RESEARCH ARTICLE

A further look of the genetic origin and singularity of the *Torbiscal* Iberian pig line

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Abstract

Although the *Torbiscal* line of Iberian pigs has been largely studied, some aspects of its history are not well known. In this paper, we used pedigree-based methods on a complete genealogy of 4,077 entries in order to get a comprehensive analysis of its four founder strains and to evaluate the expected genetic contribution of each ancestral strain to the successive generations of the composite *Torbiscal* line. Between-strain differences and specific heterotic effects on piglet weight at 50 days of age were estimated from records of 9,052 piglets born in 1,571 litters of a complete diallel cross among the four strains. Moreover, we assessed the genetic singularity of the current *Torbiscal* pigs by other three studies, based on whole genome SNP genotypes, focused on the measure of its genetic diversity and differentiation with respect to other domestic and wild pig populations. The STRUCTURE algorithm detected two uppermost levels of the whole population structure, corresponding to European and Asian ancestries. These results confirmed the exclusive European origin of the *Torbiscal* and other Iberian pigs and the admixed origin of the Duroc breed. Finally, the comparison of *Torbiscal* with a representative pool of Iberian pigs showed a maximum genetic differentiation in regions of chromosomes three and seven, including some genes related to the regulation of muscle development.

Introduction

From a publication on the seasonal influence on fecundity and litter performance in Iberian pigs (Dobao *et al.*, 1983) until a recent study on the effects of breed, feeding status, and diet on adipogenic, lipogenic, and lipolytic gene expression in growing Iberian and Duroc pigs (Benitez *et al.*, 2018), the research activity performed on the *Torbiscal* line has substantially contributed to increase the scientific knowledge of Iberian pigs. A remarkable number of papers, using this line as material, have been published in international journals concerning very different topics, including animal behaviour (Dobao *et al.*, 1984), quantitative genetics (Toro *et al.*, 1988, 2006; Pérez-Enciso & Gianola, 1992; Rodríguez *et al.*, 1994; Fernández *et al.*, 2002a, 2008a; Silió *et al.*, 2013, 2016; Muñoz *et al.*,

2017), nutrition and feeding systems (Rey *et al.*, 2006; Daza *et al.*, 2007; López-Bote *et al.*, 2008; Ayuso *et al.*, 2015), quality of meat and cured products (Hernández *et al.*, 2004; Muriel *et al.*, 2004; Carrapiso & Garcia, 2008), genetic diversity and population structure (Toro *et al.*, 1998, 2002; Alves *et al.*, 2003, 2006; Fabuel *et al.*, 2004; Rodrigáñez *et al.*, 2008); association between genetic markers and productive traits (Muñoz *et al.*, 2004; Fernández *et al.*, 2008b), functional and structural genomics (Esteve-Codina *et al.*, 2013; Óvilo *et al.*, 2014a; Gómez-Raya *et al.*, 2015) and nutrigenomics (Óvilo *et al.*, 2014b; Benítez *et al.*, 2015).

Moreover, the supply of *Torbiscal* breeding animals to Iberian pig farmers, decisively supported the partial recovery of breed census along the last three decades of the past century, after its greater crisis caused by the market rejection of fat carcasses and the eradication

of first African swine fever epidemics. The notable diffusion of the line led to the official acknowledgement of *Torbiscal* pigs as a new Iberian variety. More recently, the *Torbiscal* line has been studied in new private breeding programs for improving productive traits of Iberian pigs (Ibáñez-Escriche *et al.*, 2014, 2016).

Although the composite origin of the *Torbiscal* line has been concisely described in some of the quoted papers, the relevance of this population justifies a more comprehensive genealogical and productive characterization of its founder strains, its relatedness with these ones and its genetic singularity with respect to other pig populations, particularly with the remaining Iberian pigs. These are the objectives of the present study.

Material and methods

Animals

The *Torbiscal* line was obtained in 1963 by blending four ancient Iberian pig strains preserved by private breeders, and since 1944-45 maintained with pedigree and data recording in the experimental herd of "El Dehesón del Encinar" (Oropesa, Toledo, Spain). Two of these strains were reddish and came from the Portuguese herds of the Count of Ervideira (Evora) and Mr. Picao Caldeira (Elvas). The others, both hairless and black coated, came from the herds of the Donoso brothers (Campanario), and Mr. Fabián Lozano (Puebla de la Calzada) in Odriozola (1976). The last strain (named today *Guadyerbas*) and the *Torbiscal* line are still kept in a conservation programme. The complete pedigree of *Torbiscal* from 1944 to 2013 was analysed in this study. It consists of 4,077 entries (individual-sire-dam) including 1,411 ancestor reproducers: 312 *Ervideira* (RE), 321 *Caldeira* (RC), 237 *Campanario* (BHC), 343 *Puebla* (BHP) and 198 admixed ones.

A complete diallel cross was performed among the four quoted strains in order to test their reproductive and productive performance and the possible heterotic effects among them (García-Casco *et al.*, 2012). Here we analysed the piglet weight at 50 days of age using the available records from 9,052 piglets born in 1,571 litters representing the 16 genetic types resulting from the 4 × 4 possible crosses. The distribution of these piglets per cross and the number of sires and dams of each strain are shown in Table 1.

Pedigree analysis

A founder is defined, for pedigree analysis purpose, as an animal with no relationship to any member

Table 1. Number of animals with weight records and number of sires and dams per strain (between brackets) used in a diallel crossbreeding scheme among four Iberian strains (*Ervideira*, RE; *Caldeira*, RC; *Campanario*, BHC; *Puebla*, BHP) founders of the *Torbiscal* composite line.

Sire line (No. of sires)	Dam line (No. of dams)				
	RE (135)	RC (134)	BHC (145)	BHP (169)	Total (583)
RE (40)	1413	156	138	569	2276
RC (28)	124	1319	378	172	1993
BHC (37)	133	497	1293	168	2091
BHP (34)	404	84	110	2094	2692
Total (139)	2074	2056	1919	3003	9052

of the pedigree except its offspring. The number of discrete equivalent generations traced (EqG_i), which is the expected number of generations from the base population if generation proceeded discretely, and the coefficients of inbreeding (F_i) and coancestry (f_{jk}) were computed for each *ith* individual and for their parents *j* and *k* tracing the pedigree back to the founder animals (Woolliams & Mäntysaari, 1995; Caballero, 1995; Caballero & Toro, 2000). These parameters are closely linked to the classical concept of genetic contributions (James & MacBride, 1958), which also sustains other genealogical parameters calculated in this study, such as the effective number of founders (*Nef*) and non-founders (*Nenf*) and the founder genome equivalents (*Nge*), related among them by the expression $1/Nge = 1/Nef + 1/Nenf$ (Toro *et al.*, 2000). For some specific calculations, the entire number of traced generations (*t*) was determined for each individual as the rounded number of discrete equivalent generations. The realized effective population size was estimated from the increase coancestry over generations by measuring *IBD* (identical by descent) probabilities, through the well-known formula: $Ne = 1/2\Delta IBD$, where ΔIBD is the rate of *IBD*. ΔIBD values were calculated from individual coancestry rates by the expression $\Delta f_{jk} = 1 - \sqrt[\frac{EqGk+EqGj}{2}]{(1 - f_{jk})}$ (Cervantes *et al.*, 2011) and averaged each generation as $(1/2\overline{\Delta f_{jk}})$, being the effective census $Ne_{\Delta f} = 1/2\overline{\Delta f_{jk}}$. To take into account the possible bottlenecks, the effective number of ancestors (*fa*) or minimum number of ancestors necessary to explain the genetic diversity of a population was also calculated by each generation according to Boichard *et al.* (1997).

In order to examine the relationship between the *Torbiscal* composite line and their founder strains, the expected genetic contribution of each founder to any *Torbiscal* reproducer was measured by means of coancestry coefficients between animals and founders (James, 1972). The proportional genetic contribution of each founder to a specific generation is $p_j = \sum_{k=1}^{N} \frac{a_{jk}}{N}$

where a_{jk} is the additive relationship coefficient between founder j and animal k, being N the number of reproducers in the generation. The extension to the calculus of contributions of each one of the founder strains (S) is immediate by means of $\sum_{j \in S} p_j$ referred to the founders belonging to each one.

Moreover, to inspect whether the alleles contributing to inbreeding of *Torbiscal* animals came from specific founders, the inbreeding coefficient (F_i) can be partitioned into components due to each one of the 116 founders animals: $F_i = \sum_{j=1}^{116} F_j$, that measures, for any individual, the probability of being homozygous for a gene coming from each founder. These coefficients, calculated by using a modification of the additive matrix method for calculating inbreeding coefficients (Lacy *et al.*, 1996; Rodrigañez *et al.*, 1998), have been grouped into four components ascribable to each one of the founder strain $F_S = \sum_{j \in S} F_j$, sums of coefficients with respect to founder individuals belonging to each founder population. The sum of these four partial coefficients is equal to the overall inbreeding.

Analysis of piglet weight at 50 days of age

The animal model used in the analysis of weight records can be represented in matrix notation as

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_1\mathbf{u} + \mathbf{Z}_2\mathbf{u_M} + \mathbf{Z}_3\mathbf{c} + \mathbf{e}$$

where $\mathbf{y}$ is an $n \times 1$ vector of observations ($n= 9{,}052$); $\mathbf{X}$, $\mathbf{Z}_1$, $\mathbf{Z}_2$ and $\mathbf{Z}_3$ are known incidence matrices of order $n \times 37$, $n \times 9052$, $n \times 583$ and $n \times 1571$ relating location parameters $\boldsymbol{\beta}$, $\mathbf{u}$, $\mathbf{u_M}$ and $\mathbf{c}$, respectively, to $\mathbf{y}$; $\boldsymbol{\beta}$ is a 37×1 vector of systematic effects, including the effect of sex (2 levels: male and female), parity order (6 levels: 1st to 5th and $\geq$ 6th), number of weaned piglets per litter (11 levels), crossbreeding parameters (16 levels: mean, three individual and three maternal effects of *Caldeira; Campanario* and *Puebla* strains, six specific heterosis between strains and three reciprocal effects) according to the model of Dickerson (1969), and one regression coefficient (b_{Fd}) corresponding to the inbreeding effect of the dam; $\mathbf{u}$, $\mathbf{u_M}$ and $\mathbf{c}$ are the random vectors of additive genetic effects, maternal genetic effects and common litter environmental effects and $\mathbf{e}$ is the vector of random residual effects. Inferences about these parameters were obtained by a Bayesian procedure using the Gibbs sampling algorithm implemented in the TM software (Legarra *et al.*, 2011). The usual dispersion and location parameters were calculated using the BOA software (http://cph.uiowa.edu/boa) from saved samples of the marginal posterior distributions of the parameters of interest.

Genotypes

A total number of 61 *Torbiscal* pigs, 75 Iberian pigs of diverse Portuguese and Spanish origins, 52 Duroc from Spain, USA and several European countries, 65 European Wild boars from Spain, Tunisia, Poland and other countries, and 52 pigs of different Asian breeds (Meishan, Jiangquai, Jinhua and Xiang) were genotyped using the Porcine SNP60 BeadChip (Illumina) according to the manufacturer's recommendations. Data quality control was performed according to the following filtering criteria: *i*) call rate of the sample > 0.96, *ii*) single nucleotide polymorphisms (SNPs) with a call rate > 0.99, *iii*) GenTrain score > 0.70, *iv*) mean of the normalized r-values for the AB genotypes > 0.35. A SNP was removed if: *v*) number of inheritance errors > 5, *vi*) unknown position on the genome or mapped on chromosomes X or Y, *vii*) minor allele frequency MAF = 0. After all these editing steps only 43,693 SNPs were retained in the data set used for checking the possible admixture of Iberian with other porcine populations and for assessing their genetic diversity and differentiation.

Analysis of genetic diversity and differentiation

A Bayesian clustering method in STRUCTURE software (Pritchard *et al.*, 2000) was employed, using the quoted genotypes, to assign individuals to one of the K-clusters representing ancestral populations, or jointly to two or more populations if their genotypes indicated that they were admixed. The number K was previously determined by the method of Evanno *et al.* (2005) using a partial SNP datafile (8,738 genotypes) and a range of possible values from two to five. A warm-up of 20,000 iterations followed by 30,000 preserved samples was obtained in all the performed calculations.

We measured the genetic diversity according to Nei (1973) as the heterozygosity expected under the Hardy-Weinberg equilibrium conditions. Caballero & Toro (2002) showed as, in a metapopulation with n breeds, the total genetic diversity or expected heterozygosity ($GD_T= H_T$), may be partitioned into a within breeds component ($GD_{WS} = H_S$) and another between breeds ($GD_{BS}= H_T - H_S$):

$$H_S = 1 - \frac{1}{n}\sum_{i=1}^{n}\left(\sum_{k=1}^{m} p_{i,k}^2\right)$$

$$H_T = 1 - \sum_{k=1}^{m}\left(\sum_{i=1}^{n} \frac{p_{i,k}}{n}\right)^2$$

where $p_{i,k}$ is the frequency of allele k in the breed or line i. In the present study $n = 5$ and $m = 2$ alleles $\times$ 43,693 SNPs. An additional partition of GD_{WS} may be performed in genetic diversity within individuals (GD_{WI}) and between individuals (GD_{BI}) calculated in terms of average molecular coancestry (f) and self-coancestry

(s) according to Fabuel *et al.* (2004). Wright´s (1969) fixation index is the proportion of diversity between breeds relative to the total diversity: $F_{ST} = GD_{BS}/GD_T = (H_T-H_S)/H_T$. Hedrick (2005) has proposed to standardise it by the maximum level that can be obtained, $F_{ST(max)} = (1-H_S)/(1+H_S)$, given the heterozygosity within breeds. Thus, $F'_{ST} = F_{ST} / F_{ST(max)}$ is a measure of population differentiation relative to the maximum possible, which allows the comparison with different levels of variation. Bootstrap confidence intervals of heterozygosity and differentiation metrics were calculated using 10,000 bootstrap samples created by repeated random sampling with replacement of the *m* loci.

As proposed by Akey *et al.* (2010), the locus specific differentiation of each group was measured by unbiased estimates of pairwise F_{ST}. For each SNP and *i* population may be calculated the statistics

$$d_i = \sum_{j \neq i} \frac{F_{ST}^{ij} - E\left[F_{ST}^{ij}\right]}{SD\left[F_{ST}^{ij}\right]}$$

where $E\left[F_{ST}^{ij}\right]$ and $SD\left[F_{ST}^{ij}\right]$ are the mean and standard deviation of the F_{ST} between *i* and *j* breeds calculated from the 43,693 SNPs. For each group, d_i values were averaged for sliding overlapping windows of ten successive SNPs. Most differentiated regions were identified as the 99.5th percentile of the genome-wide distribution of the averaged d_i values. In this study we only present the results relative to the pair *Torbiscal* (*i*) and Other Iberian pigs (*j*). Gene content across candidate regions was determined using the *Sus scrofa*

genome (assembly Sscrofa11.1; https://www.ncbi.nlm.nih.gov/genome?term=sus%20scrofa).

Results

The founder strains

We computed the contribution of founder and non-founder reproducers to the pairwise coancestry between individuals of each generation *t* (rounded value of *EqG*) of *Ervideira*, *Caldeira*, *Campanario* and *Puebla* besides of the coefficients of coancestry (*f*) and inbreeding (*F*). The respective averaged coancestry coefficients increased from 0.055, 0.062, 0.054 and 0.047 in the first generation of the respective strain up to 0.199, 0.222, 0.164 and 0.186 in the last generation. The respective contributions to these values of founders and non-founders were 0.032 and 0.167 (RE), 0.061 and 0.161 (RC), 0.050 and 0.114 (BHC), and 0.048 and 0.138 (BHP). Note that the *Campanario* complete pedigree only consists of five equivalent generations instead of seven in the other strains.

The parameters N_{ef}, N_{enf}, and N_{ge} constitute an alternative to represent the information concerning genetic contributions to coancestry. The change of these parameters over successive generations of each founder strain is shown in Figs. 1a, b, c, d. The first parameter is related to the genetic contributions of founder reproducers to each generation which stabilize
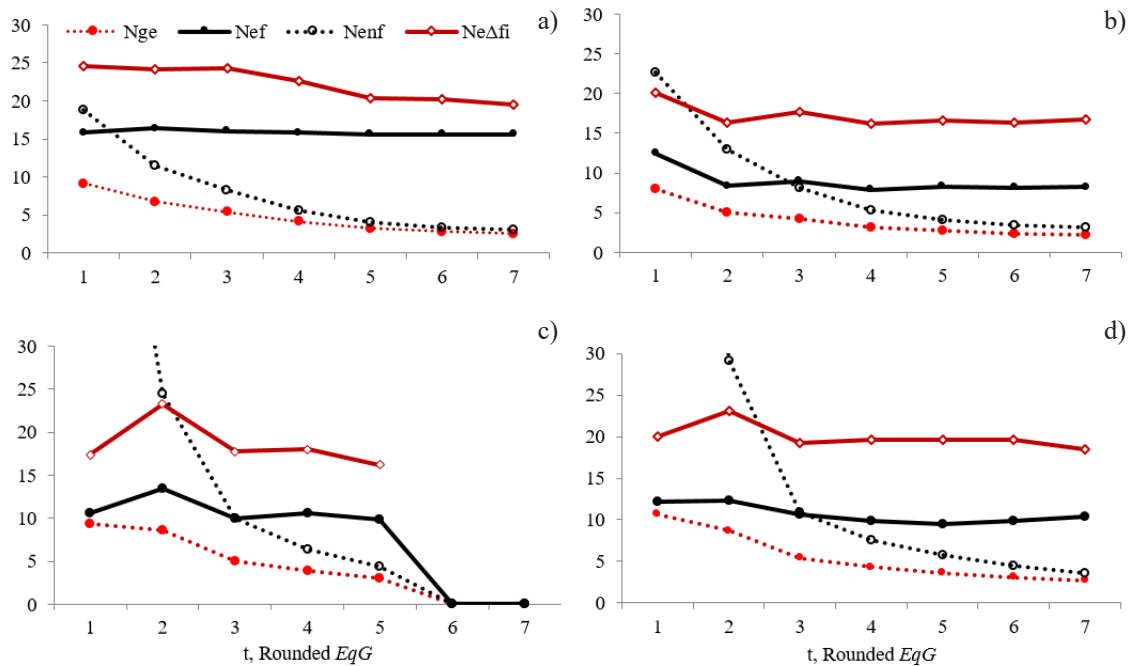

Figure 1. Changes in the founder genome equivalents (N_{ge}), effective numbers of founders (N_{ef}) and non-founders (N_{enf}) and effective population size ($N_{e\Delta fi}$) over successive rounded numbers (*t*) of equivalent discrete generations (Eq_G) of the founder strains: (a) *Ervideira*, (b) *Caldeira*, (c) *Campanario* and (d) *Puebla*.

with time. Their averaged values along the stable generations were, respectively, 15.6, 8.2, 10.0 and 10.2. In a regular system and with random mating, N_{ef} equals half the asymptotic effective population size N_e (Caballero & Toro, 2000), providing respective N_e estimates equal to 31.2, 16.4, 20.0 and 20.4. Because of the accomplishment of non-random mattings avoiding coancestry, these N_e values exceed those of effective census calculated from individual coancestry rates (N_{eAf}), represented in Fig. 1. The N_{enf} parameter depends on the contributions of non-founders, and it reflects the accumulated effects of genetic drift. Therefore it decreased in each strain from 18.8, 22.6, 76.0 and 90.0 to final values 3.0, 3.1, 4.4 and 3.6, respectively. The founder genome equivalents (N_{ge}) summarize both processes, and their values decreased along each pedigree from 9.1 to 2.5 (RE), from 8.0 to 2.3 (RC), from 9.3 to 3.0 (BHC) and from 10.7 to 2.7 (BHP). The effective number of ancestors (*fa*) is another pedigree-based metrics of genetic variability unrelated to the previous ones. Their values slightly changed between the first and last generations from 12.7 to 7.7 (RE), 10.5 to 11.7 (RC), 10.6 to 6.4 (BHC) and 12.3 to 8.4 (BHP), showing the lack of bottlenecks in these strains.

One particular aspect of the productive differences among strains was examined by the genetic analysis of piglet weight al 50 days using the data file described in Table 1. Mean and standard deviation of this trait were 11.31 and 2.54 kg. The main results obtained from the analysis are summarized in Fig. 2 and Table 2. The relationship between the number of piglets weaned per litter, including cross-fostered, and piglet weight at 50 days is represented in Fig. 2, as deviations from litters of one weaned piglet, showing a pattern with an optimum number of weaned piglets from three to seven and negative deviations for the greatest litters. Given the late weaning of litters at 50 days of age, maternal
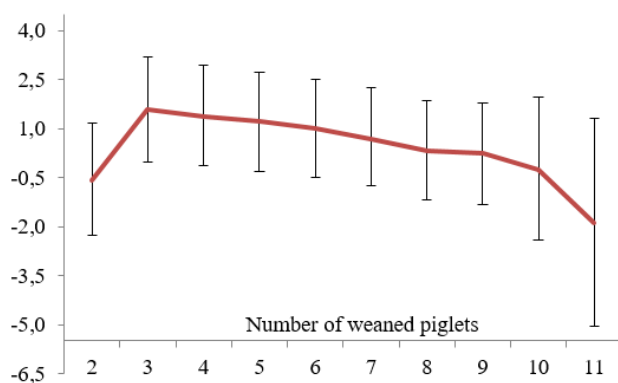
heritability (h^2_M) of this trait showed a greater magnitude than individual heritability (h^2), with a negative genetic correlation (ρ_G) between maternal and direct genetic effects. The proportion of phenotypic variance (c^2) explained by the common litter environmental effect was also important. Parity effects expressed as deviations from the first parity showed a remarkable value up to the fifth parity. Significant weight differences between males and females were also inferred. Individual and maternal strain effects were inferred as differences respect to *Ervideira,* although other contrasts have been carried out from the respective marginal posterior distributions. The only relevant strain difference among individual effects was found between *Puebla* and *Caldeira*. The other differences were lower, including the zero value their respective 95% highest posterior density intervals (95% HPD). Neither effects different from zero were found among the four maternal strains. However, the available samples of marginal posterior distributions of all the parameters of interest allow calculating additional contrasts, as between the sum of maternal and individual strain effects $[(l_i+m_i) - (l_j+m_j)]$. Measured by this procedure, the mean differences of piglet weight between *Puebla* and the other strains were 1.25 (*Ervideira*), 1.87 (*Caldeira*) and 1.21 (*Campanario*), with values of the posterior probability to be greater than zero *PProb* > 0 equal to 0.980, 0.999 and 0.973, respectively. Four out of the six specific heterotic effects on piglet weight showed significant values ranged from 0.31 to 0.86 kg, equivalent to percentages of the mean trait from 2.7 to 7.8%. However, null heterotic effects were detected between pairs *Ervideira*/*Campanario* (PM = 0.054, *PProb* > 0 = 0.583) and *Caldeira*/*Puebla* (PM = 0.034, *PProb* > 0 = 0.552). Inferred reciprocal strain effects were not different from zero.

The composition of *Torbiscal* line

Crosses between strains for founding the *Torbiscal* line were carried out from generation seven to nine. The following seven types of crosses were initially implemented: two between two strains [RE × BHP and BHC × BHP], and five between three strains [RE × (RE × BHP), RC × (RE × BHP), BHP × (RE × BHP), RE × (BHC × BHP) and BHP × (BHC × BHP)]. Other intercrosses were performed later to obtain red hairy coated pigs with contributions of the four ancestral strains, which were identified as the first individuals of the new composite line. Only 71 out of the 116 founders actually contributed to the build-up of *Torbiscal*.

The proportional genetic contribution of each strain to the successive generations of the *Torbiscal* line is represented in the Fig. 3. Note that this figure starts

Figure 2. Effect on the piglet weight at 50 days (kg) of the number of weaned littermates, represented as deviations respect to the effect of one weaned piglet per litter.

Table 2. Main statistics of marginal posterior distributions of direct (h^2) and maternal (h^2_M) heritabilities, genetic correlation between direct and maternal effect (ρ_G), coefficient of common litter environmental effect (c^2), and most relevant effects of crossbreeding parameters, gender and parity order for weight al 50 days (kg).

	PM[§]	PSD	95% HPD	PProb > 0
Variance ratios				
h^2	0.130	0.032	0.070 / 0.195	1.000
h^2_M	0.190	0.049	0.095 / 0.286	1.000
ρ_G	-0.412	0.168	-0.086 / -0.720	0.020
c^2	0.207	0.015	0.179 / 0.237	1.000
Gender effect				
Male - Female	0.605	0.043	0.522 / 0.693	1.000
Parity order				
2nd – 1st	1.204	0.102	1.013 / 1.409	1.000
3rd – 1st	1.262	0.114	1.024 / 1.473	1.000
4th – 1st	1.316	0.138	1.054 / 1.592	1.000
5th – 1st	1.315	0.164	0.989 / 1.626	1.000
≥6th – 1st	0.584	0.147	0.301 / 0.880	1.000
Strain effects				
l_{BHP} - l_{RC}	1.509	0.846	-0.192 / 3.145	0.960
Specific heterosis				
h_{RERC}	0.860	0.239	0.391 / 1.326	0.999
h_{REBHP}	0.306	0.151	0.001 / 0.588	0.979
h_{RCBHC}	0.384	0.155	0.072 / 0.680	0.992
h_{BHCBHP}	0.713	0.245	0.253 / 1.207	0.998

§ Posterior mean (PM), Posterior standard deviation (PSD), 95% highest posterior density interval (95% HPD) and Bayesian posterior probability to be greater than zero (PProb > 0).

at generation nine which is the first one where some born pigs were nominated *Torbiscal*. The values of the expected contributions of *Ervideira, Caldeira, Campanario* and *Puebla* at this generation were 0.22, 0.26, 0.24 and 0.28, respectively, and the effective number of ancestors (*f*a) equal to 20.28 (Boichard *et*
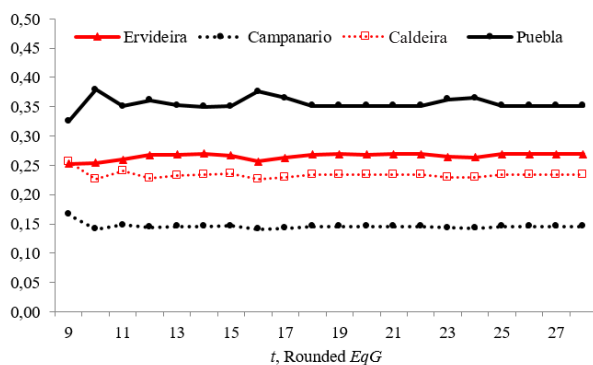

Figure 3. Expected genetic contributions to the *Torbiscal* line of the *Ervideira, Caldeira, Campanario* and *Puebla* founder strains over successive rounded numbers (*t*) of equivalent discrete generations (Eq_G).

al., 1997). The respective contributions to the last generation were 0.27, 0.23, 0.15 and 0.35, being the value of *f*a = 14.34.

The changes of average inbreeding coefficient over successive generations of *Torbiscal* line and components inbreeding ascribable to founder strains are exposed in the Fig. 4. The mean *Torbiscal* inbreeding by generation increased from F_9=0.041 to F_{28}=0.180 while the four partial components showed an analogous increase from F_9=0.007, 0.011, 0.004 and 0.019 to F_{28}=0.049, 0.043, 0.023 and 0.066.

Genetic relationships between *Torbiscal* and relevant pig populations

The application of Evanno *et al.* (2005) method to a preliminary clustering analysis of 305 individuals representing five pig populations, determined K = 2 as the number of ancestral groups on the superior hierarchical level of the data structure. This number coincides with the two well-known main focus of pig domestication that took place ten thousand years ago
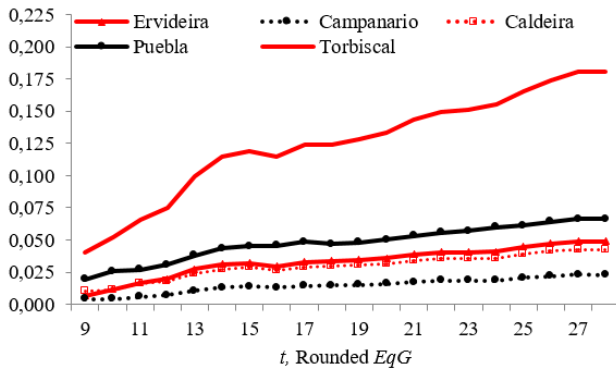
Figure 4. Partial components of *Torbiscal* inbreeding ascribable to the founder strains over successive rounded numbers (*t*) of equivalent discrete generations (Eq_G).

Table 3. SNPs based metrics of coancestries (s_i, f_{ii}), average distance between individuals (D_{ii}), inbreeding (F_i), H-W deviations (α_i) and proportion of diversity between individuals (G_i) for each population *i*.

	f_{ii}	s_i	D_{ii}	F_i	α_i	G_i
Torbiscal	0.806	0.899	0.093	0.798	-0.040	0.480
Other Iberians	0.752	0.890	0.138	0.780	0.116	0.558
European WB§	0.708	0.882	0.175	0.765	0.196	0.598
Duroc	0.681	0.859	0.178	0.718	0.116	0.558
Asian breeds	0.786	0.918	0.131	0.835	0.229	0.614

§ WB: wild boar.

in East Europe and Asia from different wild ancestors diverged 1 M years ago (Larson *et al.*, 2007). For this reason, clusters were named as European and Asian. The final results of the application of the STRUCTURE algorithm using the whole number of SNP genotypes are presented in Fig. 5. *Torbiscal*, other Iberian pigs and European Wild pigs were ascribed to a common ancestral group (European) with inferred mean proportions 1.000, 0.979 and 0.945, respectively. A similar proportion of the genome of Asian pigs (0.985) was ascribed to the other ancestral group (Asian), while the Duroc pigs showed remarkable admixing of both ancestors, with respective mean proportions of 0.671 and 0.329.

The mean expected heterozygosities (and 95% CI) of *Torbiscal*, other Iberian, European Wild pigs, Duroc and Asian pigs were 0.194 (0.193/0.196), 0.248 (0.246/0.250), 0.270 (0.268/0.272), 0.319 (0.317/0.320) and 0.218 (0.216/0.219), respectively. A more detailed partition of the genetic diversity for each *i* population is stated in Table 3. The values of individual and population self-coancestry (s_i, f_{ii}), average distance
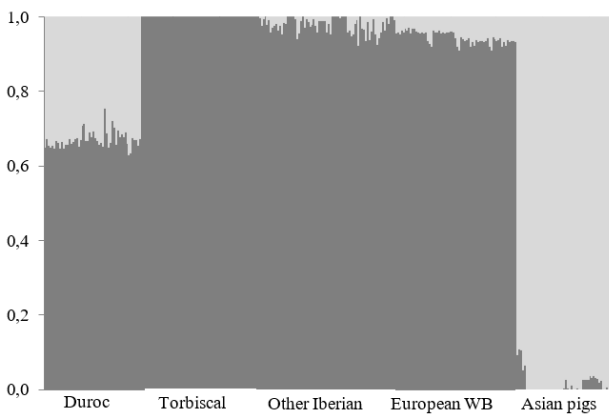

Figure 5. Bayesian probabilistic individual assignments to clusters representing European (light color) and Asian (dark color) ancestral origins. Figure built by STRUCTURE.

between individuals (D_{ii}), inbreeding (F_i), and related parameters (Fabuel *et al.*, 2004), as the Hardy-Weinberg deviations (α_i) and proportion of diversity between individuals (G_i) point out clear differences in the partition of genetic diversity among the *Torbiscal* closed line and the other pig populations.

The total heterozygosity of the whole set of five populations is $GD_T = H_T = 0.364$, with components within breeds $GD_{WS} = H_S = 0.253$ and between breeds $GD_{BS} = 0.111$. The first one also has two components, the genetic diversity within individuals $GD_{WI} = 0.110$ and between individuals $GD_{BI} = 0.143$. The GD_{BS} component is equivalent to the Nei's minimum distance between populations ($\bar{D}$). The amount of differentiation is $F_{ST} = 0.304$, the maximum possible being $F_{ST(max)} = 0.611$, so that the standardized value is in this case $F'_{ST} = 0.476$. The mean and 95% CI of pairwise values of F'_{ST} among the five populations are reported in Table 4. According to these values, the *Torbiscal* line shows a small differentiation from the groups of other Iberian pigs and European wild boars, being three and six times greater its divergence from admixed Duroc and Asian pigs, respectively.

Genetic differentiation between *Torbiscal* and other Iberian pigs

A more detailed picture of the divergence between the genomes of *Torbiscal* and the analysed pool of Iberian pigs of other origins is presented in the Manhattan plot of Fig. 6. The numbers on Y-axis correspond to d_i values between *Torbiscal* and other Iberian pigs averaged for sliding overlapping windows of ten successive SNPs. Differentiated 10 SNP windows are identified as exceeding the 99.5th percentile their whole distribution, represented as an horizontal line. Only four chromosomes (SSC 9, 10, 11 and 16) do not show significant differentiation. A total of 33 divergent regions are identified in the remaining

Table 4. Mean and 95% confidence interval of pairwise values[§] of the standardized differentiation coefficient (F'_{ST}) among the five analyzed pig populations.

	Other Iberians	European WB	Duroc	Asian pigs
Torbiscal	0.102 (0.101/0.104)	0.189 (0.187/0.192)	0.329 (0.325/0.332)	0.647 (0.643/0.651)
Other Iberians		0.131 (0.129/0.133)	0.251 (0.248/0.254)	0.588 (0.584/0.592)
European WB			0.258 (0.255/0.261)	0.550 (0.547/0.555)
Duroc				0.492 (0.488/0.496)

[§]Calculated from 43,693 SNPs and 10,000 Bootstrap samples. WB: wild boar.

14 chromosomes, being SSC6 the chromosome with a greater number (seven) of differentiated regions with a total length of 4.3 Mb. The maximum differentiation is found on SSC3 (95.45–96.50 Mb) and SSC7 (101.96–102.54 Mb). The examination of gene content using the more recent assembly of pig genome (*Sscrofa*11.1) allowed the detection of 94 protein-coding genes inside 33 divergent regions. Seven of these genes code proteins related to muscle growth, eight genes to lipid metabolism and only one (*JAG1*) involved in hair differentiation. Their symbols and chromosome positions are detailed in Table 5.

Discussion

Miguel Odriozola, who was the founder of *Torbiscal* line, and devoted much of his brilliant career to the study of Iberian pigs, classified livestock populations in two classes according to their degree of artificialness. First degree populations of farm animals are those controlled by a large number of farmers, being genetically developed by adaptation to their particular environment and production system, without any

standard racial or herd-book. Otherwise, second degree populations, with a lower environmental influence, undergo genetic changes mainly determined by a small group of breeders and resulting in modern breeds with standard racial, herd-book and data recording. In the middle of the past century, the Iberian pigs, with more than 600,000 sows, were an example of a first degree population extended over the South-West of the Iberian Peninsula. The quoted four founder strains were chosen as representative of the main varieties existing at this time: the golden type from the Alentejo (*Ervideira*), the chestnut type (*Caldeira*) and the two hairless types: the bony *Campanario* and the early fat *Puebla*. It may be stated that the history of the Iberian breed pig began with the systematic recording of pedigree and data of these four founders and the subsequent establishing of the *Torbiscal* line. The above results fulfil this particular history, highlighting genealogical and productive aspects not reported in previous studies.

The performed pedigree analyses of founder strains illustrate the way they were maintained as closed lines during five or seven generations with small effective population sizes of about $N_e \approx 20$. The respective evolution of coancestries and inbreeding coefficients along generations reflect systematic departures of random mating. The α coefficient, related to these coefficients by the expression $(1-F) = (1-f)(1-\alpha)$, indicates the degree of deviation from Hardy-Weinberg proportions in a population, and their negative values -generally observed in all the cases- point to a regular use of mating tactics for avoiding inbreeding.

The conservation of the four strains was combined with the simultaneous obtaining of growth and litter size data from a complete diallel cross. In the present study we accomplished the analysis of records of piglet weight at 50 days which confirm the positive effect of the *Puebla* strain on the early growth, previously reported for piglets at 21 days of age (Silió *et al.*, 1994) and growing pigs at 120 days (Fernández *et al.*, 2002b). However, the relative growth performance of these strains was very different in the fattening period, *Ervideira* being reported as the heaviest one at 365 days and with the same weight differences at 465 days, before the slaughter (García-Casco *et al.*, 2012). Substantial heterotic effects on weight between-
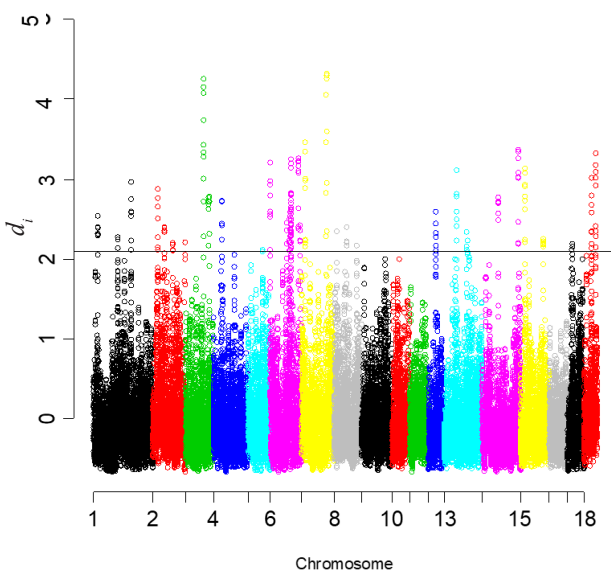

Figure 6. Genome-wide empirical distribution of d_i values for the *Torbiscal* line and the pool of other Iberian pigs. Horizontal line denotes the 99.5th percentile.

Table 5. Chromosome regions differentiated between *Torbiscal* and Other Iberian pigs with relevant gene content.

Chromosome	Region (Mb)	No of genes	Most relevant genes
1	99.20–99.34	7	*ACAA2, MYO5B*
3	95.45–96.50	10	*PPM1B, LRPPRC, PLEKHH2, ZFP36L2*
4	32.30–32.51	2	*LRP12*
6	97.46–97.91	6	*APCDD1*
6	101.67–104.97	10	*MYOM1*
17	19.60–20. 01	1	*JAG1*

strains have been estimated at different ages. Besides the values of specific heterosis here estimated, similar results have been obtained analysing diallel-cross weight records at 21, 120, 365 and 465 days (Silió *et al.*, 1994; Fernández *et al.*, 2002b; García-Casco *et al.*, 2012). Note that heterotic effects on growth up to 100 kg were greater in pigs with restricted feeding than in the pigs hand-fed to appetite according to the expected greatest heterosis in poorer environments (Fernández *et al.*, 2002). Minor between-strain differences for litter size were estimated in previous studies, being most notable the differences at the two first parities with a lower prolificacy of about -0.5 alive born piglets of the *Ervideira* sows. Specific heterotic effects on litter size were also conditional to parity order, withgreater values for the third and later parities ranging from +0.6 to +1.0 piglets alive born per litter (García-Casco *et al.*, 2012).

Miguel Odriozola was very conscious of the positive effects of the crossbreeding between the founder strains based on non-additive effects of dominance and epistasis, but he could not carry out a deep analysis of the complete diallel-cross data. Hence the *Torbiscal* line was synthesized with contributions from the four strains without exhausting the potential future changes by selection. According to the values represented in Fig. 3, the expected proportions of these contributions were unequal, although the sums of the respective proportions of the black and of the red strains were almost equal. The new composite line took advantage of some retained heterosis effects on growth and litter size (Pérez-Enciso & Gianola, 1992). As soon as the desired contributions were achieved, an empirical selection for pig growth was performed along the first fifteen years since the start of the *Torbiscal* line, based on weight records at weaning and at 240 days. The intensity of this selection was limited by the focus on the preservation of genetic variability and the simultaneous attention to other traits, such as carcass composition whose records were systematically obtained in the farm's slaughterhouse. Positive realized selection differentials and genetic responses for these traits have been estimated in the studies of Béjar *et al.* (1993) and García-Casco (1993), with rates of genetic change of 59 and 943 g/year for

weight at weaning and at 240 days, respectively. The success of this selective breeding was verified, three decades after, by independent comparative trials of the *Torbiscal* line with other Iberian strains from prestigious stockbreeders. The *Torbiscal* line showed greater growth in the fattening period, leaner body composition and greater percentages on carcasses of premium-cuts (Forero, 1999; Benito *et al.*, 2000).

Our further look to the *Torbiscal* line was also directed to the last phase of its history. We showed the main results of three studies, based on SNP genotypes, focusing on the characterization of the genetic singularity of the current animals with respect to other domestic and wild pig populations. Among these populations, the choice of other Iberian and European Wild pigs was mandatory by their respective close or remote relatedness. Two arguments justified the comparison with Duroc genotypes: Red Iberian pigs imported from Portugal and Spain in the XIX century contributed to the origin of the *Duroc–Jersey* breed in the United States (Vaughan, 1950), and nowadays the current *Duroc* breed is the only breed authorized for crossbreeding with Iberian pigs andbesides of a probable source of introgression into the Iberian genetic pool. Finally, the inclusion of genotypes from Asian pig breeds seems also necessary according to the well-known Asian influence in the genetics of most of the cosmopolitan European and American breeds. Previous studies based on mtDNA complete sequences or SNPs massive genotyping of a low number of sampled pigs (Alves *et al.*, 2009; Burgos-Paz *et al.*, 2013) have not found presence of Asian haplotypes in Iberian pigs. We performed a new whole-genome study for checking the potential admixture with Asian pigs based on an exhaustive sampling of Iberian pigs of diverse varieties and lines. The results provide a stronger support for the absence of introgression of Asian alleles in Iberian pigs, including *Torbiscal*, and European Wild boars and to their exclusive origin from European ancestors. By contrast, there is evidence of the widespread Asian influence in the other European pig breeds, although this introgression is lower in local breeds as Cinta Senese, Nera Siciliana or Mangalica

(Yang *et al.*, 2017). In comparison with the other analysed Western pig populations, *Torbiscal* pigs − maintained around 20 generations as a closed line of moderate effective size − present greater values of self-coancestries and inbreeding with lower average distance between individuals (Table 3). Permanent tactics implemented in this line avoiding mating between relatives result in an excess of observed versus expected heterozygosity (α_i<0) or more variability stocked within than between individuals (G_i<0.50). According to the genetic isolation of the four breeds (Meishan, Jiangquai, Jinhua and Xiang) included in the analysed Asian group, the partition of the genetic diversity of this group is extremely different, with the highest values of self-coancestry, inbreeding, more variability gathered between individuals, and the lowest rate between observed and expected heterozygosities. Taken the above paragraphs into account, the great genetic differentiation, measured by the F'_{ST} values, among the Asian group of breeds and the other populations is not surprising (Table 4). Moreover, the corresponding F'_{ST} values indicate that the two Iberian groups are more genetically differentiated with respect to the Duroc breed that respect to the European Wild boars, being always more differentiated the *Torbiscal* closed line than the group of other Iberian. Note that we used here the standardized F'_{ST} coefficients to allow these comparisons, based on markers with different informativeness in each group because of Iberian pigs were not considered among the breeds included in the design of the Porcine SNP60 BeadChip.

Our aim was also to investigate more precisely the genetic differentiation between *Torbiscal* and the analysed pool of other Iberian pigs. We have taken advantage of genome-wide SNP datasets for identifying footprints of the history of farm populations assignable to selection, adaptation or random genetic drift. We used a F_{ST} outliers approach (Akey *et al.*, 2010) to detect the most differentiated genome regions and then to annotate their gene content. Each point of the Manhattan plot (Fig. 6) represents the average divergence of frequencies of sliding windows of ten successive SNPs, and the most interesting outlier windows are those adjacent shaping 33 divergent chromosome regions. The longer one of these divergent regions includes 28 windows located on SSC6 (101.77−104.97 Mb) and encloses ten genes (Table 5). But only one of these genes, the *Myomesin* 1 (*MYOM1*), may be considered relevant by its implication in the myofibrillar network organisation affecting loin texture parameters in pigs (Piórkowska *et al.*, 2018). One of the regions maximally differentiated (SSC3: 95.45 - 96.50) also contains ten genes, four of them (*PPM1B, LRPPRC, PLEKHH2, ZFP36L2*) related to the regulation of the muscle development

in mammals, pigs included. These genes are outlined because a greater loin development is a well-known productive advantage of the *Torbiscal* pigs respect to the other Iberians. We report these and other genes identified in the divergent regions and related to other relevant traits (Table 5), although we understand the limitations of this study and are not able to provide a sound interpretation of its results. A deeper research combining diverse statistical approaches and more complete and accurate annotations of the pig genome will be necessary for achieving a better explanation of the genome singularity of the *Torbiscal* line. It will be the task of other geneticists involve in the conservation of the *Torbiscal* line and interested on the history of the Iberian pigs.

Acknowledgements

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