

Is heterozygotie at the “Gait Keeper” Gene an Advantage for the Trotteur Français ?

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ABSTRACT: The objective of this study was to determine the impact of DMRT3 genotype on Trotteur Français race performances. A sample of 630 genotyped horses by Illumina 50K was used. Qualification, earnings, starts, number of breaking stride races, and best records were studied. These performances were corrected by fixed environmental effects estimated on a complete data of French races including 173,176 horses. Multiple traits mixed model analysis was then used to estimate genotype effect. Selection plan was modeled to verify if selection itself may explain differences among genotypes and to understand why the TF is a rare race breed with DMRT3 polymorphism. Results show that the CC genotype is unfavorable for all race traits. But CA even if unfavorable for qualification and early earnings had an advantage for earning after 5 years (+0.41 σ_v), and this advantage cannot be explained only by selection (less than 19% overestimated).

Keywords: Horse; trot; DMRT3; SNP

Introduction

In 2012, Andersson et al. published in Nature the evidence of a SNP mutation in a major gene DMRT3 playing a role in mammals locomotion. The allele A instead of C advantages the gaited horses. This allele is fixed in standardbred, the American breed specialized in trotting and pacing races whereas it is still polymorphic in French trotters (Promerova et al. (2014)). The objective of this study is to analyze the genotype effect on performances in French races.

Materials and Methods

Performance data. All racing results from 1996 to 2011 for horses born between 1994 and 2008 (173,176 horses) were provided by the Société d'Encouragement à l'Élevage du Cheval Français, including annual earning, number of starts and number of breaking stride, life best time per kilometer and qualification test results. Genealogy was provided by IFCE (394,367 ancestors).

Genotype data. Genotyping was performed using Illumina Equine SNP50 Bechip. The sample included 630 horses (61 sires) and was the one used for the study of osteochondrosis (Teyssedre et al. (2012)). The genotype for the SNP BIEC2-620109 was used to infer the genotype at the gene mutation ($r^2=0.91$, Promerova et al. (2014)). For clarity, we called the genotype from the alleles names at the mutation rather than at the SNP (A correspond to T and C

to C). The frequency of allele C is 24.2%. For GWAS, after quality tests, 41711 SNP were retained.

Methods. The race traits were first analyzed on the whole data set to evaluate correctly genetic parameters and fixed environmental effects. The performances were continuous variables as logarithm of earning divided by the number of finished races at 2, 3, 4 and between 5 and 10 years (LAE), the number of starts at 2, 3, 4 and between 5 and 10 years (probit transformation), the best time per kilometer, the age at qualification test and binary variables as qualification test result, breaking stride in each race (0/1). The model included the fixed variables of birth year combined to gender (30 levels), and, for best time, the race track and age. The random effect included animal polygenic effect and eventually a random permanent environmental effect. Multiple trait analysis was performed. The TM software was used (Legarra and al., (2011)).

Then, to estimate SNP effect, a model was used on the genotyped sample only. The performances were pre-corrected according to the estimates of fixed effect obtained on the whole data set. The model included the fixed SNP genotype effect and a random polygenic value (5699 horses in pedigree). The genetic parameters were fixed to the estimated values obtained with the whole data set. Multiple traits were used similarly to the analysis on the whole data set ASREML software was used (Gilmour et al., (2006)). In addition, GWAS was performed using the same model with single trait analysis.

Modeling the selection process. We have put all effort to obtain unbiased estimates of genotype effect. Nevertheless, the sample is not a random sample of the population (mean performance deviates from the mean population about $\frac{3}{4}\sigma_v$) and more, selection effects may occur on performances not available for the evaluation and so not taken into account in the mixed model. A hypothesis may be that better performances observed for heterozygote horses (see results) may be due to a more permissive access to races for horses with high potentials than for horses with lower potential. More, how can we explain the polymorphism for a gene such important for race results in a specialized breed since more than one century? So, we modeled the selection scheme of Trotteur Français according to 3 hypotheses: 1) the genotype effect is as estimated (REAL), 2) the effect of AC is similar to the effect of CC (HOMO), 3) there was no genotype effects (NULL). The selection scheme included 2 steps: 1) qualification to access races (with the threshold

eventually depending on trotting ability) 2) for stallions, racing performances. A deterministic model was used.

Results and Discussion

Genetic parameters. Table 1 and 2 show the genetic parameters of most variables. For harness racing, qualification was found to be the trait with the highest heritability (0.56). The heritability of earnings was moderate and stable across age groups (0.26-0.31). Disqualification showed low heritability (0.06-0.09) and average repeatability (0.16-0.25). The genetic correlation between qualification and earnings was positive but moderate (0.44-0.62). For a given year, the different components of annual earnings were interconnected differently depending on the age group. For 3- and 4-year-olds, the earnings per finished race were negatively correlated (from -0.10 to -0.22) with the number of starts and the proportion of finished races. All the environmental correlations were strongly positive (0.32-0.64). From 5 to 10 years of age, the earnings per finished race and the proportion of finished races remained genetically (Table 2) slightly opposed (-0.19). However, because the traits were now considered over several years of operational life, the genetic ability to earn more per finished race was strongly linked to the number of starts (0.69) which demonstrates that the better a horse is then the longer its racing life is. Environmental correlations were very positive (0.45-0.93).

Table 1. Genetic parameters[§] for harness racing performances and qualification (Q).

Trait	Log(earnings/number of finished races)				Q
	2 years	3 years	4 years	5 years	
2 years	0.28 (0.01)	0.85 (0.02)	0.76 (0.02)	0.56 (0.02)	0.48 (0.03)
3 years	0.29 (0.01)	0.32 (0.01)	0.91 (0.01)	0.81 (0.02)	0.61 (0.01)
4 years	0.12 (0.02)	0.27 (0.01)	0.25 (0.01)	0.92 (0.01)	0.47 (0.01)
5 years	0.14 (0.02)	0.23 (0.01)	0.41 (0.01)	0.26 (0.02)	0.44 (0.02)
Q	0.00 (*)	0.00 (*)	0.00 (*)	0.00 (*)	0.56 (0.01)

[†]Fixed correlations.

[§]Heritability (diagonal), genetic correlation (upper triangle) residual correlation (lower triangle)

Table 2. Genetic parameters[§] for Log(earnings per finished race (LAE), number of starts (S) and proportion of finished races (F) at 5 years

Trait	LAE	S	F
LAE	0.25 (0.01)	0.69 (0.03)	-0.19 (0.05)
S	0.93 (0.02)	0.12 (0.01)	0.12 (0.05)
F	0.45 (0.03)	0.65 (0.01)	0.07 (0.01)

[§]Heritability (diagonal), genetic correlation (upper triangle) permanent environmental effect correlation (lower triangle)

Genotype effect at BIEC2-620109. The estimated genotype effects are shown in figures 1 and 2. In addition, the genotype effect did not have a significant impact on the best life recorded time, whatever the genotype. A signifi-

cant effect was observed on the age at qualification: 0.88 σ_y for the CC genotype and 0.24 σ_y for the AC genotype, i.e. 167 and 45 days later respectively.

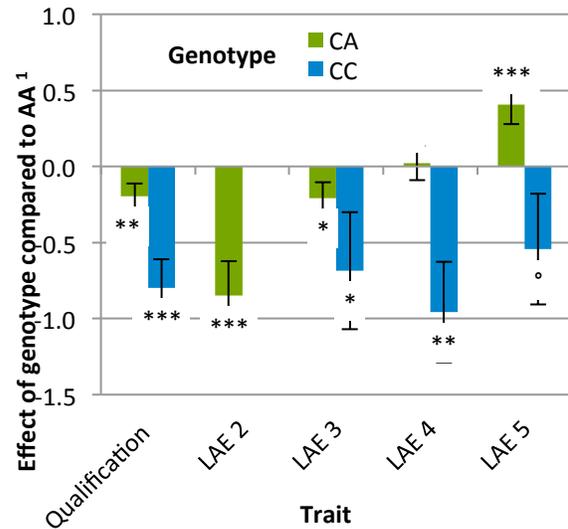


Figure 1. Effect of the genotype at SNP BIEC2-620109 on LAE at 2, 3, 4 and \geq 5 years with Harness, and qualification in deviation from the AA genotype

¹in phenotypic standard deviation units

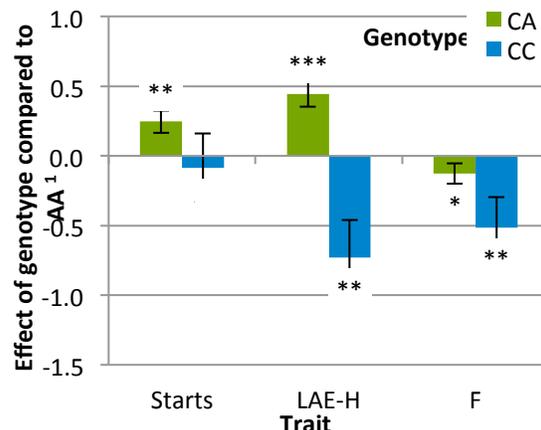


Figure 2. Effect of the genotype at SNP BIEC2-620109 on LAE, the number of starts (S) and the proportion of finished races (F) at 5 years in deviation from the AA.

The CC genotype had an extremely negative effect on all racing performance criteria, from qualification and earnings to the proportion of finished races. For heterozygous C allele carriers, the genotype effect is not always unfavorable. Qualification and early earnings were impacted with lower LAE at 2 years and, to a lesser extent, at 3 years. However, any differences disappeared at 4 years and at \geq 5 years the LAE of CA horses was actually higher than for AA horses (+0.41 σ_y). By contrast, the likelihood of being disqualified remained higher for CA horses, whatever their age, with 35% disqualified at 3 years and 27% at \geq 5 years, compared with 28% and 23% respectively for AA horses. On the other hand, whereas the median number of

starts for the whole population was 16, it was of over 22 for CA horses who had longer careers (more than 6 months longer) although they enter fewer races at 3 and 4 years.

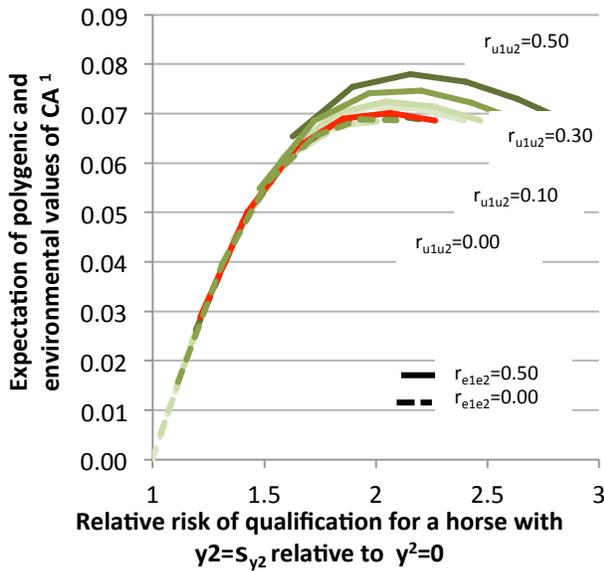


Figure 3. Expected racing performances (2) of CA horses in deviation from the AA after qualification (1) as a function of selection on easier qualification for horses assumed to have good racing performances (y^2)[§]

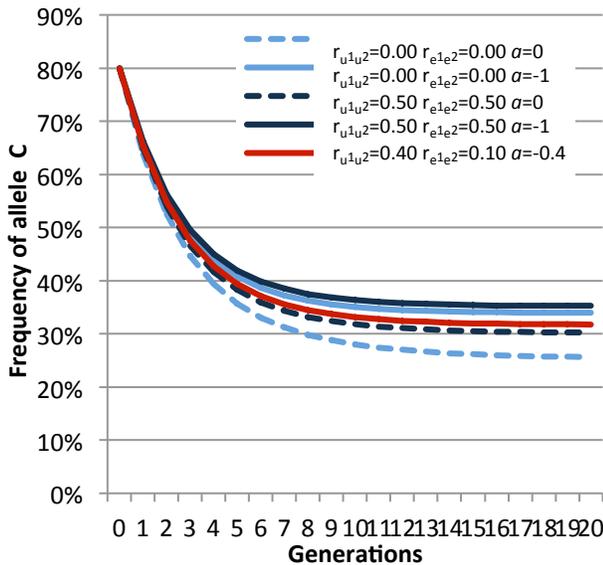


Figure 4. Variation of the frequency of allele C as a function over generations with different hypotheses for correlations between qualification (1) and racing performances (2) in REAL scenario (see text).[§]

[§] $r_{e^1e^2}$ residual correlation between (1) and (2), $r_{u^1u^2}$ genetic correlation between (1) and (2). Red line: parameters close to those estimated ($r_{e^1e^2}=0.10$, $r_{u^1u^2}=0.40$).

Modeling the selection process. The first results addressed the expected racing performances for all 3 genotypes following selection on qualification. Because of the negative effect of their genotype, CC horses had a better

polygenic value for qualification than CA or even AA horses. If the traits are genetically correlated, this difference for qualification would lead to a difference in racing performances. The same applies to the mean environmental effect. The maximal advantage for racing performances was 0.280 for CC and 0.078 for CA. It therefore appears that the polygenic and environmental differences obtained by selection do not suffice to explain the differences in performance and a direct effect of the genotype must be assumed. The direct effect would be extremely negative for CC (to overcome the 0.280 positive effect). For CA heterozygotes, the superiority due to polygenic and environmental effects only amounted to 19% of the observed superiority (0.078 vs. 0.405) so a direct positive effect, although less strong, should be assumed. Over generations, in the REAL scenario (figure 9), the frequency of the C allele decreased but leveled out at an intermediate frequency of between 25.6% and 35.2%. This scenario explains why the SNP is still polymorphic. The other scenarios lead to a decrease very sensitive to genetic parameters (HOMO) or a rapid extinction of the C allele (NULL).

GWAS analysis. Genome-wide analysis revealed 4 sets of SNPs above the significance threshold for qualification on chromosomes 11 and 14 (P-value = 7.10^{-5}), and for earnings on chromosome 3 and 18 (figure 13, P-values = 9.10^{-7} and 9.10^{-5}). These P-values are lower than those obtained for racing ability with SNP BIEC2-620109.

Conclusion

The identification of the DMRT3 gene and its major effect was a great achievement in the field of horse breeding and racing. The A allele of the mutation has been selected over the C allele in main trotting breeds because of its essential role in ambling. It was therefore surprising to discover a polymorphism in a breed such as French trotters. Based on the genotypes of 630 French trotters at a SNP associated with the DMRT3 mutation, we showed that all racing related traits were negatively affected in homozygous C allele carriers but that this was not the case for AC heterozygotes. The latter horses performed slightly less well at the qualification race but in the end achieved better results than AA in the French racing circuit which has the particularity of being based on the performances of more mature horses and also racing under saddle. This superiority of the AC heterozygote most probably explains why the polymorphism still exists in French trotters as well as the existence of other potential QTLs.

Literature Cited

Andersson, L. S., Larhammar, M., Memic? F. et al. (2012). Nature 488:642-646.
 Gilmour, A. R., Gojel, B. J., Cullis, B. R. et al (2006). ASReml User Guide Release 2.0. VSN International, UK.
 Legarra, A., Varona, L., Lopez de Maturana, E. (2011). <http://snp.toulouse.inra.fr/~alegarra>
 Promerova, M., Andersson, L. S., Juras, R. et al. (2014). Anim. Genet. Doi: 10.1111/age.12120.
 Teyssedre S., Dupuis M. C., Guerin G., et al. (2012). J. Anim. Sci. 90:45-53