Títol: Identification of carcass and meat quality QTL in a Landrace pig population selected for growth and leanness.

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Identification of carcass and meat quality QTL in a Landrace pig population selected for growth and leanness.[1]

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23 **Abstract**

24

25 The identification of quantitative trait loci (QTL) related to production
26 traits that are relevant for the pig industry has been mostly performed by using
27 divergent crosses. The main objective of the current study was to investigate if
28 these growth, fatness and meat quality QTL, previously described in diverse
29 experimental populations, were segregating in a Landrace commercial
30 population selected for litter size, backfat thickness and growth performance.
31 We have found significant QTL for carcass weight, cutlet weight, weight of ham,
32 shoulders weight and shear firmness on pig chromosome 2. Moreover,
33 significant QTL for fat thickness between the 3rd and 4th ribs (chromosome 7),
34 rib weights (chromosome 8), backfat thickness (chromosomes 8, 9 and 10) and
35 b Minolta color component (chromosomes 6 and 7) were identified. These
36 results indicate that commercial purebred populations retain a significant
37 amount of genetic variation even for traits which have been selected for many
38 generations.

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40 Key Words: Pigs, Quantitative Trait Loci, Selected Population.

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47 **Introduction**

48 Pig breeding programs have traditionally focused on growth rate and
49 leanness as major objectives in selection (Hammond and Leitch, 1998).
50 Recently, meat quality has become an important issue in genetic selection due
51 to the demands of the consumers. Marker assisted selection (MAS) might be a
52 useful tool to improve selection efficiency for meat quality traits, which are
53 usually difficult to measure. Marker linkage information required for MAS might
54 be acquired through the identification of carcass and meat quality quantitative
55 trait loci (QTL). In the recent years, F_2 crosses between divergent populations
56 have been used for locating QTL related to growth and fatness (Andersson et
57 al., 1994; Bidanel et al., 2001; Varona et al., 2002) and meat quality traits
58 (Grindflek et al. 2001; de Koning et al., 2001; Clop et al., 2003). These
59 experiments have yielded very valuable information in terms of positioning
60 major QTL which segregate in one or several of these crosses (Walling et al.,
61 2000).

62 A relevant question that has been recently addressed by several
63 scientific teams is whether QTL that have been characterized in crosses
64 between highly divergent pig breeds are also segregating in highly selected
65 commercial populations. For instance, Evans et al. (2003) analyzed eleven
66 genomic regions for which QTL had been previously reported in ten Hampshire,
67 Large White, Landrace, Pietrain and Meishan populations. Their findings
68 confirmed the existence of several of these QTL for growth, backfat and
69 carcass traits. A second analysis of this experiment with a variance component
70 method yielded similar results demonstrating the consistence of this approach
71 (de Koning et al., 2003). The main goals of our experiment were (a) to confirm

72 the existence of these previously reported carcass and meat quality QTL in one
73 highly selected Landrace population and (b) to investigate the existence of
74 additional QTL that might be specific to this population.
75
76 **Materials and Methods**
77
78 *Animal material*
79 The Landrace line used in the current work is a non-inbred maternal line
80 from the experimental farm Nova Genètica S.A. (Lleida, Spain). This line has
81 been selected by using an index that combines litter size, backfat and growth
82 performance.
83 Five boars were mated to 71 sows producing 470 F_1 individuals. An
84 average of 94 offspring was obtained from each male. Pigs were reared under
85 normal intensive conditions and feeding *ad libitum* at the experimental farm of
86 Nova Genètica. Pigs were not castrated, being slaughtered at approximately
87 179 d of age.
88
89 *Recording of phenotypical traits*
90 Both carcass and meat quality traits were recorded for each individual.
91 Carcass traits were weight at sacrifice (**W**), carcass weight (**CW**), carcass
92 length (**CL**), backfat thickness (**BFT**), Fat-o-Meter measurement of the carcass
93 lean percentage (**CaF**), fat thickness in the loin area (**LoF**), the cervical region
94 (**CeF**) and between the 3rd and 4th ribs (**RiF**), and weight of ham (**HW**),
95 shoulders (**SW**), cutlet (**CuW**), ribs (**RW**) and bacon (**BW**). Several meat quality
96 traits related to the mechanical and chemical properties of the muscle were

97 also recorded. Chemical composition of the muscle was determined from a
98 sample of the *semimembranosus* muscle. Samples were homogenized and
99 lyophilized and subsequently fat (**FC**), protein (**P**), organic matter (**OM**) and dry
100 matter (**DM**) were determined (AOAC, 1990). Mechanical characteristics
101 influencing meat texture were measured with a Texture Analyser TA.TX2
102 (Stable Micro Systems, Godalming, UK) according to the Warner-Bratzler test
103 (Moller, 1981). Samples from the *longissimus dorsi* muscle were cooked in a
104 water bath at 80 ºC for 1 h and subsequently they were cooled at room
105 temperature. Six pieces of 2 x 1 x 1 cm of each sample were cut in the same
106 direction of the muscular fibers and the following traits were recorded: shear
107 force (**SF**), which is related to the myofibrillar components of the muscle;
108 maximum shear force (**MSF**), which is related to the proportion of connective
109 tissue; total work required to shear the sample (**TWS**); and shear firmness
110 (**SFi**). Color was measured with a Minolta spectrophotometer at 24 hours post
111 mortem: lightness (**L**), red tendency (**a**), and yellow tendency (**b**) were recorded
112 in duplicate (**L2**, **a2** and **b2**) to minimize measurement errors.
113
114 *Extraction of DNA*
115 Four hundred µl blood were washed with 500 µl TE buffer (Tris-HCl
116 10mM pH = 8, and EDTA 1mM) until obtaining a white pellet of cells.
117 Subsequently, cells were lysed with 40 µg proteinase K and 400 µl buffer K
118 (KCl 50 mM, Tris-HCl 10 mM, 0.5% Tween 20) for 5 hours at 56 ºC. Genomic
119 DNA was phenol-chloroform extracted and precipitated with 25 µl NaCl 2M and
120 800 µl ethanol. After 10 minutes of centrifugation, genomic DNA was washed
121 with 70% ethanol and resuspended in 100 µl TE.

122 *Genotyping*

123 Twenty three microsatellites were selected according to their
124 chromosomal position and to their information content in the analyzed Landrace
125 population. Quantitative trait loci for carcass and meat quality traits had been
126 previously described for the chromosomal regions analyzed in the current work
127 (Table 1). The five hundred forty six pigs were genotyped for the complete set
128 of microsatellites and for the *Ryr1* gene, according to Fujii et al. (1991).
129 Amplification reactions were carried out in an ABI PRISM 877 Integrated
130 Thermal Cycler (Applied Biosystems, Warrington, UK), and were analyzed with
131 the Genescan 3.7 software in a capillary electrophoresis device with fluorescent
132 detection (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems, Warrington,
133 UK). Genotypes were stored in the Gemma database (Iannuccelli et al., 1996).

134

135 *Statistical Analyses*

136 Data were analyzed as described in Varona et al. (2001). We compare
137 two models using a Bayes Factor. First, we considered a mixed inheritance
138 model (MODEL 1):

139 $$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}_1\mathbf{u} + \mathbf{Z}_2\mathbf{q} + \mathbf{e}$$

140 where **X**, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are the incidence matrices, β are the systematic effects
141 (batch and *Ryr1* genotype), **u** are the polygenic effects, **q** are the effects
142 associated to a genome segment, and **e** are the residuals. $\mathbf{u} \sim N(0, \mathbf{A}\sigma_u^2)$, **A**
143 being the polygenic relationship matrix and σ_u^2 the polygenic genetic variance
144 and $\mathbf{q} \sim \mathrm{N}(0, \mathbf{Q}\sigma_q^2)$, **Q** being the relationship matrix associated with the genome
145 segment and σ_q^2 the polygenic genetic variance and $\mathbf{e} \sim \mathrm{N}(0, \mathbf{I}\sigma_e^2)$ and σ_e^2 is the

146 residual variance. **Q** was calculated using the algorithm described by Pérez-
147 Enciso et al. (2000b).
148 This model can be reparameterized as:
149 $$\mathbf{y} = \mathbf{X}\beta + \mathbf{e}^*$$
150 where:
151 $$\mathbf{e}^* = \mathbf{Z}_1\mathbf{u} + \mathbf{Z}_2\mathbf{q} + \mathbf{e}$$
152 consequently,
153 $$\mathbf{e}^* \sim N(0, \mathbf{V})$$
154 $$\mathbf{V} = \mathbf{Z}_1\mathbf{Q}\mathbf{Z}_1'\sigma_q^2 + \mathbf{Z}_2\mathbf{A}\mathbf{Z}_2'\sigma_u^2 + \mathbf{I}\sigma_e^2 = \sigma_p^2\left(\mathbf{Z}_1\mathbf{Q}\mathbf{Z}_1'h_q^2 + \mathbf{Z}_2\mathbf{A}\mathbf{Z}_2'h_u^2 + \mathbf{I}(1 - h_q^2 - h_u^2)\right)$$
155 where $h_u^2 = \sigma_u^2 / \sigma_p^2$ is the proportion of polygenic variation and σ_p^2 is the
156 phenotypic variance $\left(\sigma_u^2 + \sigma_q^2 + \sigma_e^2\right)$.
157 Records and parameters are jointly distributed as:
158 $$p_1(\mathbf{y}, \beta, \sigma_p^2, h_q^2, h_u^2) \propto p_1(\mathbf{y}|\beta, \sigma_p^2, h_q^2, h_u^2)p_3(\mu)p_3(\sigma_p^2)p_3(h_q^2, h_u^2),$$
159 where:
160 $p_1(\beta) = k_1$ if $\beta \in \left[-\frac{1}{2k_1}, \frac{1}{2k_1}\right]$ and 0 otherwise.
161 $p_1(h_q^2, h_u^2) = 2$ if $h_q^2 + h_u^2 \in [0,1]$ and 0 otherwise
162 $p_1(\sigma_p^2) = k_2$ if $\sigma_p^2 \in \left[0, \frac{1}{k_2}\right]$ and 0 otherwise.
163 Note that the parametric space for both heritabilities (h_q^2, h_u^2) is a triangle, and
164 the flat density to assure a volume equal to one must be two. Note also that,
165 assuming prior independence, marginal priors of h_q^2 and h_u^2 are:

166 $p_3(h_q^2) = 2 - 2h_q^2 = \text{Beta}(1,2)$

167 $p_3(h_u^2) = 2 - 2h_u^2 = \text{Beta}(1,2)$

168 The second model (MODEL 2) that we have assumed is:

169 $\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}_1\mathbf{u} + \mathbf{e}$

170 which can be reduced to:

171 $\mathbf{y} = \mathbf{X}\beta + \mathbf{e}*$

172 where:

173 $\mathbf{e}* = \mathbf{Z}_1\mathbf{u} + \mathbf{e}$

174 consequently

175 $\mathbf{e}* \sim \text{N}(0, \mathbf{V})$

176 $\mathbf{V} = \mathbf{Z}_1\mathbf{A}\mathbf{Z}_1'\sigma_u^2 + \mathbf{I}\sigma_e^2 = \sigma_p^2\left(\mathbf{Z}_1\mathbf{A}\mathbf{Z}_1'h_u^2 + \mathbf{I}(1 - h_u^2)\right)$

177 $p_2(\mathbf{y}, \beta, \sigma_p^2, h_u^2) \propto p_4(\mathbf{y}|\beta, \sigma_p^2, h_u^2)p_4(\beta)p_4(\sigma_p^2)p_4(h_u^2)$

178 where priors for β, and σ_p^2 are the same as in model 1. Prior distribution for h_u^2

179 is

180 $p_2\left(h_u^2\right) = p_1\left(h_u^2 \mid h_q^2 = 0\right) = \text{U}(0,1)$

181 U denotes a uniform distribution. It should be noted that MODEL 2 is a special

182 case of MODEL 1 when $h_q^2 = 0$.

183 According to Varona et al. (2001), the Bayes Factor of MODEL 1 against

184 MODEL 2 is:

185 $\text{BF} = \dfrac{p_1(h_q^2 = 0)}{p_1(h_q^2 = 0|\mathbf{y})} = \dfrac{2}{p_1(h_q^2 = 0|\mathbf{y})}$

186 As $p_1(h_q^2 = 0) = 2$.

187 As previously reported by Varona et al. (2001), only the analysis with the
188 complex model (MODEL 1) is required. In this situation, only the Bayesian
189 calculations with the complex model are needed. The calculation of the
190 posterior distribution $p_1\left(h_q^2 \middle| \mathbf{y}\right)$ was performed using a Gibbs sampler (Gelfand
191 and Smith, 1990) with a Metropolis-Hasting step (Hastings, 1970) used to
192 sample from the conditional distribution of the heritabilities (h_q^2, h_u^2). A total of
193 25,000 iterations was performed after discarding the first 5,000. All correlated
194 samples were used to calculate the posterior distributions using the ergodic
195 property of the chain (Gilks et al., 1996). Convergence was checked using the
196 algorithm of Raftery and Lewis (1992).

197 The posterior probability is directly related to the Bayes Factor if we
198 assume that prior probabilities of both models are 0.5. The posterior probability
199 of the QTL model is calculated as follows:

200 $$p(\mathrm{QTL}) = \frac{\mathrm{BF}}{1 + \mathrm{BF}}$$

201 In consequence, and following Kass and Raftery (1995), a Bayes factor
202 higher than 100 is associated with a 0.990 probability (****) of detecting a true
203 QTL, while factors from 10 to 100 imply probabilities between 0.909 and 0.990
204 (***). Bayes factors ranging from 3.2 to 10 are associated with probabilities
205 0.762 - 0.909 (**) and Bayes factors between 1 and 3.2 imply posterior
206 probabilities of the QTL model between 0.5 and 0.762 (*). Finally, a Bayes
207 factor < 1.0 is associated with posterior probability smaller than 0.5, and it is
208 interpreted as absence of a QTL in the analyzed region.

209

210

211 **Results and Discussion**

212

213 By using the Bayes factor, we have compared the probabilities of two
214 alternative models without selecting a null or alternative hypothesis. The Bayes
215 factor indicates which model (QTL or no QTL model) is more probable, given
216 the data, in each of the tested situations. We only used the information
217 provided by the Bayes Factor, assuming that the prior odds were 1 and the
218 probability for the QTL model and the no-QTL model were 0.5 before obtaining
219 the data. However, we analyzed the data without taking into account multiple
220 testing. Bayesian multiple testing is still a matter of intense research (Scott and
221 Berger, 2003), in special with the availability of genetic expression data from
222 microarrays. There is a straightforward alternative which is equivalent to
223 Bonferroni test. This method considers the $P(H_o)$ in all the cases equal to 0.5
224 (that is, the probability of no QTL in all the locations and all the traits is equal to
225 0.5). If we make this assumption in our QTL experiment, the prior odd for each
226 of the 280 comparisons should be 5.15×10^{-85}, making almost impossible to
227 obtain a posterior odds greater than one. Nevertheless, this prior assumption
228 would involve that there are no genes affecting any of the traits and in any of
229 the locations with probability 0.5, a hypothesis which looks very unrealistic.
230 Moreover, it would imply that all the traits are uncorrelated, and this is clearly
231 false in all the QTL experiments. Less conservative prior odds can be defined,
232 but this is not an easy task. Moreover, we believe that this statistical research is
233 clearly out out of the objectives of the paper. As a conservative rule, we
234 consider a BF of 10 to be confident on the results of the QTL. Strictly, a BF

235 greater than 1, implies that the probabilities of the QTL model is greater than
236 the no-QTL model, but we consider this as suggestive linkage.
237
238 *Genome scan analysis*
239 We have performed a QTL search for twenty-four carcass and meat
240 quality traits in a commercial Landrace pig population (Tables 2 and 3). Ten
241 genomic regions (pig chromosomes 1, 2, 3, 4, 6, 7, 8, 9, 10, 13) for which
242 growth, fatness and meat quality QTL had been previously reported (Table 1)
243 were analyzed in this experiment:
244
245 *Chromosome 2*. We have found evidence for the existence of QTL
246 affecting **SW** ****, **CuW******, **CW****, **HW****, **BW***, **W*** and **CL***. These QTL might
247 correspond to the one described by several authors for muscular mass, which
248 maps to the insulin-like growth factor 2 (*IGF*2) region and accounts for 30% of
249 the residual phenotypic variance in the F_2 generation of a Large White x Wild
250 boar cross (Andersson-Eklund et al., 1998; Jeon et al., 1999; Milan et al.,
251 2002). The identification of the causal mutation of the *IGF2* QTL, an intronic
252 polymorphism with influence on muscle mass recently described by van Laere
253 et al. (2003) will be fundamental for establishing if it is the same one that is
254 segregating in our population.
255 We did not find any QTL related to fatness in the SW2443-*IGF2* interval,
256 in spite of the fact that other authors reported QTL affecting this trait in a
257 location distal to the *IGF2* region (de Koning et al., 1999; Rattink et al., 2000).
258 This discrepancy might be attributed to the fact that we are not analyzing the
259 same chromosomal region or that this QTL is fixed in our population.

260 With regard to meat quality traits, we have found QTL for **SFi******, **SF***,
261 **FC***, **b*** and **L***. Jeon et al. (1999) found evidence of one QTL with moderate
262 effect on color on chromosome 2. The low significance of our results (**b*** and
263 **L*)** does not allow to confirm the existance of this QTL. Moreover, to the best of
264 our knowledge, there is not any report describing **SFi** or **SF** QTL on
265 chromosome 2. Since we are unable to estimate precisely the location of this
266 meat tenderness QTL, we do not know whether chromosome 2 contains one
267 single pleiotropic QTL regulating muscular mass and meat tenderness or
268 several neighboring QTL influencing these traits.
269

270 *Chromosome 6*. The genotyping of the *Ryr1* locus, which maps proximal
271 to the analyzed region, demonstrated a low frequency of the mutant allele in the
272 Landrace population (data not shown). The *Ryr1* genotype was included in the
273 statistical model as a fixed effect and showed no significance. We have
274 detected QTL involved in **b****, **OM***, **CeT***, **CaF*** and **LoF***. No meat color QTL
275 has been described in this chromosomal region to date. In contrast, QTL
276 affecting intramuscular fat and fat deposition have been reported by de Koning
277 et al. (1999) and Óvilo et al. (2000).
278

279 *Chromosome 7*. Quantitative trait loci with influence on fatness (**RiF****)
280 and color (**b**** and **a2***) have been detected in our commercial population. A
281 QTL located in this region and affecting fatness and diverse growth traits has
282 been consistently described by several authors (Bidanel et al., 2001; Rattink et
283 al., 2000; Rohrer and Keele, 1998a). Rothschild et al. (1995) and Wang et al.
284 (1998) also reported QTL for fatness and color in this region. Moreover, Óvilo et

285 al. (2002) reported a QTL with effect on color on chromosome 7. Since lipid
286 oxidation might induce a lighter meat color, we may hypothesize the existence
287 of one pleiotropic QTL regulating lipid content and meat color, although we can
288 not completely rule out the existence of two different QTL coexisting in the
289 same genomic region.

290

291 *Chromosome 8*. We found reasonable evidence for the existence of QTL
292 influencing **BFT**** and **RW****. Moreover, we detected a suggestive linkage for
293 **W***, **CW***, **CaF***, **LoF*** and **SW***. Quantitative trait loci with effects on body
294 proportion (Andersson-Eklund et al., 1998), growth and weight (Quintanilla et
295 al., 2002), growth and fatness (Bidanel et al., 2001) and fatness (Rohrer and
296 Keele, 1998a) had been previously described in this region. However, there is a
297 lack of consistency in the precise location of these QTL, a feature that suggests
298 the existence of several QTL affecting growth and fatness with a differential
299 pattern of segregation in each one of the analysed populations. In
300 consequence, although we have found significant associations between this
301 chromosomal region and growth and fatness, it is difficult to find a
302 correspondence between these QTL and the ones reported in the scientific
303 literature.

304 In addition, we have found QTL for tenderness (**SF*** and **MSF*)** and color
305 (**a***, **b***, **b2***). Since the genomic region we have analyzed corresponds to the
306 proximal region of chromosome 8, we are unable to investigate the existence in
307 our population of the meat color QTL reported by Óvilo et al. (2002) that maps
308 to the distal region of chromosome 8. Indeed, the low significance of the QTL

309 we have found might be attributable to the large distance between the region
310 we have tested region and the true location of the QTL.
311
312 *Chromosome 9*. Significant and suggestive QTL for **BFT****, **W***, **CW***,
313 **CL***, **SW***, **CuW***, **RW***, **BW*** and **a2*** have been found. Quantitative trait loci for
314 fat deposition have been described in this region (Rohrer and Keele, 1998a)
315 and might correspond to the ones we have detected. In fact, Evans et al. (2003)
316 detected QTL for growth and carcass performance at this region, in close
317 agreement with our results.
318
319 *Chromosome 10*. We have found QTL for **BFT****, **CL*** and **a2***. A QTL for
320 growth has been detected in this region by Knott et al. (1998). Rohrer and
321 Keele (1998a) reported a QTL for BFT in a distal position which might
322 correspond to the one we have found. Moreover, Evans et al. (2003) found one
323 QTL for carcass length; however, the low significance of our QTL makes
324 difficult to establish correspondences with previous experiments.
325
326 *Maintenance of genetic diversity for carcass and meat quality QTL in a*
327 *commercial population selected for growth and leanness*
328 Although a large number of QTL related to traits of economic interest
329 have been found in pigs by using crosses between divergent populations, the
330 segregation of these QTL in the highly selected populations used in commercial
331 stockbreeding has not been analyzed until recently (Evans et al., 2003;
332 Nagamine et al., 2003). In the current work, we have analyzed growth and
333 fatness traits, and an extensive repertoire of meat quality traits, mainly related

334 to meat color, chemical composition and texture, that had not been measured
335 in previous QTL experiments with commercial populations. Our results confirm
336 the existence in a Landrace population of several carcass and meat quality
337 QTL that had been previously described in divergent crosses (e. g. QTL for
338 leanness on chromosome 2, QTL for color on chromosome 7, see Tables 1, 2
339 and 3). In addition, we have found new QTL that had not been detected in
340 previous studies (QTL for weight on chromosome 13, QTL for color on
341 chromosome 9, see Tables 1, 2 and 3). These results are in good agreement
342 with similar studies undertaken by Nagamine et al. (2003) and Evans et al.
343 (2003). Nagamine et al. (2003) studied the same regions on chromosomes 4
344 and 7 that we have analyzed in our population. They analyzed five different
345 commercial populations and found evidence of segregating QTL on
346 chromosomes 4 and 7 within two and five populations, respectively. Moreover,
347 Evans et al. (2003) analyzed the same chromosomal regions tested in our
348 experiment in ten selected populations from diverse breeds. They found clear
349 evidence indicating the existence of many growth and fatness QTL in these
350 purebred populations. Quantitative trait loci affecting pH and electric
351 conductivity were also detected, demonstrating by the first time the existence of
352 meat quality QTL in commercial populations.

353 The main conclusion that can be drawn from the QTL analyses carried
354 out by us and others is that commercial populations still retain a significant
355 amount of genetic variation in spite of the fact that they have been selected for
356 a considerable period of time. This finding might be explained by the fact that
357 purebred populations are usually selected according to a diversity of criteria, a
358 feature that diminishes the probability of allele fixation for the QTL that

359 influence each single trait. Furthermore, selection criteria have changed
360 through time and introgression of foreign material in supposedly ‘pure’ breeds is
361 the rule rather than the exception. The existence of pleiotropic genes with
362 alleles that are favorably correlated to some traits and unfavorably with others
363 might also explain the maintenance of genetic diversity in selected pig
364 populations.

365 The number and significance of the QTL we have identified in this
366 Landrace purebred population were lower than the ones reported in divergent
367 crosses. This result is not surprising since divergent crosses involve breeds
368 which have largely diverged at the phenotypic and genetic levels. Obviously,
369 most of these divergent crosses are not usually performed by pig breeders and,
370 in consequence, the QTL information that they provide might have a limited
371 practical application in the pig industry. Our data show that part of this
372 information generated in divergent crosses can be extrapolated to commercial
373 populations. For instance, the chromosome 2 muscular mass QTL reported in
374 European Wild Boar x Large White and Large White x Pietrain intercrosses
375 (Jeon et al. 1999; Nezer et al. 1999) segregates in the Landrace population
376 analysed in our experiment. The existence of particular QTL alleles in purebred
377 commercial populations might be influenced by a plethora of factors such as
378 their demographic history, geographical location and breeding goals.

379 Interestingly, we have found that growth and fatness QTL are much
380 more significant and abundant than meat quality QTL in our purebred
381 population (Table 2 and 3). This result, which is in close agreement with other
382 QTL data reported in divergent crosses (Andersson-Eklund et al., 1998; Óvilo
383 et al., 2002; Varona et al., 2002), might be interpreted in the light of that meat

384 quality traits have a different genetic architecture than growth and fatness traits.
385 In fact, the average heritability values are usually higher for body composition
386 traits, such as backfat thickness (h^2 = 0.41-0.45) and carcass length (h^2 = 0.56-
387 0.57), than for meat quality traits such as pH (h^2 = 0.21), tenderness (h^2 = 0.26)
388 and color (h^2 = 0.28) (Sellier, 1998).

389 In the next years, the understanding of the genetic architecture of
390 quantitative traits will probably evolve to identification of the genetic
391 polymorphisms which influence them and the elucidation of the transcriptional
392 and post transcriptional mechanisms that regulate their expression.

393

394 **Implications**

395

396 We provide evidence of the existence of growth, fatness and meat
397 quality QTL in a Landrace commercial population. The existence of QTL in
398 commercial population reveals that they have retained a considerable amount
399 of genetic variation for traits which have been selected for many generations.
400 Our results also imply that the assumption that QTL alleles are fixed in the
401 parental lines of divergent pig crosses is an oversimplification of a complex
402 biological reality, and that genetic progress in highly selected populations is still
403 possible.

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Table 1. Chromosomal regions analysed in the current experiment and carcass and meat quality QTL reported in the scientific literature for these regions.

Chromosome	Growth and carcass QTL	Fatness QTL	Meat quality QTL	Microsatellites
1	21, 27, 25, 15, 3, 23, 12	7, 26, 25, 3, 10, 12, 27	13, 8	S0313-SW2185
2	9, 17, 18, 15, 12, 2	9, 17, 18, 7, 24	9, 7, 2	SW2443-IGF2
3	4, 23, 2		20, 8	SW72-SW2618-S0206
4	1, 14, 2, 21, 22, 12, 30, 16, 31, 3, 11	1, 14, 30, 16, 15, 3, 11	7, 22, 20, 8, 24, 6	S0214-SW35-S0001
6	3, 5, 19	7, 3, 29	7, 6, 29, 19, 20, 8	S0059-S0121
7	27, 25, 18, 28, 16, 15, 3, 23	26, 12, 7, 31, 25, 16, 15, 3, 24, 27	7, 20, 8	TNFB-SW1369
8	2, 23, 3	3	6, 20	SWR1101-SW905
9			8	SWR1848-SW21
10	11		6	SWC19-SW173-S0070
13	1, 32, 33, 3, 11	33	8	SW1056-SW398

1) Andersson et al., 1994, **2)**Andersson-Eklund et al., 1998, **3)** Bidanel et al., 2001, **4)** Casas-Carrillo et al., 1997, **5)** Clamp et al., 1992, **6)** Clop et al., 2003, **7)**de Koning et al., 1999, **8)** de Koning et al., 2001, **9)** Jeon et al., 1999, **10)** Kim et al., 2000, **11)** Knott et al., 1998, **12)** Malek et al., 2001b, **13)** Malek et al., 2001a, **14)** Marklund et al., 1999, **15)** Milan et al., 2002, **16)** Nagamine et al., 2003, **17)** Nezer et al., 1999, **18)** Nezer et al., 2002, **19)** Óvilo et al., 2000, **20)** Óvilo et al., 2002, **21)** Paszek et al., 1999, **22)** Pérez-Enciso et al., 2000a, **23)** Quintanilla et al., 2002, **24)** Rattink et al., 2000, **25)** Rohrer, 2000, **26)** Rohrer and Keele, 1998a, **27)** Rohrer and Keele, 1998b, **28)** Rothschild et al., 1995, **29)** Szyda et al., 2003, **30)** Walling et al., 2000, **31)** Wang et al., 1998, **32)** Yu et al., 1995, **33)** Yu et al., 1999.

Table 2. Bayes factors for growth, fatness and meat quality QTL identified in a commercial Landrace population.

	Chromosome									
Traits[1]	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**	**13**
W	0.814	1.932*	1.267*	0.776	1.004*	0.995	1.057*	1.335*	0.692	1.404*
CW	0.507	6.912**	1.014*	0.586	0.713	0.260	1.633*	1.853*	0.183	0.317
CL	0.748	1.514*	0.218	0.637	0.393	0.145	0.264	1.018*	1.905*	0.716
BFT	0.746	0.485	0.534	1.022*	0.770	0.811	3.890**	6.337**	7.360**	0.417
CaF	0.183	0.609	0.158	0.182	1.151*	0.587	1.591*	0.139	0.185	0.195
LoF	0.259	0.858	0.244	0.415	1.671*	0.567	1.413*	0.202	0.346	0.238
CeF	0.341	0.349	0.199	1.059*	1.192*	0.301	0.336	0.316	0.111	0.283
RiF	0.212	0.246	0.347	2.915*	0.612	5.597**	0.661	0.706	0.173	2.463*
HW	0.136	4.501**	0.684	0.177	0.375	0.204	0.933	0.841	0.223	0.888
SW	0.089	362.81****	0.138	0.978	0.241	0.281	1.373*	1.030*	0.350	0.340
CuW	0.231	3282.3****	0.147	0.791	0.201	0.246	0.501	1.158*	0.304	1.284*
RW	0.497	0.289	1.473	0.278	0.719	0.932	3.132**	1.061*	0.173	0.432
BW	0.381	1.041*	0.874	0.765	0.931	0.407	0.188	1.917*	0.466	1.672*
FC	0.179	1.003*	0.345	0.276	0.854	0.249	0.389	0.715	0.408	0.350
P	0.181	0.599	0.334	0.384	0.507	0.384	0.511	0.605	0.246	0.362
OM	0.236	0.589	0.409	0.402	2.215*	0.251	0.519	0.846	0.864	0.396
DM	0.229	0.643	2.473*	1.488*	0.120	0.146	0.364	0.712	0.190	0.244
SF	0.193	1.426*	1.451*	0.235	0.302	0.160	1.950*	0.264	0.270	0302
MSF	0.342	0.693	1.001*	0.359	0.326	0.244	1.553*	0.240	0.258	0.286

TWS	0.165	0.953	0.324	0.155	0.253	0.176	0.338	0.298	0.354	0.188
Sfi	0.243	231.78****	0.406	0.351	0.940	0.458	0.105	0.772	0.854	0.385
L	0.471	2.529*	0.257	0.310	0.966	0.492	0.227	0.409	0.509	2.245*
L2	0.114	0.738	0.334	0.253	0.322	0.421	0.393	0.733	0.240	0.691
A	1.795*	0.267	0.601	1.238*	0.300	0.360	1.162*	0.400	0.818	0.419
a2	0.755	0.498	0.661	0.897	0.580	1.113*	0.554	1.416*	1.792*	1.433*
b	0.392	1.135*	0.182	0.010	3.547**	4.528**	1.656*	0.397	0.117	0.559
b2	0.908	0.878	0.179	0.483	0.436	0.381	2.077*	0.298	0.324	0.397

**** Probability > 0.990
*** Probability from 0.909 to 0.990
** Probability from 0.762 to 0.909
* Probability from 0.5 to 0.762
[1] Phenotypic traits recorded in this experiment correspond to weight at sacrifice (**W**), carcass weight (**CW**), carcass length (**CL**), backfat thickness (**BFT**), Fat-o-Meter measurement of the carcass (**CaF**) and the loin area (**LoF**), fat thickness in the cervical region (**CeF**) and between the 3rd and 4th ribs (**RiF**), and weight of hams (**HW**), shoulders (**SW**), cutlet (**CuW**), ribs (**RW**) and bacon (**BW**); fat (**FC**), protein (**P**), organic matter (**OM**) and dry matter (**DM**) in a sample of muscle; shear force (**SF**), maximum shear force (**MSF**), total work required to shear the sample (**TWS**) and shear firmness (**SFi**); lightness (**L**), red tendency (**a**), and yellow tendency (**b**) and duplicates (**L2**, **a2** and **b2**).

Títol: Polimorfismo del gen de la malato deshidrogenasa (*MDH1*) porcino.

Autors: Oriol Vidal, Luis Gómez-Raya, Armand Sànchez, Marcel Amills.

Estatus: Resultats parcialment presentats a les Jornades ITEA 2003.

POLIMORFISMO DEL GEN DE LA MALATO DESHIDROGENASA (*MDH1*) PORCINO.

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INTRODUCCIÓN

El enzima malato deshidrogenasa soluble (MDH1) participa en la biosíntesis de los ácidos grasos catalizando la reducción del oxalacetato a malato. Esta reacción, que se acopla a la reacción catalizada por el enzima málico, resulta fundamental en el metabolismo de los ácidos grasos puesto que con la aportación de ambas se genera el poder reductor, en forma de NADPH, necesario para la biosíntesis de los mismos. Además esta ruta bioquímica posibilita la transferencia de acetil-CoA desde la mitocondria al citosol por la vía del sistema de transporte del tricarboxilato.

La MDH1 es un dímero formado por dos subunidades idénticas. Existe otra isoforma denominada MDH2 y localizada a nivel mitocondrial. El gen *MDH1* porcino ha sido mapeado en el cromosoma 3 (Wintero *et al.* 1998) y el cDNA ha sido secuenciado completamente (Trejo *et al.* 1996). En el ratón, el gen *MDH1* tiene un tamaño de 14 kb y posee 9 exones (Setoyama *et al.* 1988).

La finalidad principal de este trabajo ha consistido en secuenciar el cDNA *MDH1* de cerdos de distintas razas con el objetivo de identificar polimorfismos que puedan ser empleados en estudios de asociación con caracteres productivos, especialmente aquellos relacionados con el depósito graso.

MATERIAL Y MÉTODOS

Obtención de cDNA total porcino: Se obtuvo el RNA total a partir de muestras de hígado de cerdos de las razas Pietrain, Large White, Landrace,

Vietnamita e Ibérica. Cada muestra se congeló en nitrógeno líquido y fue pulverizada con un mortero y homogenizada mediante un politrón. El RNA total se purificó mediante el preparado *Trizol reagent* (Gibco BRL, Life Technologies) y la síntesis de cDNA se llevó a cabo mediante el kit *ThermoScript RT-PCR System kit* (Invitrogen S.A.).

Amplificación del cDNA MDH1: Los oligonucleótidos empleados en la reacción de amplificación del cDNA de la *MDH1* fueron MDH2F: 5'-GAG TGC TTG TGA CTG GAG CA-3' y MDH9R: 5'-CAG GCA GAG GAA AGA AAT TCA-3'. El perfil térmico fue de 94 ºC – 1 min, 60 ºC – 1 min, 72 ºC – 2 min durante 35 ciclos. Las condiciones de la reacción de PCR fueron de 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM de cada oligonucleótido, 2 µl de cDNA y 0.5 U de Taq DNA polimerasa (Ecogen) en un volumen final de 20 µl.

Secuenciación del producto amplificado: Los productos amplificados se secuenciaron mediante el *kit ABI PRISM Cycle sequencing kit* (Perkin Elmer Biosystems) utilizando los oligonucleótidos anteriormente citados. Las reacciones de secuenciación fueron analizadas en un aparato de electroforesis capilar *ABI PRISM 310* (Perkin Elmer Biosystems).

Genotipaje del polimorfismo C→T del exón 7 del gen MDH1: un fragmento del gen *MDH1* que incluye parte del exón 6, el intrón 6 y parte del exón 7 se amplificó mediante los oligonucleótidos MDH6F: 5'-TCA TCT GGG GAA ACC AT- y MDH7R: 5'-CTG GGG TTC CAA ACC AGA T-3'. El perfil térmico fue 94 ºC – 1 min, 61 ºC – 1 min, 72 ºC – 2 min durante 35 ciclos. Las

condiciones de la reacción de amplificación fueron 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM de cada oligonucleótido, 30 ng de DNA y 0.5 U de Taq DNA polimerasa (Ecogen) en un volumen final de 20 µl. El producto amplificado fue purificado mediante el *ExoSAP-IT kit* (Amersham Biosciences Europe GMBH) y la mutación fue genotipada mediante el *SnaPshot™ ddNTP Primer Extensión kit* (Applied Biosystems). La secuencia del oligonucleótido usado en la reacción de extensión fue SNP7, 5'-ATA TAC TGC GGC AAA AGC CAT TTG TGA CCA-3'.

Genotipaje de la inserción polimórfica LINE 1 del intrón 6 del gen MDH1: un fragmento del gen *MHD1* que incluye parcialmente el intrón 6 se amplifició mediante los oligonucleótidos MDHi6F: 5'- CCT GGG TGT CTC TCT AAG GGT G -3' y MDHi6R 5'- AGG AAA ACT GCA AAT CCT CAA AGA -3'. Estos oligonucleótidos están situados a ambos lados del elemento LINE, y el genotipaje se puede realizar identificando el tamaño del producto amplificado, de 243 pb cuando no hay inserción y de 977 pb cuando la inserción está presente. El perfil térmico fue 94 ºC – 1 min, 64 ºC – 1 min, 72 ºC – 1 min durante 35 ciclos. Las condiciones de la reacción de amplificación fueron 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM de cada oligonucleótido, 30 ng de DNA y 0.5 U de Taq DNA polimerasa (Ecogen) en un volumen final de 20 µl. Para el genotipaje se corrió el producto amplificado en un gel de agarosa al 2%.

Análisis filogenético: el análisis filogenético de las secuencia *MDH1ψ obtenida* con las secuencias de cerdo, de humano (número de accesso del ENSEMBL ENSG00000019179) y de ratón (número de accesso del ENSEMBL

ENSG00000014641) se ha llevado a cabo con el programa MEGA v2.1 (http://www.megasoftware.net), utilizando la distancia genética de Kimura con dos parámetros (Kimura 1980) para construir un árbol *neighbor-joining*.

RESULTADOS Y DISCUSIÓN

Se amplificó un fragmento del gen *MDH1* de 1 kb que abarca los exones 2 y 9 y se secuenció en diez individuos de distintas razas porcinas. Las secuencias obtenidas se compararon con el programa Multalin (Corpet 1988) y se detectó la presencia de un polimorfismo silencioso $C \rightarrow T_{759}$ en el exón 7 del gen *MDH1* (Tabla 1).

En la reacción de amplificación llevada a cabo con los oligonucleótidos MDH6F y MDH7R, que se diseñaron para el genotipaje del polimorfismo, se amplificaron tres fragmentos de 1.700 pb, 1.400 pb y 700 pb.

El alineamiento de las tres secuencias permitió determinar que los fragmentos de 0.7 kb y 1.4 kb corresponden a una región del gen *MDH1* porcino comprendida entre los exones 6 y 7. La diferencia de tamaño entre ambos fragmentos se explica por la presencia/ausencia de una inserción de 0.7 kb correspondiente a un retrotransposón *long interspersed nucleotide element* (LINE) truncado en 5' (Fig. 1). En otras palabras, la inserción del elemento LINE en el intrón 6 del gen *MDH1* es polimórfica dando lugar a dos alelos, con y sin inserción. Por otra parte, el fragmento de 1.7 kb corresponde a una secuencia que presenta una similitud nucleotídica del 84 % con la secuencia del gen *MDH1* y que posee cuatro inserciones en tándem de *short interspersed nucleotide elements* (SINE) en el intrón 6 (Fig. 1). En este caso, no parece

tratarse de otro alelo del gen *MDH1*, si no de un segundo locus *MDH1* que probablemente corresponda a un pseudogen. A continuación, pasamos a describir con mayor detalle las características estructurales de las secuencias correspondientes a los fragmentos de 1.4 kb y 1.7 kb.

1.- Alelo MDH1 con una inserción LINE (fragmento de 1.4 kb)

El elemento LINE identificado pertenece a la familia L1 tiene 722 pb y está en la posición 262 del intrón 6 del gen *MDH1*. Los elementos LINE son retrotransposones de secuencia larga (de 6 o 7 kb cuando están completas), integradas en el genoma y que forman parte del DNA altamente repetitivo (Fig. 2). Los genomas de los mamíferos tienen dos familias de LINE, la de tipo 1 (o L1) y la de tipo 2 (o L2), de las cuales solo se encuentra activa la L1. Aunque inicialmente se asumía que la familia L1 tenía un origen reciente y que empezó a retrotransponerse después de la radiación de los mamíferos, mas adelante se demostró que las copias L1 del genoma humano son muy antiguas y se insertaron antes de dicha radiación, porque hay elementos ortólogos entre humanos y otras especies de mamíferos (Smit *et al*. 1995).

En cerdo se ha descrito una familia de elementos L1, que se ha denominado L1Ss (Miller 1994), dentro de la cual hemos podido clasificar nuestra secuencia (Fig. 3). El genotipaje de esta inserción mediante un protocolo de PCR con electroforesis ha revelado que el elemento L1 descrito segrega en distintas poblaciones (Tabla 2), indicando un episodio de retrotransposición reciente que antecede a la formación de las distintas razas porcinas.

La inserción de retrotransposones en la secuencia de un gen puede alterar su función Por ejemplo, en perro, una inserción SINE en el intrón 3 del gen del receptor de la hipocretina 2 está asociada a un fenómeno de *exon-skipping* del exón 4 y a la aparición de un cuadro clínico de narcolepsia. Por otra parte, las inserciones constituyen polimorfismos de gran interés ya que permiten un genotipaje rápido y simple basado en la discriminación de los alelos por tamaño y además permiten realizar un análisis de identidad por descendencia, ya que es altamente improbable que dos retrotransposones se inserten en la misma localización genómica por azar

2.- Locus MDH1ψ (fragmento de 1.7 kb)

Tal como hemos comentado, el fragmento de 1.7 kb parece corresponder a un segundo locus *MDH1ψ* parálogo de *MDH1*. Aunque la secuencia identificada es parcial y no permite realizar un análisis filogenético en profundidad, el fragmento secuenciado se agrupa con la secuencia *MDH1* de cerdo (Fig. 4). Además, hemos observado la presencia de una deleción de 2 pb en el exón 7 (codón 78) que desplaza el marco de lectura y provoca la aparición de un codón de parada prematura de la traducción, lo cual indica que se trata de una copia no funcional. La similitud nucleotídica entre *MDH1* y *MDH1ψ* es del 84% si se comparan ambas secuencias excluyendo las distintas inserciones LINE y SINE identificadas (Fig. 5). La similitud con las secuencias *MDH1* funcionales de ratón y humano fue de 63.4% y 63.2%. Si se compara la secuencia *MDH1ψ* con las secuencias parálogas *MDH1* de porcino, ratón y humano excluyendo el intrón 6 (comparación de las secuencias correspondientes a los exones 6 y 7), la similitud nucleotídica es más elevada,

presentando valores del 93%, 87% y 85% respectivamente (Fig. 5). En definitiva, y a pesar de que la secuencia obtenida es sólo parcial, nuestros datos sugieren la existencia de un pseudogen *MDH1*ψ en el genoma porcino que probablemente ha surgido a través de un fenómeno de duplicación del gen *MDH1*. La presencia del intrón 6 en la secuencia *MDH1*ψ parece descartar que se trate de un pseudogen procesado.

Por otra parte cabe destacar que la secuencia *MDH1*ψ presenta 4 inserciones SINE en tándem y localizadas en el intrón 6. Dichas inserciones se han denominado SINE1, SINE2, SINE3 y SINE4 (Fig. 1). En porcino se han descrito tres familias de SINE: los *porcine repetitive element* 1 (PRE-1) (Singer *et al*. 1987) y los *artiodactyl repetitive element* 1 *and* 2 *porcine* (ARE-1P y ARE-2P) (Alexander *et al*. 1995). El análisis Blastn de las 4 inserciones ha permitido determinar que pertenecen a la familia PRE-1. En la Fig 6 se observa el alineamiento de las 4 inserciones SINE1 a 4 y la secuencia del SINE PRE-1 descrita por Singer *et al*. (1987) (Nº de accesso del Genbank Y00104). Puede verse que la similitud nucleotídica es mayor entre SINE1 vs SINE3 y entre SINE2 vs SINE4 que entre cualquiera de las otras combinaciones posibles, lo cual sugiere que la existencia de 4 inserciones consecutivas se debe a la duplicación de un bloque ancestral de dos inserciones en tándem.

Las inserciones LINE o SINE tienden a provocar fenómenos de recombinación desigual o ilegítima, tal como sucede por ejemplo en el caso del gen *KIT*, que en algunos haplotipos presenta dos copias e incluso 3 debido a la recombinación entre dos elementos LINE flanqueantes (Giuffra *et al*. 2002). En el caso de la secuencia *MDH1*ψ, nuestros datos sugieren que a partir de una copia del SINE se adquiere una segunda copia, en tándem, por recombinación

desigual. El grado de divergencia entre estas copias indica que este proceso es anterior a un segundo evento de recombinación desigual, en el cual se adquieren las dos copias adicionales.

En definitiva, en el presente trabajo hemos caracterizado la existencia de dos polimorfismos en el gen *MDH1* porcino y hemos identificado una secuencia, que hemos denominado *MDH1*ψ, compatible con la existencia de un pseudogen *MDH1*ψ en el genoma porcino. El próximo paso consistiría en realizar análisis de asociación entre los polimorfismos encontrados en el gen *MDH1* y caracteres productivos así como el cartografiado del locus *MDH1*ψ mediante un panel de células somáticas híbridas irradiadas.

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Tabla1. Frecuencias alélicas para la mutación identificada, calculada a partir de 10 individuos de cada raza. La posición de la mutación del gen *MDH1* (indicada mediante subíndices) está referida a la secuencia con número de acceso U44846.

GEN	MUTACION	RAZA		
		Large White	Piétrain	Landrace
MDH1	C_{759}	0,63	0,31	0,29
	T_{759}	0,37	0,69	0,71

Tabla 2. Frecuencias alélicas para el polimorfismo generado por la presencia de una inserción de tipo L1Ss. Las frecuencias fueron calculadas en 118 cerdos de las razas Piétrain, Large White, Landrace, Ibérico y Meishan.

Polimorfismo LINE 1		LINE	NO LINE
Raza	Piétrain (N=24)	0,54	0,46
	Large White (N=24)	0,50	0,50
	Landrace (N=24)	0,30	0,70
	Ibérico (N=27)	0,10	0,90
	Meishan (N=19)	0,20	0,80

A

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TCCTCAACTCAGTATCCAGATGTCAACCATGCCAAGGTGAAACTGCAGGCAAAGGAAGTTGGTGTTTATG
AAGCTGTGAAGGATCACAGCTGGCTGAAGGAGAGTTCATCACGGTGAGAAAATCCAGGAGCGCTCTTC
ACAGCCAAACATCAAGAACTCTTAAGAGAAACACACCACCTTACTGACCTGGGTGTCTCTCTAAGGGTGG
TGGGAGGAAACTCAAGGACAGCATCCTTTGATGAGTGCATATTTCTTTTGGGGATCATACTAAGCCAGTC
ATAATTCAGTCTGATCTGTTGTATAGCCATCAAGATTGATTGTTAAAGTGAGAAAAAGCTAAATCGCCTC
CAGTCATTCAGCTGTCTTACAGAGATCCATTTCTTTTTTTTTTTTTTTTTTTTTATNAATATTATTTTC
CCACTGTACAGCAAGGGGGTCAGGTCATCCTTAGATGTATACATTGCAGTTACAGTTTTTTTCCCCACCC
TTTCGTCTGTTGCGACATGAGTATCTAGACATAGTTCTCAATGCTATTCAGCAGGATCTCCTTATAAATC
TATTCTAGGNAGTGTCTGATAAGCCCAAGCTTCCGATCCCTCCCACTCCCTCCCCCTCCCATNAGGNANC
CANAAGTCTCTTCTCCAAGTCCATGATTTCTTTTCTGAGGAGATGTTCATTTGTGCTGGATATTAGATTC
CAGTTATAAGGGATATCANAGGNATTNGTCTTNGTCTTCTGGCTCATTCACTCAATATGAGATTCTCTAG
TTCCATCCATGTTGCTGCAAATGGCATTATGTCATCCTTTTTTATGGCTGAGTAGTATCCCATTGTGTAT
ATATACCACATCTTCCGAATCCAATCATTTGTCGATGGACATTTGGATTGTTTCCATGTCCTGGCTATTG
TGAATAGGGCTGCAATGAACATGAGGGTGCATGTGTCTCTTTTAAGTAGGGCTTTGACCGGATAGATGCC
CAAGAGTGGGATTGCAGGGTCATATGGAAGTTCTATGTATAGATTTCTAAGGTATCTCCAAACTGTTCTC
CATAGTGGCTGTACCAGTTTACATTCCCACCAGCAGTGCAGGAGGGTTCCCTTTAGACATCCATTTCTTT
GTATGAAGTGTGGTAAACTTTCTTTGAGGATTTGCAGTTTTCCTTTTCAGGAGGACTGGCGTTTACAACT
GAAATAGATTAAAAGGCTGACATAGGGTAGTTGCCTGACCTCGGAGGGGACACATTAAGCTGTGGTTTG
CAGTCCTTCACCCCAGGAGTAATACTTCTCTCTCTGCCTGCCCCACCCAGACTGTGCAGCAGCGCGGTGC
TGCTGTCATCAAGGCTCGAAAACTGTCCAGTGCAATGTCTGCGGCAAAAGCCATTTGTGACCACGTCAGA
GAC
```

Secuencia del gen *MDH1* que incluye los exones 6 y 7, el intrón 6 y la inserción LINE L1Ss. En azul están indicadas dos repeticiones directas, tal como es habitual encontrar al principio y al final de los retrotransposones LINE.

B

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TCCTCAACTCAGTATCCAGATGTCAACCATGCCAAGGTGAAACTGCAGGCAAAGGAAGTTGGTGTTTATG
AAGCTGTGAAGGATGACAGCTGGCTCAAGGAGAGATCATCATGGTGAGAAAAATCCGGAGCACTCTTC
ACAACTGAGCATCAAGAACTCTTGAGAGGAGGAGTTCCCTTCGTGGCTCAGTGGTTAACGAACCCAACTA
CGATCCATGAGGATGTGGGTTTGATCCCTGGCCTCTCTCAGTGGGGTAAGGATCTGGTATTGCTGCGAGC
TGTGGTCTAAGTCTCAGACATGGCTCAGATCTCACATTGCTGTGGCTGTGGCATAGGCTGGCAGTGGTAG
CTCCAATGTGACCCCTAGCCTGGGAGCCTCCATGTGCCGTAGGTGCGACCCTAAAACAAAAAGAACTCTT
GAGAGGAGTTCCCATCATGGCTCAGCAGAAACGAATCTGACTGGTATACATGAGGACTCATGTTCAATTC
CTGGTCTCTCTCAGTGGGTTAAGGAATCGGCATTGCCATGAGCTGTGGTGTAGGTCGCAGGCCAGCGGCT
ACAGCTCTGATTAAACCCCTAGCCTGGGAATATCCATATACCACTAACGCAGCCCTAAAAAGACAAAAAA
CAAACAAAAGAACTCTTGAAAGACACACCACCTTAGTGACCTGGGTGTCTCTCTAAGGAGGAAACTCAA
GGATAGCAGCCTTTGATATGATCCTTTGGGGATCATATTAATCCAGTTATAATCCAGTCTGATCTGTTGT
GTGGCCATCAAGATTGATTGTTAGGGAGTTCCCGTCATGACTCAGTGGGTTACAAAACCAACTAGTATTC
ATAGAATGCAGGTTAGATCTTTGGCCTCACTCAGTGGGTTAAGGATTTAGCATTGCTGTGAGCTATGGTA
TAGGTTGCAGAAGAGGCTCAGATCCCAAGTTGTGGTGGCTCTGACATAGGCCAACAGCTCCAGCTCCAAT
TTGACCCCTAGCCTGGGAACTTACATTTGCCACAGGTGTAGCCCTAAAAAAAATAAAATAAAAAGGCAAA
AAAAAAAACAAAAACAAAAAACAAAAAACAAAACGGAGTTCCCATCGTGGCACAGCAGAAACAAATCTGA
CTAGGAACCATGAGGTTGTGGGTTCGATCCCTGGCCTCACTCAGTGGGTTGGGGTTCTGGCATTGCTGTG
AGCTGTGGTGTAGGTCGCAGTCGAGGTTCGGATCTGGCGTTGCTGTGGCTGTGGTTTAGACTGGCAGCTG
TAGCTCTGATTCGACCCCTAGCCTGGGAACCTCCATGTGCCGCGGGTGCAGCCCCCAAAAAAAGGACAAA
AAGACAAATAAAATAAAATAAAATAAATAAAAATAAAAAGGGTTTGTTAAAGTGGAAAAAAACTAGATC
GCCTCTAGTCATCCAGCTATCTTACAGAGATTCATTTCTTTGTATGAAGTGTGGATTTGTATTTCCCTTT
GCAAGAAGACTGGAGCTTACAAGTGAAATAAATTTAATAAGGCCACATGAGGTAGTTGCGTGATTTCAGA
GGGGACACATTAAGCCATGATTGTGGCCTTTCTCCCCAGGAGTAGCAATGCTCCCTCTGCCTGCCAAACC
CACACTGTGCAGCAGTGCGATGCTGTTGTCATCAAGGCTCGAAAACCGTCCAGTGCAATGTCTGCAAAAA
GCCATCTGTCACCACATCACAGAC
```

Secuencia *MDH1Ψ* que incluye los exones 6 y 7, el intrón 6 y 4 inserciones SINE en tándem, de la familia PRE-1.

Figura 1. Secuencia parcial y estructura de (A) fragmento de 1,4 kb correspondiente a un alelo del gen *MDH1* con una inserción LINE en el intrón 6 y (B) fragmento de 1,7 kb correspondiente a un pseudogen *MDH1* con 4 inserciones SINE en el intrón 6.

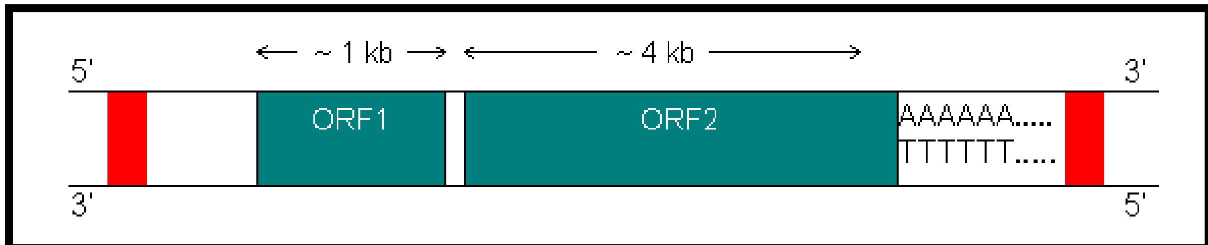

Figura 2. Organización básica de los LINE. ORF1 y ORF2 son *open reading frames*. La proteína producto del ORF1 se denomina p40, y es una *DNA binding protein*. El ORF2 codifica una transcriptasa reversa y una endonucleasa, necesarias para la retrotransposición de la secuencia. Las regiones de color rojo son repeticiones directas producidas durante el proceso de inserción. En la especie humana de 60 a 100 elementos L1 directores tienen la secuencia entera y contienen un promotor de la RNA polimerasa II. Son elementos capaces de producir transcriptasa reversa activa, responsable de nuevas retrotransposiciones.

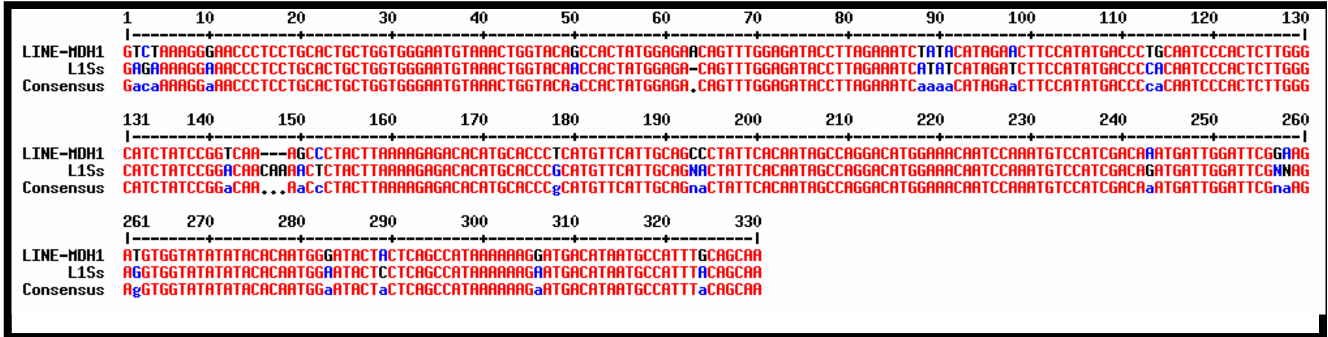

Figura 3. Alineamiento entre un fragmento del elemento LINE-L1 identificado en el intrón 6 del gen *MDH1* y la secuencia del elemento LINE L1Ss descrito por Miller (1994, número de acceso X15411).

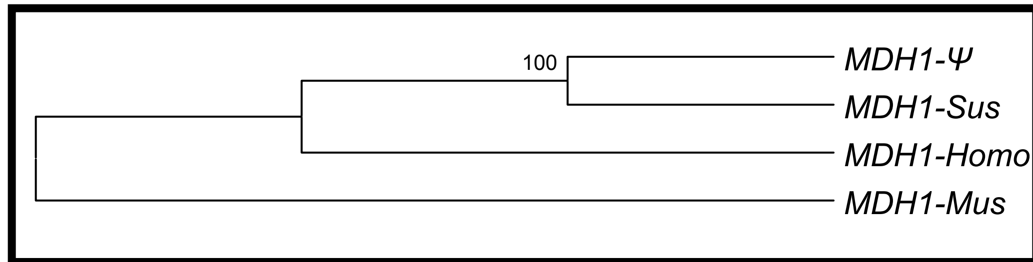

Figura 4. Análisis filogenético de un fragmento de la secuencia *MDH1Ψ* (exón 6 al 7) con las secuencias *MDH1* de cerdo, humano (número de acceso del ENSEMBL ENSG00000014641) y ratón (número de acceso del ENSEMBL ENSMUSG00000019179).

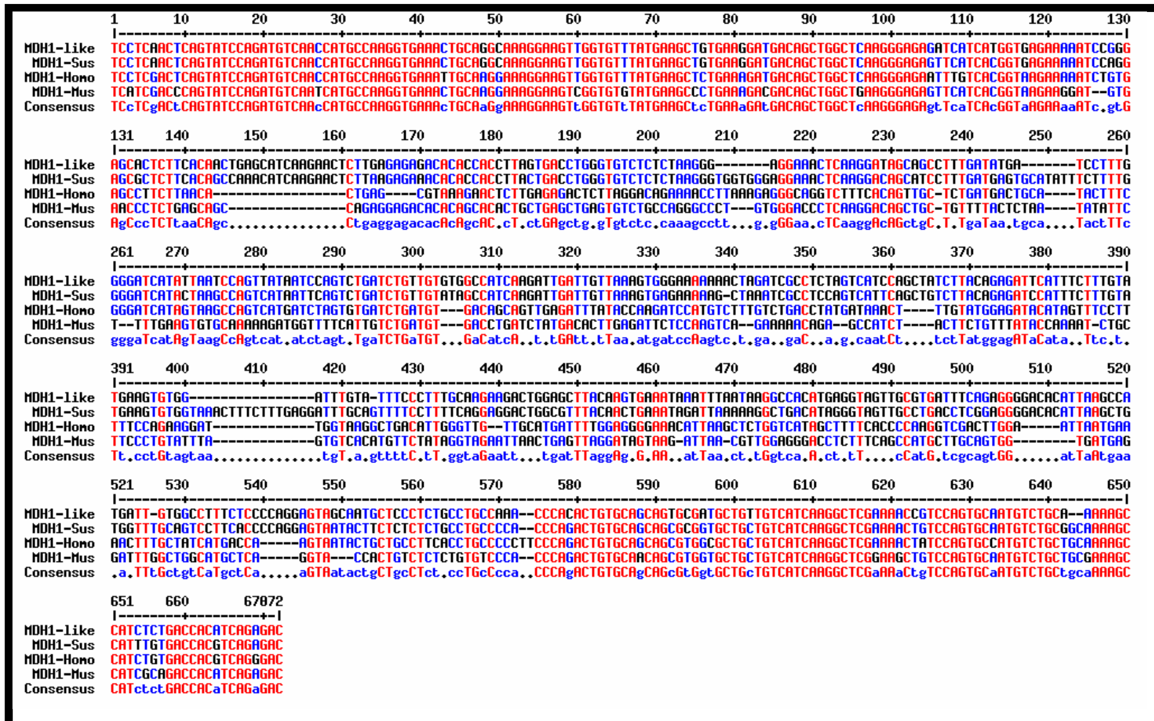

Figura 5. Alineamiento de diversas secuencias correspondientes al gen *MDH1* porcino (exón 6 al 7), humano (número de acceso del ENSEMBL ENSG00000014641) y murino (número de acceso del ENSEMBLENSMUSG00000019179) y al pseudogen *MDH1Ψ* porcino. En las secuencias porcinas se han excluido los elementos repetitivos para maximizar el alineamiento.

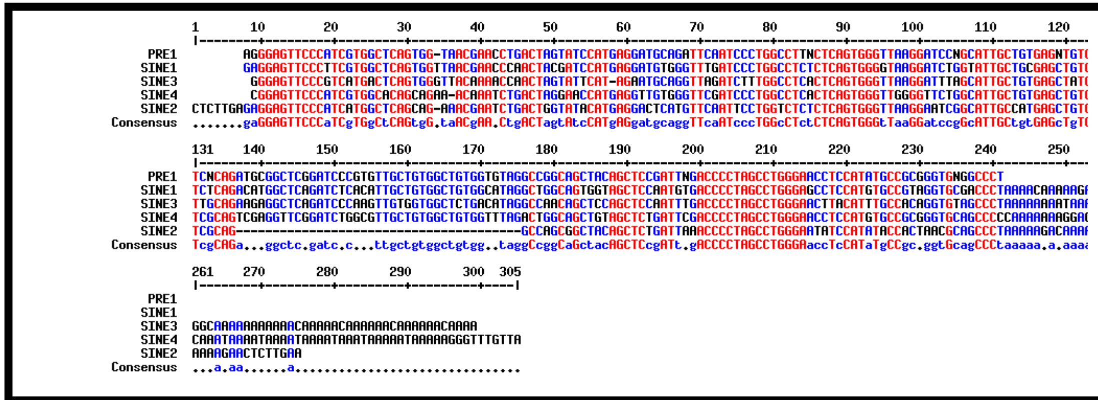

Figura 6. Alineamiento las 4 inserciones SINE en tándem identificadas en el intrón 6 del pseudogen *MDH1Ψ* porcino (SINE 1 a 4) con la secuencia de un SINE PRE-1 (número de acceso Y00104) descrita por Singer y col. (1987).