Pervasive selection pressure in wild and domestic pigs

J. Leno-Colorado[1], S. Guirao-Rico[1,2], M. Pérez-Enciso[1,3,4], S. E. Ramos-Onsins[1*]

[1] Centre for Research in Agricultural Genomics (CRAG) Consortium CSIC-IRTA-UAB-UB. 08193 Bellaterra, Spain.

[2] Institute of Evolutionary Biology, CSIC-Universitat Pompeu Fabra, Barcelona, Spain.

[3] Universitat Autònoma de Barcelona, Department of Animal Science, Bellaterra, Spain.

[4] Institut Català de Recerca I Estudis Avançats (ICREA), Carrer de Lluís Companys 23, Barcelona, Spain.

*Corresponding author: sebastian.ramos@cragenomica.es

ABSTRACT

Animal domestication typically affected numerous polygenic quantitative traits, such as behavior, development and reproduction. However, uncovering the genetic basis of quantitative trait variation is challenging, since they are caused by small allele-frequency changes. To date, only a few causative mutations related to domestication processes have been reported, strengthening the hypothesis that small effect variants have a prominent role. So far, approaches on domestication have been limited to the detection of the global effect of domestication on deleterious mutations and on strong beneficial variants, ignoring the importance of variants with small selective effects. To overcome these difficulties, here we propose to estimate the proportion of beneficial variants based on the asymptotic MacDonald Kreitman (MK) method, according to estimates of variability based on frequency spectrum. We applied this approach to the pig species, analyzing 46 complete genome sequences from 20 European wild boars, 6 Iberian and 20 Large White pigs at different molecular scales: gene, metabolic pathway and whole-genome.

Descriptive variability analyses on pig populations indicate that domestic and wild pig populations do not differ in nonsynonymous fixed mutations. Instead, most variants are shared among them, despite that the phenotypes of wild and domestic individuals are clearly divergent. Additionally, asymptotic MK plots based on summary statistics show that small effects variants may affect the final calculation of α, the proportion of beneficial mutations. The distribution of fitness effects inferred with Approximate Bayesian Computation analysis indicates that both wild and domestic pigs display an important quantity of deleterious mutations at low frequency (~83% of total mutations) and a high number of nearly-neutral mutations (~17%) that may have a significant effect on the evolution of domestic and wild populations. Exclusive mutations show that recent demographic changes have severely affected the fitness of populations, especially of the local Iberian breed. Finally, the median proportion of the strong favorable mutations are very scarce in all cases ($\leq$ 0.2%). The median estimated alpha values (weak and strong favorable) are 0.9% for wild and domestic pigs.

Keywords: Domestication, Distribution of fitness effects, Proportion of beneficial mutations, Approximate Bayesian calculation, Polygenic selection, Population genomics

INTRODUCTION

Domestic animal histories are evolutionary experiments that have often lasted for millennia, with the result of dramatic phenotypic changes to suit human needs. In addition, domestic species can be structured into subpopulations (breeds) that are partly or completely genetically isolated and can display a wide catalog of specific phenotypes. Therefore, they offer a material of utmost interest to study the interplay of demography and accelerated adaptation. However, as their demographic history can be quite complex, many events remain unknown or poorly documented nowadays.

The pig (*Sus scrofa*) is a particularly interesting species because of its domestication history and its relatively well-annotated genome. *S. scrofa* originated in Southeast Asia ~ 4 MYA and spread throughout Eurasia ~1.2 MYA, colonizing all climates except the driest (Frantz et al. 2013). Subsequently, the pig was domesticated from local wild boars independently in both Asia and Europe ~9,000 years ago. To complicate the story, modern European domestic pig breeds were crossed with Asian domestic pigs during the late 17th century and onwards. In breeds such as Large White (LW), approximately 30% of the genome is estimated to be of Asian origin (Bosse, Megens, Madsen, et al. 2014). Nevertheless, some local European breeds, such as the Iberian breed (IB), were spared genetic contact with Asian pigs and no evidence of genetic introgression has been found in this breed (Alves et al. 2003, Esteve-Codina et al. 2013). Moreover, domestic breeds have different recent demographic histories. For instance, the IB breed suffered a dramatic reduction of its effective population size during the last century (Alves et al. 2006), whereas commercial breeds such as Duroc or LW have been introgressed with Asian pigs (Bosse, Megens, Frantz, et al. 2014).

Differences in the effective population size, demographic histories and artificial selective pressures between pig populations could result in differences among their evolutionary rates. In addition to possible differences in the rate of evolution between populations, there may be differences in the rate of evolution between genes within genomes. For instance, it is known that the strength of the selection is affected by the position of the genes in the networks in which they participate. Genes that are more central in a network and are more connected with other genes are more evolutionarily constrained, while peripheral genes are more prone to be under adaptive selection (Fraser et al. 2002; Hahn and Kern 2005; Montanucci et al. 2011; Alvarez-Ponce and Fares 2012). Furthermore,

it has been observed that the evolutionary rate, within a metabolic pathway, increases as we move downstream, possibly because upstream genes are more pleiotropic, since they are involved in more functions and hence these genes are probably more conserved (Rausher, Miller, and Tiffin 1999; Riley, Jin, and Gibson 2003; Livingstone and Anderson 2009; Ramsay, Rieseberg, and Ritland 2009).

So far, the nature of the underlying genetic changes caused by domestication and ensuing artificial breeding is still under debate. While the most prevalent view is that regulatory changes have been targeted (Anderson 2013), several other studies underline the influence of protein coding changes (Rubin et al. 2012). Other authors have reported an increase in the rate of deleterious mutations in domestic pigs compared to their wild counterparts (Cruz, Vilà, and Webster 2008; Renaut and Rieseberg 2015; Pérez-Enciso et al. 2016; Leno-Colorado et al. 2017). Others, as in Makino et al. (2018) detected a general pattern of reduction of variability in domestic populations in relation to their wild counterpart, and a higher nonsynonymous/synonymous ratio across the frequency spectrum. These patterns were compatible with the effect of strong bottlenecks in domestic populations and the higher accumulation of deleterious mutations. Interestingly, the same authors observed that the opposite trend has been observed in pigs. Moreover, most of the previous studies have focused on genes of major effect with clear signals of selective sweeps. In those studies, the hallmarks of positive selection were detected by a valley of reduced variation and/or population differentiation that spans a relatively large region (e.g., Amaral et al. 2011, Rubin et al. 2012, Frantz et al. 2013, Wilkinsonet al. 2013) but also by haplotype structure and homozygosity blocks (e.g., Fang et al. 2011, Bosse et al. 2012, Li et al. 2013). Some of these studies have detected recent breed specific signals of selection attributed to the domestication process (Li et al. 2014, Kim et al. 2015). Nevertheless, the signals were too scarce to explain the domestication process. Other studies have tried to elucidate the effect that domestication has at a whole-genome scale and on the fitness of individuals of domestic populations (e.g., Cruz et al. 2008, MacEachern et al. 2009, Kono et al. 2016, Perez-Enciso et al. 2016, Makino et al. 2018, Chen et al. 2018, Orlando and Librado 2019). For instance, an excess of deleterious variants has been observed in a number of domestic animal and plants (e.g., contrasting nonsynonymous versus synonymous polymorphism ratios, Chen et al. 2018, using the MacDonald framework, MacEachern et al. 2009, contrasting ancestors with ancient DNA, Orlando and Librado 2019, combining the frequency of polymorphisms with functional effects and divergence, Kono et al. 2016, Makino et al. 2018).

Kono et al. (2016) and Perez-Enciso et al. (2016) found an excess of detrimental variants affecting phenotypes of interest, suggesting, as we previously mention above, that protein sequence may have a stronger influence than regulatory changes in the domestication process. Kono et al. (2016) also showed that null alleles are uncommon in domestic animal species (also reviewed by Anderson 2013), suggesting that phenotypic changes involved in domestication are produced by the accumulation of consecutive mutations that modify these functions under selection. Finally, the possible presence of beneficial mutations during the domestication process has also been reported (Perez-Enciso 2016).

Here, we are interested in determining the proportion and the selective effects of protein-coding variants in wild and domestic pig genomes to understand their role in the domestication process. Particularly, we aimed to test the role of both new and extant mutations in the domestication process and whether the phenotypes associated with domestic breeds are the product of a large number of variants with weak selective effects, as suggested by previous results. To achieve this, we have investigated the differential effects of selection on coding sequences at the different molecular scales (gene, metabolic pathway and whole-genome) in two domestic and one wild pig population using the McDonald-Kreitman framework (McDonald and Kreitman 1991, Eyre-Walker 2006, Fay 2011) and have inferred the distribution of fitness effects (DFE) while taking into account the effect of different demographic scenarios. Interestingly, the analysis was performed using variability estimators that allow including positions with missing data (Ferretti, Raineri, and Ramos-Onsins 2012).

Our results support the hypothesis that changes in allele frequencies in coding variants with weak positive selective effect have been relevant for pig domestication, as evidenced by a relatively high number of nonsynonymous variants segregating at medium and high frequencies and by the obtained estimates of the Distribution of Fitness Effects in domestic pig populations.

MATERIALS AND METHODS

Biological samples

We analyzed a sample of 46 pig (*Sus scrofa*) genomes (Table S1). These pigs correspond to European wild boars (WB, n = 20) and domestic pigs, which are represented by the Iberian Guadyerbas (IB, n = 6) and LargeWhite (LW, n = 20) breeds. These two domestic breeds were selected because they have very different interesting features: IB is a local breed that has been under weak artificial selection intensity and with no documented evidence of Asian introgression. LW, in contrast, is a commercial breed undergoing strong artificial selection with a deliberate admixture with Asian pigs (Bosse, Megens, Madsen, et al. 2014; Groenen 2016). To analyze the divergence between the different breeds, we used the consensus ancestral reference sequence obtained from combining the information from several *Sus* species (*S. barbatus*, *S. cebifrons*, *S. verrucosus*, *S. celebensi*, approximately 4.2 MYA of divergence) and the African warthog (*Phacochoerus africanus*, around ~10 MYA of divergence) as an outgroup, as detailed in Bianco et al. (2015). The sequences are available in public databases (Rubin et al. 2012; Ramírez *et al.* 2014; Bianco *et al.* 2015; Frantz et al. 2015; Moon *et al.* 2015, Esteve-Codina *et al.* 2013, Leno *et al.* 2017) and were downloaded from the short read archive (SRA, http://www.ncbi.nlm.nih.gov/sra).

Mapping and genotyping analysis

Raw reads for each pig genome were mapped against the reference genome assembly (Sscrofa10.2, Groenen et al. 2012) using *BWA mem* option (H. Li and Durbin 2009). PCR duplicates were removed using *SAMtools rmdup* v 0.1.19 (H. Li et al. 2009) and mapped reads were realigned around indels with the *GATK IndelRealigner* tool (McKenna et al. 2010). Genotype calling was performed with *SAMtools mpileup* and *bcftools call* v 1.3.0 (H. Li et al. 2009) for each individual separately. We set a minimum (5x) and a maximum depth (twice the average sample's depth plus one) to call a SNP. Base quality was set to 20 (*P*-value=1e-2). Homozygous blocks (regions of contiguous positions with the same nucleotide as the reference genome) were also called, following the same criteria (i.e., minimum and maximum coverage and base quality) as with the SNPs and using *samtools depth* utility, *BEDtools* (Quinlan 2014) and custom scripts. This resulted in a *gVCF* file per individual with the combined information about variant calls and non-varying

158 positions. Next, each *gVCF* file was converted into a fasta file and all fasta files were subsequently
159 merged to obtain a multindividual *gVCF* file (Pérez-Enciso et al. 2016), which comprised the
160 whole set of the SNPs of the 46 pigs.

161

162 **Analysis of the population structure of the samples**

163 A principal component analysis (PCA) was performed using the total number of SNPs to analyze
164 the population structure. First, genotypes were converted to alternative allele frequency, being 0
165 for the homozygous reference genotype (0/0), 0.5 for the heterozygous genotype (0/1) and 1 for
166 the homozygous alternative genotype (1/1). For cases of missing genotype (./.), these were
167 replaced by the average SNP frequency across all individuals. We used the function *tcrossprod()*
168 from R version 3.3.1 (2016) to obtain the matrix of covariates from the frequencies matrix. Finally,
169 we obtained the principal components from the Eigen-value decomposition with the R function
170 *eigen()*.

171

172 **Estimation of levels and patterns of variability**

173 Genetic diversity and divergence per pig population were estimated using *mstatspop* software
174 (Nevado, Ramos-Onsins, and Perez-Enciso 2014; Bianco et al. 2015; Guirao-Rico et al. 2018,
175 available from the authors, https://github.com/cragenomica/mstatspop). The *multi-VCF* file was
176 converted into a *tfasta* (transposed *fasta*) file and *mstatspop* was run on either the whole genome,
177 using 5 Mb windows and at each functional coding region. We used four different estimators of
178 nucleotide variability that takes into account missing data (Ferretti, Raineri, and Ramos-Onsins
179 2012): Watterson (Watterson 1975), Tajima (Tajima 1983), Fu&Li (Fu and Li 1993) and
180 Fay&Wu's estimators (Fay and Wu 2000). Specifically, variability was estimated using the
181 Ferretti, Raineri, and Ramos-Onsins (2012) expression:

$$\hat{\theta} = \frac{1}{L}\sum_{x=1}^{L}\sum_{i=1}^{n_x-1} i\omega_{i,n_x}\xi_i(x), \quad \frac{1}{L}\sum_{x=1}^{L}\sum_{i=1}^{n_x-1}\omega_{i,n_x} = 1$$

182

183 *(Equation 1)*

where (ω_i) is the weight for the different variability estimators such as $\omega_i = n/(i(n-i)(1+\partial_{i,n-i}))$ for the Watterson estimator, $\omega_i = n/(1+\partial_{i,n-i})$ for the Tajima estimator (both for folded spectrum), $\omega_i = i$ for the Fay&Wu estimator and $\omega_i = 1$, $\omega_{i>1} = 0$ for the Fu&Li estimator (Achaz 2009).

Filtering for artifactual effects

A preliminary analysis of the variability showed a moderate negative correlation (~ 0.3) between the levels of variability and divergence and the proportion of missing data for each gene. To eliminate this artifactual correlation, we plotted the estimators of variability and divergence versus the ratio of missing data and eliminated those genes that showed a ratio of missing data greater than 0.3. Since this filtering was not enough to completely remove the bias, we also removed genes with extreme values of variability and divergence (higher than 99% quantile of the total genes). The remaining ~13,500 genes (70% of the total annotated genes) showed a low or null correlation with missing data and were used in the present analysis (Table S2).

Estimation of the proportion of adaptive of variants

Under the neutral model, the majority of polymorphisms segregating in a population are neutral and only a small number of positively selected variants segregates for a short time on their way to loss or fixation. Hence, most of the positive selected variants are only observed as fixed variants. In addition, functional positions (nonsynonymous positions) are constrained compared to nonfunctional positions (synonymous positions), and hence their evolutionary ratios are smaller. In the neutral scenario, polymorphism and divergence (excluding the adaptive fixed variants) are proportional to the mutation rate and to the constriction factor in the case of nonsynonymous positions (McDonald and Kreitman 1991, Eyre-Walker 2006, Fay 2011). That is:

$$\frac{\theta_n}{\theta_s} = \frac{(1-\alpha)K_n}{K_s},$$

(Equation 2)

where θ_n the nonsynonymous variability, θ_s is the synonymous variability, K_n the nonsynonymous divergence, K_s the synonymous divergence and α is the proportion of adaptive variants that have

been fixed. To estimate the proportion of nonsynonymous substitutions that are adaptive (α) we reorder the previous expression:

$$\alpha = 1 - \frac{K_s}{K_n}\frac{\theta_n}{\theta_s}$$

(Equation 3)

A higher ratio of nonsynonymous to synonymous divergence versus polymorphisms suggests that positive selection has fixed adaptive variants ($\alpha > 0$) and the opposite case ($\alpha < 0$) suggests the presence of deleterious mutations segregating in the population.

If we consider that weak deleterious mutations are segregating in the population, we expect that their relative proportion will be higher at lower frequency variants and low or null for fixed deleterious mutations. Following the same notation as in equation 3:

$$\frac{\theta_{in}(1-\beta i)}{\theta_{is}} = \frac{(1-\alpha-\beta d)K_n}{K_s},$$

(Equation 4)

where i refers to the frequency at which the calculation of variability is estimated, βi is the proportion of weakly deleterious polymorphic mutations at frequency i, βd is the proportion of weakly deleterious fixed mutations. $\beta d < \beta i$ was assumed at any frequency. Then, solving for the proportion of fixed adaptive variants (α):

$$\alpha = 1 - \beta d - (1-\beta i)\frac{K_s}{K_n}\frac{\theta_{in}}{\theta_{is}}$$

(Equation 5)

We see that in case calculating α without considering the effects of deleterious mutations, it would be underestimated depending on the frequency at which the estimates of variability are calculated. If we assume that the detrimental variants would never be fixed, a good estimator of α using equation 3 would be the one that estimates variability based on high frequencies, as it would hardly contain detrimental mutations. This is in agreement with the arguments used in Messer and Petrov 2013.

Similarly, if we also consider that weak positively selected variants are segregating in the population, we expect that their relative proportion, compared to neutral ones, is higher at higher frequencies:

$$\frac{\theta_{in}(1-\beta i-\gamma i)}{\theta_{is}} = \frac{(1-\alpha-\beta\,\mathrm{d}-\gamma d)K_n}{K_s},$$

(Equation 6)

where γi is the proportion of weakly advantageous polymorphic mutations at frequency i, and γd is the proportion of weakly advantageous fixed mutations. Again, solving for the proportion of fixed adaptive variants (α):

$$\alpha + \gamma d = 1-\beta d-(1-\beta i-\gamma i)\frac{K_s}{K_n}\frac{\theta_{in}}{\theta_{is}}$$

(Equation 7)

In this case, the presence of adaptive variants segregating in the population would affect the estimates of variability based on high frequency variants when using equation 3, which would result in an underestimation of the proportion of fixed adaptive variants (α). Note that adaptive variants stabilized at intermediate frequencies are not considered in this approach, which can be an important source of adaptation considering the infinitesimal model.

If we focus on the effects on the polymorphisms, equation 6 suggests that the ratio of nonsynonymous to synonymous polymorphisms would increase due to mutations having both positive and negative effects. It is expected that the number of mutations with negative selection coefficients would rapidly decrease as we move to intermediate and high frequencies, while the opposite trend is expected for mutations with positive selection coefficients. Hence, higher ratios of nonsynonymous to synonymous polymorphisms at higher frequencies may be explained by the presence of advantageous mutations segregating in the population.

Furthermore, in cases where two populations are from the same species and no fixed mutations between them we can estimate the possible differential effect of the selection (positive and

negative) at any frequency among populations from the ratios of synonymous to nonsynonymous polymorphisms of the two populations ($R\_\beta\gamma i$):

$$\frac{\theta_{in1}(1-\beta i1-\gamma i1)}{\theta_{is1}} = \frac{\theta_{in2}(1-\beta i2-\gamma i2)}{\theta_{is2}}$$

and

$$\frac{(1-\beta i1-\gamma i1)}{(1-\beta i2-\gamma i2)} = \frac{\theta_{is1}\theta_{in2}}{\theta_{in1}\theta_{is2}} = R\_\beta\gamma i$$

(Equation 8)

In addition, a comparison of the $R\_\beta\gamma i$ values calculated using different variability estimators (hereafter $R\_\beta\gamma i$ pattern) can be used to inform about the effects of the different types of selection. Importantly, different demographic effects (e.g., bottlenecks) together with the presence of mutations with small selective effects may also disturb the ratios of variability and hence must be taken into account when interpreting the results.

The effect of linkage disequilibrium between selective (detrimental or adaptive) and neutral variants should not affect the expected estimate of the proportion of adaptive variants, as it would affect both synonymous and nonsynonymous positions in the same proportion. On the contrary, the interaction of variants with opposite selective effects would possibly reduce the effect of selection and would have a significant consequence on the estimation of adaptive fixed variants (Hill and Robertson 1966; Booker and Keightley 2018).

Bootstrap analysis

Nonparametric bootstrap analysis was performed to estimate the null distribution of the α statistic for each variability estimator and pig population. In each case, coding positions for synonymous and nonsynonymous (separately) were randomly chosen with replacement and the α statistic was calculated as in equation 2. This process was repeated 100 times.

Simulations

We carried out forward simulations using the software *SLiM* (Haller and Messer 2017) in order to assess the interaction of the different selective effects and demographic factors affecting the evolution of pig populations during domestication. We explored the expected values of nucleotide diversity, divergence, α and $R\_\beta\gamma$ under 63 different scenarios. For each scenario, we simulated three populations corresponding to wild, domestic and an outgroup species. We first simulated nine different scenarios that were classified into three main groups: i) standard neutral model (SNM); ii) a model with negative selection (NS) and iii) a model with positive selection (PS). For the models with selection, we let that selection operate from the ancestral species to the present time. Each group of scenarios (SNM, NS and PS) was simulated with a constant effective population size for the three populations or with a reduction or an expansion of the effective population size in the branch leading to domestic pigs. A second group of simulations was performed under more complex scenarios. In those simulations, we incorporated the combined effect of negative and positive selective effects (using gamma and exponential distributions for the selective coefficients, respectively) plus demographic effects such as expansion and reduction of the effective population size in the domestic simulated populations and with or without migration from the wild into the domestic populations (in total 54 complex simulated scenarios). Figure S1 shows a general scheme for the simulated populations and Table S3A-B shows the parameter values used in these simulations. The obtained results were analyzed using the *mstatspop* software.

Approximate Bayesian computation (ABC) analysis

We used the ratio of the estimates of nucleotide variability ($\theta n/\theta s$) per nucleotide for nonsynonymous versus synonymous positions (Fu&Li, Watterson, Tajima and Fay&Wu) and of divergence (*Kn/Ks*) to infer the distribution of fitness effects (DFE) in coding regions. We compared three evolutionary models that differ in the shape of the DFE using the algorithm proposed by Tataru et al. (2017), which are the following: (i) model A: a model with a deleterious gamma DFE with the mean and the shape of the gamma distribution as model parameters, (ii) model C: a model with a gamma distribution of deleterious variants with two parameters (shape and mean) and an exponential distribution of beneficial variants with one parameter (mean), and the additional parameter of the proportion of beneficial versus deleterious variants, and (iii) model D: a model with a discrete distribution of a priori values of possible selective coefficients (positive

and negative) and considering as parameters the proportion of each (positively and negatively selected mutations). Some of the additional parameters, such as demographic or linkage effects, were considered as nuisances, while others, such as errors in the polarity of unfolded mutations, were fixed. Table S4 shows the parameters and the prior distributions used in the analysis. We used *polyDFEv2* (Tataru et al 2019) to obtain the expected unfolded site-frequency spectrum (SFS). With the aim of performing the ABC using summary statistics, the code of *polyDFEv2* was slightly modified in order to print the SFS and the parameters for a large number of conditions. For each model, one million iterations were run using different parameter conditions and the resulting SFS for each condition were kept to later calculate the ratios of variability, divergence and the α statistic. ABC analysis was performed using the R library *abc* (Csillery et al. 2012). We performed a cross validation analysis to evaluate the ability of the approach to distinguish between models using the *cv4postpr()* function, as suggested in the *abc* library documentation. The confusion matrix indicated that these three models were quite distinguishable with a probability of true classification from model A versus C/D of 0.70, from model C versus A/D of 0.60 and from model D versus A/C of 0.67, with a tolerance of 0.05 (Table S5). Posterior probabilities of each model given the observed data were obtained using the *postpr()* function and considering a multinomial logistic and a rejection approach. Additionally, a goodness of fit analysis, which compares the median of the distance between the accepted summary statistics and the observed ones, was also performed to select the best model. Once the best model was chosen, the ability to infer the parameters of the model was assessed using the *cv4abc()* function. Prediction errors for the parameter inference of each model are shown in Table S6 and Figure S2. The parameters of the best model were inferred with the *abc()* function using a local linear regression and a rejection approach. Posterior predictive simulations were performed with the α statistic (instead of with the ratios of variability and of divergence, to avoid circularity in the analysis) to determine whether the simulated data generated from the estimated parameter of our best model resembled the observed data (1000 replicates). Finally, the α values can be simply estimated using equation 10 from Tataru et al. (2017), which is a simple proportion of positive selective coefficients (s) values in the case of the discrete distribution.

Pathway analysis

We downloaded the complete list of pathways and genes of *Sus scrofa* from KEGG v.20170213 (http://www.genome.jp/kegg/, Kanehisa et al. 2008). The list contained 471 pathways and 5480 genes. The median and mean number of genes per pathway was 26 and 43, respectively, and ranged from 1 to 949. We filtered the pathways according to their size, removing pathways with less than 10 and more than 150 genes in order to discard pathways that were not informative or too generic and complex. The final list contained 171 pathways and 3449 genes.

To analyze the selection pressure of each gene according to its position in the pathway, we obtained different topological parameters. For that, we first downloaded the XML file of each pathway from KEGG v.20170213. These files were analyzed with the *iGraph* R package (Csardi G. and Nepusz T. 2006) to obtain the topological descriptors of each gene in each pathway. For each gene, three different measures were computed: *betweenness* (number of shortest paths going through a vertex), *in-degree* (number of in-going edges) and *out-degree* (number of out-going edges). These parameters are measures of the importance of a gene within a pathway: *betweenness* is a centrality feature, *in-degree* suggests the facility of a protein to be regulated and *out-degree* reflects the regulatory role of a protein. We tested whether negatively and positively selected genes differed in any of these statistics using a nonparametric Wilcoxon rank test, due to the extreme leptokurtic distributions involved.

365 **RESULTS**

366 **Predominance of shared variants and similar selective effects on pig populations**

367 We found a total of 6,684,142 SNPs in autosomes, with 149,440 SNPs of these located in coding
368 regions. We found that 12.5% of the SNPs in the coding regions are shared among the three
369 populations, 32.2% are shared between at least two populations, 31.2% are exclusive to LW, 2.2%
370 are exclusive to IB and 34.4% are exclusive to WB (Table 1). Based on the PCA analysis and using
371 the total number of SNPs, we found that the individuals of each breed cluster together and are well
372 separated from other breeds (Figure S3). The proportion of private SNPs in each population is in
373 accordance with its specific demographic history (Esteve-Codina et al. 2013, Bosse, Megens,
374 Madsen, et al. 2014; Bosse, Megens, Frantz, et al. 2014).

375

376 For each breed, each coding position was classified as polymorphic, fixed (i.e., different alleles
377 from the outgroup) or ancestral allele (i.e., same allele as in the outgroup), with the aim of
378 identifying those variants that appeared previously or posteriorly to the domestication process
379 (Table 2). Surprisingly, we found very few fixed mutations between populations, indicating that
380 the phenotypic traits of each population are not associated with fixed coding variants. Similarly,
381 we found very few fixed coding variants in domestic (IB or LW) versus wild (WB). There are few
382 variants fixed in the domestic that are polymorphic in the wild population, suggesting that these
383 variants were previously present in wild breeds or, alternatively, were transferred into WB by gene
384 flow from introgressed domestic breeds. Most of the variants that are exclusive of a single breed
385 are polymorphic, which is in agreement with the recent origin of these variants. We found a large
386 number of fixed variants in the IB that are polymorphic in LW and WB, likely due to a reduction
387 of the effective population size of the IB breed. The ratio of nonsynonymous to synonymous
388 polymorphism was always lower than one and with similar values in the three populations
389 regardless of the variability estimator used. This result suggests that apparently, there are no
390 differential effects of selection between domestic and wild populations.

391

392 **Limited influence of genomic context and the network topology on selective patterns**

The heterogeneity in the recombination rate, the gene density, the %GC and the distribution of CpG islands across the genome can affect the local levels of variability. A previous study on the IB breed detected a strong correlation between recombination and variability, although no correlation was observed between variability and gene density or GC content (Esteve-Codina et al. 2013). However, the effect of these factors on the estimation of the proportion of adaptive nonsynonymous mutations (α) has not been previously studied. We observed no correlation between α and recombination, gene density, missing rate, %GC and CpG in any of the three breeds (P-values > 0.01).

Next, we investigated the effect of gene network topology on the selective patterns. It has been claimed that topology limits the 'evolvability' of genes and that highly connected genes are more constrained and, consequently, less likely to be targets of positive selection. We compared the network topology features (*betweenness*, *out-degree* and *in-degree*) of genes within pathways regarding the value of α, grouping genes with positive versus negative α values. We found that genes with negative α values show significant large values of the *betweenness* statistic in the three pig breeds compared to genes with positive α values (*P*-value < 0.01; Figure S4). LW and WB (but not IB, possibly because the low sample size) show significant values (*P*-values < 0.01) of the *in-degree* statistic for genes with negative α values compared to genes with positive *a* values. However, we did not observe significant differences in the *out-degree* values between genes with negative and positive *a* values in any of the three breeds (Figure S4). These results suggest that, in the three breeds, genes that are more central in a pathway are more evolutionary constrained compared to peripheral genes. In addition, in LW and WB, the genes that are more constrained tended to have a higher number of upstream genes that regulated them, which is also in agreement with the central position of these genes in the pathway. We did not observe significant differences in *in-degree* statistic in the IB breed between genes with negative and positive *a* values, perhaps because of a relaxation of functional constraints as a consequence of the reduction of its effective population size.

Levels of nucleotide variation at protein coding regions is compatible with the history of the surveyed pig populations and with positive selection

To assess the selective effect of domestication, we first studied the pattern of variation at synonymous and nonsynonymous positions using four estimators of variability that differentially weight the SNP frequencies (See Material and Methods). Estimates of the levels of variability per nucleotide at the genome level using different estimators are shown in Figure 1 and detailed in Table S7. We expect that, under the Standard Neutral Model (SNM), all estimates of variability should be similar while differences among them may indicate demographic and/or selective effects. We observed that the levels of variability are different for each estimator within breeds and also for the same estimator for different breeds. However, we observed a similar ratio of nonsynonymous to synonymous polymorphisms for all estimators of variability, suggesting that demographic effects are responsible for the differences in the levels of variability (Figure 1). The less variable population is the IB breed, which shows far fewer singletons compared to WB and LW, probably as a consequence of the reduction of its population size. Note than in all three populations, high-frequency variants are proportionally more abundant than those at intermediate frequencies, which would be compatible with the accepted demographic history of the surveyed populations (i.e., introgression in the LW) but also with the presence of pervasive positive selection in all three populations.

α's and $R\_\beta\gamma$ ratios might show a differential effect of selection due to domestication

The differential effect of selection in the domestic and wild populations can be studied by comparing the α values. Figure 2 and Table S8 show the genome-wide α values calculated using the four variability estimators for each population. As expected, the α values are negative when α is calculated using the estimate of variability based on low-frequency variants ($\alpha_{Fu\&Li}$), probably reflecting the relatively high proportion of deleterious versus neutral mutations that are segregating at low frequencies. We observed a similar value of $\alpha_{Fu\&Li}$ in all populations, suggesting a similar proportion of segregating deleterious mutations, irrespective of the domestication process or other demographic events (Figure 2A). Moreover, we observed less negative values of α, or even positive (for LW in Figure 2A), when α is calculated based on variants at high frequencies, according to expectations that point to a progressive elimination of deleterious mutations at higher frequencies. Nevertheless, the pattern of α (i.e., the comparative a values calculated using the four different variability estimators within each population) is very different in each population. WB

and IB show very low negative values of α for most of the estimators of variability, except for $\alpha_{Fay\&Wu}$ in WB, which is zero. LW is the only breed that shows a linear increase of the α negative values across the SFS, being even positive when calculated based on high frequencies ($\alpha_{Fay\&Wu}$).

We did not observe similar patterns of α between domestic breeds compared to WB (Figure 2A). The differences in the ratio of synonymous to nonsynonymous variability between the two different breeds is summarized by the $R\_\beta\gamma$ ratio (Figure 3A). We observed deviations from $R\_\beta\gamma = 1$ when the ratio was calculated based on high-frequency variants ($\alpha_{Fay\&Wu}$). Although the ratio of the two populations is difficult to interpret because of their different underlying demographics, some trends can be observed. WB shows an excess of nonsynonymous variants segregating at intermediate frequencies (WB-IB, WB-LW), which might be explained by a past bottleneck that increased deleterious mutations at intermediate frequencies. In addition, the $R\_\beta\gamma$ ratio in LW-IB shows an incremental pattern from low to high frequencies, which is compatible with an increase of nonsynonymous beneficial variants on their way to fixation in LW.

α's and $R\_\beta\gamma$ ratios based on from exclusive and shared polymorphisms might reflect changes in selection patterns before and after domestication

We observed a high ratio of nonsynonymous to synonymous exclusive singletons ($\alpha_{Fu\&Li}$, Figure 2B), suggesting that they have deleterious effects in all populations. Nevertheless, the values of α calculated based on intermediate frequency variants (α_{Tajima}) in the WB and IB populations are lower than to those based on low-frequency variants, which point to the action of positive selection maintaining nonsynonymous variants at higher frequencies in the case of WB and to an attenuated effect of deleterious mutations in IB due to a population size decline. In the case of LW, the observed pattern of α is similar to that calculated with all SNPs (Figure 2B). Likewise, the R_βγ statistic shows the same pattern as that calculated using all SNPs but with a higher magnitude of its value (Figure 3B).

On the other hand, the α values based on shared variants are in general more moderate (closer to zero) than those based on exclusive or all SNPs (Figure 2C), likely because shared nonsynonymous

polymorphisms are older and hence, expected to be more functionally constrained than exclusive ones. Additionally, the values of α based on singletons ($\alpha_{Fu\&Li}$) are less negative than those based on intermediate-frequency variants. Again, this pattern might indicate that selection is involve in the increase of the ratio nonsynonymous to synonymous polymorphisms up to intermediate frequencies. The $R\_\beta\gamma$ statistic shows similar patterns than those observed for all variants but with values much closer to 1, indicating a small or moderate selective effect on the shared variants compared to all variants (Figure 3C).

When we calculated the α values from shared variants only between the two domestic breeds, we found an inverse pattern regarding that calculated from all SNPs in each population, with high positive values of α based on low frequencies and very negative values when α is calculated based on high-frequency variants (Figure 2D). Some possible explanations might be the active elimination of new nonsynonymous variants to preserve differences among breeds ($\alpha_{Fu\&Li}$) and either the effect of the ancestral population structure (wild versus domestic), or the presence of nonsynonymous variants targeted by the process of domestication that shifts them toward high frequencies ($\alpha_{Fay\&wu}$).

Absolute values of α are dependent of the molecular scale but patterns remain similar

In addition to the genome-wide analysis, α was estimated using three additional molecular scale levels: i) gene level, ii) genes within windows of 5 Mb, and iii) genes within the same pathway. Figure 4 shows the median of the distributions of the α values for each scale level. In general, the distribution of α values estimated at the genome-wide level are concordant with those estimated at the gene level, genes within windows and pathway level in each breed. However, differences in the value of α within each breed are notorious depending on the scale level examined. The median estimates of α are generally lower at the gene scale level and most of them are very negative, while at the genome-wide scale, the α values are closer to zero. However, the distribution of α values can have a large variance at the gene scale since few variants are used for its estimation. We identified the regions and pathways that showed extreme α values (Table S9 and S10). We found a large number of genes having $\alpha = 1$ (highest value) because the number of polymorphic nonsynonymous variants per gene was zero. We also found a moderately high correlation of α

values between breeds (rho ~ 0.7, Pearson correlation using pathways) suggesting that in general, these breeds are under similar selective effects. For shared and exclusive variants, we generally observed the same pattern, from genes to whole-genome, that is, larger α values at the gene level and closer to zero α values at the larger scale. Only shared variants of the IB breed exhibited similar α values from genes to the whole-genome scale. The differences in absolute α values could be explained because of the nature of this ratio statistic, in which the mean can be more displaced to more negative values due to a reduced number of functional variants (*i.e.,* few or null segregating nonsynonymous variants).

Simulated data under scenarios that include the joint effect of demography and selective events were more similar to the observed data

We used computer simulations to study how the different demographic and selective events occurred during domestication affected nucleotide variation. We simulated populations mimicking the process of domestication using *SLiM* software (Haller and Messer 2017) coupled with several demographic events, including changes of the population size and/or migration. We analyzed the genome-wide patterns of α and the $R\_\beta\gamma$ statistic produced by 63 simulated scenarios that included different demographic events and selective forces acting separately (simple scenarios) or jointly (complex scenarios). The results of the simulation study are summarized in Figures S5-S46. The observed patterns of α calculated from all variants in the surveyed populations are not compatible with simple scenarios that only consider demographic or positive selection forces (Figure S5). Rather, simulated α trends (irrespective of the magnitude of α) fit a scenario with a predominant effect of negative selection (Figure S5). However, the $R\_\beta\gamma$ statistic do not fit any of the simulated simple scenarios (Figure S6). When more complex scenarios were considered, the general trends of the patterns of α generated by scenarios with both negative and positive selection resembled those observed in WB and LW (Figures S7-S13). Notice that this is true only for those scenarios that include some demographic or migration events (Figures S10-S12). On the other hand, the IB population would fit a scenario without positive selection and with a recent population size reduction (Figure S11). The trends in the $R\_\beta\gamma$ statistic are, in broad strokes, concordant with the conclusions extracted from the comparison between the observed and simulated patterns of α (Figures S13-S18).

The observed patterns of α values from exclusive variants are different from those based on the total variants in WB and IB populations (Figure S19). These patterns cannot be fully explained by any of the complex simulated scenarios proposed when considering all variants, except for the IB population, which might be compatible with a scenario with negative selection and population size reduction (Figures S22-S26). The observed $R\_\beta\gamma$ are also compatible with the scenarios combining both types of selection and population size reduction (WB-IB) or with scenarios that include migration (WB-LW; Figures S27-S32). Finally, the observed patterns of α and $R\_\beta\gamma$ statistics calculated from shared variants are also compatible with scenarios having deleterious plus beneficial mutations (Figures S33-S46).

A model that assumes a discrete distribution of beneficial and deleterious mutations would fit better the observed data in the ABC analysis

We used an approximate Bayesian computation (ABC) analysis to infer the DFE separately for each population using the ratios of nonsynonymous to synonymous variants obtained from the whole-genome analysis (see Materials and Methods). Three different models implemented in *polyDFE2* software (Tataru et al. 2019) were tested. Model A, which assumes a gamma distribution of deleterious mutations; model C, which assumes a gamma distribution for deleterious mutations and an exponential distribution for beneficial mutations; and model D, that assumes a discrete distribution of beneficial and deleterious mutations. In these models, we included demographic and linkage effects as nuisance parameters (Tataru et al. 2017). Goodness of fit (GoF) analysis, which is a measure of the adjustment of the prior chosen models to the data, revealed that the simulated data under the different models do not always fit well to the observed data (Table S11A), suggesting that the real data fit only to a very restrictive parameter range of each model. This is especially pronounced for exclusive variants, where the simulations under the different models fit only marginally to the observed data. Indeed, the model with a wider parameter range versus the real data is usually the discrete model D. However, if we also take into account the values of the posterior probabilities, which is the probability assigned to each model relative to the other models of the analysis, we found that the best fit model differed among populations (model C to WB and LW and model D to the IB breed; Table S11A). Finally, posterior predictive

572 analysis indicated that the observed α values (Figure 5) and variability
573 nonsynonymous/synonymous ratios (Figure S47) cannot be obtained using the estimated
574 parameters of any model, although they were closer to model D. The parameters of the DFE
575 inferred for each population are shown in Table S12. The obtained results indicate that the DFE is
576 quite similar among all three populations, which is not entirely surprising because they share a
577 long-term history. Despite there is a lot of uncertainty in the inferred estimates, the obtained results
578 show that the DFE contains a large fraction of very deleterious variants, with approximately 83%
579 of the variants being strongly deleterious ($S \leq -200$; considering the posterior distribution and using
580 the rejection method), and with approximately 17% of the variants being neutral, weak beneficial
581 and weak deleterious mutations ($-2 \leq S \leq +2$). The median proportion of adaptive mutations (α)
582 estimated from the discrete distribution (by summing weak and strong beneficial proportion of
583 mutations in Model C) is approximately 0.9% (Table S12A).

584

585 **The inferred DFE is different when based on exclusive and shared variants**

586 Although the inference of the DFE is going to be distorted by choosing only a subsection of the
587 variants (e.g., exclusive variants are mostly very recent), we considered that it can give some clues
588 about past events that could be more related to the domestication processes. Regarding to exclusive
589 variants, the simulations under the three different models show a low fit to the observed data.
590 Indeed, in some cases, the GoF is less than 1% (Table S11B). Although the posterior probability
591 is higher for model C, the posterior predictive simulations show that none of the models can
592 reproduce well the observed data. In general, the posterior predictive simulations under model D
593 yield more similar values to the observed data. However, only the posterior predictive simulations
594 under this model are reasonably similar to the observed data for the LW breed (Figure 6, Figure
595 S48). The results obtained for shared variants are very similar to those considering the total
596 variants, supporting the hypothesis that shared mutations have a predominant effect (compared to
597 exclusive variants) at the whole genome. Finally, the median proportion of adaptive variants (α)
598 estimated from the discrete distribution (Model C) gave similar results to the total mutations, but
599 surprisingly estimated a slightly higher proportion for the Iberian breed (Table S12C; Figure S49-
600 S50).

601

DISCUSSION

The polygenic nature of the domestication traits in animals often precludes identifying their underlying genes since the domestic phenotypes are caused by subtle allele-frequency changes of variants distributed throughout the genome that are very difficult to detect. In addition, the study of the effects of selection using genome sequences that contain a nonnegligible fraction of missing data is challenging and needs the use of appropriate methods to account for these positions. Statistics that exploit the frequency of the variants while accounting for missing data are particularly appropriate for such analyses.

Here, we provide a novel approach that combines the use of different estimators of variability that account for missing data with the asymptotic approach proposed by Messer and Petrov (2013) and Uricchio et al. 2019). This approach allowed us to study and to interpret the effects of the domestication process on genomic variation as an alternative to the use of the full SFS. Although it can be less precise (we used only four statistics to capture the entire trend of α across the SFS), it helps to reduce the variance and facilitates the visual interpretation. The analyses performed here include an exhaustive comparative study of the observed patterns of functional versus neutral diversity in domestic pigs and wild boars, a forward simulation study for several diverse evolutionary scenarios and the inference of DFE parameters given different selective models.

Note that the DFE was inferred using Bayesian calculations (ABC) instead of exact Bayesian or Likelihood methods. Despite ABC requires additional steps and validation analysis and is in general less precise, it allows contrasting models and inferring parameters from complex datasets or data containing missing information (Beaumont et al. 2002).

Selection pressure on pigs and the process of domestication

A number of analyses with the aim to explain the process of domestication have been performed already, using the MacDonald and Kreitman extension methods, or using other estimates such as variability or divergence at functional or synonymous positions (MacEachern et al. 2009, Kono et al. 2016, Makino et al. 2018). For instance, Kono et al. (2016) analyzed derived frequencies in

631 domesticated barley populations at different functional classes (deleterious, tolerated) and
632 observed a higher quantity of deleterious variants at low frequencies compared to tolerated or to
633 synonymous mutations. Makino et al. (2018) investigated the ratio of functional to neutral variants
634 at different frequencies in domestic and in wild populations of several animal and plant species,
635 including Asian and European pigs. They observed generally lower levels of synonymous and
636 nonsynonymous variation and a higher ratio of functional to neutral variants in the domestic
637 species compared with their wild counterparts. This ratio was negatively correlated with the
638 frequency of the variants, consistent with a higher number of detrimental variants in domestic
639 populations. The authors claimed that these patterns were compatible with the expected effect of
640 a bottleneck as a consequence of domestication, and with an increase in nonsynonymous variants
641 produced by the lesser efficacy of purifying selection at smaller population sizes, although the
642 presence of positive selection (hitchhiking) was not discarded. However, the opposite pattern was
643 observed in European wild boars and domestic pigs (a higher variability and a lower ratio of
644 nonsynonymous to synonymous in domestic pig populations compared to wild boars). The authors
645 argue that this can be explained by the highly variable patterns produced by bottlenecks, the strong
646 population contraction of European wild boars during the last glaciation and the presence of gene
647 flow between wild and domestic populations.

648

649 As in Makino et al. (2018), we do not observe an increase in functional diversity in domestic versus
650 wild populations. This result may also be explained by several recent events occurring in these
651 populations: (i) differences in the recent history of local and commercial domesticated populations,
652 that is, high inbreeding in Iberian local pigs but recent gene flow of the commercial pigs with
653 Asian pigs; (ii) demographic effects in the wild boar population that may have reduced their
654 diversity (Groenen et al 2016) or increase the variance of the patterns (see simulations, Figures
655 S5-S46); (iii) differential adaptive forces in local versus commercial pigs, with a recent high
656 selective pressure in this last population.

657

658 The two domestic breeds analyzed here have very different recent histories: the IB is a local
659 Spanish breed (Guadyerbas) that suffered a strong bottleneck during the 1970s (Esteve-Codina et
660 al. 2013) and with no evidence of introgression whereas the LW breed was admixed with pigs of

Asian origin (Bosse, Megens, Madsen, et al. 2014). Currently, approximately 20–35% of the LW genome has been estimated to be of Asian origin (Groenen et al. 2012, Bosse et al. 2012, Frantz et al. 2015, Bianco et al. 2015, Ai et al. 2015). Accordingly, the IB breed shows the lowest levels of synonymous and nonsynonymous variation among the breeds studied, probably because of its small effective population size and because the individuals from the IB sample come from a very closed population of pigs. However, we expected to find a higher variability in LW compared to WB due to the process of Asian introgression that this breed has undergone. Surprisingly, we detected very similar levels of variability between them. However, the high levels of variability were observed for variants that belonged to different frequency ranges in these two populations: singletons in WB and in high-frequency derived alleles in LW. This difference may be due mainly to the effects of gene flow in LW but also in some extend to the selective programs applied to the commercial breed.

Domestication hallmarks at pig coding regions

The paucity of fixed variants at coding positions in the three breeds indicates that the observed heritable phenotypic differences among the breeds are either due to: i) few selective sweeps, ii) positive selection at noncoding functional regions which were not analyzed in this work, iii) changes in the frequencies of nonsynonymous variants without being fixed. In the first hypothesis, we expect that domestication process should fix the adaptive variants for those genes underlaying the phenotypes of interest. However, we found no fixed variants between domestic breeds and wild pigs, although they show the right breed phenotype. We checked the α values for those genes that were previously reported to show signals of positive selection using other approaches (Groenen 2016). We found that these genes show little or no nonsynonymous polymorphisms or fixed variants (Table S13). This absence of variability is typical from regions under selective sweeps, although not necessarily implicating that these genes are the targets of domestication since there are no variants fixed or close to fixation at their coding regions. We only found significant values of α over zero at gene KIT in the IB breed, genes IGF2R and JMJD1C in the LW breed and gene LRRTM3 in the WB population. The second hypothesis implies that the functional regions implicated in domestication would be out of coding regions (promotors, enhancers, etc. (e.g., Li et al 2018, Rubin et al 2012, Anderson 2012). However, although being a promising hypothesis, we

691 did not analyze those regions because it requires a very accurate analysis of homology and their
692 associated functionality, which is very complicated at the genome level, especially for non-model
693 species. The third hypothesis suggests that the domesticated phenotype is caused by a moderate
694 change in the frequency of a relatively large number of variants with small selective effects. In this
695 case, depending on the size of the selective effect, the number of fixed shared variants may be
696 significant at the genome level, or alternatively, there would be only changes in the frequencies of
697 the variants without reaching fixation. In the last case, the functional variants involved in
698 domestication should be segregating in the analyzed populations. These positively selected
699 variants segregating at high frequencies, together with the presence of deleterious mutations also
700 segregating at low frequencies, would be reflected as an excess of non-neutral compared to
701 divergence. Hence, negative α values calculated using statistics based on high frequencies variants
702 should be observed in cases where there is a significant proportion of positive selection variants
703 that have not yet being fixed. We have observed this pattern in WB and the IB breed, but not in
704 LW. Furthermore, the estimation of DFE from ABC analysis showed that the global proportion of
705 beneficial mutations (weak and strong) is relatively small (~0.9%) and similar in all wild and
706 domestic populations. Nevertheless, this proportion of mutations is substantial in absolute
707 numbers. Although speculative, these mutations may change the fate of these populations that are
708 affected by natural or artificial selection.

709

710 We expected that shared variants between populations would be enriched by selective pressures
711 that predate domestication. Although they may reflect biological constraints at the species level,
712 these shared polymorphisms can also be the source of phenotypic variation in a polygenic selective
713 scenario such that a change in their frequencies (in an infinitesimal scenario) would result in the
714 observed phenotypic differences among the breeds. Furthermore, private variants (those
715 segregating only in one breed) may reflect recent and breed-specific selective hallmarks and hence,
716 would be responsible for the observed differences between domesticated and wild breeds. In both
717 cases, shared and exclusive polymorphisms are contributing to the differences in the SFS between
718 functional and nonfunctional positions. We expect that adaptive changes would increase the ratio
719 of nonsynonymous to synonymous polymorphisms and that this should be reflected as an increase
720 in the negative value of the α statistic. Our simulated domestication process indicates that the effect
721 of positive selection irrespective of being either strong and affecting a small percentage of variants

or weak and affecting a large percentage of variants is not reflected as marked changes in the estimated patterns of α. This could be due to the short time since the change in the DFE occurred but also by the interaction of positive and negative selection and demographic processes. In fact, the observed α patterns are compatible with demographic effects (population size reduction in WB and IB and gene flow in LW) but also with the effect of positive selection in LW. Finally, the obtained results in the ABC analysis based on total variants show a clear genome-wide effect of the action of purifying selection. We also observed a minor effect of purifying selection in IB and WB when the analysis was performed based on exclusive variants, which suggest a reduction of the population size of these two populations. Nevertheless, we had some difficulties in adjusting the observed data to pertinent DFE models, especially when the analysis was performed based on exclusive variants. Although the models used here are very simple and contain few parameters, the real observations contained high heterogeneity that could not be fitted to these models. The reasons may be technical, conceptual (undetected correlations that distort model assumptions) or biological (too simplistic models to explain the real data). In any case, a model that assumes a discrete distribution of beneficial and deleterious mutations (model D) seems to generally explain the observed data better. The change of the DFE when the analysis was performed based on shared variants is undistinguishable from that based on all variants, indicating that exclusive variants should be more useful to detect the effects of the change of selective effects.

Final remarks

The observed patterns of variability are compatible with the presence of deleterious mutations segregating in all three breeds and with weak signals of positive selection. Nevertheless, when the variants are split into shared and exclusive, we observed patterns that are in line with the simulated data under different demographic scenarios joint with the action of positive and negative selection. We found a clear effect of deleterious mutations at low-frequency variants and a mild effect of positive selection at high frequencies. Additional analyses contrasting evolutionary models that consider the effects of standing variation whose effect change under domestication may shed more light and will help to understand the patterns of variation due to the domestication process.

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TABLES

Table 1. *Number of SNPs (in whole genome, in genes and in coding regions), classified by its presence in each population.*

Table 2. *Combinations of SNPs from coding regions according to its allelic status in each population (A: Ancestral allele, F: Fixed allele, P: Polymorphic allele). SNPs that are missing in any of the populations are not considered in this table.*

FIGURES

Figure 1. Levels of variation at synonymous (A) and nonsynonymous (B) sites for each pig population and variability estimates and for shared and exclusive variants. WB; wild boar population; IB, Iberian breed; LW, Large White breed.

Figure 2. Estimates of α for each pig population. Total variants (A), exclusive variants (B), shared variants (C) and shared variants between IB and LW (D). Bootstrap intervals at 95% are indicated by a line at each bar. WB; wild boar population; IB, Iberian breed; LW, Large White breed.

Figure 3. Estimates of R_βγ for each pig population and for all, exclusive and shared variants. WB; wild boar population; IB, Iberian breed; LW, Large White breed.

Figure 4. Estimates of the median values of α for each pig population, different molecular scales and for all, exclusive and shared variants. For each population, the order of different α's is: Fu&Li, Watterson, Tajima and Fay&Wu. WB; wild boar population; IB, Iberian breed; LW, Large White breed.

Figure 5. Posterior distribution of the α values for total variants. Four different estimators of alpha (Fu&Li, Watterson, Tajima and Fay&Wu, see Materials and Methods) are used. Box plots indicate simulated distributions of α values. Red line indicates observed α values.

Figure 6. Posterior distribution of the α values for exclusive variants. Four different estimators of alpha (Fu&Li, Watterson, Tajima and Fay&Wu, see Materials and Methods) are used. Box plots indicate the simulated distributions of α values. Red line indicates observed α values.

SUPPLEMENTARY MATERIAL

See Supplementary Material file added to see the Tables (S1-S13) and Figures (S1-S50).

Table 1. Number of SNPs present in the whole genome, genes and coding regions and classified according to their presence in pig populations.

	Number of SNPs	Shared between WB, IB and LW	Shared between WB and IB	Shared between WB and LW	Shared between IB and LW	Exclusive of WB	Exclusive of IB	Exclusive of LW
Whole-genome	24,869,699	7,293,787	666,927	4,017,107	138,378	4,239,052	385,504	8,128,944
Genes	6,684,142	1,964,562	98,433	1,152,555	48,351	1,138,370	100,550	2,181,321
Coding regions	149,440	18,611	3,252	25,044	1,177	51,432	3,356	46,568

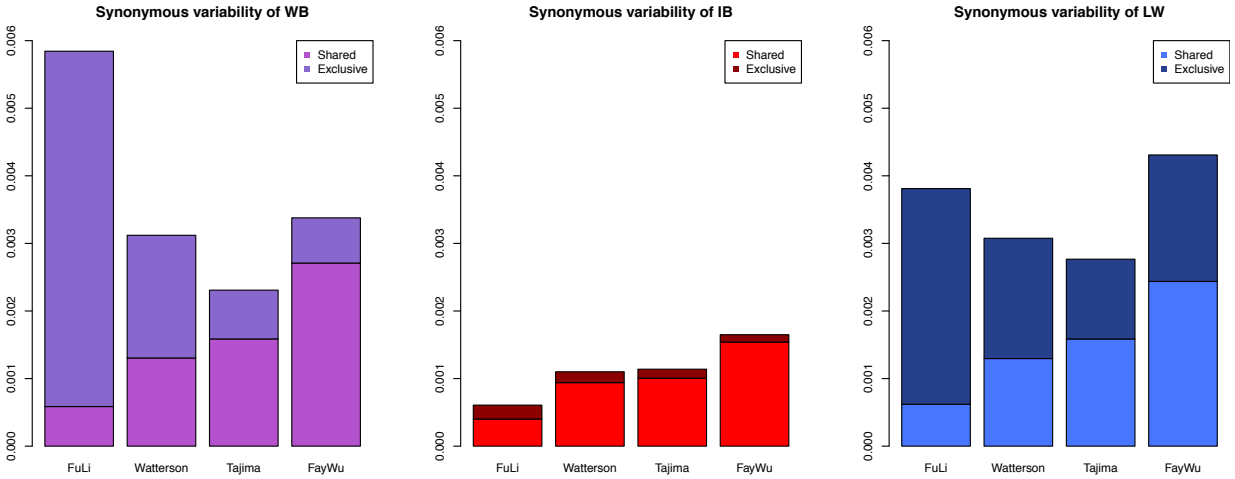
Table 2. Number of synonymous and nonsynonymous SNPs for different combinations of the allelic status. SNPs that are missing in any of the populations are not considered in this table.

IB	LW	WB	Synonymous	Non-synonymous
F	F	F	20297	9342
P	P	P	11712	7597
A	A	F	0	0
A	F	A	0	0
F	A	A	3	5
A	A	P	30314	20988
A	P	A	26027	15035
P	A	A	1833	1588
A	F	F	0	0
F	A	F	1	0
F	F	A	1	0
A	P	P	10128	7930
P	A	P	1676	1254
P	P	A	700	363
A	F	P	11	1
A	P	F	0	2
F	A	P	30	30
P	A	F	0	0
F	P	A	8	4
P	F	A	1	1
F	P	P	4924	2378
P	F	P	242	139
P	P	F	81	52
F	F	P	1140	489
F	P	F	4911	2073
P	F	F	38	22
			114078	69293

F: position with a fixed derived variant. **P**: Polymorphic position. **A**: position with the ancestral varian

Figure 1

A



B

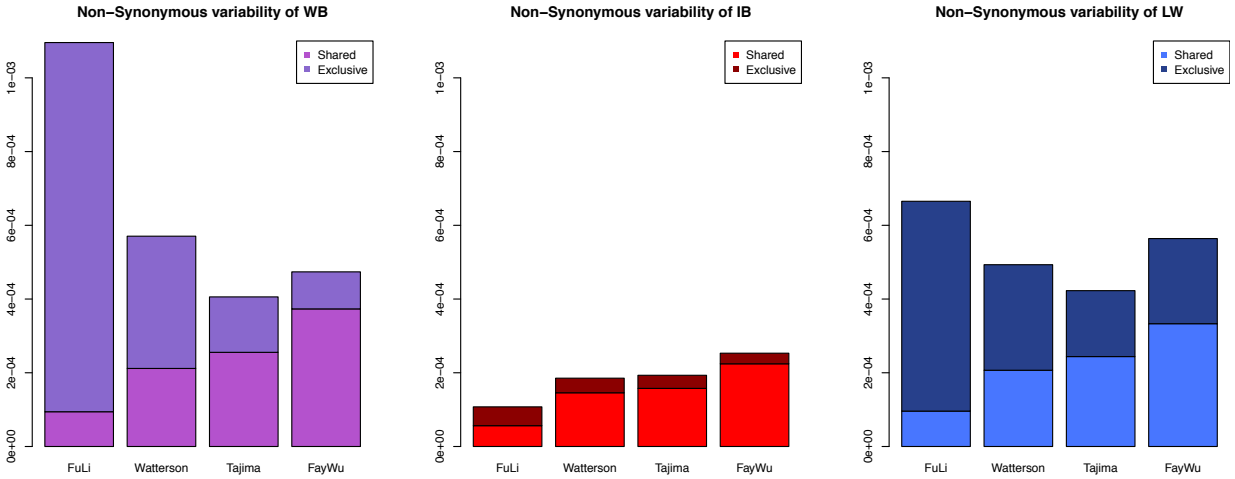


Figure 2

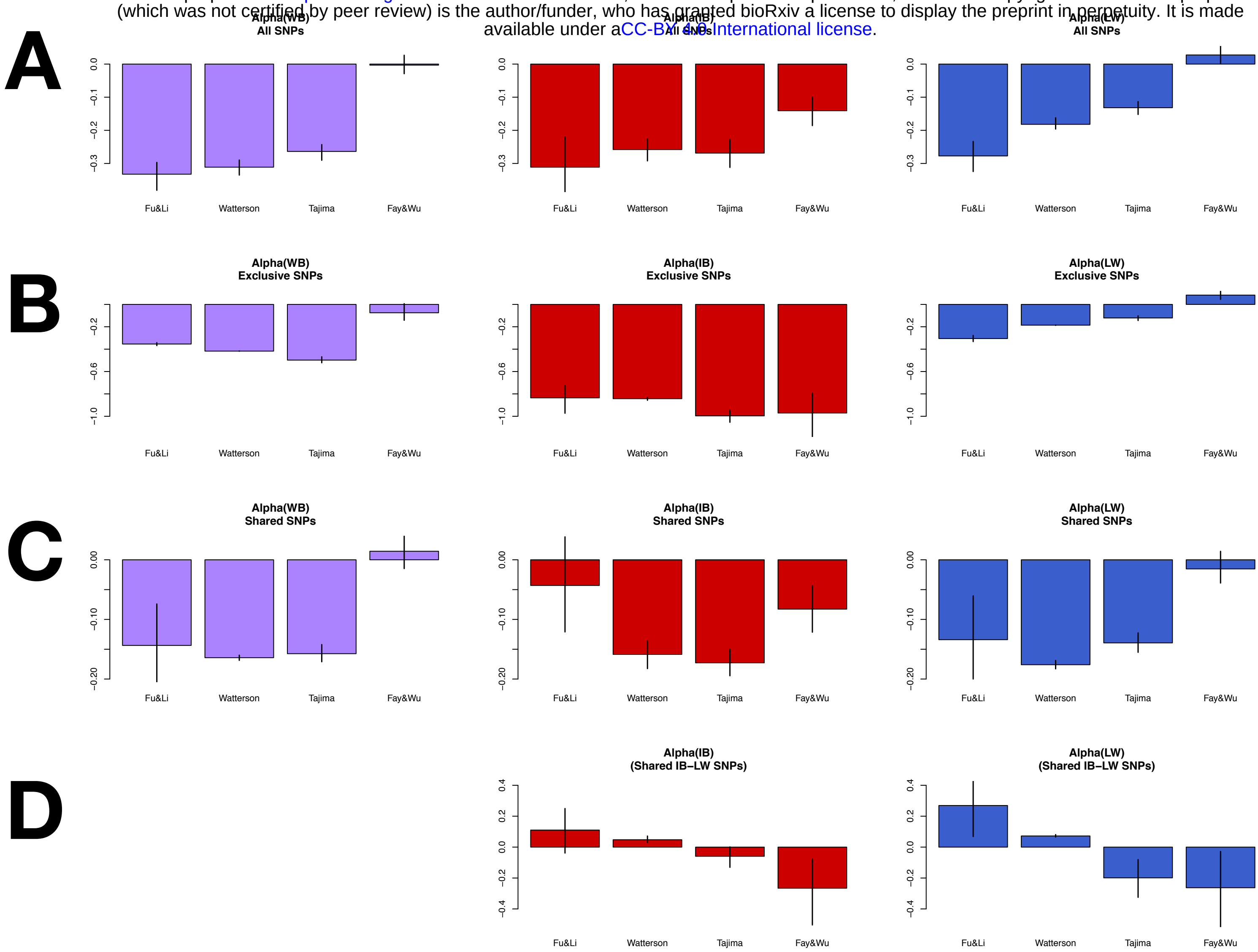
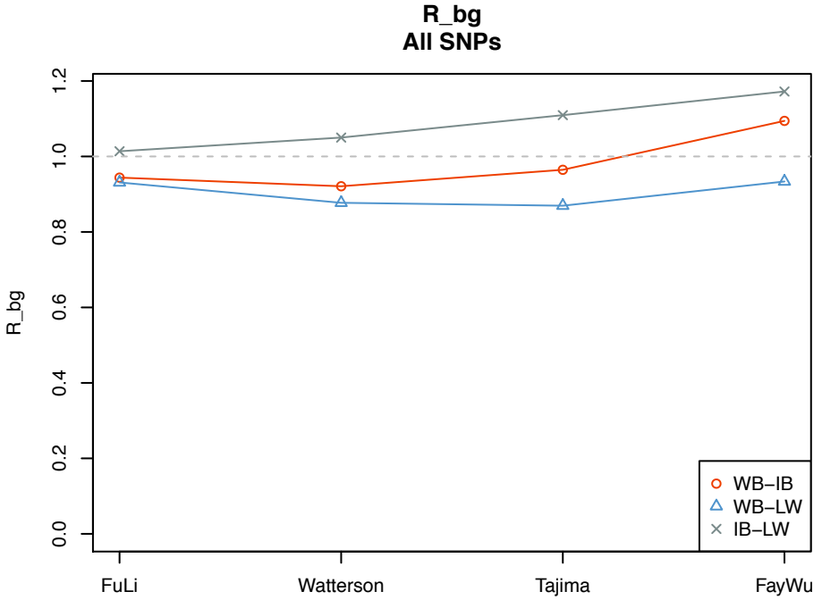


Figure 3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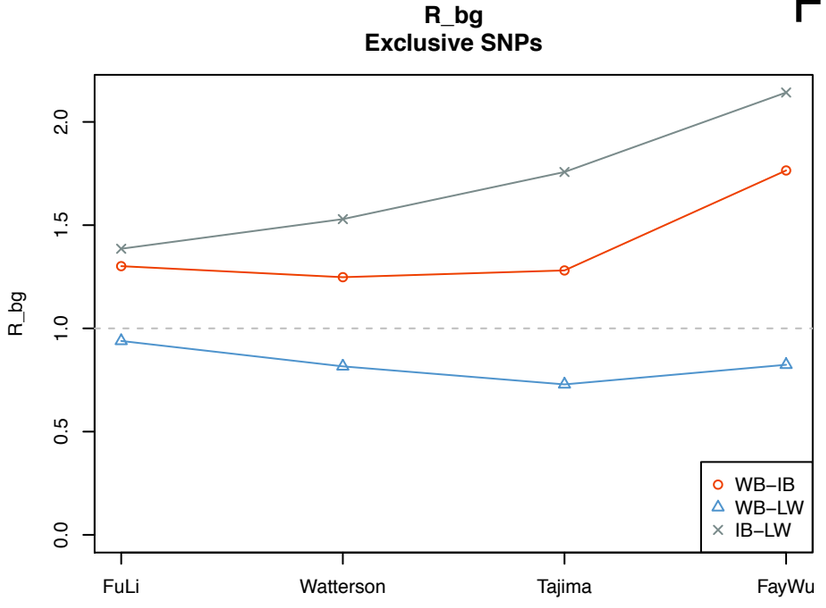
All SNPs
R_bg
0.0
0.2
0.4
0.6
0.8
1.0
1.2
WB–IB
WB–LW
IB–LW
FuLi
Watterson
Tajima
FayWu


R_bg
Exclusive SNPs
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WB–IB
WB–LW
IB–LW
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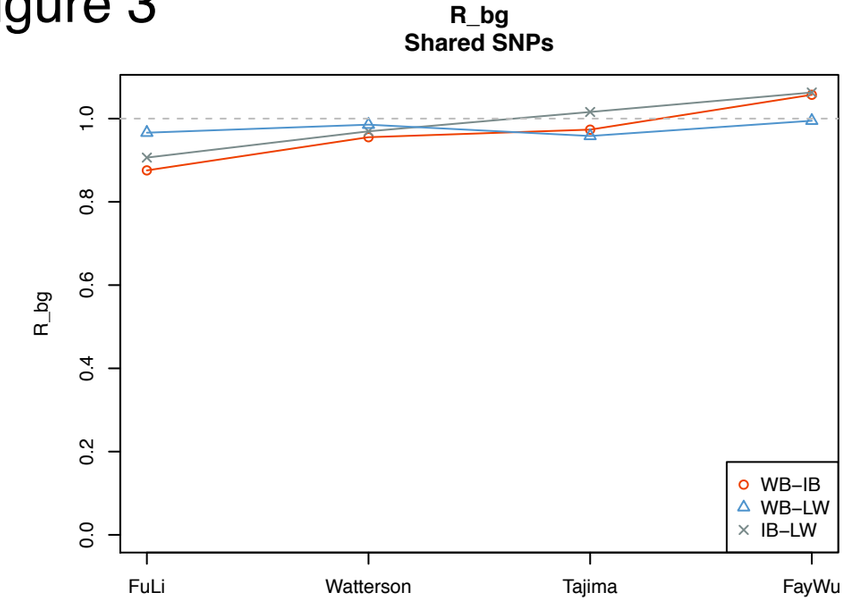
Watterson
Tajima
FayWu


R_bg
Shared SNPs
R_bg
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WB–IB
WB–LW
IB–LW
FuLi
Watterson
Tajima
FayWu

Figure 4

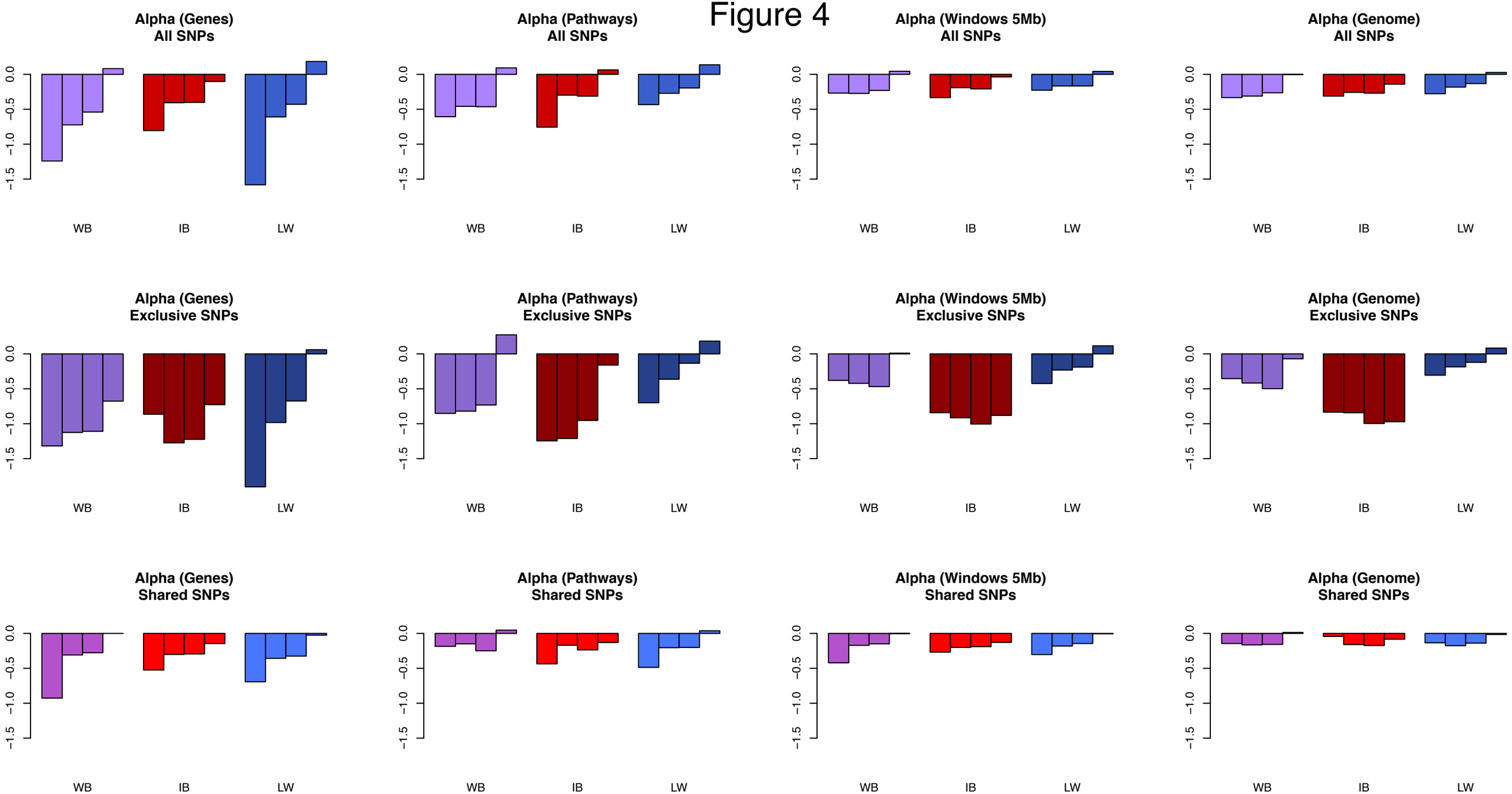

Alpha (Genes)
All SNPs
Alpha (Pathways)
All SNPs
Alpha (Windows 5Mb)
All SNPs
Alpha (Genome)
All SNPs
Alpha (Genes)
Exclusive SNPs
Alpha (Pathways)
Exclusive SNPs
Alpha (Windows 5Mb)
Exclusive SNPs
Alpha (Genome)
Exclusive SNPs
Alpha (Genes)
Shared SNPs
Alpha (Pathways)
Shared SNPs
Alpha (Windows 5Mb)
Shared SNPs
Alpha (Genome)
Shared SNPs
0.0
−0.5
−1.0
−1.5
WB
IB
LW

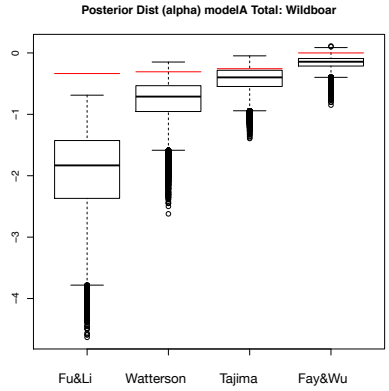
Figure 5

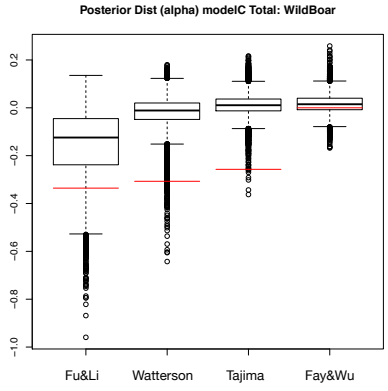
model A (only deleterious)

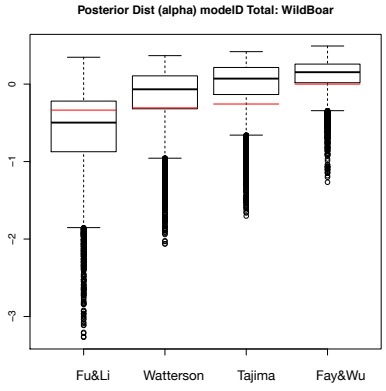
model C (deleterious plus beneficial)

model D (deleterious plus beneficial, discrete distribution)

WB







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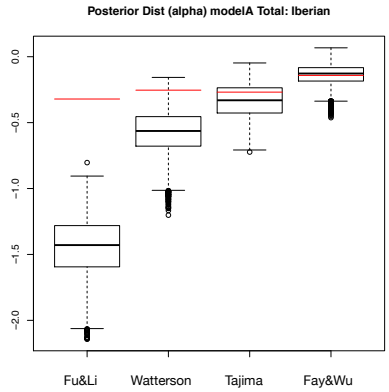

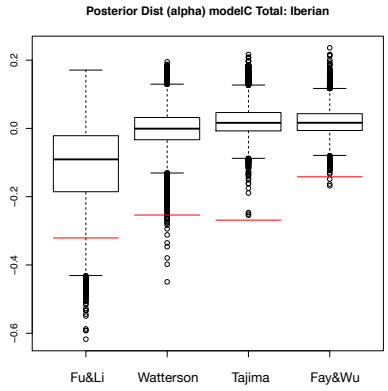

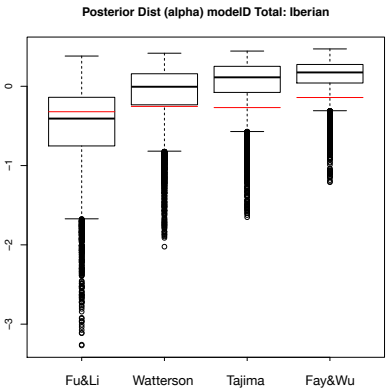

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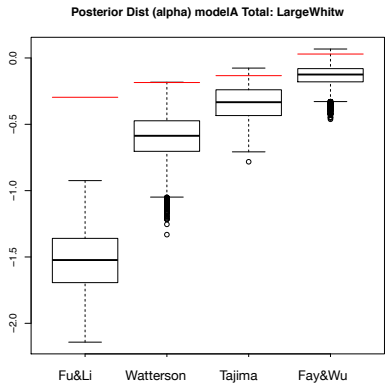
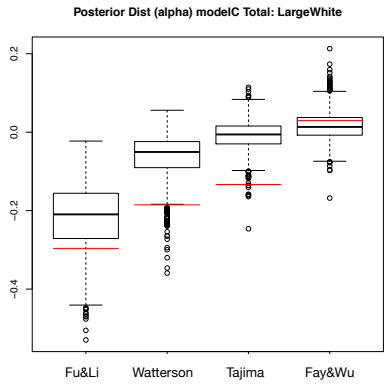




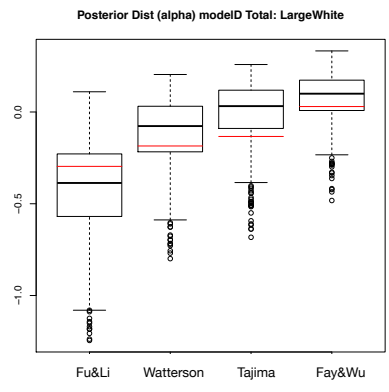


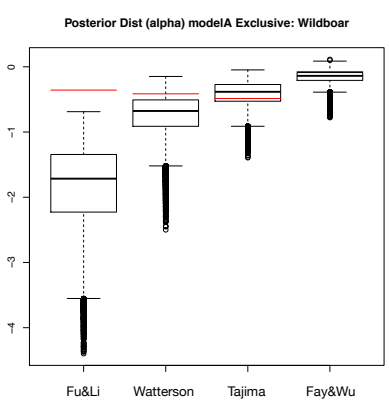
Figure 6

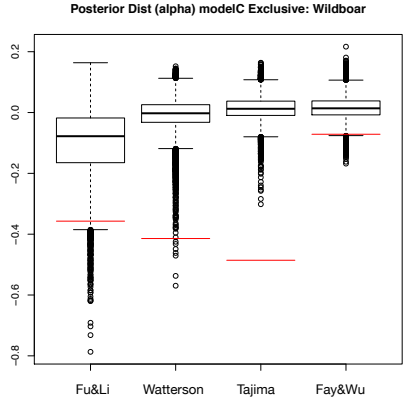
model A (only deleterious)

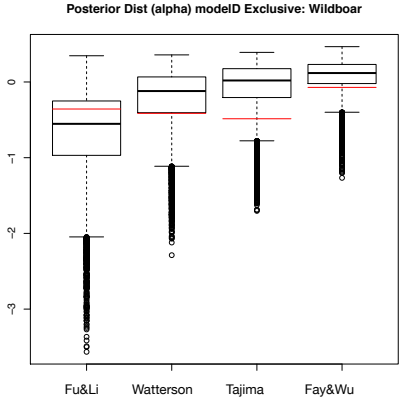
model C (deleterious plus beneficial)

model D (deleterious plus beneficial, discrete distribution)

WB







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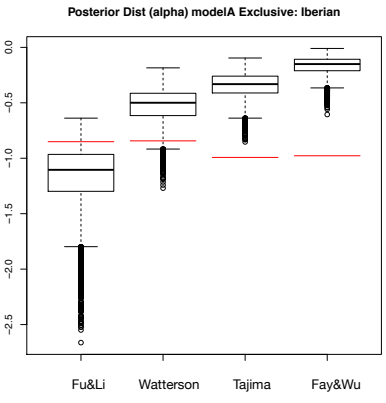

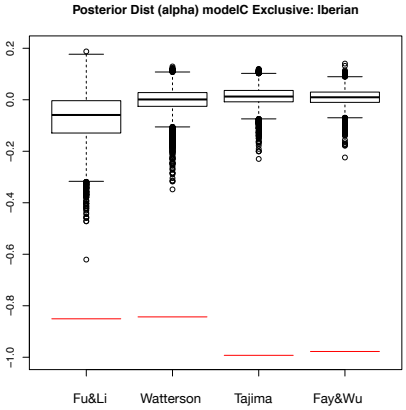

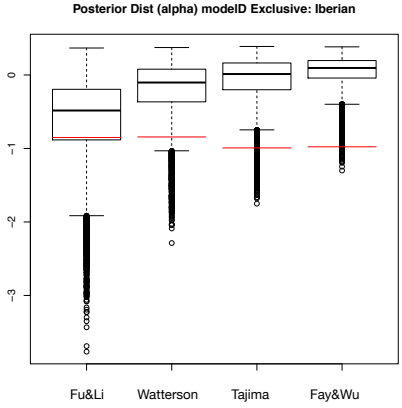

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