PIGMEAT QUALITY : EXPERIMENTAL STUDY ON THE *RN* MAJOR LOCUS

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SUMMARY

This report gives preliminary results of an experimental investigation on the *RN* locus influencing technological quality of meat in pigs. Further evidence is given on the existence of this major gene, its effect on the "Napole" technological yield (RTN) and the relationship between RTN and muscle glycolytic potential.

INTRODUCTION

In 1986, Naveau studying a new meat quality measurement, the so-called "Napole" technological yield (RTN) (Naveau *et al.*, 1985), suggested the existence of the *RN* major locus affecting meat quality. A segregation analysis of RTN records collected in two commercial lines confirmed the rejection of the polygenic inheritance hypothesis when tested against a mixed genetic hypothesis (Le Roy *et al.*, 1990). Maximum likelihood estimate of the deviation between the means of homozygotes reached about 3 phenotypic standard deviations, the unfavourable allele RN^-, responsible for low RTN, being totally dominant over the normal one rn^+.

However, these results obtained after statistical analysis of field data needed to be confirmed. So, a multipurpose experiment was initiated in 1990 by INRA in order to add evidences about the existence of this major gene, to estimate its effects on productivity, to create homozygous RN^-RN^- and rn^+rn^+ lines for further research, and to search for marker genes of the *RN* locus. A presentation is made in this paper of the experimental design and analysis of its first results.

EXPERIMENTAL DESIGN

Overall description

The animals are originating from the Laconie line, created by the Pen ar Lan breeding company. This synthetic line selected since 1973 was founded on with Hampshire, Piétrain and Large White breeds in equal proportions. It is one of the populations in which the *RN* locus was discovered (Le Roy *et al.*, 1990). The experimental animals were bred at Le Magneraud INRA farm located near Surgères (Charente Maritime).

Two subgroups were initially created : a tester group expected to be homozygous rn^+rn^+ and a tested group where both alleles RN^- and rn^+ are segregating. The protocol comprised 3 steps. In 1990 and 1991, expected heterozygous animals born from presumably homozygous RN^-RN^- and rn^+rn^+ parents were intercrossed to produce a segregating population of RN^-RN^-, RN^-rn^+ and rn^+rn^+ individuals sharing similar polygenic background. In 1992 and 1993, males and females from this segregating population were progeny tested using the tester group in the view of determining their genotype. In 1994 and 1995, offspring from proven homozygous parents will be produced in a "diallel" cross for accurately comparing the 3 *RN* genotypes.

This multigenerational design offered the opportunity of testing again the major gene hypothesis in a "planified" situation and produced informative families for marker identification (offspring of RN^-rn^+ by rn^+rn^+). As regards the third step, the preliminaries were needed for two reasons. The first was to maximise, using a progeny test, our confidence in the homozygosity of the third-step parents. The second was to avoid any confusion between mean polygenic value and major locus genotype, the RN^-RN^- and rn^+rn^+ animals mated in the final step sharing the same parents.

First step

The tester and tested lines were respectively produced from 4 rn^+rn^+ and 5 RN^-RN^- sows who were mated to 6 rn^+rn^+ boars and transferred from the Pen ar Lan farm to Le Magneraud. These 15 animals were chosen in the Laconie population after analysis of their progeny measured on RTN. The genotypes probabilities of these animals were estimated using a simplified segregation analysis as described by Elsen and Le Roy (1989). Note that the 2 alleles RN^- and rn^+ were supposed to be segregating in both sexes in this analysis. The consistency of predicted genotypes of parents, mates and grand parents was checked before the final choice.

The tester line was founded on 4 males and 8 females, coming from the 4 rn^+rn^+ females, who gave 5 and 34 tester males and females. The tested line was founded on the RN^-rn^+ offspring of the 5 RN^-RN^- sows (6 and 19 heterozygous sires and dams) who gave birth to the males and females subsequently submitted to progeny test.

Second step

Assuming that the 6 boars and 19 sows founders were heterozygous, the 1/4, 1/2, 1/4 Mendelian proportions of *RN-RN-*, *RN-rn+* and *rn+rn+* were expected in their offspring. However, only a small part of these offspring was progeny tested (16 sons and 43 daughters). In order to avoid a random loss of homozygous individuals, a preselection of the progeny tested animals was performed based on an individual *in vivo* measurement of muscle glycolytic potential (GP) (Monin and Sellier, 1985). The choice of this criterion is based on a number of observations (Fernandez and Tornberg, 1991; Wassmuth and Glodek, 1992) indicating that the *RN-* carriers show a large increase in GP of "white" muscles (Fernandez *et al.*, 1990; Le Roy *et al.*, 1994).

METHODS

Biological methods

The Napole technological yield (RTN) was measured as described by Naveau *et al.* (1985). The RTN is an indicator of technological yield of cured-cooked ham processing estimated on a 100g sample of *semi membranosus* muscle removed from the carcass. Note that a slight change was introduced as compared with the initial method, the sample being taken 24h after slaughter and not on the slaughter line.

The muscle glycolytic potential (GP) was measured on *longissimus dorsi* sample taken *in vivo* (Talmant *et al.*, 1989). The GP is the sum of the main compounds likely to produce lactic acid *post mortem* and was determinated using enzymatic methods (Monin and Sellier, 1985).

Statistical methods

A segregation analysis was performed on the progeny-test data (2nd step) following Elston and Stewart (1971). Based on maximum likelihood techniques, it estimates genetic and environmental effects under the hypotheses of polygenic or mixed (major gene + polygenes) inheritances, and tests the polygenic inheritance (H_0) against the mixed one (H_1).

Under the general hypothesis H_1, the likelihood was written as :

$$M_1 = \prod_{i=1}^{n} \sum_{s_i=1}^{3} p_{s_i} \int_{u_i} f(u_i) \prod_{j=1}^{m_i} \int_{v_{ij}} g(v_{ij}) \prod_{k=1}^{l_{ij}} \sum_{r_{ijk}=1}^{3} P(R_{ijk} = r_{ijk} / s_i) h_{r_{ijk}} (y_{ijk} / u_i, v_{ij})$$

where y_{ijk} is the RTN of the k^{th} progeny of the j^{th} mate of the i^{th} parent; n is the number of tested parents; m_i is the number of mates of the i^{th} parent; l_{ij} is the number of progeny of the ij^{th} family; s_i and r_{ijk} are the major locus genotype of the i^{th} tested parent and its jk^{th} progeny; p_s is the probability of the genotype s in the parental population and P(R=r/s) is 1, 1/2 or 0 depending on s and r; u_i is the i parental effect; v_{ij} is the ij mate effect; f, g and h_r are normal densities $N(0,\sigma_u^2)$, $N(0,\sigma_v^2)$ and $N(\mu_r,\sigma_e^2)$ respectively. A sex effect was included in the h density (females or castrates).

It must be noted that the tested parents are males or females. Consequently, the effect u_i covers simultaneously the polygenic value (for males and females) and the permanent non genetic maternal effect (for females). In the same way, the effect v_{ij} comprises the polygenic plus the whole environmental dam effects when considering tested sows, the polygenic sire effect plus the litter effect when considering tested boars.

Assuming complete dominance at the *RN* locus and homozygosity of the tester mates, 8 parameters were estimated under H_1 (2 means, 3 variances, 2 frequencies and a sex effect) against 5 under H_0 (1 mean, 3 variances and a sex effect). Following Wolfe (1971), the likelihood ratio test $£=-2Ln(M_0/M_1)$ is distributed under H_0 as a χ_6^2. The likelihoods were maximised using a quasi Newton algorithm of the NAG Fortran Library (E04JBF).

RESULTS

Until now, a total of 905 progeny from 50 tested parents were recorded for the RTN. The table 1 summarizes the segregation analysis results. The likelihood ratio £ widely exceeds 16.8, the 1% threshold of the χ_6^2, and the polygenic inheritance is rejected. The major gene effect (7.4 points) is similar to the previous estimate of 8.3 points found in the model of complete dominance tested by Le Roy *et al.* (1990). However, the 2 estimated means $\mu 1$ and $\mu 3$ are lower than those obtained in the above study. We hypothesize that the change in the time of muscle sampling (24h after slaughter) could explain this difference. The sex effect is close to the previous estimate although it concerns the comparison between females and castrates and not between females and entire males. The residual variance is also similar to the previous estimate but a high value is obtained for the σ_u^2 variance. This result is probably due to ambiguous definition of the parental effect u_i as described above.

Table 1 : Results of segregation analysis : ML estimates of the parameters *

Hypothesis	H_0 (polygenic model)	H_1 (mixed model)
μ_0	84.60	
μ_1 (RN-RN- and RN-m+)		81.79
μ_3 (m+m+)		89.21
σ_e (residual)	3.82	2.99
σ_u (parent)	2.74	1.27
σ_v (mate)	1.73	1.64
p_1 (RN-RN-)		0.36
p_2 (RN-m+)		0.38
sex (female)	0.48	0.42

*Likelihood ratio : £ = 72.5***

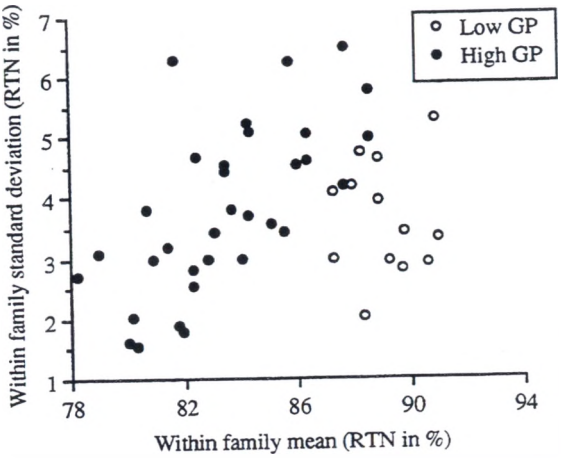
Table 2 : Results of the progeny test

GP	Characteristics of the family			Genotype probability		GP	Characteristics of the family			Genotype probability	
	N	Mean	Std	-/-	-/+		N	Mean	Std	-/-	-/+
Males											
159	25	87.2	3.0	0.01	0.00	353	16	85.6	3.4	0.33	0.39
173	20	90.9	5.3	0.00	0.32	365	10	80.3	1.6	1.00	0.00
178	19	88.3	4.7	0.00	0.27	366	19	80.2	2.0	1.00	0.00
179	18	87.3	4.1	0.00	0.97	370	15	84.3	5.2	0.00	1.00
188	16	91.0	3.3	0.00	0.01	377	18	85.7	6.2	0.00	1.00
342	28	80.7	3.8	0.81	0.19	380	14	80.0	1.6	1.00	0.00
342	27	82.5	4.7	0.03	0.97	390	16	80.9	3.0	1.00	0.00
349	26	81.7	6.3	0.00	1.00						
Females											
161	15	89.2	3.0	0.00	0.01	321	19	83.1	3.4	0.89	0.10
170	25	88.0	4.2	0.00	0.12	322	24	86.3	5.1	0.00	1.00
182	11	88.3	2.0	0.00	0.00	323	23	83.4	4.4	0.02	0.98
183	21	88.9	4.6	0.00	0.02	323	16	82.3	2.5	1.00	0.00
183	13	90.5	2.9	0.00	0.01	329	18	88.5	5.7	0.00	1.00
193	16	89.7	3.4	0.00	0.02	334	14	83.7	3.8	0.33	0.66
195	13	88.8	3.9	0.00	0.02	336	17	78.3	2.7	1.00	0.00
201	16	89.7	2.8	0.00	0.00	348	21	85.1	3.6	0.38	0.47
302	20	83.5	4.5	0.04	0.96	362	21	87.7	6.5	0.00	1.00
306	25	81.8	1.9	1.00	0.00	365	13	84.3	3.7	0.49	0.49
309	21	84.4	5.1	0.00	1.00	381	20	82.8	3.0	0.99	0.00
315	15	88.5	5.0	0.00	0.65	383	21	86.0	4.5	0.00	1.00
316	22	87.6	4.2	0.00	0.97	384	21	79.0	3.1	1.00	0.00
316	19	82.0	1.8	1.00	0.00	431	24	81.4	3.2	1.00	0.00
317	21	86.4	4.6	0.00	1.00	434	20	84.0	3.0	0.96	0.03
317	23	82.3	2.8	1.00	0.00						

GP=glycolytic potential of the tested parent (in µmol/g); N=number of progeny in the family; Mean and Std=within family RTN mean and standard deviation (in %); -/-=*RN-RN-* and -/+=*RN-rn+*

Detailed results of the progeny test are given in table 2 for parents tested on more than 10 progeny. The genotype probabilities were calculated as described by Ott (1974). The classification is most often clear and easily explained by the characteristics of the offspring RTN distributions : high mean and small variance for *m+m+* parents, low mean and small variance for *RN-RN-* parents, intermediate mean and high variance for *RN-rn+* parents. A representation of these tendencies is given in the figure 1.

Figure 1 : Relationship between the within-family means and standard deviations



The Mendelian proportions were not respected in the tested parents, with 36% of RN^-RN^-, 38% of RN^-rn^+ and 26% of rn^+rn^+ (deviation significant at the 5% level). The preselection of parents on their own GP can be incriminated. Assuming that RN^- is not fully dominant on rn^+ for GP, extreme animals may have been retained after this preselection, with a correlated increase in the proportion of RN^-RN^- versus RN^-rn^+. Another explanation could be false genotyping for some founders animals of the tester or tested group.

The correspondance between muscle glycogen content and RTN classification was perfect but for one male (#4) without clear explanation of this discrepancy.

CONCLUSIONS

On the whole these progeny test results added new evidence for the segregation of 2 alleles at a major locus influencing technological yield of pigmeat. The preliminary analyses presented were done in a simplified way, in particular for the description of the genealogical structure of the population. Indeed, the tested parents were assumed to be unrelated and the repetition of some of the tester mates was neglected. A more complete analysis accounting for the true genealogical structure and including the progeny test results of the tester line will be performed when all data will be available.

With the progeny test of heterozygous individuals, a reference population for the mapping of the *RN* locus was generated. After a strict choice of progeny on their genotype, we got 10 informative families with a minimum of 10 progeny per heterozygous parent. The expected identification of a marker gene will definitely prove the major gene hypothesis and will give a valuable tool for further research and practical applications.

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