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Is the porcine *RN* locus a pleiotropic QTL? A Bayesian marker assisted segregation analysis

Norbert Reinsch*, Christian Looft, Iris Rudat, Ernst Kalm

Institut für Tierzucht und Tierhaltung der Christian-Albrechts-Universität zu Kiel, Olshausenstraße 40, 24098 Kiel, Germany

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Abstract – The question of possible pleiotropic effects of the porcine *RN* locus on production traits was addressed. Chromosome 15 microsatellite data, mapping results and performance data from the Kiel *RN* mapping experiment were used to compare heterozygous RN^-/rn^+ carrier animals and rn^+/rn^+ non-carriers. Progenies from 14 Hampshire × Piétrain sires, all of which were heterozygous at both the *RN* and *RYR1* loci, were recorded for 24 traits. Dams were either Landrace Large White crossbreds or Landrace or Large White purebreds. The number of progeny with valid records ranged from 323 to 418, depending on the trait. A mixed polygenic-major gene inheritance model was applied, using a Bayesian approach. Reflectance and terminal pH were lowered in carriers. Additionally, a 2.42 mm increase in muscle depth in the loin, an enlargement of loin eye area by 1.39 cm^2 and a 0.46 % increase in estimated lean meat content due to the RN^- allele were observed. It was shown that the increased lean meat content was not high enough to outweigh the economic loss from poor technological yield. The proportion of genetic variance in progeny which could be attributed to the RN locus ranged from 5 to 55 %. More than 40 % of the total genetic variance in eye muscle depth was generated by the joint action of *RN* and *RYR1*. This is discussed with respect to uniformity of end products in a crossbreeding scheme. The results suggest that not only loci which are involved in glycogen metabolism, but also those which are related to muscular development, may be regarded as possible candidate genes for the *RN* locus. © Inra/Elsevier, Paris

major genes / pig meat quality / chromosome substitution effects / Gibbs sampler

* Correspondence and reprints
E-mail: nreinsch@tierzucht.uni-kiel.de

Résumé – Analyse Bayésienne de ségrégation, assistée par marqueurs, pour savoir si le locus *RN* chez le Porc a des effets pléïotropiques. On a abordé la question de la possibilité d'effets pléïotropiques du locus *RN* chez le Porc. Des données concernant des microsatellites au chromosome 15, des résultats de cartographie et des données de performances issus de l'expérience de localisation du gène *RN* à Kiel ont été utilisés pour comparer des porteurs hétérozygotes RN^-/rn^+ et des non porteurs rn^+/rn^+. Les descendances de 14 verrats Hampshire × Piétrain, tous doubles hétérozygotes aux locus *RN* et *RYR1* ont été analysés pour 24 caractères. Les mères ont été soit des croisées Landrace × Large-White soit des pures Landrace ou Large-White. Le nombre de descendants avec données valides a varié de 323 à 418 suivant le caractère. Un modèle à hérédité mixte polygénique-gène majeur a été appliqué. On en a inféré, suivant la méthode Bayésienne, aux distributions marginales a posteriori, grâce à l'échantillonnage de Gibbs. Les résultats ont montré que la réflectance est diminuée d'environ une unité chez les porteurs et que le pH ultime a été abaissé pour le filet et pour le jambon. Les résultats les plus importants ont été que l'allèle RN^- a entraîné un accroissement de 2,42 mm de l'épaisseur du filet, un accroissement de la surface du filet de 1,39 cm^2 et un accroissement de 0,46 % de la teneur en viande maigre. Il a été montré que l'accroissement de la teneur en viande maigre n'est pas assez grand pour contrebalancer la perte économique provenant du mauvais rendement technologique. La proportion de variance génétique chez la descendance croisée attribuable au locus *RN* a varié de 5 à 55 %. Plus de 40 % de la variance génétique de l'épaisseur de noix a été expliqué conjointement par les génotypes *RN* et *RYR1*. Ce résultat est discuté par rapport à l'uniformité qui est souhaitée chez les produits terminaux. La conclusion est que les différents allèles au QTL devraient être assemblés dans différentes lignées de manière à fabriquer des hétérozygotes chez les produits terminaux, quand les homozygotes sont défavorables ou quand les hétérozygotes sont difficiles à obtenir par sélection. Les résultats suggèrent que d'autres loci que ceux impliqués dans le métabolisme du glycogène, peuvent être considérés comme candidats pour le locus *RN*, par exemple ceux concernés par le développement musculaire.

gène majeur / qualité de viande / porc / effet de gène / échantillonnage de Gibbs

1. INTRODUCTION

Two alleles (RN^- and rn^+) have been found to segregate at the porcine *RN* locus in purebred Hampshires and in Hampshire crossbreds. This gene was discovered by Naveau [19] by its effect on the Rendement Technologique NAPOLE (RTN [20]). 'Hampshire-type' meat [18] from carriers of the unfavourable RN^- allele is known to exhibit an increased muscle glycogen content, lowered terminal pH and an increased cooking loss. The ability of muscle glycogen to bind water is similar to protein in fresh muscle tissue, but this water is set free during the cooking process and generates the increased loss [17]. Measurements of technological yield in cured cooked ham processing as well as the glycolytic potential in muscle tissue (weighted sum of concentrations of lactate and its preliminary stages in muscle energy metabolism: glucose, glucose-6-phosphate and glycogen) showed a bimodal distribution in pig populations with both alleles segregating [4, 19]. This observation is explained by the dominant mode of inheritance [8, 19]. All these facts were the foundation for the mapping of the *RN* locus to chromosome 15 in the neighbourhood of the microsatellite markers SW906, SW936 and SW120 [12, 14–16, 24].

The financial loss as a consequence of any kind of heat processing was estimated to reach 5.29 DM per carcass (50 % lean meat content) of a carrier animal [3]. This penalty from the primary gene effects may be reason enough to select against the unfavourable dominant RN^- allele. However, breeders would like to know if there are additional costs or benefits from such a selection because of the existence of pleiotropic effects on other traits such as lean meat content. Moreover, knowledge of pleiotropic effects may be helpful to make a more educated guess on possible candidate genes in positional cloning studies.

In a preliminary report [9] marginally significant effects of the *RN* genotype on body composition characteristics and growth traits were found in animals from a synthetic line. An examination of sensory traits [10] showed additive and favourable effects of RN^- on flavour as well as an undesired dominant impact on tenderness, juiciness and mellowness, whereas aspect was affected only in heterozygotes. In Hampshire (Swedish Landrace × Swedish Yorkshire) crossbreds [13] a stronger taste and smell was found in heterozygous carrier animals as well as a greater acidity and a beneficial effect on Warner-Bratzler shear force. Recently Le Roy et al. [11] presented new results from the Laconie synthetic line. The weight of ham and loin was significantly increased in carriers and backfat thickness was significantly reduced when measured at back and rump. The *RN* effect on carcass lean content as measured during grading failed to be significant, while the error probability for *RN* effects on an estimate of lean content, made from the weight of several cuts, almost reached the significance threshold (5.3 %).

In order to contribute more information on pleiotropic RN^- effects this investigation makes use of the chromosome 15 microsatellite data, mapping results and performance data from the Kiel *RN* mapping experiment [22]. Carriers and non-carriers of the RN^- allele from litters of 14 boars, which were heterozygous at the *RN* locus and at the *RYR1* locus, were compared. A Bayesian approach was developed to combine phenotypic and genetic marker information for genotype assignment of progeny and to analyse 24 traits related to growth, carcass quality and meat quality. Additionally, results on the proportion of genetic variance attributable to the *RN* and *RYR1* loci are given.

2. MATERIALS AND METHODS

2.1. Animals and genotypes

Performance data were recorded from progeny of the Kiel *RN* mapping experiment. Fourteen Hampshire–Piétrain crossbred sires known to be heterozygous both at the *RN* locus and the *RYR1* (Ryanodine receptor) locus contributed female and male castrated offspring to the experimental families. These boars were mated to homozygous rn^+/rn^+ Landrace × Large White, Landrace and Large White sows to produce progeny with expected proportions of 50 % RN^-/rn^+ and 50 % rn^+/rn^+ genotypes. The assumption of homozygosity of all sows is based on the fact that Feddern [3] could not find any animals with high glycolytic potential within both dam lines. Furthermore, there is no literature report, to the authors' knowledge, of the occurrence of the RN^- allele

in other breeds than Hampshires or Hampshire crossbreds. Terminal *musculus longissimus dorsi* glycogen content of all offspring was determined from a meat sample drawn 24 h after slaughter between the 13th and 14th ribs. All 14 boars had two types of extreme offspring, with either low or high muscle glycogen content, and were therefore classified as heterozygous. Genotypes at the *RYR1* locus were determined with a DNA probe. Since most of the sows were homozygous stress resistant *NN* at the *RYR1* locus, the proportions of *Nn* and *NN* genotypes among offspring were both about 50 %. The number of observations from progeny being homozygous stress sensitive *nn* was below 10 for all traits. Unbalancedness with respect to sires within the two generation family structure of the experiment can be seen in *figure 1*. Sires and dams originated from a commercial crossbreeding programme. All progeny were fattened under usual production conditions at the Hohenschulen experimental piggery of the Kiel University until finishing groups reached an average liveweight of approximately 110 kg. More details on the Kiel *RN* experiment, especially on glycogen measurement, marker genotypes and map construction, can be found in Reinsch et al. [22].

2.2. Traits

A set of 24 traits was investigated, concerning growth, carcass quality and meat quality. Growth is described by carcass length and a series of weights: birthweight, weight at weaning, weight at beginning of the fattening period, endweight and slaughterweight. Dressing was computed as the ratio of slaughterweight and endweight. Six different fatness traits were available: backfat thickness measured at three different positions of the carcass (neck, middle and end of the back), the average of these three values, loin fat area and backfat thickness measured with an optical probe after slaughter for commercial grading. Carcass meatiness is described by eye muscle thickness and lean meat content, both commercial grading results, loin eye area, and additionally by a score (1–5) of belly meat content. The pH of the *musculus longissimus dorsi* (*m. l. dorsi*) between the 13th and 14th ribs was measured approximately 1 h after slaughter (single measurement) and 24 h after slaughter (average of three measurements). Conductivity was measured in the same muscle 1 and 24 h after slaughter. Terminal pH (24 h post mortem) in the ham was measured in the *musculus semimembranosus* and is an average of two values. Reflectance (*m. l. dorsi*) was measured during grading with a Fat-O-Meater probe. Meat brightness (average of two measurements) of the *m. l. dorsi* was taken 24 h post mortem at a cross section of the carcass between the 13th and 14th ribs. For measuring meat brightness, pH values and conductivity an Opto-Star apparatus, a pH-Star and a LF-Star device (Mathäus, Pöttmes, Germany) were used, respectively. For each trait the number of progeny with valid observations is given in *table II*.

2.3. Competing hypotheses

The contrast between RN^-/rn^+ and rn^-/rn^+ genotypes is equivalent to the sum $a+d$, where a is half the difference between the genotypic values of the two types of homozygotes and d is the deviance of the heterozygotes from the

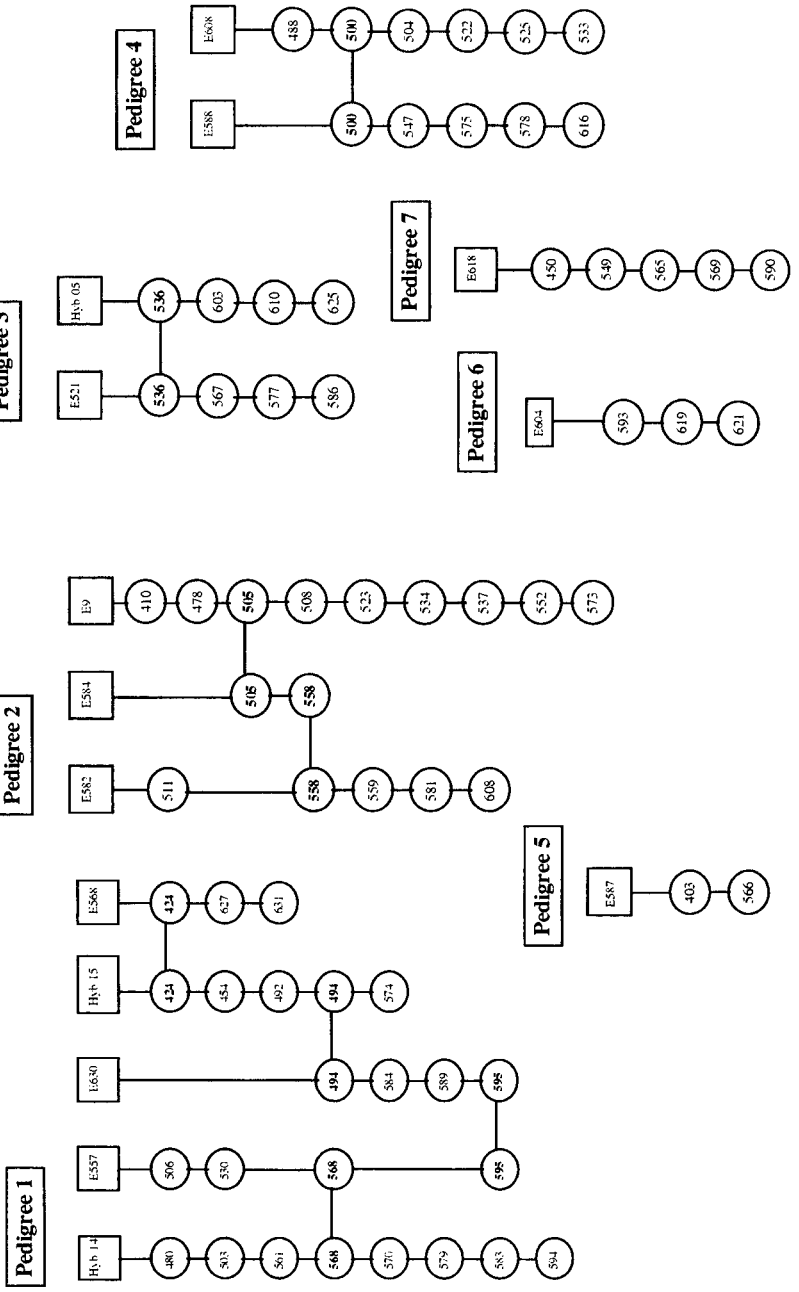

Figure 1. Families with sires (squares) heterozygous at the *RN* locus and homozygous dams (circles).

mean of both homozygotes [2]. Thus we expect a zero contrast if there are no pleiotropic effects at all or, alternatively, if a equals minus d. There is only one genetic situation with a equal to minus d, that is complete dominance of rn^+ over rn^+. However, this case is only of interest when selection is aimed at a population where all three genotypes are present, such as purebred Hampshires. In the German pig industry Hampshires are usually used to produce Hampshire Piétrain crossbreds as terminal sires which are mated to either Landrace or Landrace × Large White sows. Consequently only RN^-/rn^+ and rn^+/rn^+ genotypes are observed in the target genotypes of such a crossbreeding scheme. Though all three possible genotypes are of scientific interest, the contrast under investigation is currently the most important aspect of RN pleiotropy from a short term commercial point of view under these circumstances. Hence two competing hypotheses are to be judged:

H_1: either no effect at all of the RN^- allele or complete dominance of rn^+; and H_2: all other modes of inheritance with $a + d$ different from zero.

2.4. Statistical analysis

A mixed polygenic-major gene inheritance model was applied. Using a Bayesian approach, marginal posterior distributions of the contrast mentioned above and other important model components were generated via a Gibbs sampling scheme [5, 6]. This scheme may briefly be summarized as follows.

At the beginning of each cycle a genotype was drawn for each progeny from a discrete distribution conditional on phenotypic or genetic marker information for that animal, its performance in a particular trait, and values for all fixed and random effects from the previous round of the Gibbs cycle. Since sires were known to be heterozygous carriers and dams were known to be homozygous non-carriers it was not necessary to draw genotypes for parent animals or gene frequencies. Fixed and random effects were then drawn from proper conditionals, and the effects of both genotypes were treated like any other fixed effect in this step. Finally, new values for the residual variance, polygenic variance and variances for other random effects were generated from inverted Chi-square distributions.

In order to execute the first step of the Gibbs cycle it was necessary to assign a prior probability r for being a RN^- carrier to each progeny. *Musculus longissimus dorsi* glycogen content (its distribution is shown in *figure 2*) was used as a phenotypic marker: offspring with low glycogen content up to the 30 % percentile of the glycogen distribution (7 $\mu mol \cdot g^{-1}$) were classified as rn^+/rn^+ and those with glycogen content above the 70 % percentile (100 $\mu mol \cdot g^{-1}$) as RN^-/rn^+, with values of 0 and 1 for r, respectively. The genotype of all other offspring with glycogen content within a medium region of uncertainty (7 $\mu mol \cdot g^{-1}$ < glycogen content < 100 $\mu mol \cdot g^{-1}$) was treated as unknown. So far the assignment of genotypes was identical to the procedure we used to map the RN gene [22]. The bounds of the uncertainty region are of course somewhat arbitrary. However, it has been shown that mapping results obtained by using these bounds were in good agreement with the findings of other researchers and insensitive to variation of these bounds [22]. For further treatment of all animals within the uncertainty region we made use of our male map of chromosome 15 and microsatellite marker data from seven different loci on this chromosome

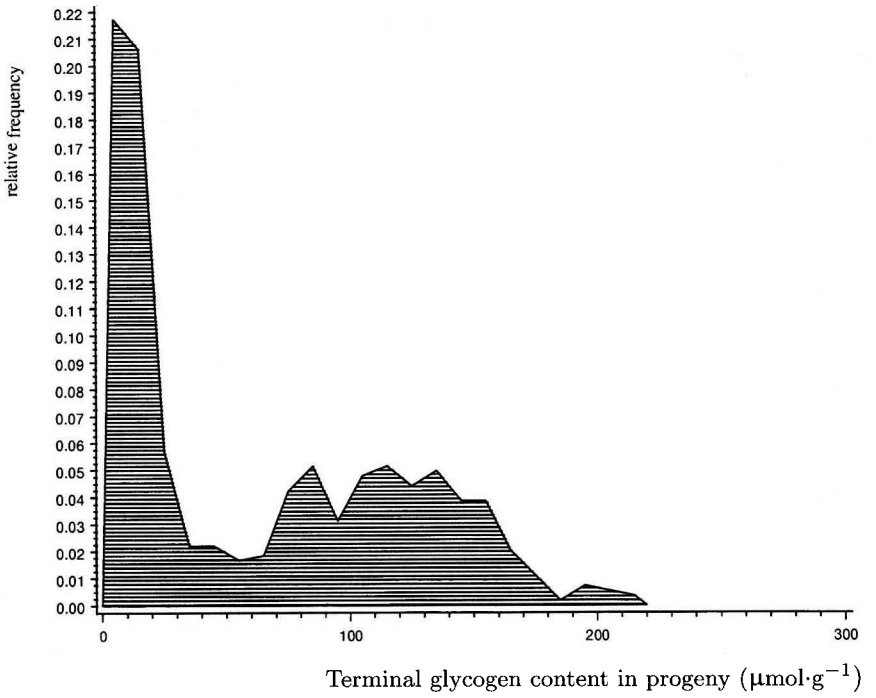

Figure 2. Distribution of glycogen measurements of progeny ($n = 496$).

(see *figure 3* for marker loci and male multipoint map distances). For each of those progeny the smallest informative marker interval bracketing the *RN* locus was used to derive the risk r of having received a RN^- allele from its sire. In most cases r was fairly close to 0 or 1. In those cases where either a recombination had occurred within the interval SW120–SW906 (*RN* is mapped exactly in the middle of this interval) or where no marker information was available at all an r-value of 0.5 was used since all progeny analysed are descendants of a mating between an RN^-/rn^+ and an rn^+/rn^+ genotype.

All models applied to the different traits comprised an effect for *RN* genotype, various fixed effects, one uncorrelated random common environmental effect and one polygenic effect. The corresponding design matrices and parameter vectors are denoted by $\mathbf{X}_1$, $\mathbf{X}_2$, $\mathbf{Z}_1$, $\mathbf{Z}_2$ and $\mathbf{b}_1$, $\mathbf{b}_2$, $\mathbf{u}_1$ and $\mathbf{u}_2$, respectively. In contrast to the other design matrices $\mathbf{X}_1$ is unknown prior to analysis. The current realizations of $\mathbf{b}_1$, $\mathbf{b}_2$, $\mathbf{u}_1$ and $\mathbf{u}_2$ during iteration are symbolized by b_1, b_2, u_1 and u_2. Variance components for common environment, polygenic component and residuals, $\boldsymbol{\sigma}_c^2$, $\boldsymbol{\sigma}_a^2$ and $\boldsymbol{\sigma}_e^2$, have transient values s_c^2, s_a^2 and s_e^2. During the first step of the Gibbs cycle from each single observation y^* the difference $y = y^* - x_2' b_2 - z_1' u_1 - z_2' u_2$ was computed, where x_2', z_1' and z_2' are rows of the design matrices $\mathbf{X}_2$, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ for a particular observation. Then two probabilities p_1 and $p_2 = 1 - p_1$ were calculated for each offspring:

$$p_1 = \frac{r \cdot \phi(y|\mu_1, s_e^2)}{r \cdot \phi(y|\mu_1, s_e^2) + (1 - r) \cdot \phi(y|\mu_2, s_e^2)}$$

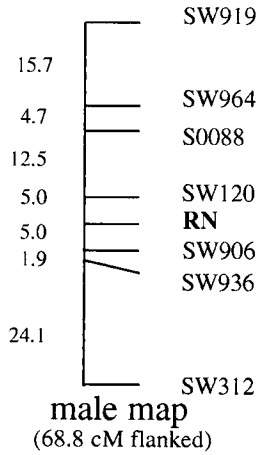

Figure 3. Markers and map distances on chromosome 15, results from the Kiel *RN* experiment.

Here μ_1 and μ_2 are the components of b_1, i.e. the effects of both genotypes which were generated during the previous iteration and $\phi(y|\mu_1, s_e^2)$ and $\phi(y|\mu_2, s_e^2)$ are probability densities from normal distributions at the location y with variance s_e^2 and means μ_1 and μ_2, respectively. A genotype was drawn for each progeny in each round of the Gibbs cycle from a discrete distribution with probabilities p_1 and $p_2 = 1 - p_1$. In those cases, where r has an extreme value of 0 or 1 (equivalent to an extreme glycogen measurement) p_1 is identical to r and remains constant from iteration to iteration for a particular observation; in all other cases p_1 is a data dependent modification of r changing from cycle to cycle. The prior probability r itself is, of course, independent of the trait analysed. The values for r were not recalculated at the beginning of each Gibbs cycle, since the paternal linkage phases between markers and also between markers and *RN* were known to almost certainty, based on 300 progeny informative for *RN* and 496 progeny with marker data available [22].

Sampling of genotypes is equivalent to sampling one of all possible $\mathbf{X}_1$ matrices. Therefore the computations proceeded by an update of the mixed model equations using the last sampled version of $\mathbf{X}_1$. Finally new vectors b_1, b_2, u_1 and u_2 for fixed and random effects were sampled from normal conditionals and components s_c^2, s_a^2 and s_e^2 from inverted Chi-square distributions as described for Bayesian analysis of mixed models with flat priors by, e.g. Wang et al. [27], Jensen et al. [7] and Janss et al. [6].

Fixed effects in all cases comprised *RYR1* genotype, sex, line of dam and a seasonal effect, which was defined as month of birth for birthweight, month at weaning day for weaning weight and as month of slaughter for all other traits. A parity of dam effect $(1, \geq 2)$ as well as a linear regression on litter size was fitted to early growth traits. Growth traits were corrected for age (linear and quadratic) and carcass traits for slaughterweight. Litter, day of slaughter and pen were used as random uncorrelated common environmental effects for early growth traits, meat quality traits and all other traits, respectively. For exact model specifications for all traits see *table I.*

Table I. Type of common environmental (c^2) effect and fixed effects with number of factor levels for all models applied to various traits.

Trait	c^2 effect	*RN*	Month	*RYR1*	Sex	Line of dam	Parity of dam	Linear regression on	Linear regression on	Quadratic regression on
Birthweight	litter	2	11	3	2	3	2	litter size	–	–
Weight at weaning	litter	2	10	3	2	3	2	litter size	age	age
Starting weight	litter	2	11	3	2	3	2	litter size	age	age
Endweight	pen	2	11	3	2	3	–	–	age	age
Slaughter weight	pen	2	11	3	2	3	–	–	age	age
Carcass length	pen	2	11	3	2	3	–	–	age	age
Meat quality	day of slaughter	2	11	3	2	3	–	–	–	–
Dressing	pen	2	11	3	2	3	–	–	slaughterweight	–
Carcass composition	pen	2	11	3	2	3	–	–	slaughterweight	–

Computation of probabilities for alternative coupling phases for marker loci in sires and of risk values r for progenies were performed with our own FORTRAN program. RISKOFIT software, a derivative of LMMG [21] was used for the Gibbs sampler. The UNIVARIATE and AUTOREG procedures of the SAS package [25] were used to compute the mean and the standard deviation of the marginal posterior distribution of the contrast between RN^- carriers and non-carriers and to check for serial correlations and other properties. Non-parametric estimates of the marginal posterior probability density functions as well as 95 % confidence intervals for these functions were calculated with the lifetable method as implemented in the LIFETEST procedure [25]. One long Gibbs chain with one million cycles was run for each trait. From each 50th cycle the contrast $\mu_1 - \mu_2$ was written to a file, along with other model parameters of interest, and saved for post Gibbs analysis. Since the first 20 000 iterations were discarded (burn-in plus a wide safety margin), results originate from 19 600 single values per trait. CPU time varied somewhat from trait to trait and was roughly 16 h when a 100 MH Pentium PC was used and 5 h on a SUN ultra-sparc workstation. For all estimated means the probability mass of a highest posterior content region bounded by zero on either side was determined from a normal distribution curve, which was used to approximate the posterior distribution, with mean and variance estimated from the Gibbs sample. A contrast is reported as significant if this probability mass exceeded 95 %.

Finally for all traits with a significant result another long chain was generated. A total of 80 000 values was sampled for each of three parameters from four million post Gibbs cycles per trait: the difference between *RN* genotypes, the difference between *RYR1* genotypes, and the polygenic variance. From these data, samples for six population parameters were generated: samples of the QTL variance due to the *RN* locus, of the QTL variance due to the *RYR1* locus, of the total genetic variance, of the ratio of *RN* variance and total genetic variance, of the ratio of *RYR1* variance and total genetic variance, and of the polygenic heritability hp^2. In order to obtain a realization of the total genetic variance v_g both QTL variances and the polygenic variance from one cycle were summed:

$$v_g = v_{RN} + v_{Ryr} + v_a$$

By analogy, samples for the polygenic heritability were generated as ratios of the polygenic variance and the sum of polygenic, common environmental (*table I*), and residual variances:

$$hp^2 = \frac{v_a}{v_a + v_c + v_e}$$

From each sampled contrast between *RN* genotypes an observation for the *RN* variance v_{RN} was computed as

$$v_{RN} = \frac{1}{2}\left[\mu_1 - \frac{1}{2}(\mu_1 + \mu_2)\right]^2 + \frac{1}{2}\left[\mu_2 - \frac{1}{2}(\mu_1 + \mu_2)\right]^2 = \frac{1}{4}(\mu_1 - \mu_2)^2$$

Observations for the *RYR1* variance v_{RYR} were computed in exactly the same manner, replacing the contrast between *RN* genotypes by the contrast

between *NN* and *Nn* genotypes. This corresponds to a situation where all boars are doubly heterozygous (Nn, RN^-/rn^+), all sows are doubly homozygous (NN, rn^+/rn^+) and the RN^- and n alleles in final crossbred progeny are found exclusively in heterozygous genotypes with a frequency of 0.5. Since the standard Piétrain boar used by the industry is homozygous stress sensitive, the n allele at the *RYR1* locus has been eradicated to a large extent in dam lines, and the RN^- frequency in the sire lines, from where our animals originate, seems to be high [22], such a genetic situation can be regarded as close to reality.

3. RESULTS AND DISCUSSION

Examination of serial correlations showed that uncorrelated values for the difference between genotypes were obtained by the sampling of each 50th realization from the Gibbs chain. Posterior means and posterior standard deviations are shown in *table II*. Because of uncorrelatedness, the corresponding Monte Carlo errors are just the ratio of standard deviation and square root of the size of the Gibbs sample. All posterior distributions turned out to be highly symmetric and could be very well approximated by a normal distribution. In *figure 4* a typical non-parametric estimate of the distribution curve is shown as an example, together with its 95 % confidence limits. These confidence limits show the good determination of the distribution curve, especially in the tails. A joint plot of this curve and its normal approximation show them to be almost identical (*figure 4*).

Small but significant effects were found for pH values measured 24 h after slaughter (*table II*). Reflectance was significantly lowered in RN^- carriers with a difference of nearly one unit. The most important results, however, are a highly significant 2.4 mm increase in eye muscle thickness, an enlargement of loin eye area of 1.39 cm^2 and a 0.46 % increase in estimated lean meat content. Neither conductivity nor any growth trait was affected by *RN* genotype.

An impact of *RN* on optical meat properties was also found by Le Roy et al. [11] and Lundström et al. [13], who reported an increase in several surface reflectance measurements, taken 1 day and 4 days after slaughter. Although these traits are not important economically, knowledge of *RN* effects may lead to the development of optical procedures for RN^- detection and quality control, e.g. at the slaughterhouse. For all traits with a significant *RN* effect the estimated difference between heterozygotes at the *RYR1* locus and homozygous stress resistant animals is shown in *table IV*.

The entire range of fatness traits showed no response at all to RN^-. Le Roy et al. [11] found evidence for the reverse: backfat thickness was reduced in carriers. It should be noted here that it is not possible to distinguish between pleiotropic effects of the *RN* locus itself and the pleiotropic effects of the *RN* region, i.e. all coupled loci close to *RN*. Thus the *RN* region in one experiment may show other characteristics than in another experiment.

In the experiment described by Le Roy et al. [11] the genotypes of all recorded progeny were fully determined by the homozygosity of their parents. In all investigations, where at least one parent of the recorded animals is heterozygous the assignment of genotypes to progeny may be another source of different experimental results. Lundström et al. [13] reported a rate of 16 %

Table II. Number of observations, marginal posterior mean of difference between *RN* genotypes (positive sign denotes higher trait values for carriers), corresponding standard deviation of the marginal posterior distribution (standard error of estimates), and probability mass of highest posterior content (HPC) interval bounded by zero. Grey background indicates a significant result.

Trait	Number of observations	Posterior mean of contrast	Standard deviation	Probability mass of HPC interval (%)
Birthweight (kg)	418	−0.039	0.028	83.2
Weight at weaning (kg)	391	−0.044	0.107	32.3
Starting weight (kg)	416	−0.231	0.275	60.1
Endweight (kg)	418	−0.087	0.561	12.4
Slaughterweight (kg)	418	−0.085	0.438	15.4
Dressing (%)	418	−0.0628	0.143	34.0
Carcass length (cm)	417	−0.1	0.3	32.8
pH 1 h after slaughter, *m. l. dorsi*	418	0.002	0.002	68.8
Conductivity 1h after slaughter, *m. l. dorsi* (mS/cm)	416	0.61	0.68	62.5
Reflectance, *m. l. dorsi*	414	−0.86	0.32	99.3
pH 24 h after slaughter, *m. semimembranosus*	417	−0.17	0.013	100
pH 24 h after slaughter, *m. l. dorsi*	417	−0.07	0.007	100
Conductivity 24 h after slaughter, *m. l. dorsi* (mS/cm)	394	0.18	0.19	64.0
Meat brightness	418	0.25	0.58	33.8
Eye muscle depth (grading) (mm)	334	2.42	0.53	100
Lean meat content (grading) (%)	333	0.46	0.20	97.7
Loin eye area (cm^2)	338	1.39	0.63	97.3
Backfat thickness, grading (mm)	334	0.07	0.19	29.7
Backfat thickness, neck (mm)	334	0.06	0.42	12.1
Backfat thickness, middle of back (mm)	334	0.57	0.35	90.1
Backfat thickness, end of back (mm)	334	0.05	0.32	13.8
Backfat thickness, average (mm)	334	0.24	0.28	60.6
Loin fat area (cm^2)	338	0.09	0.26	27.8
Belly fatness score	334	−0.04	0.18	17.2

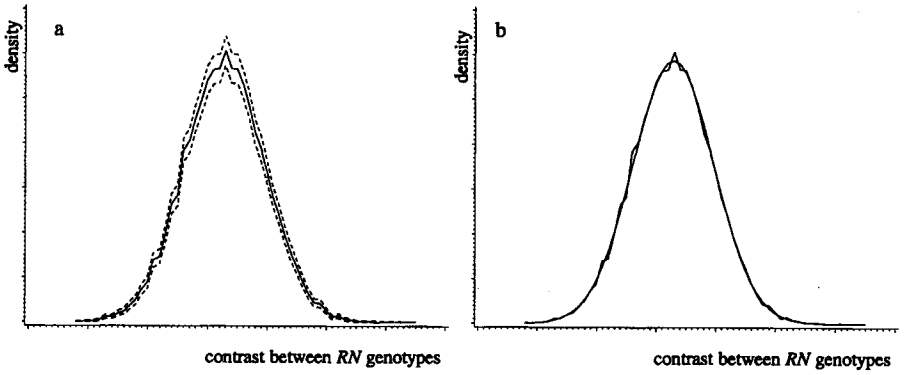

Figure 4. Estimates of the marginal posterior distribution of the difference between carriers and non-carriers. The trait is terminal pH in the loin. Non-parametric curve, generated with the LIFETEST procedure, together with its 95 % confidence interval (**a**). The non-parametric curve and the normal density curve are almost identical (**b**).

misclassified *RN* genotypes, when *RTN* was used for classification instead of glycolytic potential. A region of uncertainty, where the observed phenotype generates more or less equal likelihoods for carriers and non-carriers, is a problem of every phenotypic measure which may be used as a classification criterion. This study is an attempt to overcome this problem by combining phenotypic measures, genetic markers and a proper statistical procedure, which takes account of uncertain genotypes. Despite many genotypes being fixed or known with high probability, there was still room for changes in the number of carriers from iteration to iteration. In the analysis of lean meat content, for example, the minimum and maximum number of carriers were 142 and 201, respectively, with an average value of 160. This variation is due to animals with uncertain genotypes because of a recombination within the flanking marker interval or because of low marker informativeness. For these progeny the choice of their genotype completely rests on their performance in a specific trait. Another way to exploit glycogen content as a major source for genotype discrimination would have been a multivariate analysis, combining glycogen and other traits. However, incorporating glycogen as a phenotypic marker is easier to implement without ignoring important information. The close normal approximation of the posterior probability curve for the contrast between genotypes suggests that a regression analysis would have provided similar results with regard to differences between genotypes. A Bayesian or a maximum likelihood approach, however, is also well suited for an examination of variance components.

When considering *figure 2* the within-genotype variance of glycogen content seems to be affected by *RN*. This phenomenon may be explained by two specific features of glycogen measurements: first, the large difference between low (non-carriers) and high (carriers) glycogen values, and second, the fact that the variability in non-carriers is limited by zero. A variance effect of *RN* seems not to exist or is at least far away from being obvious when glycolytic potential

is considered instead of glycogen content [3]. This is the reason why variance effects of *RN* were regarded as glycogen specific and have not been further investigated in this study.

Benefits from increased lean meat content are not high enough to outweigh financial losses from decreased technological yield and to justify the use of the RN^- allele in future pig breeding. *Figure 5* illustrates a calculation revealing this fact: 1.47 kg of fresh lean meat from a carrier animal is necessary in order to obtain 1 kg of processed lean meat, compared to 1.41 kg of fresh lean meat from a non-carrier (conversion factors of 0.68 and 0.71 are from Feddern [3]). If we assume a carrier with 55 % lean meat content, then a non-carrier, which is genetically identical except at the *RN* locus, has 54.5 % lean meat content, according to our results. Therefore there are 2.67 kg of slaughterweight needed to produce the same amount of 1 kg processed lean meat, compared to 2.59 kg from a non-carrier. This translates to costs of 12.60 DM and 12.22 DM per kg processed lean meat, using carcasses from carriers or non-carriers, respectively (4.72 DM per kg carcass weight). The cost difference of 0.38 DM is still positive though somewhat lower than in a situation where both genotypes have an equal lean meat content of 55 % (0.52 DM). The weight of bones and fat per kg processed lean meat is reduced by 0.02 kg in non-carriers, a side effect which is neglected in these calculations as well as the increased drip loss in carriers [13].

The proportion of the total genetic variance explained by the *RN* locus (*table III*) ranges from 5 % for lean meat content to 55 % for terminal pH in

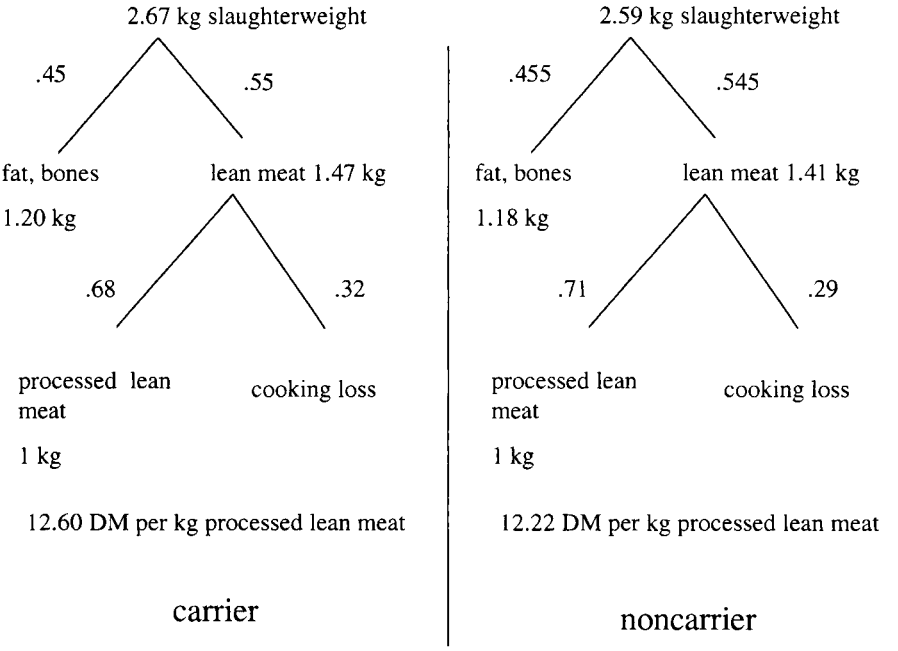

Figure 5. Comparison of costs per kg processed lean meat from carriers and non-carriers.

the loin. The proportion of the genetic variance which can be attributed to *RYR1* as the second QTL known to be segregating in this population also reaches considerable levels up to 20 % for eye muscle depth. Thus the joint action of both QTLs generates more than 40 % of the total genetic variance in eye muscle depth, though only those two genotypes which are of practical importance are assumed to occur at each locus. The estimate of the polygenic heritability for eye muscle depth is rather low at 16.5 % (*table IV*) compared to the estimates for loin eye area and lean meat content (62.9 and 36.0 %).

Table III. Genetic variances due to *RN* and *RYR1* (v_{RN}, v_{RYR}), total genetic variance (v_g), proportions of the total genetic variance explained by *RN* and *RYR1*, respectively (v_{RN}/v_g, v_{RYR}/v_g), and polygenic heritabilities (hp^2). Figures are marginal posterior means.

Trait	v_{RN}	v_{RYR}	v_g	v_{RN}/v_g	v_{RYR}/v_g	hp^2
Reflectance	0.212	0.047	1.34	0.210	0.051	0.094
Terminal pH, ham	0.00687	0.0000489	0.0200	0.369	0.003	0.080
Terminal pH, loin	0.00114	0.000136	0.00222	0.547	0.064	0.077
Eye muscle depth (mm)	1.18	1.01	5.82	0.237	0.201	0.165
Loin eye area (cm^2)	0.578	1.16	30.7	0.022	0.041	0.629
Lean meat content (%)	0.0642	0.174	1.65	0.048	0.128	0.360

Table IV. Marginal posterior means for the difference between heterozygotes at the *RYR1* locus and homozygous stress resistant animals, the corresponding standard deviation of the posterior distribution, and the probability mass of the highest posterior content interval bounded by zero.

Trait	Posterior mean of difference	Standard deviation	Probability mass of HPC interval (%)
Reflectance	0.29	0.32	63.8
Terminal pH, ham	0.003	0.014	15.8
Terminal pH, loin	−0.022	0.007	99.9
Eye muscle depth (mm)	1.94	0.51	100
Loin eye area (cm^2)	2.05	0.64	99.9
Lean meat content (%)	0.81	0.20	100

In the four-way crossbreeding scheme that is considered here, the *RN* and *RYR1* alleles are combined in such a way that terminal sires are almost exclusively doubly heterozygous for these two loci. Since eye muscle depth can be regarded as a type trait it can be expected that Piétrain × Hampshire crossbred boars will exhibit a high degree of uniformity, not only with respect to muscle depth measurements, but also when they are visually scored. However, this uniformity is obviously lost in the target genotype of the crossbreeding scheme. The *RN–RYR1* example clearly demonstrates that commercially selected pig populations exist, where knowledge on QTLs may be used to increase uniformity of crossbreds, rather than only to increase the mean performance level.

Furthermore, it throws some fundamental light on the potential impact of QTL detection on crossbreeding schemes in livestock species: the crossing of highly inbred lines as a means for variance reduction is not feasible in animal breeding because of inbreeding depression. An alternative approach towards uniformity may exploit knowledge on existing QTLs by crossing lines which are homozygous at a number of known QTLs. Homozygosity may be for the same or for alternative alleles in different lines, depending on pleiotropic effects on the entire set of breeding goal traits. Alleles to be fixed in sire lines are, of course, those with favourable effects on production but unfavourable effects on reproduction. Alternative QTL alleles should be assembled in different lines in such a way that 100 % heterozygosity is achieved in end products in cases where homozygosity is not desired or cannot be reached by selection within a short time.

An interesting fact is that the chromosomal region of the bovine *MH* locus (muscular hypertrophy) and also the neighbourhood of the *RN* locus were shown to be homologous to the human chromosome 2 [1, 23, 26]. Therefore the bovine *MH*, if identified, is probably a candidate for the porcine *RN*. Apart from this, the results from this study along with the findings of Le Roy et al. [11], who found an increased weight of ham and loin, both suggest that all loci, which are involved in muscle tissue development, may be regarded as new members in the assembly of candidate genes for the *RN* locus.

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