome-wide heritability in 695 red grouse (*Lagopus lagopus scotica*) genotyped at 384 SNPs. Silva *et al.* (2017) estimated quantitative genomic parameters in 1,898 house sparrows (*Passer domesticus*) genotyped at 6,348 SNPs and in 825 collared flycatchers (*Ficedula albicollis*) genotyped at 38,689 SNPs. These examples confirmed the usefulness of the method in new cases and with fewer

109 individuals. Although we did not perform a thorough meta-analysis, there was a trend
110 among these studies for an increase, among and within studies, of heritability estimates'
111 standard errors with decreasing sample size and SNP amount. Nevertheless, and
112 surprisingly considering the number of studies performing genomic analyses since the
113 beginning of the genomic era, there are relatively few applications of genomic data to
114 estimate heritability, genetic correlations and chromosome partitioning, using smaller
115 datasets.

116 Here, we assessed heritability, genetic correlations, and chromosome partitioning
117 for four phenotypic traits, namely tarsus length, wing length, bill length, and body mass,
118 in a collection of 494 individuals of Corsican blue tits (*Cyanistes caeruleus ogliastrae*)
119 from three closely located sites in Corsica (Figure 1). Based on mitochondrial genetic
120 and phenotypic data, blue tits in Corsica and Sardinia have been qualified as a
121 subspecies, *Cyanistes caeruleus ogliastrae* (Kvist *et al.* 2004). The focal populations are
122 ideal for quantitative genetic study because of the availability of pedigree, phenotypic
123 and genetic data gathered through a long-term study (Charmantier *et al.* 2016). Based
124 on microsatellite and SNP genetic data, important gene flow together with significantly
125 low genetic differentiation have been suggested between Corsican populations (Porlier
126 *et al.* 2012; Szulkin *et al.* 2016). Phenotypic variance for the four aforementioned traits
127 has been characterized as quantitative and moderately to highly heritable (Charmantier
128 *et al.* 2004b; Blondel *et al.* 2006; Charmantier *et al.* 2016). The latest published results
129 based on 17 to 27 years of monitoring, provided population-specific significant
130 heritability ranging from 0.43 to 0.57 for tarsus length, 0.20-0.33 for wing length, 0.18-
131 0.34 for bill length, and 0.22-0.32 for body mass. All trait combinations showed

significant additive genetic covariance (COV_A) in at least one population; in particular COV_A s between wing length and tarsus length and between wing length and body mass were significantly positive (Delahaie et al 2017). Lastly, from 18 to 25% of young have been identified as extra-pair offspring in these Corsican blue tit populations (Charmantier et al. 2004a), which may lead to slight underestimation of trait heritability from a field-based social pedigree (Charmantier & Réale 2005).

The general objective of this study was to test the effectiveness of GRM to estimate heritability and genetic correlations among traits and to perform heritability partitioning while using a relatively small sample size of Corsican blue tits. We produced a dataset of 494 Corsican blue tits, from three sites (sample sizes of 110, 185 and 199), genotyped at nearly 50,000 filtered SNPs derived from RAD-seq and phenotyped for four quantitative phenotypic traits (tarsus length, wing length, bill length, and body mass). We looked for erroneous links in the social pedigree (PED-social) using the genetic data, in order to build a corrected social pedigree (PED-corrected). We estimated heritability using both GCTA (Yang *et al.* 2011a) and *MCMCglmm* (Hadfield 2010) for the 494 genotyped individuals (with the three sites pooled or separately), based on GRM, PED-social and PED-corrected. Heritability differences between PED-corrected and PED-social were computed in order to infer the possible effects of pedigree errors on estimates of traits heritability (notably underestimation originating from extra-pair copulations (Charmantier & Réale 2005)). Comparisons between heritability estimated using PED-corrected and GRM aimed at defining the effect of the greater precision and information content of the GRM. We estimated the effect of the number of SNPs on tarsus length heritability estimates, in order to discuss the SNP

density needed to recover much of the additive genetic variance. Finally, we estimated genetic correlations between traits and we partitioned heritability between chromosomes, although these quantitative genomic tests may challenge our sample size and probably require much larger sample size to produce accurate results. We discuss the usefulness and limitations of these heritability inferences in small samples from wild populations of non-model species.

Methods

Study sites, bird monitoring and phenotypic data

Data were collected in three locations (E-PIRIO, D-MURO and E-MURO) in Corsica (France, Figure 1, Table 1). Approximately six kilometers separate D-MURO from E-MURO and 27 km separate these sites from E-PIRIO. The landscape of these sites is dominated either by the evergreen holm oak *Quercus ilex* (E-PIRIO and E-MURO) or by the deciduous downy oak *Q. pubescens* (D-MURO). All sites were monitored as part of a long-term research programme for 20 (E-Muro), 24 (D-Muro) and 42 years (E-Pirio) until 2017. A total of 360 nest-boxes were monitored and 26,650 birds were ringed. Capture and handling of the birds was conducted under permits provided by the Centre de Recherches sur la Biologie des Populations d'Oiseaux (CRBPO) and by the Direction Départementale des Services Vétérinaires (DDSV).

Each year, nest boxes were visited at least once a week during the reproductive period (from early April to late June). Breeding blue tits were captured in nest boxes during the feeding of their young, and banded (if not already banded earlier) with a

unique metal ring provided by the CRBPO. Nestlings were also banded before fledging at 9-15 days old.

Four phenotypic traits were measured on male and female breeders: tarsus length (from the intertarsal joint to the most distal undivided scute on the tarsometarsus), flattened wing length, bill length (from the anterior end of the nares to the tip of the upper mandible), and body mass. Phenotypic differences among individuals from the different sites and used in genomic heritability estimates are presented in Table 1 and Supplementary material 1 (see (Charmantier *et al.* 2016) for a review of phenotypic divergence in these sites). Five to 20µl of blood were sampled from a small neck vein or from a wing vein from adult breeders for later DNA extraction. Blood was stored at 4°C in Queen's buffer (Seutin et al. 1991).

DNA extraction, RAD-sequencing, SNP calling and data filtering

We selected a set of 494 individuals captured between the years 2010-2016, from which we extracted DNA from collected blood. The individuals were captured from three sites (E-PIRIO, n = 185; D-MURO, n = 199; E-MURO, n = 110; Table 1). Individuals were chosen according to the presence of several phenotypic measurements and a blood sample. DNA extraction was achieved using Qiagen DNeasy Blood & Tissue kits. DNA extractions were randomized across sites. DNA was quantified using first a NanoDrop ND8000 spectrophotometer and then a Qubit 2.0 fluorometer with the DNA HS assay kit (Life Technologies). DNA quality was checked on agarose gels.

Library preparation using restriction-site-associated DNA sequencing (RAD-seq; Baird *et al.* 2008) with the enzyme SbfI was done by MGX (CNRS, Montpellier). Each

individual was identified using a unique 6 nucleotide tag and individuals were multiplexed in equimolar proportions by groups of 36 individuals. Each library was sequenced on one of 20 lanes of an Illumina HiSeq 2000 (libraries also included blue tit individuals from another site that was not analyzed in this study).

Raw sequences were inspected with *FastQC* (Andrews 2010) for quality controls. Potential fragments of Illumina adapters were trimmed with *Cutadapt* (Martin 2011), allowing for a 10% mismatch in the adapter sequence. Reads were filtered for overall quality, demultiplexed and trimmed to 85bp using *process_radtags*, from the *Stacks software pipeline V1.39* (Catchen et al. 2013), , allowing for one mismatch in the barcode sequence. BWA-MEM 0.7.13 (Li & Durbin 2009) was used to map individual sequences against the reference genome of the great tit (Laine et al. 2016) and to produce *sam* files using default options. Samtools 0.1.19 (Li et al. 2009) was used to build and sort *bam* files. Back in *Stacks V1.39*, we used *pstacks* to treat *bam* files, align the reads into matching stacks, infer loci, and detect SNPs at each locus. We used a minimum depth of coverage (m) of 5, the SNP model, and alpha = 0.05 (chi square significance level required to call a heterozygote or homozygote). *cstacks* was used to build the catalogue of loci using n = 3 (number of mismatches allowed between sample loci when build the catalog). *sstacks* was used to match loci against the catalog. *populations*, the last *Stacks* program used here, genotyped individuals. Loci were retained if genotyped in at least 90% of individuals (all individuals from all sites grouped), with heterozygosity per site ≤ 0.60 , and with individual minimal read depth of 10 (“na” replaced genotypes below a read depth of 10). *VCFtools* (Danecek et al. 2011) was used for further filtering of loci for a minimum average read depth of 20 across all

genotypes, and a maximum average read depth of 100 across all genotypes. Individuals were genotyped for at least 90% of all loci; a filter would have otherwise been implemented to remove individuals with low genotyping rate. The dataset was filtered to retain loci with minimum allele frequency larger than 5% ($MAF \geq 0.05$). Subsequently the dataset was pruned for linkage disequilibrium with the plink command *LD indep 50 5 2*.

Population genetic structure and genome wide relatedness matrix

Data were converted to several formats using the R package *radiator* (Gosselin 2017). Expected heterozygosity per site and *Fst* between sites were estimated using *Genodive* (Meirmans & van Tienderen 2004). A PCA resolving genetic distance between individuals was calculated using the R packages *gdsfmt* and *SNPRelate* (Zheng et al. 2012).

Genome wide relatedness matrices (GRM) were computed with GCTA (Yang et al. 2010; 2011a) using the markers found on autosomes (*i.e.* excluding markers from sex chromosomes). We estimated GRMs for each of the three sites and for the three sites pooled. The GRM based on the three sites pooled was represented using a heatmap and histograms in order to depict relatedness diversity in the different sites (Figure 2). Finally, we computed several other GRMs for the unique purpose of chromosome partitioning (detailed in the corresponding section).

Social pedigree

Social pedigrees (PED-social) were constructed using the *pedantics* R-package (Morrissey & Wilson 2009), on the basis of the complete pedigree generated from the

whole long-term monitoring period. We first included all ringed individuals and assigned their mother and father based on observational data at capture. Unknown parents of a given nest were coded using a dummy identity in the PED-social to preserve sibship information. PED-social were pruned for each dataset studied (each population separately, and the combined pedigree of the three sites pooled) to retain only genotyped individuals and their ancestors (see supplementary material 2 for detailed characteristics of the pedigree).

Comparison between social pedigree and genome wide relatedness matrix and creation of a corrected social pedigree

We compared the PED-social and GRM for the 494 genotyped individuals mainly in order to infer the potential presence of erroneous pedigree relationship (particularly parent-offspring and sibling relationship). Indeed, errors may occur in social pedigrees, essentially due to extra-pair paternities, but also observational errors such as misreading of bird ring, successive egg-laying by different females in the same nest box, and brood parasitism. Such errors, notably extra-pair paternities, can downwardly bias estimates of heritability (Charmantier & Réale 2005). We illustrated the concordances and discrepancies between PED-social and GRM using a biplot (Figure 2D). We then created corrected social pedigrees (PED-corrected) on the basis of discrepancies found between the PED-social and the GRM. For pairs of individuals having PED-social relatedness values of 0.5 (full-sibs or parent-offspring relationships) but GRM relatedness estimates below 0.1, we assumed that these relationships corresponded to erroneous parent-offspring relationship due to extra-pair paternity and assigned them a

0 in PED-corrected. For PED-social relatedness values of 0.5 but GRM estimates between 0.15 and 0.35, we assumed they corresponded to full-sibs being half-sibs and assigned them a value of 0.25 in PED-corrected. We also corrected false half-sibs in the pedigree (pairs of individuals having a 0.25 relatedness value in the PED-social but a GRM estimates smaller than 0.1) and assigned them a 0 relatedness values. We acknowledge that probably some more relationships could have been corrected based on discrepancies between PED-social and GRM, but the distribution of GRM values below 0.15 were largely overlapping and it would have been difficult to assign correct PED-corrected values. Using a method such as the one implemented in the R package *sequoia* (Huisman 2017) would certainly have allowed the correction of these potential errors as well. Another potential limitation comes from the fact that ancestors of the genotyped individuals in the pedigree were not genotyped and therefore erroneous relationships between these individuals were impossible to correct.

Inference of heritability

We used both a frequentist and a Bayesian method to estimate heritability of the four traits. The frequentist method was implemented in GCTA. The Bayesian framework was implemented in the *MCMCglmm* R-package (Hadfield 2010). Bayesian inference is renowned to have a clear advantage over the other existing methods since the use of posterior distributions propagates the errors in estimates derived from animal models (Morrissey et al., 2014).

GCTA was used to estimate heritability for each trait, separately for each of the four GRMs. We here used best linear unbiased estimates (BLUPs) for each individual

and phenotype since we could not implement more complex models in GCTA. BLUPs were estimated for the entire dataset of 494 genotyped individuals using a generalized linear mixed model with the *MCMCglmm* function, integrating a random effect for the observer and permanent environment effects accounting for multiple measurements of the same individual. Differences between sites and sex were also accounted for by adding these two factors as fixed effects. We then extracted the posterior mode of the BLUPS for each individual, and ran GCTA for each of the four GRMs and the four traits.

MCMCglmm was used to estimate heritability for each trait, separately for each of the four GRMs, the four PED-social and the four PED-corrected. Sex was integrated as a fixed effect in all models. Site was integrated as a fixed effect in the models that included all the individuals from the different sites. Measurer, identity and animal (corresponding either to the GRM, PED-social or PED-corrected matrix) effects were incorporated as random factors in order to partition the phenotypic variance into its observer, permanent environment and additive genetic components. Running models based on the GRMs, we used both BLUPs (to compare with GCTA) and alternatively all phenotypic measurements. For the models using GRMs and BLUPs, the only random factor was the animal effect (ie. the GRM) as permanent environmental and observer effects were already taken into account in the BLUPs. For the models based on PED-social and PED-corrected, we did not use BLUPs (since it could bias the analysis and/or give anticonservative results, Hadfield et al. 2010) but instead used all phenotypic measurements. We used identical parameters, priors and iterations for each estimate. Random effects included additive genetic effects (V_A) estimated through the inclusion of pedigree data, permanent environmental effects (V_{PE}) accounting for repeated

measurements of the same individual, measurer identity controlling for any potential confounding measurer effect (V_{OBS}) and residual variance (V_R). The models used can be described as follow:

$$Y = \mu + Xb + Z_A a + Z_{PE} pe + Z_O obs + e \text{ Equation 1}$$

Equation 1 describes the animal models run on phenotypic traits with Y the vector of phenotypic observations for all individuals and μ the vector of mean phenotypes. Xb stands for the fixed effects (containing sex and site for the models on all individuals, and sex only for within site models). Z_A , Z_{PE} and Z_O correspond to the random factors: additive genetic (a), permanent environment (pe), and measurer (obs) random effects, respectively. e is the vector of residual errors. Posterior distributions were composed of 1,000 values per parameter. We used 120,000 iterations per model with sampling every 100 steps and with 20,000 discarded burn-in iterations. We used slightly informative priors to facilitate convergence, with $V = V_P / (r + 1)$, $nu = 1$, V_P being the phenotypic variance, and r the number of random factors (results were quantitatively and qualitatively similar using uninformative priors, $V = 1$, $nu = 0.002$, but convergence takes a bit longer). We checked the models graphically. We verified that autocorrelations were less than 0.05. We finally reported posterior median, posterior mode, and 95% credible interval.

Inference of the effect of the number of SNPs on heritability estimates

We inspected whether the number of SNPs included resulted in i) an increase in heritability estimation precision (approximated *via* standard deviation, and LRT

(Likelihood Ratio Test) in the case of GCTA) and ii) an increase in heritability (median) via saturation of the GRMs by SNPs in linkage disequilibrium with loci most likely causative of the phenotypic variation (Gienapp et al. 2017). We reported the median and standard deviation values of tarsus length heritability inferred using GCTA and *MCMCglmm* for several GRMs produced using a variable number of SNPs. Using GCTA, we analyzed 991 GRMs made up of a decreasing number of randomly chosen SNPs from the entire dataset, by step of 50 SNPs from the total number of SNPs. To confirm GCTA results, we analyzed a smaller number of 25 GRMs with *MCMCglmm* (much less than for GCTA since the Bayesian analysis was much more time consuming than the frequentist one) made from randomly chosen SNPs, concentrated mainly between 1000 to 10,000 SNPs (since GCTA indicated that the rate of improvement in estimates was particularly concentrated between these numbers).

Inference of genetic correlations

We used bivariate models in GCTA and in *MCMCglmm* in order to estimate genetic correlations between each of the four traits. These bivariate models were achieved using the same data, same fixed and random effects, and same number of iterations in the case of *MCMCglmm*, as for the univariate models. For *MCMCglmm*, we used slightly

informative priors with $V = \begin{bmatrix} \frac{V_P}{r} & 0 \\ 0 & \frac{V_P}{r} \end{bmatrix}$ and $nu = 2$ with V_P being the phenotypic variance, and r the number of random factors.

Chromosome partitioning

Finally, we partitioned heritability across the chromosomes using GCTA, using two methods: i) fitting the univariate GCTA model simultaneously on each GRM corresponding to each autosome; ii) fitting n times, where n is the number of autosomes, the univariate GCTA model simultaneously on two GRMs, one GRM computed for the focal chromosome and the other GRM computed using the other autosomes pooled. In both cases (i) and (ii), several microchromosomes (22, 27, 28, 25LG1, 25LG2, LGE22) were pooled into one artificial autosome because they had too few SNPs. When a model did not converge, we discarded the smallest autosomes and ran the model again. We did so until the model would converge.

Results

Phenotypes

Among the 494 genotyped individuals, 494, 493, 470, and 488 were measured for tarsus length, wing length, bill length, and body mass, respectively (Table 1, Supplementary material 1). The sex ratio of genotyped individuals was near 0.5, with 246 females out of 494 individuals. Phenotypic traits varied slightly between sexes and between sites, justifying the inclusion of sex and site effects in the models partitioning phenotypic variance.

SNP calling and population genetic structure

The median number of reads per individuals was 6,066,514. The median read depth per individual and per locus was 56. The Stacks program *population* outputted 52,783 loci

totaling 96,009 SNPs. After MAF pruning, we retained 41,986 loci totaling 68,114 SNPs. After LD pruning, we retained 38,030 loci totaling 49,682 SNPs. 47,865 of these SNPs were on autosomes. The number of filtered SNPs per chromosome ranged from 5523 (chromosome 2) to 56 (LGE22).

The heterozygosity was 0.205, 0.205 and 0.204 in E-PIRIO, D-MURO and E-MURO, respectively (Supplementary material 3). Genetic differentiation between sites estimated using an *Fst* index was low yet significant, ranging from 0.006 to 0.008 (*p*-value < 0.001; Supplementary material 3). The two first axes of the PCA (Figure 2A) explaining each 0.98% and 0.93% of the genotypic variance, and the heatmap of the GRM (Figure 2B), depicted the low genetic structure between the three sites.

Genome wide relatedness matrix and social pedigree

The GRM (Figure 2C) mostly included non-related (or distantly related) individuals. Zooming in the histogram (Figure 2C) shows the presence of related individuals with parent-offspring or full sib-like links (grey), and half-sib-like links (blue). Comparing the relatedness values from the GRM to the one from the PED-social showed great consistency between the two matrices (Figure 2D). However, as expected (and shown elsewhere, Charmantier and Réale 2005), 112 links out of 858 known links presented PED-social relatedness higher than GRM relatedness by at least 0.10. Among these, 39 links exhibited PED-social relatedness of 0.5 but GRM relatedness of 0, and 36 links showed PED-social relatedness of 0.25 but GRM relatedness of 0. In addition, the GRM allowed the identification of many links that cannot be documented in the PED-social because of immigration, incomplete sampling or extra-pair paternity (Pemberton 2008).

Notably, we found 460 links with PED-social relatedness was equal to 0 but GRM relatedness larger than 0.1. In total, we corrected 83 erroneous PED-social relatedness values (corresponding to ca. 10% of the total number of links known) thanks to the GRM analysis to create the PED-corrected.

Heritability estimates

In general, heritability measures based on PED-social, PED-corrected and GRM, using BLUPs or all the measurements, and with *MCMCglmm* or GCTA, were highly consistent for the same trait (Table 2, Figure 3; see supplementary material 4 for variance estimates associated with the other random factors). The variations were very high among the three localities, for which sample sizes were very small. When pooling the three localities, variations between methods and credible intervals decreased.

Considering estimates from *MCMCglmm* and models fitting all phenotypic measurements, there was a consistent trend for lower heritability when using PED-social compared to GRM (Figure 3). For example, tarsus length and body mass heritability were lower by 22% and 31%, respectively, when using PED-social compared GRM. Specifically, there was a trend for lower heritability when using PED-social than PED-corrected. For example, tarsus length and body mass heritability were lower by 7% and 11%, respectively, when using PED-social compared to PED-corrected. There was also a trend for lower heritability when using PED-corrected compared to GRM. For example, tarsus length and body mass heritability were lower by 15% and 22%, respectively, when using PED-social compared to PED-corrected.

Heritability estimates were slightly higher when using BLUPs instead of all the phenotypic measures, together with the GRM, in *MCMCglmm* (e.g. only 3% higher for tarsus length, but 24% higher for body mass). Furthermore, credible intervals were slightly higher when using BLUPs. Using BLUPs and the GRM in GCTA, heritability estimates were relatively similar to the one obtained using *MCMCglmm* on all the phenotypic measures and either the GRM or the PED-corrected.

The number of SNPs included to compute GRMs influenced the tarsus length heritability estimates using either GCTA on BLUPs or *MCMCglmm* on all of the phenotypic measures (Figure 4). For this trait, heritability sharply increased from a handful of SNPs to approximately 5,000 SNPs. Heritability increased slowly from approximately 5,000 to 15,000 SNPs and then reached a plateau along which a very slow increase was however apparent. It is also worth mentioning a similarly shaped increase of likelihood ratio tests from GCTA.

Genetic correlations between traits

In general, genetic correlations between traits calculated with the different methods, were consistent for the same pair of trait (Table 3). However, the credible intervals were generally large, with 13 of the 30 intervals including 0. The largest estimates and smallest credible intervals were obtained for the genetic correlation between tarsus length and body mass, the most heritable traits. For each pair of traits there was a trend for a higher genetic correlation when using all measures than BLUPs.

Chromosome partitioning

When fitting the univariate GCTA model simultaneously on each autosome, the correlation between heritability and chromosome length was positive for tarsus length ($r^2 = 0.26$; p -value = 0.009), being the most heritable traits of this study. The correlation was non-significant for body mass ($r^2 = 0.08$; p -value = 0.20), wing length ($r^2 = 0.03$; p -value = 0.41), and bill length ($r^2 = 0.00$; p -value = 0.85). Standard error intervals for chromosome scaled heritability did not include zero only for chromosomes 1A and 3 for tarsus length (Table 4, Figure 5), and for chromosome 6 for body mass. In the case of tarsus length, no autosome had to be removed to enable the convergence of the model fitting all the autosomes simultaneously. In contrast, for wing length, bill length and body mass, the microchromosomes group had to be removed and respectively one, 12 and three additional autosomes (by order of increasing size) had to be removed to enable convergence of the models. When fitting the univariate GCTA model separately for each autosome versus the rest of the genome, several heritabilities were likely overestimated since the total heritability was larger than with the previous method ($h^2 = 1.47$ vs 0.84 for tarsus length, 0.85 vs 0.36 for wing length, 1.11 vs 0.31 for bill length, and 0.95 vs 0.62 for body mass).

Discussion

Genomic analyses offer great opportunities for measuring relatedness precisely among individuals from natural populations and hence estimating heritability and genetic correlations among traits in the wild (Edwards 2013; Gienapp et al. 2017). Heritability partitioning along the genome can also help in identifying regions explaining the additive genetic variation for polygenic traits (Yang et al. 2011b). Determining trait heritability and

its partitioning along the genome, and understanding trait genetic covariances, are also essential first steps to detect individual loci in the genome that contribute to trait differences between individuals, using for example genome wide association or linkage mapping (Schielzeth & Husby 2014). Although such approaches have been recently tested in a few datasets of well-studied wild organisms (Robinson et al. 2013; Stanton-Geddes et al. 2013; Bérénos et al. 2014), we require more explorations on whether smaller datasets could be used for such procedures and on how they compare with the classic animal model approach based on long-term pedigrees. In this context, we used both social pedigrees (PED-social) and genome wide relatedness matrices (GRM), to compute heritability estimates, genetic correlations, and chromosome partitioning for several phenotypic quantitative traits (tarsus length, wing length, bill length, and body mass) in a rather small dataset of individuals sampled in wild populations of Corsican blue tits and genotyped at nearly 50,000 RADseq derived SNPs. We discuss the power of such relatively small GRM, which may be typically produced while studying wild populations of non-model species, to examine the aforementioned quantitative genetic parameters.

Our main findings were i) a high congruence between heritability estimated using GRM, PED-corrected and PED-social. However, the median heritability obtained using the GRM was generally slightly higher than using PED-corrected and PED-social, which may be attributed to absence of erroneous links, greater precision and higher information content of the GRM. ii) The number of markers was not an issue for computing the GRM and estimating heritability, when approximately 15,000 SNPs were reached. iii) Genetic correlations among traits and chromosome partitioning were less

likely than heritability estimates to be very informative or robust given the large credible intervals and the number of non-significant estimates, most likely due to power issues (insufficient number of SNPs per chromosome in the case of the micro-chromosomes and insufficient number of individuals). We thereafter discuss the usefulness and limitations of inferences of quantitative genomic parameters, including heritability, in relatively small sample sizes, with a medium number of genetic markers, for wild populations of non-model species.

Comparing PED-social and GRM

The GRM obtained differed in two aspects from the PED-social (Fig 2D): higher density and relatedness variance on one hand, and no pedigree errors on the other hand. The first major difference was that the GRM enabled estimating relatedness among individuals that were not connected in PED-social (Figure 2D; 460 GRM relatedness values were higher than PED-social relatedness values by at least 0.1). This concerned individuals at every degree of relatedness but obviously primarily among individuals from disconnected families. Therefore, GRM increased the depth and also the size of the pedigree. This illustrates the usefulness of GRM in cases for which a dense and deep pedigree is difficult to obtain due to for example dispersal, large population size compared to the fieldwork capacity, and family structure (Pemberton 2008). Furthermore, GRM allows capturing much more variance in realized relatedness among individuals, both close and more distantly related (Figure 2D), which may also increase the power of such GRM-based estimates in large and open populations.

The second major difference between PED-social and GRM was that several individuals had much higher relatedness in PED-social than in the GRM, most likely indicating errors in PED-social. The most common cases consisted of wrongly assigned parentages (relatedness of 0.5 in PED-social and 0 in GRM), half sibs most likely originating from extra-pair paternities (relatedness of 0.5 in PED-social and 0.25 in GRM), and the spread of such errors in the pedigree (other smaller relatedness in GRM than in PED-social). Using this comparison between GRM and PED-social, we created a PED-corrected in which these aforementioned erroneous links, accounting for approximately 10% of the PED-social links, were corrected. This proportion of erroneous linked corrected in our dataset should be expected since 18 to 25% of young have been previously identified as extra-pair offspring in these Corsican blue tit populations (Charmantier et al. 2004a).

Heritability estimates from PED-social, PED-corrected and GRM

Heritability estimated for tarsus length (0.62 to 0.81 depending on the method, for three sites pooled, see Table 2) and body mass (0.34-0.61) were on the upper limit compared to previous estimates in this species and for other close species (Jensen et al. 2008; Husby et al. 2011; Postma 2014; Delahaie et al. 2017) while heritability for wing length (0.26-0.34) and bill length (0.14-0.21) were rather on the lower ranges usually found. Here again it should be noted that we did not optimize the animal models by integrating factors that may have contributed to explain trait variation, for example to account for daily and seasonal variation in body mass. Moreover, it has been observed that the number of individuals included can affect the heritability estimates (Silva et al. 2017).

Credible intervals and standard errors for heritability estimates were much larger than in previous studies based on larger sample size (Robinson et al. 2013; Stanton-Geddes et al. 2013; Bérénos et al. 2014; Silva et al. 2017). The credible intervals per site were larger than for the three sites pooled, suggesting the decrease of power with decreasing sample size. Similarly, Silva et al. (2017) obtained increased standard errors when decreasing sample size. This most likely suggests that the number of individuals used here conferred limited power.

Overall, when using *MCMCglmm* and models using all of the phenotypic measurements, heritability estimates were relatively similar for GRM, PED-corrected and PED-social for the same trait (Figure 3). These results are in line with previous comparisons realized with much larger sample size (Robinson et al. 2013; Bérénos et al. 2014). However, unlike these previous studies showing very small differences in heritability based on PED-social and GRM, our estimates based on GRM were larger than using PED-social and to a lesser extent, than using PED-corrected, for tarsus length and for body mass, but not for wing length and bill length. Here, wing length heritability was 0.28 using PED-social, 0.26 using PED-corrected and 0.26 using GRM (Table 2). In turn, tarsus length heritability obtained from GRM was 18% higher than from PED-corrected and the one from PED-corrected 8% higher than from PED-social. Similarly, body mass heritability obtained from GRM was 29% higher than from PED-corrected and the one from PED-corrected 12% higher than from PED-social. The difference between PED-corrected and PED-social for these two traits may be attributable to the erroneous links in PED-social. Indeed, it has been estimated that PED-social containing 5 to 20% of extra-pair paternities result in underestimated

heritability by up to 17%, in blue tits (Charmantier & Réale 2005). Then, the differences observed between heritability estimated using GRM and PED-corrected are likely to originate from higher density of the GRM and the fact that GRM incorporate more variance in relatedness for a given class of kinship. Further changing the pedigree by not only correcting erroneous existing links but also informing previously unidentified links (eg. linking previously unknown extra-pair fathers to their offspring) based on the genetic data, could also decrease the heritability difference between PED-corrected and GRM.

Heritability estimates, and their credible intervals, based on BLUPs were slightly higher compared to estimates based on models using all phenotypic measurements when using *MCMCglmm* (this was not true for GCTA). This is not surprising given that BLUPs are known to artificially increase the precision of the measure and therefore increase statistical significance (Hadfield et al. 2010). While the difference was low for tarsus and bill length (3% and 5% higher, respectively), the difference was particularly pronounced for wing length and body mass (31% and 24%, respectively). One speculation is that wing length and body mass are less repeatable than tarsus length and bill length and that our BLUPs did not adequately take into account individual variance.

Effect of the number of SNPs on the heritability estimates based on GRM

The number of SNPs had a relatively minor effect on heritability estimates, from approximately 15,000 SNPs (Figure 4). This is concordant with what has been shown in previous studies (Stanton-Geddes et al. 2013; Béréanos et al. 2014). Of course, the effect

of the number of SNPs on the heritability estimation depends on several parameters (e.g. genome size, LD across the genome, LD among markers, genetic architecture of the focal trait, polymorphism of the markers, genotyping quality, missing data) and is not transposable from one study to another. These days, the number of markers may no longer be a problem, given the rise of the accessibility of many genomic tools. However, the genotyping quality and missing data occurrence may be challenging for studies using RADseq and GBS methods, as noted by (Gienapp et al. 2017). Here, we prioritized the quantity and the quality of the RAD markers over the number of individuals, as well as genotyping only individuals with high quality DNA, ending up with a relatively high read depth and low missing rate. Future studies aiming to use RADseq derived markers to estimate heritability should also find a tradeoff between the number of genomic markers and the number of genotyped individuals. A formal simulation study determining such a tradeoff between numbers of individuals and of markers and markers read-depth (directly linked to quality) would be highly valuable for guiding the design of RADseq analyses in the context of quantitative genomics.

Genetic correlations using PED-social, PED-corrected and GRM

Genetic correlations obtained using GRM, PED-social and PED-corrected had large credible intervals, preventing us to make robust interpretations, except that our sample size was probably too small to obtain acceptable credible intervals. This was partly expected since bivariate models are known to be data hungry. While such lack of power is a recurrent issue when estimating genetic correlations even with much larger sample sizes (Vattikuti et al. 2012; Visscher et al. 2014; Ni et al. 2017), it was nevertheless a

disappointment while comparing these results to the one from (Bérénos et al. 2014), in which the credible intervals were relatively small. The median values of genetic correlations for these traits were in line with previous studies in other bird species (Teplitsky et al. 2014).

Chromosome partitioning based on GRM

Chromosome partitioning of heritability of tarsus length was on par with what has been observed in previous studies, *i.e.* increasing heritability of quantitative traits with increasing number of genes per chromosome (Yang et al. 2011b; Robinson et al. 2013; Santure et al. 2013, 2015; Bérénos et al. 2015; Wenzel et al. 2015; Silva et al. 2017). Similarly to several of these previous studies, there were few chromosomes for which standard error did not include zero. Of particular interest, chromosomes 3 and 1A showed high heritability for tarsus length. These chromosomes may be of particular interest for future studies investigating the genomic bases of this quantitative trait variation in blue tits. Chromosome 3 was already identified in a recent study on morphology of Dutch and British great tits (Santure et al. 2015) as explaining high heritability for tarsus length. In contrast, chromosome 1A explained a low proportion of genetic variance in this last study. These kind of discrepancies might be congruent with the fact that this same study (Santure et al. 2015) also showed very little consistency between the variance explained by each chromosome for great tit populations from the Netherlands and from the United-Kingdom.

Regarding the three other traits analyzed, the chromosome partitioning results appeared much less robust since several chromosomes had to be removed (7, 18, and

9 autosomes removed, for wing length, bill length and body mass, respectively) to enable models' convergence. The need for such chromosomes removals has previously been reported (Wenzel et al. 2015; Silva et al. 2017) and has received detailed consideration by Kemppainen & Husby (2018). In addition, the relationship between the number of genes and the heritability explained by chromosomes were deviating from theoretical expectations of increasing heritability with increasing chromosome size for these polygenic traits. For comparison, Bosse et al. (2017) showed a significant increase of the proportion of additive genetic variance of bill length explained by a chromosome in relation to chromosome size in great tits. Such deviation may not have been affected by removing chromosomes (Kemppainen & Husby 2018) but most likely originated from a limited power of the small sample size, alongside relatively small heritabilities (Kemppainen & Husby 2018).

Conclusion on the usefulness of GRM based heritability measures using small samples from wild populations

Overall, our study reveals that RADseq data on around 50k SNPs (as advised by Berenos et al 2014) for around 500 phenotyped individuals can provide estimates of heritability that are close to, and probably more accurate than, estimates based on social pedigree data from seven years monitoring for more than 1600 individuals (as included in the PED-social used here). This opens very interesting avenues in the field of quantitative genetics since estimating heritability and genetic correlations in the wild have long been restricted to study systems where long-term monitoring is feasible. RADseq data could allow the estimation of quantitative genetic parameters for a much

655 lower cost in terms of time (years) spent in the field, although individual capture and
656 phenotyping will most likely remain time consuming. Using GRM on few individuals gives
657 the power to estimate such parameters on virtually any species even though no long-
658 term pedigree is available (Gienapp 2017). This will notably allow moving on from
659 individual study to more comparative approaches and answer important questions such
660 as understanding the spatial variation of evolutionary potential or the role of evolutionary
661 constraints in phenotypic evolution. Such a genomic approach also provides the
662 possibility to explore genetic covariance between traits as well as chromosome
663 partitioning to confirm the polygenic architecture for phenotypic traits classically
664 measured in birds. Less than 500 individuals appears however to provide insufficient
665 power to correctly estimate either genetic covariances between traits or contribution of
666 individual chromosomes to overall heritability.

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Data archiving

Data (Plink files, PED-social, and repeated and BLUPs phenotypic traits values) are available from the Dryad Digital Repository: doi:10.5061/dryad.k6r1mk8

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conception of the study: AC & CP. Field work: AC, BD & CP. Lab work & bioinformatics: CP. Quantitative genetic/genomics inferences: CP & BD. Writing of the manuscript: CP. Critically revised and improved the manuscript: AC, BD & CP.

Figure captions

Figure 1. A- Map of the sampling locations in Corsica, including a zoom-in of the area where the populations are located B- D-Muro and E-Muro and C- E-Pirio (see table 1 for details).

Figure 2. Genetic structure and relatedness among individuals. A- Biplot of the two first axes of a principal component analysis of genetic distances between individuals, B- Heatmap of the genome wide relatedness matrix (GRM) among the 494 genotyped individuals, C- histogram of genome wide relatedness (GRM) among individuals, with a zoom-in highlighting particularly high relatedness values, and D- Biplot of genome wide relatedness (GRM) and social pedigree relatedness from the field observations (PED-social). In B, C and D, color-coding is based on the empirical relatedness distribution and is only indicative.

Figure 3. A- Heritability estimates using the GRM, the PED-social or the PED-corrected, based on either BLUPS or the entire phenotypic measurements, and implementing models in GCTA or *MCMCglmm*, for the 494 genotyped individuals from the three populations pooled. B, C & D- Relationship between heritability inferred for the four traits for PED-social, PED-corrected and GRM using *MCMCglmm*, using the model with all phenotypic measurements in *MCMCglmm*. Errors bars correspond to 95% credible interval for *MCMCglmm* and to 95% confidence intervals for GCTA.

Figure 4. Effect of the number of SNPs used to build the GRM on the tarsus length heritability estimated with GCTA & BLUPs (A) and *MCMCglmm* & all the phenotypic measures (B). Circles represent the median value. Bars represent standard errors for GCTA and 95% credible interval for *MCMCglmm*. Black curves correspond to LOESS fits. The log likelihood ratio of GCTA estimates is color-coded.

Figure 5. Chromosome partitioning. The univariate GCTA model was fitted simultaneously on each autosome. Linear correlation between the number of genes per chromosome and the GCTA estimates of heritability per chromosome for the four phenotypic traits. Names of the largest chromosomes, and of the chromosomes having the highest heritability, are given.

867 **Tables**

868 Table 1. Geographic coordinates, number of genotyped individuals (N), and trait mean values ($\pm$ SE) for this sample, for
 869 each of the three sites and for the entire dataset. The phenotypic variance (V_p) is also given for each trait.

Site	Latitude	Longitude	N	Sex	Tarsus length (mm)				Wing length (mm)				Bill length (mm)				Body mass (g)			
					N	mean	SE	V_p	N	mean	SE	V_p	N	mean	SE	V_p	N	mean	SE	V_p
E-Pirio	42.376	8.750	185	male	98	16.4	0.5		98	63.5	1.6		90	6.4	0.3		97	9.4	0.4	
				female	87	15.9	0.6		87	60.6	1.2		82	6.6	0.4		86	9.2	0.4	
				both	185	16.1	0.6	0.25	185	62.2	2.0	4.91	172	6.5	0.4	0.16	183	9.3	0.4	0.27
D-Muro	42.551	8.923	199	male	95	16.6	0.4		95	63.3	1.6		92	6.4	0.3		92	9.8	0.4	
				female	104	16.1	0.6		104	60.8	1.3		99	6.5	0.3		104	9.5	0.5	
				both	199	16.3	0.5	0.24	199	62.0	1.9	4.38	191	6.4	0.3	0.16	196	9.6	0.5	0.27
E-Muro	42.589	8.967	110	male	55	16.5	0.5		55	63.4	1.5		53	6.4	0.3		54	9.7	0.4	
				female	55	16.0	0.4		54	60.8	1.1		54	6.7	0.5		55	9.5	0.4	
				both	110	16.3	0.5	0.25	109	62.1	1.8	3.84	107	6.6	0.4	0.19	109	9.6	0.4	0.24
Total			494	male	248	16.5	0.4		248	63.4	1.6		235	6.4	0.3		243	9.6	0.4	
				female	246	16.0	0.5		245	60.7	1.2		235	6.6	0.4		245	9.4	0.5	
				both	494	16.2	0.5	0.26	493	62.1	1.9	4.43	470	6.5	0.3	0.17	488	9.5	0.5	0.30

870 Table 2. Heritability estimates (h^2) and confidence (GCTA) / credible (*MCMCglmm*) intervals (95%CI) for the four traits,
871 using the GRM, the PED-social or the PED-corrected, based on either BLUPS or the entire measures, and implementing
872 models in GCTA or *MCMCglmm*, for all the individuals or each of the three sites separately. Number of individuals used
873 (N).

Relatedness	Data	Implementation	Site	N	Tarsus length			Wing length			Bill length			Body mass		
					N	h^2	95%CI	N	h^2	95%CI	N	h^2	95%CI	N	h^2	95%CI
GRM	BLUPS	GCTA	3 pop pooled	494	489	0.72	0.5 0.94	493	0.26	0 0.48	469	0.16	0 0.4	488	0.48	0.25 0.72
			E-Pirio	185	182	0.64	0.28 1	185	0.25	0 0.6	171	0.11	0 0.46	183	0.37	0 0.74
			D-Muro	199	197	0.73	0.38 1	199	0.46	0.04 0.87	191	0.22	0 0.64	196	0.41	0 0.88
			E-Muro	110	110	1	0.66 1	109	0.36	0 0.84	107	0.59	0 1	109	1	0.69 1
GRM	BLUPS	<i>MCMCglmm</i>	3 pop pooled	494	489	0.81	0.63 0.92	493	0.34	0.11 0.58	469	0.2	0.06 0.52	488	0.61	0.38 0.83
			E-Pirio	185	182	0.74	0.44 0.95	185	0.31	0.08 0.66	171	0.26	0.05 0.52	183	0.58	0.19 0.78
			D-Muro	199	197	0.8	0.42 0.96	199	0.49	0.19 0.91	191	0.3	0.71 0.07	196	0.58	0.11 0.86
			E-Muro	110	110	0.93	0.65 0.98	109	0.49	0.1 0.82	107	0.35	0.11 0.95	109	0.9	0.55 0.98
GRM	All measures	<i>MCMCglmm</i>	3 pop pooled	494	489	0.79	0.65 0.87	493	0.26	0.11 0.5	469	0.19	0.03 0.37	488	0.49	0.33 0.64
			E-Pirio	185	182	0.72	0.39 0.89	185	0.2	0.06 0.42	171	0.13	0.02 0.35	183	0.39	0.09 0.57
			D-Muro	199	197	0.84	0.54 0.89	199	0.48	0.18 0.73	191	0.29	0.06 0.49	196	0.17	0.04 0.57
			E-Muro	110	110	0.86	0.61 0.91	109	0.37	0.07 0.55	107	0.36	0.05 0.6	109	0.66	0.42 0.78
PED-corrected	All measures	<i>MCMCglmm</i>	3 pop pooled	494	489	0.67	0.49 0.8	493	0.26	0.13 0.41	469	0.21	0.06 0.3	488	0.38	0.24 0.48
			E-Pirio	185	182	0.54	0.22 0.78	185	0.12	0.04 0.3	171	0.14	0.03 0.27	183	0.25	0.07 0.41
			D-Muro	199	197	0.68	0.33 0.83	199	0.5	0.19 0.61	191	0.22	0.08 0.35	196	0.32	0.09 0.44
			E-Muro	110	110	0.74	0.41 0.84	109	0.28	0.06 0.4	107	0.18	0.05 0.43	109	0.85	0.43 0.95
PED-social	All measures	<i>MCMCglmm</i>	3 pop pooled	494	489	0.62	0.46 0.73	493	0.28	0.13 0.37	469	0.14	0.06 0.23	488	0.34	0.22 0.42
			E-Pirio	185	182	0.52	0.22 0.71	185	0.12	0.03 0.26	171	0.10	0.03 0.22	183	0.22	0.06 0.4
			D-Muro	199	197	0.6	0.27 0.72	199	0.37	0.16 0.52	191	0.09	0.03 0.19	196	0.29	0.11 0.41
			E-Muro	110	110	0.68	0.38 0.78	109	0.19	0.05 0.35	107	0.11	0.04 0.37	109	0.4	0.17 0.53

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875 Table 3. Genetic correlations (rG), and confidence (GCTA) / credible (*MCMCglmm*) intervals (95%CI), between traits
876 estimated for the individuals from the three site pooled, using GRM, the PED-social or the PED-corrected, based on either
877 BLUPS or all the measures, and implementing models in GCTA or *MCMCglmm*.

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		Tarsus length	Tarsus length	Tarsus length	Wing length	Wing length	Bill length
		Wing length	Bill length	Body mass	Bill length	Body mass	Body mass
GCTA	rG	0.40	0.37	0.48	-0.06	0.22	0.13
	BLUPs	0.05	0.05	-0.14	0.23	-0.86	-0.49
	GRM	0.95	0.75	0.88	0.73	0.86	0.67
<i>MCMCglmm</i>	rG	0.41	0.32	0.52	0.10	0.21	0.03
	BLUPs	0.05	-0.08	-0.09	0.20	-0.57	-0.28
	GRM	0.95	0.67	0.73	0.75	0.48	0.66
<i>MCMCglmm</i> all measures	rG	0.48	0.45	0.56	0.50	0.50	0.33
	BLUPs	0.05	0.16	0.06	0.37	-0.08	0.05
	GRM	0.95	0.81	0.80	0.76	0.87	0.71
<i>MCMCglmm</i> all measures PED-corrected	rG	0.48	0.43	0.57	0.46	0.49	0.52
	BLUPs	0.05	0.13	0.00	0.40	-0.12	0.06
	GRM	0.95	0.79	0.81	0.78	0.89	0.74
<i>MCMCglmm</i> all measures PED-social	rG	0.46	0.52	0.61	0.42	0.41	0.37
	BLUPs	0.05	0.17	0.16	0.38	-0.13	0.09
	GRM	0.95	0.77	0.79	0.82	0.79	0.72

879 Table 4. Chromosome partitioning of the heritability estimated for the four phenotypic traits using GCTA. Partitioning was
880 achieved either i) fitting all the chromosomes simultaneously or ii) considering one chromosome versus all of the other
881 autosomes, iteratively for each chromosome. Several micro-chromosomes were grouped (22, 27, 28, 25LG1, 25LG2,
882 LGE22) and referred as “micros”. “na” refers to the sets of chromosomes that have been removed to enable the
883 convergence of the model fitting simultaneously all the chromosomes (see methods). The underlined values do not
884 include 0 in their standard error intervals.

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Chromosome name	SNPs	Genes	Tarsus		Wing length				Bill length				Body mass					
			All chromosomes simultaneously		One chromosome versus the other, iteratively		All chromosomes simultaneously		One chromosome versus the other, iteratively		All chromosomes simultaneously		One chromosome versus the other, iteratively		All chromosomes simultaneously		One chromosome versus the other, iteratively	
			Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE
1	4784	1131	0	0.16	0	0.14	0.01	0.16	0.01	0.14	0	0.15	0	0.13	0.03	0.16	0.07	0.14
2	5523	1287	0.06	0.17	0.01	0.15	0	0.17	0	0.16	0	0.16	0	0.15	0	0.19	0	0.16
3	4383	1096	0.17	0.16	0.21	0.14	0	0.15	0	0.14	0.03	0.16	0.02	0.14	0.07	0.15	0.08	0.13
4	2869	736	0	0.13	0	0.11	0.05	0.13	0.16	0.12	0.06	0.13	0.12	0.12	0.01	0.13	0.04	0.11
4A	1428	343	0	0.09	0	0.08	0	0.1	0	0.09	0.05	0.1	0.11	0.09	0.01	0.09	0.01	0.08
1A	2638	853	0.21	0.13	0.24	0.11	0	0.12	0	0.11	0.13	0.13	0.17	0.12	0.04	0.12	0.06	0.11
5	2902	940	0.04	0.12	0	0.1	0	0.12	0	0.11	0	0.13	0	0.11	0.09	0.13	0.1	0.11
6	1818	513	0.04	0.11	0.11	0.1	0.01	0.1	0	0.09	0.01	0.1	0.07	0.09	0.12	0.11	0.17	0.1
7	1907	488	0.06	0.11	0.21	0.1	0.01	0.11	0.06	0.1	0	0.12	0	0.1	0.07	0.11	0.11	0.1
8	1718	492	0.04	0.1	0.11	0.09	0	0.1	0	0.09	0	0.11	0	0.1	0.01	0.1	0.04	0.1
9	1689	434	0	0.11	0	0.09	0.09	0.1	0.16	0.1	0.03	0.1	0.05	0.09	0.02	0.1	0.01	0.09
12	1573	343	0	0.1	0	0.08	0.04	0.09	0.04	0.08	0	0.1	0.01	0.08	0	0.08	0	0.09
13	1371	322	0.03	0.08	0.08	0.08	0.01	0.08	0.02	0.08	0	0.09	0.03	0.08	0	0.09	0.08	0.08
10	1177	394	0.07	0.09	0.1	0.08	0	0.08	0	0.07	na	na	0.14	0.08	0	0.1	0	0.07
11	1309	364	0.03	0.09	0.12	0.09	0.05	0.09	0.11	0.08	na	na	0.02	0.08	0.08	0.09	0.01	0.08
14	1364	396	0.05	0.09	0.08	0.08	0	0.08	0	0.08	na	na	0.04	0.08	0	0.09	0	0.08
15	1004	358	0	0.08	0	0.07	0	0.08	0	0.07	na	na	0	0.08	0.03	0.08	0.01	0.07
17	974	277	0.02	0.08	0.16	0.08	0	0.09	0	0.08	na	na	0.07	0.07	0.03	0.08	0.05	0.07
18	1114	318	0	0.09	0	0.08	0	0.09	0	0.08	na	na	0	0.08	0	0.08	0	0.07
19	849	311	0	0.07	0	0.06	0.05	0.08	0.1	0.07	na	na	0.01	0.06	0.01	0.08	0.1	0.08
21	632	260	0	0.07	0	0.06	0	0.07	0	0.06	na	na	0	0.07	0	0.07	0	0.07
24	846	175	0.02	0.08	0.04	0.07	0.02	0.07	0.08	0.07	na	na	0	0.07	0	0.08	0	0.07
20	1301	341	0	0.09	0	0.08	0	0.09	0	0.08	na	na	0.17	0.09	na	na	0.01	0.07
26	802	262	0	0.07	0	0.06	0.02	0.07	0.04	0.07	na	na	0.01	0.06	na	na	0	0.07
23	521	232	0	0.07	0	0.06	na	na	0	0.06	na	na	0.07	0.06	na	na	0	0.06
micros	1368	874	0	0.1	0.02	0.09	na	na	0.05	0.08	na	na	0	0.09	na	na	0	0.09

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Supplementary material

Supplementary material 1. Box plots showing sex- and site-specific median and variation in tarsus length, wing length, bill length and body mass for the 494 genotyped individuals. The first four letters code for the site and the last letter codes for the sex (F: female; M: male).

Supplementary material 2. Social pedigree characteristics.

Supplementary material 3. Heterozygosity (H_e) in E-Piro, D-Muro and E-Muro and F_{ST} among the three sites (F_{ST} above diagonal and p-value below the diagonal).

Supplementary material 4. Variance estimates associated to the other random factors.

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Supplementary material 2.

	Maternities	Paternities	Full-sibs	Maternal half-sibs	Paternal half-sibs	Max Depth
Pooled sites	333	333	88	47	35	14
E-Pirio	115	115	27	15	9	14
E-Muro	117	117	23	18	17	7
D-Muro	116	116	34	13	9	6

Supplementary material 3.

	F_{ST}			
	He	E-Pirio	D-Muro	E-Muro
E-Pirio	0.205	--	0.006	0.008
D-Muro	0.205	p < 0.001	--	0.007
E-Muro	0.204	p < 0.001	p < 0.001	--

Supplementary material 4.

Variance associated to the permanent environment effect.

		Tarsus			Wing			Bill			Mass		
		Vpe	95% CI		Vpe	95% CI		Vpe	95% CI		Vpe	95% CI	
GRM	Pooled	0.04	0.01	0.07	0.65	0.4	1.09	0.03	0.01	0.05	0.02	0.01	0.06
GRM	E-Pirio	0.03	0.01	0.11	0.65	0.24	1.03	0.03	0.01	0.05	0.03	0.01	0.09
GRM	D-Muro	0.03	0.01	0.09	0.73	0.24	1.42	0.02	0	0.03	0.08	0.01	0.11
GRM	E-Muro	0.02	0.01	0.07	0.44	0.12	0.97	0.02	0.01	0.07	0.01	0	0.06
PED-corrected	Pooled	0.03	0.01	0.06	0.5	0.2	0.9	0.02	0.01	0.04	0.02	0.01	0.05
PED-corrected	E-Pirio	0.03	0.01	0.12	0.5	0.18	0.96	0.02	0	0.04	0.02	0.01	0.09
PED-corrected	D-Muro	0.02	0.01	0.1	0.38	0.12	1.1	0.01	0	0.03	0.02	0.01	0.08
PED-corrected	E-Muro	0.02	0.01	0.08	0.45	0.12	0.88	0.02	0.01	0.07	0.02	0.01	0.07

Variance associated to the observer effect.

		Tarsus			Wing			Bill			Mass		
		Vobs	95% CI		Vobs	95% CI		Vobs	95% CI		Vobs	95% CI	
GRM	Pooled	0.01	0.01	0.02	0.23	0.12	0.52	0.05	0.02	0.1	0.01	0.01	0.03
GRM	E-Pirio	0.01	0	0.03	0.21	0.08	0.46	0.02	0.01	0.06	0.01	0	0.03
GRM	D-Muro	0.01	0.01	0.04	0.36	0.15	1.05	0.07	0.03	0.16	0.02	0.01	0.05
GRM	E-Muro	0.01	0.01	0.04	0.26	0.11	0.72	0.04	0.01	0.09	0.02	0.01	0.08
PED-corrected	Pooled	0.01	0.01	0.02	0.24	0.11	0.53	0.05	0.02	0.1	0.02	0.01	0.04
PED-corrected	E-Pirio	0.01	0.01	0.03	0.21	0.08	0.45	0.02	0.01	0.06	0.01	0	0.03
PED-corrected	D-Muro	0.01	0.01	0.04	0.35	0.15	0.97	0.07	0.03	0.17	0.01	0.01	0.05
PED-corrected	E-Muro	0.01	0.01	0.04	0.33	0.09	0.8	0.04	0.02	0.09	0.03	0.01	0.08

Variance associated to the residuals.

		Tarsus			Wing			Bill			Mass		
		Vresiduals	95% CI		Vresiduals	95% CI		Vresiduals	95% CI		Vresiduals	95% CI	
GRM	Pooled	0.03	0.03	0.04	1.22	1.14	1.35	0.1	0.08	0.1	0.13	0.12	0.14
GRM	E-Pirio	0.03	0.03	0.04	1.68	1.46	1.95	0.11	0.09	0.13	0.14	0.13	0.17
GRM	D-Muro	0.03	0.03	0.04	0.9	0.77	1.04	0.06	0.05	0.07	0.14	0.12	0.16
GRM	E-Muro	0.02	0.02	0.03	1.12	0.97	1.39	0.09	0.07	0.11	0.09	0.07	0.1
PED-corrected	Pooled	0.03	0.03	0.04	1.26	1.13	1.35	0.09	0.08	0.1	0.13	0.12	0.14
PED-corrected	E-Pirio	0.03	0.03	0.04	1.62	1.42	1.93	0.11	0.09	0.13	0.14	0.13	0.17
PED-corrected	D-Muro	0.03	0.03	0.04	0.88	0.77	1.03	0.06	0.05	0.07	0.13	0.12	0.16
PED-corrected	E-Muro	0.02	0.02	0.03	1.18	1	1.39	0.09	0.07	0.11	0.09	0.07	0.11