Pervasive selection pressure in wild and domestic pigs

J. Leno-Colorado[1], S. Guirao-Rico[12], M. Pérez-Enciso[134], 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 (≤ 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,

70 it has been observed that the evolutionary rate, within a metabolic pathway, increases as we move
71 downstream, possibly because upstream genes are more pleiotropic, since they are involved in
72 more functions and hence these genes are probably more conserved (Rausher, Miller, and Tiffin
73 1999; Riley, Jin, and Gibson 2003; Livingstone and Anderson 2009; Ramsay, Rieseberg, and
74 Ritland 2009).

75 So far, the nature of the underlying genetic changes caused by domestication and ensuing artificial
76 breeding is still under debate. While the most prevalent view is that regulatory changes have been
77 targeted (Anderson 2013), several other studies underline the influence of protein coding changes
78 (Rubin et al. 2012). Other authors have reported an increase in the rate of deleterious mutations in
79 domestic pigs compared to their wild counterparts (Cruz, Vilà, and Webster 2008; Renaut and
80 Rieseberg 2015; Pérez-Enciso et al. 2016; Leno-Colorado et al. 2017). Others, as in Makino et al.
81 (2018) detected a general pattern of reduction of variability in domestic populations in relation to
82 their wild counterpart, and a higher nonsynonymous/synonymous ratio across the frequency
83 spectrum. These patterns were compatible with the effect of strong bottlenecks in domestic
84 populations and the higher accumulation of deleterious mutations. Interestingly, the same authors
85 observed that the opposite trend has been observed in pigs. Moreover, most of the previous studies
86 have focused on genes of major effect with clear signals of selective sweeps. In those studies, the
87 hallmarks of positive selection were detected by a valley of reduced variation and/or population
88 differentiation that spans a relatively large region (e.g., Amaral et al. 2011, Rubin et al. 2012,
89 Frantz et al. 2013, Wilkinsonet al. 2013) but also by haplotype structure and homozygosity blocks
90 (e.g., Fang et al. 2011, Bosse et al. 2012, Li et al. 2013). Some of these studies have detected recent
91 breed specific signals of selection attributed to the domestication process (Li et al. 2014, Kim et
92 al. 2015). Nevertheless, the signals were too scarce to explain the domestication process. Other
93 studies have tried to elucidate the effect that domestication has at a whole-genome scale and on
94 the fitness of individuals of domestic populations (e.g., Cruz et al. 2008, MacEachern et al. 2009,
95 Kono et al. 2016, Perez-Enciso et al. 2016, Makino et al. 2018, Chen et al. 2018, Orlando and
96 Librado 2019). For instance, an excess of deleterious variants has been observed in a number of
97 domestic animal and plants (e.g., contrasting nonsynonymous versus synonymous polymorphism
98 ratios, Chen et al. 2018, using the MacDonald framework, MacEachern et al. 2009, contrasting
99 ancestors with ancient DNA, Orlando and Librado 2019, combining the frequency of
100 polymorphisms with functional effects and divergence, Kono et al. 2016, Makino et al. 2018).

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

109

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

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

126

127

128 **MATERIALS AND METHODS**

129 **Biological samples**

130 We analyzed a sample of 46 pig (*Sus scrofa*) genomes (Table S1). These pigs correspond to
131 European wild boars (WB, n = 20) and domestic pigs, which are represented by the Iberian
132 Guadyerbas (IB, n = 6) and LargeWhite (LW, n = 20) breeds. These two domestic breeds were
133 selected because they have very different interesting features: IB is a local breed that has been
134 under weak artificial selection intensity and with no documented evidence of Asian introgression.
135 LW, in contrast, is a commercial breed undergoing strong artificial selection with a deliberate
136 admixture with Asian pigs (Bosse, Megens, Madsen, et al. 2014; Groenen 2016). To analyze the
137 divergence between the different breeds, we used the consensus ancestral reference sequence
138 obtained from combining the information from several *Sus* species (*S. barbatus*, *S. cebifrons*, *S.*
139 *verrucosus*, *S. celebensi*, approximately 4.2 MYA of divergence) and the African warthog
140 (*Phacochoerus africanus*, around ~10 MYA of divergence) as an outgroup, as detailed in Bianco
141 et al. (2015). The sequences are available in public databases (Rubin et al. 2012; Ramírez *et al.*
142 2014; Bianco *et al.* 2015; Frantz et al. 2015; Moon *et al.* 2015, Esteve-Codina *et al.* 2013, Leno *et*
143 *al.* 2017) and were downloaded from the short read archive (SRA,
144 http://www.ncbi.nlm.nih.gov/sra).

145

146 **Mapping and genotyping analysis**

147 Raw reads for each pig genome were mapped against the reference genome assembly (Sscrofa10.2,
148 Groenen et al. 2012) using *BWA mem* option (H. Li and Durbin 2009). PCR duplicates were
149 removed using *SAMtools rmdup* v 0.1.19 (H. Li et al. 2009) and mapped reads were realigned
150 around indels with the *GATK IndelRealigner* tool (McKenna et al. 2010). Genotype calling was
151 performed with *SAMtools mpileup* and *bcftools call* v 1.3.0 (H. Li et al. 2009) for each individual
152 separately. We set a minimum (5x) and a maximum depth (twice the average sample's depth plus
153 one) to call a SNP. Base quality was set to 20 (*P*-value=1e-2). Homozygous blocks (regions of
154 contiguous positions with the same nucleotide as the reference genome) were also called,
155 following the same criteria (i.e., minimum and maximum coverage and base quality) as with the
156 SNPs and using *samtools depth* utility, *BEDtools* (Quinlan 2014) and custom scripts. This resulted
157 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:

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

182

183 *(Equation 1)*

184 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
185 the Watterson estimator, $\omega_i = n/(1+\partial_{i,n-i})$ for the Tajima estimator (both for folded spectrum), $\omega_i =$
186 i for the Fay&Wu estimator and $\omega_i = 1$, $\omega_{i>1} = 0$ for the Fu&Li estimator (Achaz 2009).

187

188 **Filtering for artifactual effects**

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

197

198 **Estimation of the proportion of adaptive of variants**

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

207 $$\frac{\theta_n}{\theta_s} = \frac{(1-\alpha)K_n}{K_s},$$

208 *(Equation 2)*

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

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

213 $$\alpha = 1 - \frac{K_s}{K_n}\frac{\theta_n}{\theta_s}$$

214 *(Equation 3)*

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

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

221 $$\frac{\theta_{in}(1-\beta i)}{\theta_{is}} = \frac{(1-\alpha-\beta d)K_n}{K_s},$$

222 *(Equation 4)*

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

227 $$\alpha = 1 - \beta d - (1-\beta i)\frac{K_s}{K_n}\frac{\theta_{in}}{\theta_{is}}$$

228 *(Equation 5)*

229 We see that in case calculating α without considering the effects of deleterious mutations, it would
230 be underestimated depending on the frequency at which the estimates of variability are calculated.
231 If we assume that the detrimental variants would never be fixed, a good estimator of α using
232 equation 3 would be the one that estimates variability based on high frequencies, as it would hardly
233 contain detrimental mutations. This is in agreement with the arguments used in Messer and Petrov
234 2013.

235

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

239 $$\frac{\theta_{in}(1-\beta i-\gamma i)}{\theta_{is}}=\frac{(1-\alpha-\beta\,\mathrm{d}-\gamma d)K_n}{K_s},$$

240 *(Equation 6)*

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

244 $$\alpha+\gamma d=1-\beta d-(1-\beta i-\gamma i)\frac{K_s}{K_n}\frac{\theta_{in}}{\theta_{is}}$$

245 *(Equation 7)*

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

251

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

259

260 Furthermore, in cases where two populations are from the same species and no fixed mutations
261 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

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

305

306 **Approximate Bayesian computation (ABC) analysis**

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

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

345

346 **Pathway analysis**

347 We downloaded the complete list of pathways and genes of *Sus scrofa* from KEGG v.20170213
348 (http://www.genome.jp/kegg/, Kanehisa et al. 2008). The list contained 471 pathways and 5480
349 genes. The median and mean number of genes per pathway was 26 and 43, respectively, and ranged
350 from 1 to 949. We filtered the pathways according to their size, removing pathways with less than
351 10 and more than 150 genes in order to discard pathways that were not informative or too generic
352 and complex. The final list contained 171 pathways and 3449 genes.

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

364

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**

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

401

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

420

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

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

439

440 **α's and $R_\beta\gamma$ ratios might show a differential effect of selection due to domestication**

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

453 and IB show very low negative values of α for most of the estimators of variability, except for
454 $\alpha_{Fay\&Wu}$ in WB, which is zero. LW is the only breed that shows a linear increase of the α negative
455 values across the SFS, being even positive when calculated based on high frequencies ($\alpha_{Fay\&Wu}$).

456

457 We did not observe similar patterns of α between domestic breeds compared to WB (Figure 2A).
458 The differences in the ratio of synonymous to nonsynonymous variability between the two
459 different breeds is summarized by the $R_\beta\gamma$ ratio (Figure 3A). We observed deviations from $R_\beta\gamma$
460 = 1 when the ratio was calculated based on high-frequency variants ($\alpha_{Fay\&Wu}$). Although the ratio
461 of the two populations is difficult to interpret because of their different underlying demographics,
462 some trends can be observed. WB shows an excess of nonsynonymous variants segregating at
463 intermediate frequencies (WB-IB, WB-LW), which might be explained by a past bottleneck that
464 increased deleterious mutations at intermediate frequencies. In addition, the $R_\beta\gamma$ ratio in LW-IB
465 shows an incremental pattern from low to high frequencies, which is compatible with an increase
466 of nonsynonymous beneficial variants on their way to fixation in LW.

467

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

470 We observed a high ratio of nonsynonymous to synonymous exclusive singletons ($\alpha_{Fu\&Li}$, Figure
471 2B), suggesting that they have deleterious effects in all populations. Nevertheless, the values of α
472 calculated based on intermediate frequency variants (α_{Tajima}) in the WB and IB populations are
473 lower than to those based on low-frequency variants, which point to the action of positive selection
474 maintaining nonsynonymous variants at higher frequencies in the case of WB and to an attenuated
475 effect of deleterious mutations in IB due to a population size decline. In the case of LW, the
476 observed pattern of α is similar to that calculated with all SNPs (Figure 2B). Likewise, the R_βγ
477 statistic shows the same pattern as that calculated using all SNPs but with a higher magnitude of
478 its value (Figure 3B).

479

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

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

489

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

498

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

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

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

520

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

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

542

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

552

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

555 We used an approximate Bayesian computation (ABC) analysis to infer the DFE separately for
556 each population using the ratios of nonsynonymous to synonymous variants obtained from the
557 whole-genome analysis (see Materials and Methods). Three different models implemented in
558 *polyDFE2* software (Tataru et al. 2019) were tested. Model A, which assumes a gamma
559 distribution of deleterious mutations; model C, which assumes a gamma distribution for
560 deleterious mutations and an exponential distribution for beneficial mutations; and model D, that
561 assumes a discrete distribution of beneficial and deleterious mutations. In these models, we
562 included demographic and linkage effects as nuisance parameters (Tataru et al. 2017). Goodness
563 of fit (GoF) analysis, which is a measure of the adjustment of the prior chosen models to the data,
564 revealed that the simulated data under the different models do not always fit well to the observed
565 data (Table S11A), suggesting that the real data fit only to a very restrictive parameter range of
566 each model. This is especially pronounced for exclusive variants, where the simulations under the
567 different models fit only marginally to the observed data. Indeed, the model with a wider parameter
568 range versus the real data is usually the discrete model D. However, if we also take into account
569 the values of the posterior probabilities, which is the probability assigned to each model relative
570 to the other models of the analysis, we found that the best fit model differed among populations
571 (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

602
603 **DISCUSSION**

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

611

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

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

625

626 **Selection pressure on pigs and the process of domestication**

627 A number of analyses with the aim to explain the process of domestication have been performed
628 already, using the MacDonald and Kreitman extension methods, or using other estimates such as
629 variability or divergence at functional or synonymous positions (MacEachern et al. 2009, Kono et
630 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

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

673

674 **Domestication hallmarks at pig coding regions**

675 The paucity of fixed variants at coding positions in the three breeds indicates that the observed
676 heritable phenotypic differences among the breeds are either due to: i) few selective sweeps, ii)
677 positive selection at noncoding functional regions which were not analyzed in this work, iii)
678 changes in the frequencies of nonsynonymous variants without being fixed. In the first hypothesis,
679 we expect that domestication process should fix the adaptive variants for those genes underlaying
680 the phenotypes of interest. However, we found no fixed variants between domestic breeds and
681 wild pigs, although they show the right breed phenotype. We checked the α values for those genes
682 that were previously reported to show signals of positive selection using other approaches
683 (Groenen 2016). We found that these genes show little or no nonsynonymous polymorphisms or
684 fixed variants (Table S13). This absence of variability is typical from regions under selective
685 sweeps, although not necessarily implicating that these genes are the targets of domestication since
686 there are no variants fixed or close to fixation at their coding regions. We only found significant
687 values of α over zero at gene KIT in the IB breed, genes IGF2R and JMJD1C in the LW breed and
688 gene LRRTM3 in the WB population. The second hypothesis implies that the functional regions
689 implicated in domestication would be out of coding regions (promotors, enhancers, etc. (e.g., Li et
690 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

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

740

741 **Final remarks**

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

750

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759

760 **LITERATURE CITED**

761 Achaz G. 2009. "Frequency Spectrum Neutrality Tests: One for All and All for One." *Genetics*
762 183: 249–58. https://doi.org/10.1534/genetics.109.104042.

763 Ai H, Fang X, Yang B, Huang Z, Chen H, Mao L, et al. 215 "Adaptation and possible ancient
764 interspecies introgression in pigs identified by whole- genome sequencing." *Nat Genet.*
765 2015;47:217–25.

766 Alvarez-Ponce, David, and Mario A. Fares. 2012. "Evolutionary Rate and Duplicability in the
767 Arabidopsis Thaliana Protein-Protein Interaction Network." *Genome Biology and Evolution*
768 4 (12): 1263–74. https://doi.org/10.1093/gbe/evs101.

769 Alves, E,, C Ovilo, C Rodríguez, and L Silió. 2003. "Mitochondrial DNA sequence variation and
770 phylogenetic relationships among Iberian pigs and other domestic and wild pig
771 populations." *Animal Genetics* 34:319-324.

772 Alves, Estefania, A I Fernández, Carmen Barragán, C Ovilo, C Rodríguez, and L Silió. 2006.
773 "Inference of Hidden Population Substructure of the Iberian Pig Breed Using Multilocus
774 Microsatellite Data." *Spanish Journal of Agricultural Research* 4 (1): 37–46.
775 http://www.inia.es/gcontrec/pub/alves-fernandez-barragan-..._1141287881015.pdf.

776 Amaral AJ, Ferretti L, Megens HJ, Crooijmans RPMA, Ni H, Ramos-Onsins SE, Perez-Enciso
777 M, Schook LB, Groenen MAM. 2011 "Genome-wide footprints of pig domestication and
778 selection revealed through massive parallel sequencing of pooled DNA." *PLoS One.*
779 2011;6(4):e14782. doi:10.1371/journal.pone.0014782

780 Andersson L. 2012 "How selective sweeps in domestic animals provide new insight into
781 biological mechanisms." J Intern Med. 2012;271:1–14.

782 Andersson L. 2013 "Molecular consequences of animal breeding." *Curr Opin Genet Dev*.
783 23(3):295-301. doi: 10.1016/j.gde.2013.02.014.

784 Beaumont MA, Zhang W, Balding DJ. Approximate Bayesian computation in population
785 genetics. Genetics. 2002;162(4):2025-2035.

786 Bianco, Erica, Bruno Nevado, Sebastian E. Ramos-Onsins, and Miguel Pérez-Enciso. 2015. "A
787 Deep Catalog of Autosomal Single Nucleotide Variation in the Pig." *PLoS ONE* 10 (3): 1–
788 21. https://doi.org/10.1371/journal.pone.0118867.

789 Booker, Tom R, and Peter D Keightley. 2018. "Understanding the Factors That Shape Patterns of
790 Nucleotide Diversity in the House Mouse Genome." *Molecular Biology and Evolution* 35
791 (12): 2971–88. https://doi.org/10.1093/molbev/msy188.

792 Bosse, Mirte, Hendrik-Jan Megens, Laurent A. F. Frantz, Ole Madsen, Greger Larson, Yogesh
793 Paudel, Naomi Duijvesteijn, et al. 2014. "Genomic Analysis Reveals Selection for Asian
794 Genes in European Pigs Following Human-Mediated Introgression." *Nature*
795 *Communications* 5: 4392. https://doi.org/10.1038/ncomms5392.

796 Bosse, Mirte, Hendrik-Jan Megens, Ole Madsen, Laurent A. F. Frantz, Yogesh Paudel, Richard
797 P. M. A. Crooijmans, and Martien A. M. Groenen. 2014. "Untangling the Hybrid Nature of
798 Modern Pig Genomes: A Mosaic Derived from Biogeographically Distinct and Highly
799 Divergent Sus Scrofa Populations." *Molecular Ecology* 23 (16): 4089–4102.
800 https://doi.org/10.1111/mec.12807.

801 Bosse, Mirte, Hendrik Jan Megens, Ole Madsen, Yogesh Paudel, Laurent A. F. Frantz, Lawrence
802 B. Schook, Richard P.M.A. Crooijmans, and Martien A.M. Groenen. 2012. "Regions of
803 Homozygosity in the Porcine Genome: Consequence of Demography and the
804 Recombination Landscape." *PLoS Genetics* 8 (11).
805 https://doi.org/10.1371/journal.pgen.1003100.

806 Chen J, Ni P, Li X, Han J, Jakovlić I, Zhang C, Zhao S. 2018 "Population size may shape the
807 accumulation of functional mutations following domestication." *BMC Evolutionary Biology*
808 18:4 DOI 10.1186/s12862-018-1120-6

809 Cruz F, Vilà C, and Webster MT. 2008. “The Legacy of Domestication: Accumulation of
810 Deleterious Mutations in the Dog Genome.” *Molecular Biology and Evolution* 25 (11):
811 2331–36. https://doi.org/10.1093/molbev/msn177.

812 Csardi G., and Nepusz T. 2006. “The Igraph Software Package for Complex Network Research.”
813 *InterJournal, Complex Systems* 1695. http://igraph.org.

814 Csilléry K, Olivier François O. and Blum MGB. 2012 " abc: an R package for approximate
815 Bayesian computation (ABC) " Methods in Ecology and Evolution 2012, 3, 475–479.

816 Esteve-Codina, Anna, Yogesh Paudel, Luca Ferretti, Emanuele Raineri, Hendrik-Jan Megens,
817 Luis Silió, María C Rodríguez, Martien A. M. Groenen, Sebastian E. Ramos-Onsins, and
818 Miguel Pérez-Enciso. 2013. “Dissecting Structural and Nucleotide Genome- Wide
819 Variation in Inbred Iberian Pigs.” *BMC Genomics* 14 (148): 1. https://doi.org/10.1186/1471-
820 2164-14-148.

821 Eyre-Walker, Adam. 2002. “Changing Effective Population Size and the McDonald-Kreitman
822 Test.” *Genetics* 162: 2017–24.
823 http://www.genetics.org/content/genetics/162/4/2017.full.pdf.

824 Eyre-Walker A. 2006 "The genomic rate of adaptive evolution." *Trends Ecol Evol* 21(10):
825 569–575.

826 Fang M, Larson G, Ribeiro HS, Li N, Andersson L. 2011 "Contrasting mode of evolution at a
827 coat color locus in wild and domestic pigs." *PLoS Genet.* 5:e1000341.

828 Fay, Justin C, and Chung-I Wu. 2000. “Hitchhiking Under Positive Darwinian Selection.”
829 *Genetics* 155: 1405–13.
830 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1461156/pdf/10880498.pdf.

831 Fay JC, Wyckoff GJ, Wu CI. 2001 "Positive and negative selection on the human ge-
832 nome." *Genetics* 158(3):1227–1234.

833 Ferretti, Luca, Emanuele Raineri, and Sebastian E. Ramos-Onsins. 2012. “Neutrality Tests for
834 Sequences with Missing Data.” *Genetics* 191 (4): 1397–1401.
835 https://doi.org/10.1534/genetics.112.139949.

836 Fisher, R. A. 1919. “XV.—The Correlation between Relatives on the Supposition of Mendelian

837 Inheritance." *Transactions of the Royal Society of Edinburgh* 52 (02): 399–433.
838 https://doi.org/10.1017/S0080456800012163.

839 Frantz, Laurent A. F., Joshua G. Schraiber, Ole Madsen, Hendrik-Jan Megens, Mirte Bosse,
840 Yogesh Paudel, Gono Semiadi, et al. 2013. "Genome Sequencing Reveals Fine Scale
841 Diversification and Reticulation History during Speciation in Sus." *Genome Biology* 14 (9):
842 R107. https://doi.org/10.1186/gb-2013-14-9-r107.

843 Frantz, Laurent A. F., Joshua G. Schraiber, Ole Madsen, Hendrik-Jan Megens, Alex Cagan,
844 Mirte Bosse, Yogesh Paudel, Richard P. M. A. Crooijmans, Greger Larson, and Martien A.
845 M. Groenen. 2015. "Evidence of Long-Term Gene Flow and Selection during
846 Domestication from Analyses of Eurasian Wild and Domestic Pig Genomes." *Nature*
847 *Genetics* 47 (10): 1141–48. https://doi.org/10.1038/ng.3394.

848 Fraser, Hunter B, Aaron E Hirsh, Lars M Steinmetz, Curt Scharfe, and Marcus W Feldman.
849 2002. "Evolutionary Rate in the Protein Interaction Network." *Science* 296 (5568): 750–52.
850 https://doi.org/10.1126/science.1068696.

851 Fu, Yun-Xin, and Wen-Hsiung Li. 1993. "Maximum Likelihood Estimation of Population
852 Parameters." *Genetics* 134: 1261–70.
853 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1205593/pdf/ge13441261.pdf.

854 Groenen, Martien A. M. 2016. "A Decade of Pig Genome Sequencing: A Window on Pig
855 Domestication and Evolution." *Genetics, Selection, Evolution : GSE Sel Evol* 48 (23): 1–9.
856 https://doi.org/10.1186/s12711-016-0204-2.

857 Groenen, Martien A. M., Alan L. Archibald, Hirohide Uenishi, Cristopher K. Tuggle, Yasuhiro
858 Takeuchi, Max F. Rothschild, Claire Rogel-Gaillard, et al. 2012. "Analyses of Pig Genomes
859 Provide Insight into Porcine Demography and Evolution." *Nature* 491 (7424): 393–98.
860 https://doi.org/10.1038/nature11622.

861 Guirao-Rico, Sara, Oscar Ramirez, Ana Ojeda, Marcel Amills, and Sebastian E. Ramos-Onsins.
862 2018. "Porcine Y-Chromosome Variation Is Consistent with the Occurrence of Paternal
863 Gene Flow from Non-Asian to Asian Populations." *Heredity* 120 (1): 63–76.
864 https://doi.org/10.1038/s41437-017-0002-9.

865 Hahn, Matthew W., and Andrew D. Kern. 2005. "Comparative Genomics of Centrality and

866 Essentiality in Three Eukaryotic Protein-Interaction Networks." *Molecular Biology and*
867 *Evolution* 22 (4): 803–6. https://doi.org/10.1093/molbev/msi072.

868 Haller, Benjamin C., and Philipp W. Messer. 2017. "SLiM 2: Flexible, Interactive Forward
869 Genetic Simulations." *Molecular Biology and Evolution* 34 (1): 230–40.
870 https://doi.org/10.1093/molbev/msw211.

871 Hill, W G, and A Robertson. 1966. "The Effect of Linkage on Limits to Artificial Selection."
872 *Genetical Research* 8 (3): 269–94. http://www.ncbi.nlm.nih.gov/pubmed/5980116.

873 Kanehisa, Minoru, Michihiro Araki, Susumu Goto, Masahiro Hattori, Mika Hirakawa, Masumi
874 Itoh, Toshiaki Katayama, et al. 2008. "KEGG for Linking Genomes to Life and the
875 Environment." *Nucleic Acids Research* 36 (SUPPL. 1): 480–84.
876 https://doi.org/10.1093/nar/gkm882.

877 Kim H, Song KD, Kim HJ, Park W, Kim J, Lee T, et al. 2015 "Exploring the genetic signature of
878 body size in Yucatan miniature pig." *PLoS One.* 10:e0121732.

879 Kono TJY, Fu F, Mohammadi M, Hoffman PJ, Liu C, Stupar RM, Smith KP, Tiffin P, Fay JC,
880 Morrell PL. 2016 "The Role of Deleterious Substitutions in Crop Genomes." *Mol. Biol.*
881 *Evol.* 33(9):2307–2317. https://doi:10.1093/molbev/msw102

882 Leno-Colorado, Jordi, Nick J Hudson, Antonio Reverter, and Miguel Pérez-Enciso. 2017. "A
883 Pathway-Centered Analysis of Pig Domestication and Breeding in Eurasia." *G3* 7 (7):
884 2171–84. https://doi.org/10.1534/g3.117.042671.

885 Li C, Zou C, Cui Y, Fu Y, Fang C, Li Y, Li J, Wang W, Xiang H, Li C. 2018 "Genome-wide
886 epigenetic landscape of pig lincRNAs and their evolution during porcine domestication."
887 Epigenomics. 2018 Dec;10(12):1603-1618. doi: 10.2217/epi-2017-0117.

888 Li, Heng, and Richard Durbin. 2009. "Fast and Accurate Short Read Alignment with Burrows-
889 Wheeler Transform." Journal Article. *Bioinformatics* 25 (14): 1754–60.
890 https://doi.org/10.1093/bioinformatics/btp324.

891 Li, Heng, Bob Handsaker, Alec Wysoker, Tim Fennell, Jue Ruan, Nils Homer, Gabor Marth,
892 Gonçalo R. Abecasis, Richard Durbin, and Subgroup Genome Project Data Processing.
893 2009. "The Sequence Alignment/Map Format and SAMtools." Journal Article.
894 *Bioinformatics* 25 (16): 2078–79. https://doi.org/10.1093/bioinformatics/btp352.

895 Li, Mingzhou, Shilin Tian, Long Jin, Guangyu Zhou, Ying Li, Yuan Zhang, Tao Wang, et al.
896 2013. “Genomic Analyses Identify Distinct Patterns of Selection in Domesticated Pigs and
897 Tibetan Wild Boars.” *Nature Genetics* 45 (12): 1431–38. https://doi.org/10.1038/ng.2811.

898 Li M, Tian S, Yeung CK, Meng X, Tang Q, Niu L, Wang X, Jin L, Ma J, Long K, Zhou C, Cao
899 Y, Zhu L, Bai L, Tang G, Gu Y, Jiang A, Li X, Li R. 2014 "Whole-genome sequencing of
900 Berkshire (European native pig) provides insights into its origin and domestication." *Sci*
901 *Rep.* 4:4678. doi: 10.1038/srep04678.

902 Livingstone, Kevin, and Stephanie Anderson. 2009. “Patterns of Variation in the Evolution of
903 Carotenoid Biosynthetic Pathway Enzymes of Higher Plants.” *Journal of Heredity* 100 (6):
904 754–61. https://doi.org/10.1093/jhered/esp026.

905 MacEachern S, McEwan J, McCulloch A, Mather A, Savin K, Goddard M. 2009 "Molecular
906 evolution of the Bovini tribe (Bovidae, Bovinae): is there evidenceof rapid evolution or
907 reduced selective constraint in Domestic cattle?" *BMC Genomics* (10) *179;* https:// doi:
908 10.1186/1471-2164-10-179.

909 Makino T, Rubin CJ, Carneiro M, Axelsson E, Andersson L, Webster MT. 2018 "Elevated
910 Proportions of Deleterious Genetic Variation in Domestic Animals and Plants." *Genome*
911 *Biol Evol.* 10(1):276-290. https://doi: 10.1093/gbe/evy004.

912 McDonald, J H, and M Kreitman. 1991. “Accelerated Protein Evolution at the Adh Locus in
913 Drosophila.” *Nature* 351: 652–54.

914 McKenna, Aaron, Matthew Hanna, Eric Banks, Andrey Sivachenko, Kristian Cibulskis, Andrew
915 Kernytsky, Kiran Garimella, et al. 2010. “The Genome Analysis Toolkit: A MapReduce
916 Framework for Analyzing next-Generation DNA Sequencing Data.” *Genome Research* 20
917 (9): 1297–1303. https://doi.org/10.1101/gr.107524.110.

918 Messer, Philipp W., and Dmitri A. Petrov. 2013. “Frequent Adaptation and the McDonald-
919 Kreitman Test.” *Proceedings of the National Academy of Sciences of the United States of*
920 *America* 110 (21): 8615–20. https://doi.org/10.1073/pnas.1220835110.

921 Montanucci, Ludovica, Hafid Laayouni, Giovanni Marco Dall’Olio, and Jaume Bertranpetit.
922 2011. “Molecular Evolution and Network-Level Analysis of the N-Glycosylation Metabolic
923 Pathway across Primates.” *Molecular Biology and Evolution* 28 (1): 813–23.

https://doi.org/10.1093/molbev/msq259.

Moon, Sunjin, Tae-Hun Kim, Kyung-Tai Lee, Woori Kwak, Taeheon Lee, Si-Woo Lee, Myung-Jick Kim, et al. 2015. “A Genome-Wide Scan for Signatures of Directional Selection in Domesticated Pigs.” *BMC Genomics* 16 (1): 1–12. https://doi.org/10.1186/s12864-015-1330-x.

Nevado, Bruno, Sebastian E. Ramos-Onsins, and Miguel Perez-Enciso. 2014. “Resequencing Studies of Nonmodel Organisms Using Closely Related Reference Genomes: Optimal Experimental Designs and Bioinformatics Approaches for Population Genomics.” *Molecular Ecology* 23 (7): 1764–79. https://doi.org/10.1111/mec.12693.

Orlando L, Librado P. 2019 "Origin and Evolution of Deleterious Mutations in Horses." *Genes* 10, 649; doi:10.3390/genes10090649

Pérez-Enciso, M., G. de los Campos, N. Hudson, J. Kijas, and A. Reverter. 2016. “The ‘Heritability’ of Domestication and Its Functional Partitioning in the Pig.” *Heredity* 118: 160–68. https://doi.org/10.1038/hdy.2016.78.

Quinlan, Aaron R. 2014. “BEDTools: The Swiss-Army Tool for Genome Feature Analysis.” *Current Protocols in Bioinformatics / Editoral Board, Andreas D. Baxevanis ... [et Al.]* 47 (January): 11.12.1-11.12.34. https://doi.org/10.1002/0471250953.bi1112s47.

Ramírez, Oscar, William Burgos-Paz, Encarna Casas, Maria Ballester, Erica Bianco, Iñigo Olalde, Gabriel Santpere, et al. 2014. “Genome Data from a Sixteenth Century Pig Illuminate Modern Breed Relationships.” *Heredity* 114 (2): 175–84. https://doi.org/10.1038/hdy.2014.81.

Ramsay, Heather, Loren H Rieseberg, and Kermit Ritland. 2009. “The Correlation of Evolutionary Rate with Pathway Position in Plant Terpenoid Biosynthesis.” *Molecular Biology and Evolution* 26 (5): 1045–53. https://doi.org/10.1093/molbev/msp021.

Rausher, Mark D, Richard E Miller, and Peter Tiffin. 1999. “Patterns of Evolutionary Rate Variation Among Genes of the Anthocyanin Biosynthetic Pathway.” *Molecular Biology and Evolution* 16 (2): 266–74. https://watermark.silverchair.com/mbev_16_02_0266.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAdwwggHYBgkqhkiG9w0BBwagggHJMIIB

953 xQIBADCCAb4GCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMB_IVBQyX1n-
954 F9I4pAgEQgIIBj8r0BkU_bdeIWEuY_7bUxMFToA8Yo-26snF_yPY.

955 Renaut, Sebastien, and Loren H. Rieseberg. 2015. "The Accumulation of Deleterious Mutations
956 as a Consequence of Domestication and Improvement in Sunflowers and Other Compositae
957 Crops." *Molecular Biology and Evolution* 32 (9): 2273–83.
958 https://doi.org/10.1093/molbev/msv106.

959 Riley, Rebecca M., WeI Jin, and Greg Gibson. 2003. "Contrasting Selection Pressures on
960 Components of the Ras-Mediated Signal Transduction Pathway in Drosophila." *Molecular*
961 *Ecology* 12 (5): 1315–23. https://doi.org/10.1046/j.1365-294X.2003.01741.x.

962 Rubin, Carl-Johan, Hendrik-Jan Megens, Alvaro Martinez Barrio, Khurram Maqbool, Shumaila
963 Sayyab, Doreen Schwochow, Chao Wang, et al. 2012. "Strong Signatures of Selection in
964 the Domestic Pig Genome." *Proceedings of the National Academy of Sciences of the United*
965 *States of America* 109 (48): 19529–36. https://doi.org/10.1073/pnas.1217149109.

966 Tajima, Fumio. 1983. "Evolutionary Relationship of DNA Sequences in Finite Populations."
967 *Genetics* 105: 437–60.
968 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1202167/pdf/437.pdf.

969 Uricchio LH, Petrov DA, Enard D. 2019 "Exploiting selection at linked sites to infer the rate and
970 strength of adaptation." *Nat Ecol Evol*. 3(6):977-984. doi: 10.1038/s41559-019-0890-6.

971 Watterson, G.A. 1975. "On the Number of Segregating Sites in Genetical Models without
972 Recombination." *Theoretical Population Biology* 7 (2): 256–76.
973 https://doi.org/10.1016/0040-5809(75)90020-9.

974 Wilkinson S, Lu ZH, Megens HJ, Archibald AL, Haley C, Jackson IJ, Groenen MA, Crooijmans
975 RP, Ogden R, Wiener P. 2013 "Signatures of diversifying selection in European pig
976 breeds." PLoS Genet. 2013 Apr;9(4):e1003453. doi: 10.1371/journal.pgen.1003453.

977 Zeder, Melinda A. 2012. "The Domestication of Animals." *Journal of Anthropological Research*
978 68 (2): 161–90. https://doi.org/10.3998/jar.0521004.0068.201.

979

980 **TABLES**

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

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

986

987 **FIGURES**

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

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

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

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

1000 **Figure 5**. Posterior distribution of the α values for total variants. Four different estimators of alpha
1001 (Fu&Li, Watterson, Tajima and Fay&Wu, see Materials and Methods) are used. Box plots indicate
1002 simulated distributions of α values. Red line indicates observed α values.

1003 **Figure 6**. Posterior distribution of the α values for exclusive variants. Four different estimators of
1004 alpha (Fu&Li, Watterson, Tajima and Fay&Wu, see Materials and Methods) are used. Box plots
1005 indicate the simulated distributions of α values. Red line indicates observed α values.

1006

1007 **SUPPLEMENTARY MATERIAL**

1008 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

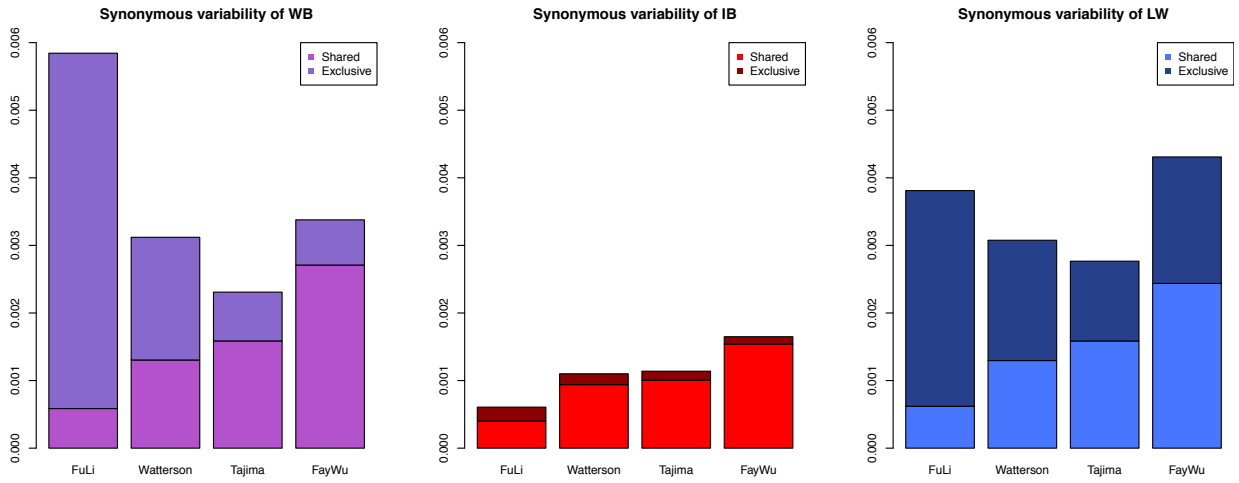
Table 2. Number of synonymous and nonsynonymous SNPs for different combinations of the allelic status. SNPs that are missing in any of the populations are not considered in this table.

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


Synonymous variability of WB
Synonymous variability of IB
Synonymous variability of LW
Shared
Exclusive
0.000
0.001
0.002
0.003
0.004
0.005
0.006
FuLi
Watterson
Tajima
FayWu

B

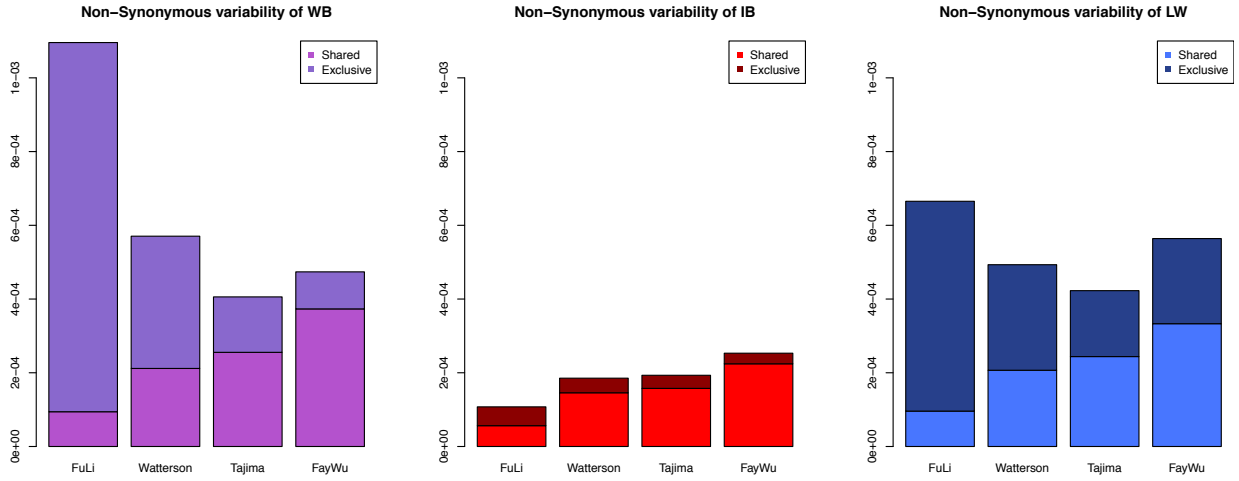

Non−Synonymous variability of WB
Non−Synonymous variability of IB
Non−Synonymous variability of LW
Shared
Exclusive
0e+00
2e−04
4e−04
6e−04
8e−04
1e−03
FuLi
Watterson
Tajima
FayWu

Figure 2

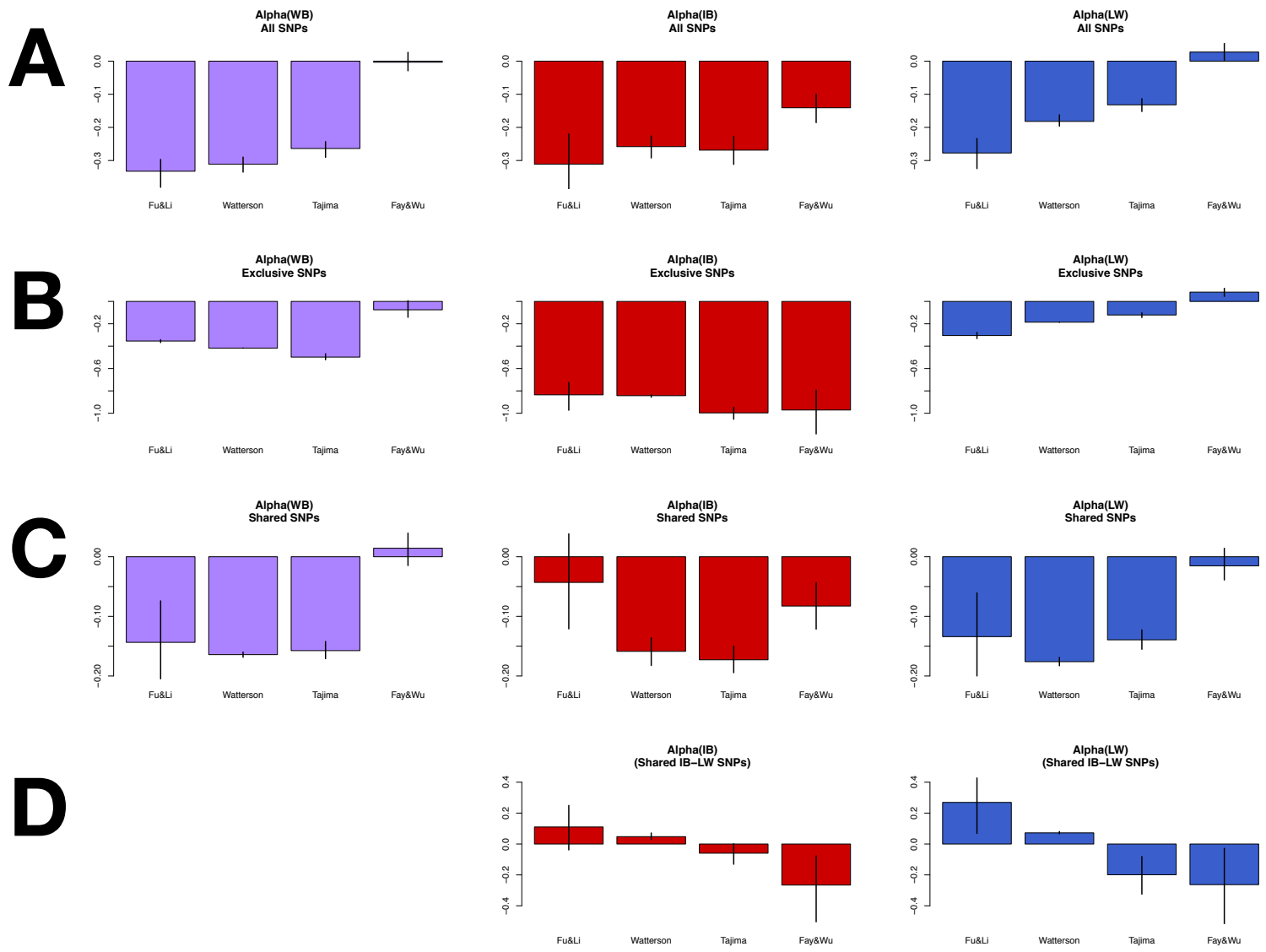

A
Alpha(WB)
All SNPs
Alpha(IB)
All SNPs
Alpha(LW)
All SNPs
B
Alpha(WB)
Exclusive SNPs
Alpha(IB)
Exclusive SNPs
Alpha(LW)
Exclusive SNPs
C
Alpha(WB)
Shared SNPs
Alpha(IB)
Shared SNPs
Alpha(LW)
Shared SNPs
D
Alpha(IB)
(Shared IB−LW SNPs)
Alpha(LW)
(Shared IB−LW SNPs)
Fu&Li
Watterson
Tajima
Fay&Wu

Figure 3

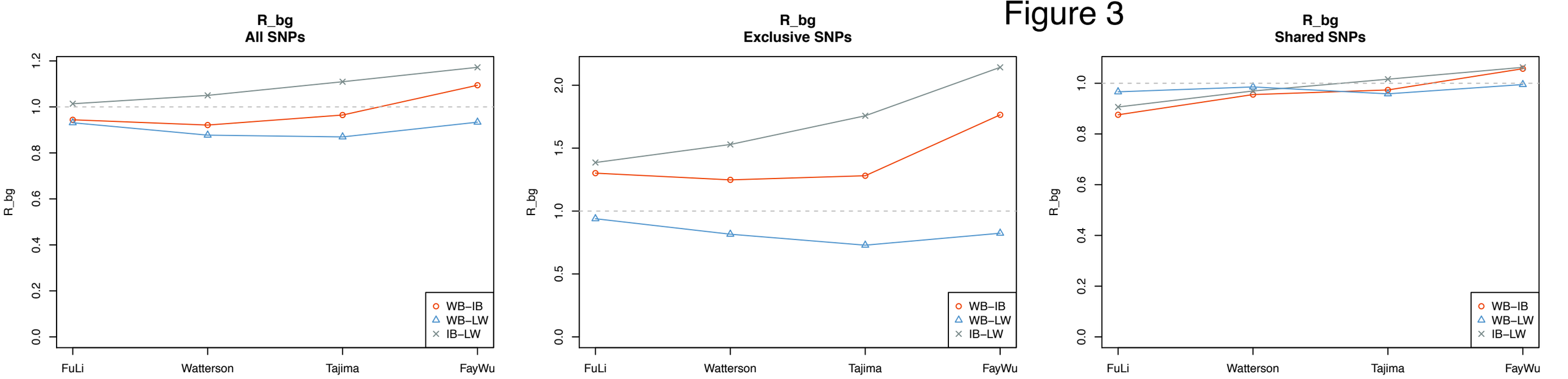

R_bg
All SNPs
R_bg
Exclusive SNPs
R_bg
Shared SNPs
R_bg
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.5
1.5
2.0
FuLi
Watterson
Tajima
FayWu
WB–IB
WB–LW
IB–LW

Figure 4

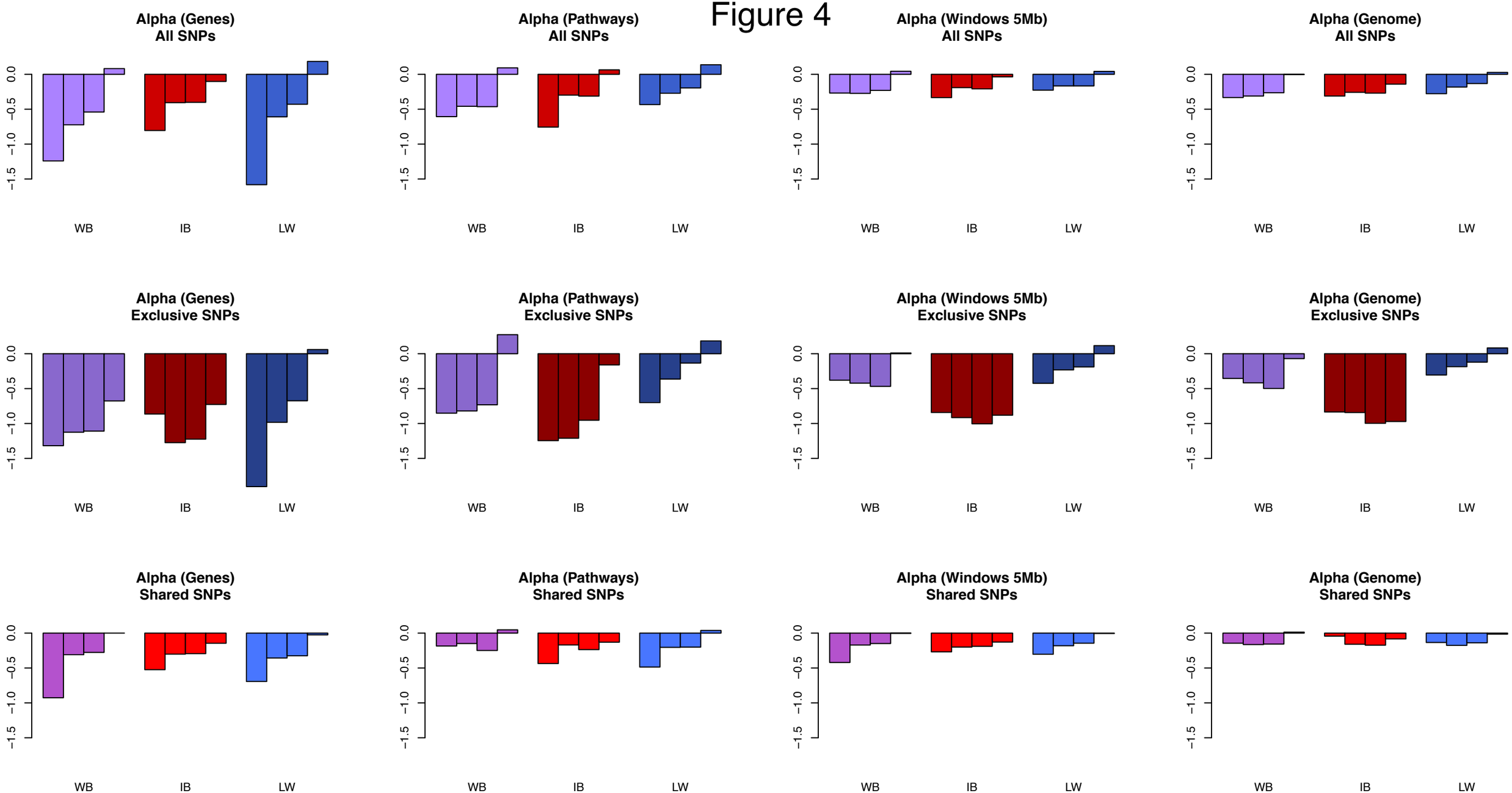

Alpha (Genes)
All SNPs
Alpha (Pathways)
All SNPs
Alpha (Windows 5Mb)
All SNPs
Alpha (Genome)
All SNPs
Alpha (Genes)
Exclusive SNPs
Alpha (Pathways)
Exclusive SNPs
Alpha (Windows 5Mb)
Exclusive SNPs
Alpha (Genome)
Exclusive SNPs
Alpha (Genes)
Shared SNPs
Alpha (Pathways)
Shared SNPs
Alpha (Windows 5Mb)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

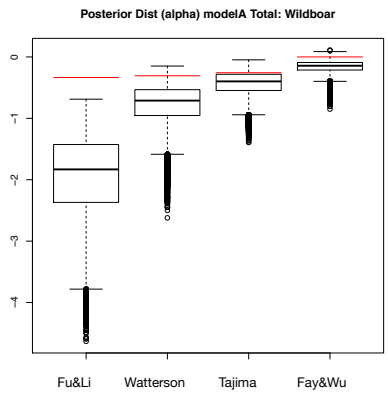
Figure 5

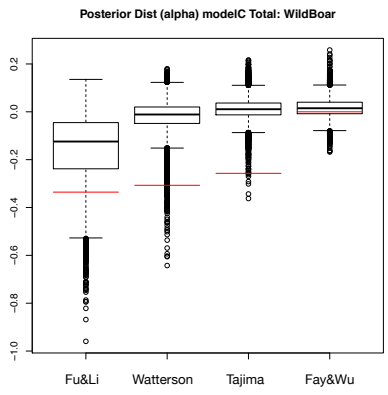
model A
(only deleterious)

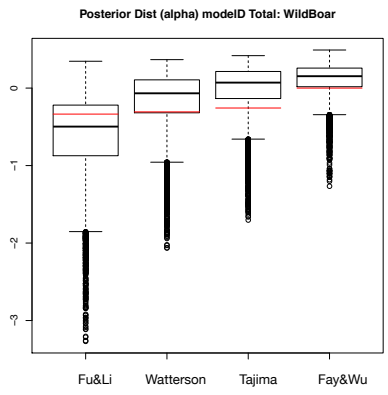
model C
(deleterious plus beneficial)

model D
(deleterious plus beneficial, discrete distribution)

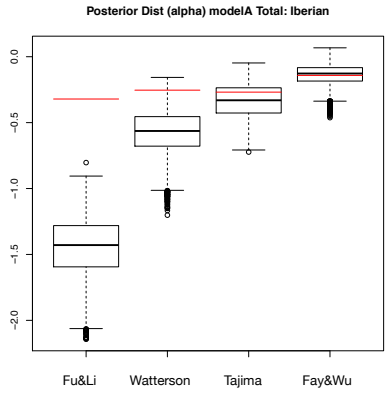
WB

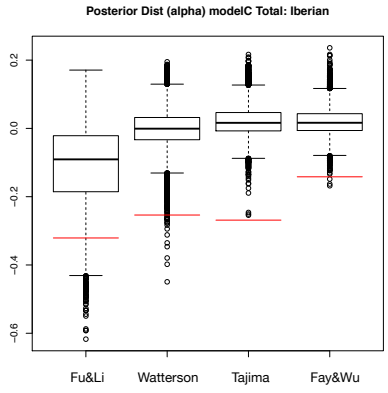


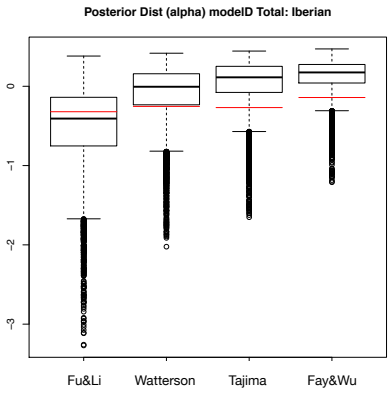




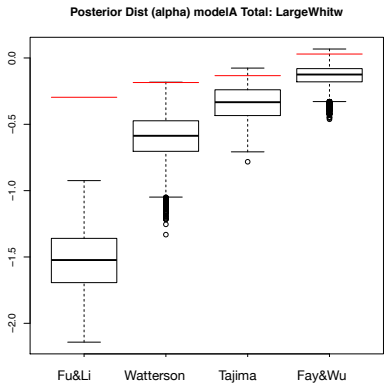
IB

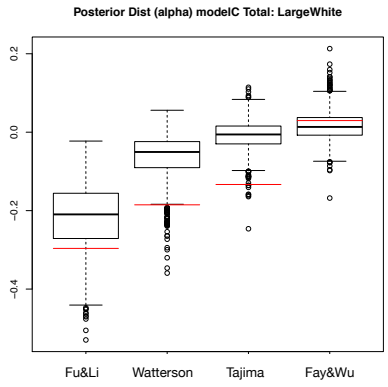






LW





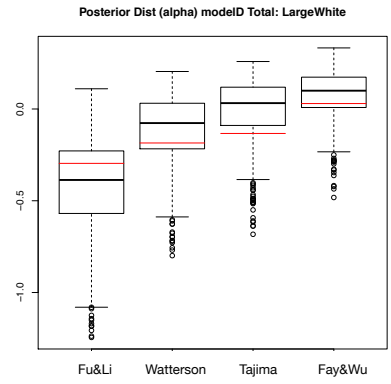


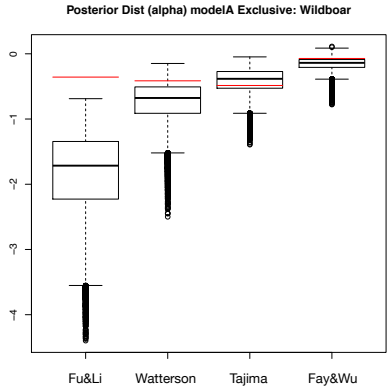
Figure 6

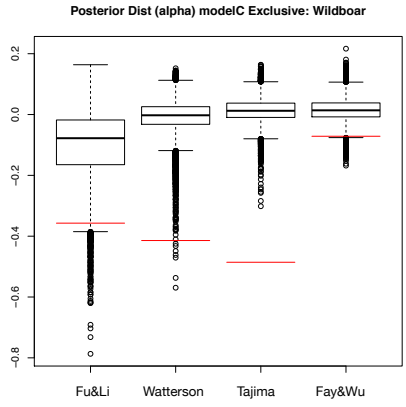
model A
(only deleterious)

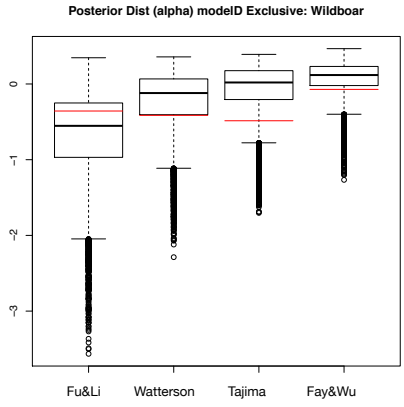
model C
(deleterious plus beneficial)

model D
(deleterious plus beneficial, discrete distribution)

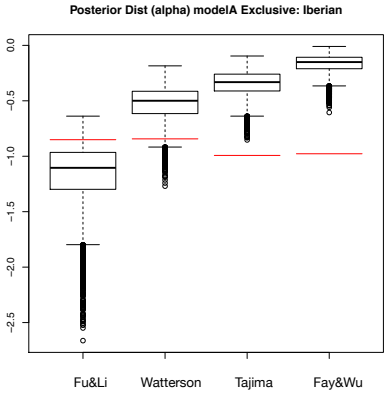
WB

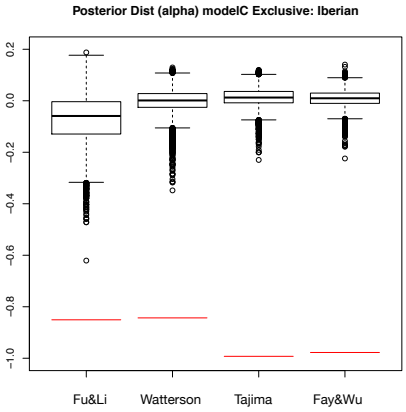


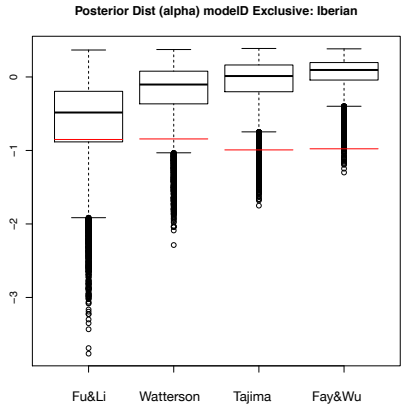
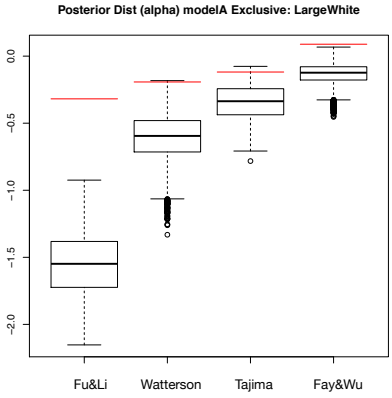
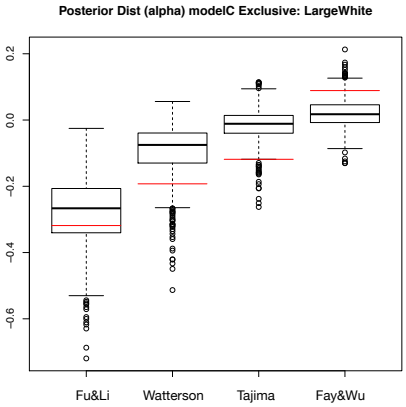
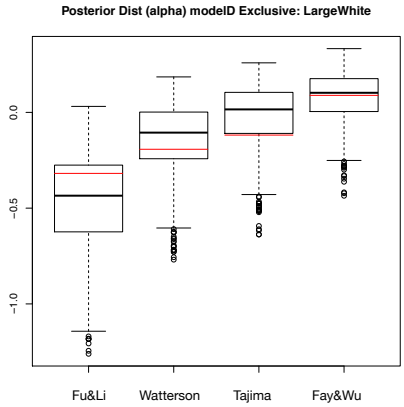




IB



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