On the Detection of Imprinted Quantitative Trait Loci in Experimental Crosses of Outbred Species

Dirk-Jan de Koning,[1] Henk Bovenhuis and Johan A. M. van Arendonk[2]

Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, Wageningen University, 6700 AH Wageningen, The Netherlands

Manuscript received September 28, 2001
Accepted for publication March 22, 2002

ABSTRACT

In this article, the quantitative genetic aspects of imprinted genes and statistical properties of methods to detect imprinted QTL are studied. Different models to detect imprinted QTL and to distinguish between imprinted and Mendelian QTL were compared in a simulation study. Mendelian and imprinted QTL were simulated in an F_2 design and analyzed under Mendelian and imprinting models. Mode of expression was evaluated against the H_0 of a Mendelian QTL as well as the H_0 of an imprinted QTL. It was shown that imprinted QTL might remain undetected when analyzing the genome with Mendelian models only. Compared to testing against a Mendelian QTL, using the H_0 of an imprinted QTL gave a higher proportion of correctly identified imprinted QTL, but also gave a higher proportion of false inference of imprinting for Mendelian QTL. When QTL were segregating in the founder lines, spurious detection of imprinting became more prominent under both tests, especially for designs with a small number of F_1 sires.

PARENTAL genomes undergo modifications during gametogenesis. The result is that some genes inherited from one parent are not completely expressed, if at all. This phenomenon of genomic imprinting has been shown to influence several genes and traits in animals (including humans, Morison *et al.* 2001) as well as plants (Alleman and Doctor 2000) and insects (Lloyd *et al.* 1999).

Genome scans have revealed a number of genes or quantitative trait loci (QTL) contributing to genetic variation in many species. Genome scans can also be used to search for imprinted QTL provided that the parental origin of alleles can be traced back from the F_2 to the F_1 parents (Knott *et al.* 1998). This prerequisite excludes F_2 crosses between inbred lines because the F_1 parents are all heterozygous for the same marker alleles. Methods to detect imprinted QTL have been described for outbred crosses by Knott *et al.* (1998) and successfully applied to genome scans by Jeon *et al.* (1999) and in a modified form by de Koning *et al.* (2000). Nezer *et al.* (1999) used a maximum-likelihood algorithm to detect QTL with specific LOD scores for imprinted QTL against Mendelian QTL. The quantitative genetics of imprinted QTL and the statistical properties of tests to detect imprinted QTL and distinguish between Mendelian and imprinted QTL have not been studied in great detail. In this study, we first outline some of the quantitative genetic aspects of a (partially) imprinted QTL and subsequently we describe the results of a simulation study. The objective of the simulation study was twofold: (1) determine empirically the power for detection of imprinted QTL in outbred F_2 designs under Mendelian or imprinting models and (2) quantify the risk of spurious detection of imprinted QTL under different tests.

THEORY

Quantitative genetics of an imprinted gene: For a Mendelian gene with additive effect a and dominance effect d and with frequency p for the positive allele A and q for the negative allele B, the population mean under random mating is

$$M = a(p - q) + 2pqd \quad (1)$$

(Falconer and Mackay 1996). The average effect of allele substitution α is

$$\alpha = a + d(q - p). \quad (2)$$

The single gene variance is

$$V_G = 2pq[a + d(q - p)]^2 + (2pqd)^2 \quad (3)$$

(Falconer and Mackay 1996).

Now consider a biallelic gene with partial maternal imprinting (preferential expression of paternally inherited allele). This imprinting effect (i) will be apparent in the two groups of heterozygous individuals (AB and BA, first allele coming from sire). The genetic value for AB individuals can be denoted $d + i$ and for BA individuals as $d - i$. The population mean is identical to (1) but the average allele substitution effect has to

[1]*Present address:* Roslin Institute, Midlothian, EH25 9PS, United Kingdom.

[2]*Corresponding author:* Animal Breeding and Genetics Group, WIAS, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands. E-mail: johan.vanarendonk@alg.vf.wag-ur.nl

be specified for the sex through which the allele will be transmitted:

$$\alpha\♂ = a + i + d(q - p)$$
$$\alpha\♀ = a - i + d(q - p) = \alpha\♂ - 2i. \quad (4)$$

The single gene variance becomes

$$V_{Gi} = [p^2a^2 + pq(d + i)^2 + pq(d - i)^2 + q^2a^2] - [a(p - q) + 2dpq]^2$$
$$= 2pq[a + d(q - p)]^2 + 2pqi^2 + (2pqd)^2. \quad (5)$$

When there is complete imprinting, i will be equal to a and d will be zero. For a gene with exclusive paternal expression $\alpha\♀$ becomes zero and the paternal allele substitution effect ($\alpha\♂$) becomes $2a$. For complete imprinting, the single gene variance V_{Gi} (5) reduces to

$$V_{Gi} = 4pqa^2. \quad (6)$$

Detection of imprinted QTL in outbred F_2 designs: The analyses of crosses between outbred species are based mainly on the line-cross methodology proposed by Haley *et al.* (1994), assuming that the founder lines may segregate at the marker loci, but are fixed for alternative alleles at the QTL. Assuming Mendelian expression, an additive effect (a) and a dominance effect (d) are estimated using least squares as

$$y_j = m + ap_{a_j} + dp_{d_j} + e_j, \quad (7)$$

where y_j is the trait score of individual j, m is the population mean, a and d are the estimated additive and dominant effects of a putative QTL at the given location, p_{a_j} is the conditional probability of animal j to carry two alleles of line 1, p_{d_j} is the conditional probability of animal j to be heterozygous, and e_j is the residual error. The calculations of these probabilities and QTL effects are described in detail by Haley *et al.* (1994).

To test for imprinting, Knott *et al.* (1998) added the contrast between the two types of heterozygous individuals as an additional component to model (7):

$$y_j = m + ap_{a_j} + dp_{d_j} + ip_{i_j} + e_j. \quad (8)$$

Variables are as in (7), with the extension that i is the estimated imprinting effect and p_{i_j} is the conditional probability that individual j is heterozygous and inherited the line 1 allele from its sire. de Koning *et al.* (2000) proposed a reparameterization of (8) by introducing the conditional probabilities that an individual inherited a line 1 allele through its sire (p_{pat}) or through its dam (p_{mat}):

$$p_{pat} = p_a + p_i$$
$$p_{mat} = p_a - p_i. \quad (9)$$

Model (8) can be rewritten with a specific maternal and paternal QTL component as

$$y_j = m + a_{pat}p_{pat_j} + a_{mat}p_{mat_j} + dp_{d_j} + e_j, \quad (10)$$

where a_{pat} is the paternally inherited QTL effect and a_{mat} is the maternally inherited QTL effect. Models (8) and (10) are identical in terms of total variance explained by the model. de Koning *et al.* (2000) proposed to scan the genome with reduced imprinting models with exclusive paternal or maternal expression:

$$y_j = m + a_{pat}p_{pat_j} + e_j$$
$$y_j = m + a_{mat}p_{mat_j} + e_j. \quad (11)$$

SIMULATION STUDY

Simulation details: The outline of the simulation study is comparable to that of Alfonso and Haley (1998), who investigated the effect of mating design and segregation of QTL alleles in the founder lines on the power of detecting Mendelian QTL. F_1 individuals were generated by random mating of 20 sires from line 1 to 80 different dams (4 dams per sire) from line 2, each having five offspring. For most of the simulations 20 F_1 sires and 80 F_1 dams (4 dams per sire) were randomly mated to produce 400 F_2 offspring (5 offspring per dam). We also simulated an extreme design, where only 2 F_1 sires were mated to 80 F_1 dams (40 dams per sire). Marker data were simulated for all animals for a 100-cM chromosome with 11 evenly spaced markers. To have fully informative markers with regard to line of origin as well as optimal distinction of parental origin for the marker alleles in the F_2, eight alleles were simulated for every marker, with four line-specific alleles segregating at equal frequencies in the two founder lines. An additive, a dominant ($a = d$), a paternally expressed, or a maternally expressed biallelic QTL was simulated at 46 cM. Founder lines were either fixed for alternative QTL alleles or segregating at frequencies of 0.80 and 0.20 for the positive allele in lines 1 and 2, respectively. Imprinted QTL were simulated with exclusive uniparental expression and no dominance (*i.e.*, complete imprinting). The phenotype of an individual was further determined by 10 unlinked biallelic QTL, each with an effect of 0.25 and segregating at a frequency of 0.5 in both founder lines, giving an expected additive genetic variance of 0.31 (Alfonso and Haley 1998). An additional environmental component was sampled from a normal distribution with a variance of 0.47 and added to the genetic (QTL) value of an individual to obtain the phenotype (Alfonso and Haley 1998). QTL effects were varied between 0.25 and 1.0. The simulated QTL effects and their expected genetic variances following Equation 3 or 5 for the different genetic models are summarized in Table 1. One thousand replicates were simulated and analyzed for every alternative. For every mating design, an alternative with-

TABLE 1

Variance (σ^2_{qtl}) and proportion of total variance (h^2_{qtl}) explained by the simulated QTL in the F_2, under different genetic models

QTL effect	Additive QTL		Dominant QTL		Imprinted QTL	
	σ^2_{qtl}	(h^2_{qtl})	σ^2_{qtl}	(h^2_{qtl})	σ^2_{qtl}	(h^2_{qtl})
1	0.50	0.39	0.75	0.49	1.00	0.56
0.75	0.28	0.27	0.42	0.35	0.56	0.42
0.50	0.13	0.14	0.19	0.19	0.25	0.24
0.25	0.03	0.04	0.05	0.06	0.06	0.07

out a QTL was simulated to validate the use of chromosome-wide 5% significance thresholds.

Analyses: For every replicate, the coefficients of line origin were estimated following Haley *et al.* (1994). Subsequently, the best Mendelian QTL was estimated using (7) and the best imprinted QTL were estimated using both the paternal and maternal models of (11), respectively. For each of these three models, a chromosome-wide 5% threshold against the H_0 of no QTL was imposed to claim a significant QTL. Thresholds were obtained by permutation tests (Churchill and Doerge 1994) with 10,000 permutations for every 20th replicate and subsequent averaging over the 50 thresholds. For the significant replicates of the reduced imprinting models (11), imprinting was tested in the following manner:

Alternative a: H_0, Mendelian QTL ($i = 0$ or $a_{pat} = a_{mat}$); H_1, imprinted QTL. It was tested whether a full model (Equations 8 and 10) explained significantly more variance than a Mendelian model (7). This test, which is referred to as F_{Mend}, was performed at the best QTL position from the reduced model. F_{Mend} is an F-test with 1 d.f. in the numerator and $n - 4$ (n is the number of F_2 individuals) d.f. in the denominator. This test was first described by Knott *et al.* (1998), with the exception that in this study F_{Mend} is carried out against a Mendelian QTL at the position of the best imprinted QTL, which is not necessarily the best position of the Mendelian QTL.

Alternative b: H_0, imprinted QTL (*e.g.*, H_0: $a_{mat} = d = 0$ when evaluating a model with exclusive paternal expression); H_1, Mendelian QTL. It was tested, at the position of the best imprinted QTL, whether the specific reduced model (11) explained the same amount of variance as the full model (8 and 10) at that position. This test, which is referred to as $F_{red,}$ is an F-test with 2 d.f. in the numerator and $(n - 4)$ d.f. in the denominator. For both alternatives a and b, a tabulated F value corresponding to $P = 0.05$ was imposed to respectively infer (a) or reject (b) imprinting.

Alternative c: Imprinting was inferred when both a and b pointed toward imprinting (H_0 was rejected under alternative a but under alternative b H_0 was not rejected).

RESULTS

Detection of imprinted QTL: The results of the simulations with imprinted QTL are summarized in Table 2. All replicates showed significant QTL under both the Mendelian and the correct imprinting model for QTL effects of 0.50 or larger (Table 2). However, for a QTL effect of 0.25, only 83% of the replicates showed significant QTL under a Mendelian model while under the imprinting model all replicates showed significant QTL.

When founder lines were segregating for the positive QTL allele with frequencies of 0.80 and 0.20, respectively, the Mendelian model had 40% lower power compared to the correct imprinting model to detect imprinted QTL with an effect of 0.25 (Table 2).

Under the extreme design with two F_1 sires, there was consistently more power to detect maternally expressed QTL compared to paternally expressed QTL (Table 2). Across all simulations, F_{Mend} had better power to correctly identify imprinted QTL for larger QTL effects, while F_{red} had higher power to distinguish imprinted QTL for smaller QTL effects.

The estimates of QTL effects and position were comparable for the Mendelian and imprinting analyses for all simulated imprinted QTL, although the estimates from the Mendelian analyses had higher standard deviations (Table 2).

Detection of Mendelian QTL: The results for simulations without a QTL confirmed that using the 5% chromosome-wide thresholds for the H_0 of no QTL was sufficient to keep the type I error <5% (Table 3). When founder lines were fixed for different QTL alleles, all replicates showed significant QTL for effects >0.50 under both the Mendelian and imprinting models. Under the F_{Mend} imprinting is inferred if H_0 is rejected; *i.e.*, the column in Table 3 represents the type I error for that specific test. However, under F_{red} imprinting is inferred if H_0 is accepted and H_1 is rejected, *i.e.*, the type II error. Both F_{Mend} and F_{red} performed generally well in identifying the simulated QTL as being Mendelian for QTL effects of 0.50 and 0.75. The proportion of spuriously identified imprinted QTL was higher for purely additive QTL compared to dominant QTL (Table 3). Applying both thresholds restricted the spurious detection of imprinting to 5% of the replicates or less.

When founder lines were segregating at 0.80 and 0.20, respectively, the power to detect QTL was reduced (Table 3). There was little difference in power between the paternal and maternal imprinting models. The proportion of replicates with spurious imprinting was up to 11% for F_{Mend} and 22% for F_{red} (Table 3). Imposing both tests to infer imprinting kept the proportion of spurious imprinting <6%. Analyses with QTL effects between

TABLE 2

Detection and characterization of imprinted QTL

Simulation details		Power[b]		Estimated effects[c]		QTL position[c]		Imprinting inferred		
No. males/ females F_1	QTL effect[a] (frequency)	Mend.	Imp.[d]	Mend. $\hat{a} \pm$ SD[e]	Imp.[d] $\hat{a} \pm$ SD[e]	Mend. cM $\pm$ SD	Imp.[d] cM $\pm$ SD	F_{Mend}[e]	F_{red}[f]	Both[g]
20/80	P 0.75 (1.0/0.0)	1.0	1.0	0.75 ± 0.07	0.75 ± 0.06	46 ± 2.4	46 ± 1.4	1.0	0.96	0.96
	P 0.50 (1.0/0.0)	1.0	1.0	0.50 ± 0.07	0.50 ± 0.05	46 ± 4.2	46 ± 2.1	1.0	0.96	0.96
	P 0.25 (1.0/0.0)	0.83	1.0	0.28 ± 0.05	0.25 ± 0.05	46 ± 11.3	46 ± 6.8	0.97	0.95	0.92
	P 0.75 (0.8/0.2)	0.95	0.99	0.46 ± 0.11	0.45 ± 0.11	46 ± 7.1	46 ± 4.2	0.98	0.94	0.93
	P 0.50 (0.8/0.2)	0.82	0.98	0.33 ± 0.08	0.31 ± 0.08	46 ± 10.3	46 ± 6.6	0.94	0.93	0.90
	P 0.25 (0.8/0.2)	0.33	0.74	0.22 ± 0.06	0.18 ± 0.04	47 ± 17.4	46 ± 12.9	0.61	0.70	0.59
	M 0.75 (0.8/0.2)	0.97	1.0	0.46 ± 0.10	0.45 ± 0.09	47 ± 8.1	46 ± 3.6	0.99	0.94	0.94
	M 0.50 (0.8/0.2)	0.85	0.99	0.33 ± 0.07	0.30 ± 0.06	46 ± 11.4	46 ± 6.3	0.97	0.93	0.91
	M 0.25 (0.8/0.2)	0.33	0.74	0.22 ± 0.06	0.18 ± 0.04	47 ± 17.3	46 ± 13.3	0.59	0.7	0.56
2/80	P 0.75 (0.8/0.2)	0.84	0.85	0.51 ± 0.29	0.50 ± 0.29	46 ± 6.7	46 ± 4.0	0.85	0.80	0.80
	P 0.50 (0.8/0.2)	0.76	0.84	0.36 ± 0.20	0.34 ± 0.19	46 ± 9.4	46 ± 5.5	0.82	0.79	0.77
	P 0.25 (0.8/0.2)	0.45	0.69	0.24 ± 0.10	0.20 ± 0.10	47 ± 15.1	46 ± 11.0	0.60	0.64	0.56
	M 0.75 (0.8/0.2)	0.99	1.0	0.46 ± 0.10	0.45 ± 0.08	46 ± 7.0	46 ± 3.5	1.0	0.95	0.95
	M 0.50 (0.8/0.2)	0.88	0.99	0.32 ± 0.07	0.30 ± 0.06	47 ± 10.4	46 ± 5.8	0.97	0.94	0.93
	M 0.25 (0.8/0.2)	0.37	0.78	0.22 ± 0.06	0.18 ± 0.04	48 ± 19.2	46 ± 13.4	0.64	0.74	0.62

QTL were simulated under an imprinting model and analyzed under Mendelian (Mend.) and imprinting (Imp.) models for 400 F_2 individuals with different designs, QTL effects, and allele frequencies.

[a] P, paternally expressed QTL effect; M, maternally expressed QTL effect (frequency of positive QTL allele in F_0).

[b] Proportion of replicates significant at the 5% chromosome-wide level against the H_0 of no QTL.

[c] Estimates and empirical standard deviations, calculated with the replicates that exceed the 5% chromosome-wide significance level.

[d] Analyzed under the appropriate reduced model (Equation 12).

[e] Proportion of replicates significant at the 5% chromosome-wide level and for which a full model explains significantly more variance ($P < 0.05$) than a Mendelian QTL at the position of the best QTL under the respective model.

[f] Proportion of replicates significant at the 5% chromosome-wide level, for which a full model does not explain significantly more variance ($P < 0.05$) than a QTL with a single parental effect at the position of the best imprinted QTL.

[g] Proportion of replicates where tests of both full *vs.* Mendelian (F_{Mend}) and reduced *vs.* full (F_{red}) indicate imprinting.

0.50 and 0.25 revealed that detection of spurious imprinting, when applying only F_{red}, was as high as 29% of the replicates for a QTL effect of 0.35 (data not shown). For smaller QTL effects, the proportion of spurious imprinted replicates decreased as a result of lower power to detect any QTL effect.

For the extreme design, with only two F_1 sires and segregating founder lines, the power to detect QTL under the Mendelian model was lower than that for the design with 20 F_1 sires, for effects of 0.50 and 0.75 (Table 3). F_{Mend} gave levels of spurious imprinting up to 35%, whereas F_{red} indicated imprinting for 24% of the replicates (Table 3). Even when both tests were imposed, spurious imprinting was detected for up to 13% of the replicates under the model with maternal expression (Table 3).

Estimated QTL effects: When founder lines were segregating at 0.80 and 0.20, respectively, the estimated dominance effects were much smaller than the estimated additive effects (data not shown). The estimates of the additive effect were empirically shown to follow

$$\hat{a} = \Delta f \times a, \tag{12}$$

where $\hat{a}$ is the estimated QTL effect, Δf is the difference in allele frequency between the founder lines, and a is the simulated QTL effect. The estimated dominance effects were empirically shown to be proportional to the squared difference in allele frequency between the founder lines,

$$\hat{d} = \Delta f^2 \times d, \tag{13}$$

where $\hat{d}$ is the estimated QTL effect and d is the simulated dominance effect. This shows clearly that the power to detect dominance effects is compromised when founder lines are segregating.

Further analyses: Results of additional simulations of additive QTL for a population of 800 F_2 individuals as well as for a mating design with five F_1 sires and 16 F_1 dams are summarized in Table 4.

For the design with 800 F_2 individuals, there was better power to detect smaller QTL effects individuals, both under fixation and segregation of founder lines, compared to a design with 400 F_2 individuals. For QTL effects between 0.25 and 0.75 and fixation of founder lines, there was considerably less spurious imprinting compared to the design with 400 F_2 individuals (Tables 3 and 4). However, for a QTL effect of 0.15, up to 32% of the replicates showed spurious imprinting following

TABLE 3

Detection and characterization of Mendelian QTL

Simulation details		Power[b]			Imprinting inferred					
					Maternal			Paternal		
No. males/ females F_1	QTL effect[a] (frequency)	Mend.	Mat.	Pat.	F_{Mend}[c]	F_{red}[d]	Both[e]	F_{Mend}[c]	F_{red}[d]	Both[e]
20/80	No QTL	0.05	0.05	0.05	0.03	0.05	0.03	0.03	0.05	0.03
	A 0.75 (1.0/0.0)	1.0	1.0	1.0	0.05	0.00	0.00	0.05	0.00	0.00
	A 0.50 (1.0/0.0)	1.0	1.0	1.0	0.06	0.01	0.00	0.06	0.01	0.00
	A 0.25 (1.0/0.0)	0.85	0.64	0.62	0.07	0.28	0.06	0.06	0.27	0.05
	D 0.75 (1.0/0.0)	1.0	1.0	1.0	0.05	0.0	0.00	0.05	0.00	0.00
	D 0.50 (1.0/0.0)	1.0	0.99	1.0	0.05	0.01	0.00	0.05	0.00	0.00
	D 0.25 (1.0/0.0)	0.96	0.61	0.60	0.06	0.15	0.04	0.06	0.13	0.04
	A 0.75 (0.8/0.2)	0.99	0.94	0.91	0.11	0.11	0.06	0.08	0.07	0.02
	A 0.50 (0.8/0.2)	0.91	0.72	0.71	0.07	0.24	0.06	0.08	0.22	0.06
	A 0.25 (0.8/0.2)	0.37	0.25	0.38	0.05	0.19	0.05	0.06	0.22	0.06
	D 0.75 (0.8/0.2)	0.98	0.88	0.87	0.09	0.09	0.03	0.10	0.06	0.02
	D 0.50 (0.8/0.2)	0.9	0.66	0.67	0.08	0.19	0.06	0.08	0.17	0.05
	D 0.25 (0.8/0.2)	0.39	0.24	0.25	0.07	0.17	0.06	0.07	0.19	0.06
2/80	No QTL	0.05	0.05	0.04	0.03	0.04	0.03	0.03	0.04	0.03
	A 0.50 (1.0/0.0)	1.0	0.99	0.99	0.06	0.01	0.00	0.05	0.01	0.00
	A 0.75 (0.8/0.2)	0.92	0.94	0.82	0.33	0.17	0.13	0.28	0.05	0.03
	A 0.50 (0.8/0.2)	0.83	0.77	0.71	0.18	0.24	0.13	0.21	0.17	0.10
	A 0.25 (0.8/0.2)	0.40	0.28	0.32	0.07	0.21	0.07	0.09	0.24	0.09
	D 0.75 (0.8/0.2)	0.89	0.82	0.77	0.35	0.15	0.12	0.32	0.07	0.04
	D 0.50 (0.8/0.2)	0.83	0.65	0.67	0.19	0.19	0.12	0.21	0.12	0.06
	D 0.25 (0.8/0.2)	0.47	0.30	0.32	0.09	0.20	0.08	0.09	0.20	0.07

QTL were simulated under a Mendelian model and analyzed under Mendelian (Mend.) and imprinting [maternal/paternal (Mat./Pat.)] models for 400 F_2 animals with different designs, QTL effects, and allele frequencies.

[a] A, additive QTL; D, dominant QTL with $a = d$ (frequency of positive QTL allele in F_0).

[b] Proportion of replicates significant at the 5% chromosome-wide level against the H_0 of no QTL.

[c] Proportion of replicates significant at the 5% chromosome-wide level and for which a full model explains significantly more variance ($P < 0.05$) than a Mendelian QTL at the position of the best QTL under the respective model.

[d] Proportion of replicates significant at the 5% chromosome-wide level and for which a full model does not explain significantly more variance ($P < 0.05$) than a QTL with a single parental effect at the position of the best imprinted QTL.

[e] Proportion of replicates where both tests of full *vs.* Mendelian (F_{Mend}) and reduced *vs.* full (F_{red}) indicate imprinting.

F_{red} under the model with maternal expression (Table 4). Under segregation of founder lines, there was considerable spurious imprinting for a QTL effect of 0.25, indicating that also for larger F_2 populations spurious detection of imprinting can be a problem. For the design with five F_1 sires, the proportion of spuriously detected imprinted QTL was lower compared to the design with two F_1 sires, but still considerably higher compared to the design with 20 F_1 sires (Table 4).

DISCUSSION

Detection of imprinted QTL: For imprinted and Mendelian QTL with the same QTL effect there was a higher power to detect imprinted QTL as compared to Mendelian QTL (Tables 2 and 3). This is not surprising given that the variance explained by an imprinted QTL is larger than that of a Mendelian QTL (Table 1). It could, however, be argued that, on average, the effects of imprinted genes are expected to be smaller than those for Mendelian genes, because for an imprinted gene, only one allele is expressed.

For smaller QTL effects and when founder lines are segregating for the same QTL alleles, it was demonstrated that the reduced imprinting models had higher power to detect imprinted QTL than standard Mendelian models (Table 2). Consequently, it is not surprising that performing QTL analyses with reduced imprinting models reveals imprinted QTL that remained undetected under a Mendelian model as found by de Koning *et al.* (2001). However, for practical situations this would imply testing three different models. We did not impose an additional Bonferroni correction for these tests. Since the three models are correlated, it would be over-

TABLE 4

Additional analyses for additive Mendelian QTL

Simulation details					Imprinting inferred					
		Power[b]			Maternal			Paternal		
No. males/ females F_1	QTL effect[a] (frequency)	Mend.	Mat.	Pat.	F_{Mend}[c]	F_{red}[d]	Both[e]	F_{Mend}[c]	F_{red}[d]	Both[e]
20/160	0.50 (1.0/0.0)	1.0	1.0	1.0	0.06	0.00	0.00	0.06	0.00	0.00
	0.25 (1.0/0.0)	0.99	0.90	0.91	0.07	0.12	0.04	0.05	0.12	0.04
	0.15 (1.0/0.0)	0.67	0.49	0.45	0.08	0.32	0.08	0.06	0.25	0.05
	0.50 (0.8/0.2)	0.99	0.94	0.90	0.10	0.10	0.05	0.07	0.06	0.02
	0.25 (0.8/0.2)	0.66	0.47	0.47	0.08	0.26	0.07	0.07	0.26	0.07
5/80	0.75 (0.8/0.2)	0.97	0.84	0.83	0.20	0.18	0.11	0.15	0.05	0.03
	0.50 (0.8/0.2)	0.87	0.74	0.68	0.12	0.26	0.10	0.10	0.20	0.07
	0.25 (0.8/0.2)	0.41	0.27	0.30	0.06	0.21	0.06	0.06	0.23	0.06

QTL were simulated under a Mendelian model and analyzed under Mendelian (Mend.) and imprinting [maternal/paternal (Mat./Pat.)] models for 800 (20/160) and 400 (5/80) F_2 animals with different QTL effects and allele frequencies.

[a] Additive QTL (frequency of positive QTL allele in F_0).

[b] Proportion of replicates significant at the 5% chromosome-wide level against the H_0 of no QTL.

[c] Proportion of replicates significant at the 5% chromosome-wide level, for which a full model explains significantly more variance ($P < 0.05$) than a Mendelian QTL at the position of the best QTL under the respective model.

[d] Proportion of replicates significant at the 5% chromosome-wide level, for which a full model does not explain significantly more variance ($P < 0.05$) than a QTL with a single parental effect at the position of the best imprinted QTL.

[e] Proportion of replicates where both tests of full *vs.* Mendelian (F_{Mend}) and reduced *vs.* full (F_{red}) indicate imprinting.

conservative to count every new model as an additional test. We recommend fitting both the maternal and paternal models separately without an additional Bonferroni correction, accepting a small increase in type I error.

For the design with an extremely low number of F_1 sires and the QTL allele segregating in the founder lines, there is considerably less power to detect paternally expressed QTL compared to maternally expressed QTL. This is because with only two F_1 sires, there is an increased risk that one or both F_1 sires are homozygous for their QTL alleles or have a different phase between line origin and QTL effect. The number of F_1 parents is an important factor to take into account when founder lines are not fixed for their QTL.

Detection of Mendelian QTL: Alfonso and Haley (1998) performed an extensive simulation study on the detection of Mendelian QTL in F_2 designs. The estimated powers in Table 3 correspond generally well with those reported by Alfonso and Haley (1998). The estimated QTL effects reported by Alfonso and Haley (1998) follow approximately the expectations denoted in (11) and (12).

Detection of spurious imprinted QTL: The simulations of the Mendelian QTL showed that spurious detection of imprinting is a serious problem for smaller QTL effects, when founder lines are segregating, and for mating designs with a low number of F_1 sires (Table 3). Obviously, design is not an issue when founder lines are completely fixed for their QTL alleles, but for experimental crosses in livestock this is not a very likely scenario. For most scenarios, the test of Knott *et al.* (1998) (alternative a) is more conservative, while F_{red}, similar to de Koning *et al.* (2000), is more liberal and can give higher rates of spurious imprinting. However, F_{Mend} gave the highest rates of spurious imprinting for larger QTL effects in designs with two F_1 sires. This shows clearly that both tests have their flaws, although F_{Mend} performs better on average for the scenarios considered in this study. For smaller QTL effects, the H_0 of F_{red} appears to be too robust against a purely additive Mendelian QTL. Imposing both tests to infer imprinting kept the level of spurious imprinting <6% for the design with 20 F_1 sires. This could be an ad hoc solution to control the spurious detection of imprinting, but better alternatives should be investigated (*e.g.*, Lee *et al.* 2001). Imposing both tests to the simulations with imprinted QTL resulted in a proportion of correctly identified imprinted QTL that was close or equal to the smaller of the two proportions identified by the individual tests (Table 2). This indicates that the power to detect imprinted QTL would not be greatly affected by imposing both tests.

The designs with only two or five F_1 sires resulted in high proportions of spuriously imprinted QTL, even when both tests (alternative c) were imposed (Tables 3 and 4). Although the detection of imprinted QTL was reasonable compared to the design with 20 F_1 sires, the results for the Mendelian QTL clearly indicate that

these designs are unsuitable for the detection of imprinted QTL when founder lines are segregating. It is not straightforward to provide a yardstick for the minimum number of F_1 parents of each sex that should be used to circumvent the risks of detection of spurious imprinting. However, the results here indicate that with only two or five F_1 sires, not only the power to detect QTL is affected, but also the risk of detection of spurious imprinting is increased. Although this study focused on the effect of mating design in the F_1, the results are also applicable for the mating design of the F_0. In practice, it might seem cost effective to restrict the number of F_0 and F_1 parents to the minimum number that is necessary to obtain the desired number of F_2 individuals. Our study, however, indicates that this is not the best strategy when one of the objectives of a study is to test for imprinting effects.

In our simulation study we considered a relatively simple pedigree structure, which facilitated the use of regression methods and enabled a large-scale simulation study. Due to the approximations involved in regressions methods, one may want to explore data from real QTL experiments in more detail with more advanced methods that can handle complex pedigree structures (*e.g.*, Hoeschele *et al.* 1997; Sillanpää and Arjas 1999). The results obtained in our study will also apply to the more advanced methods of analysis.

In the simulation study, we used fully informative markers and complete imprinting to prevent effects other than those under evaluation from causing any differences in results between models. Both assumptions are unlikely to be met in the analysis of experimental crosses between outbred lines. Uninformative markers lead to an increase in the effective average marker spacing, resulting in a generally lower power. In cases where line origin can be derived, but parental origin cannot, this might compromise correct characterization of the QTL. When a QTL displays partial imprinting, the power to distinguish between imprinted and Mendelian QTL will be a function of the difference between the paternally and maternally inherited alleles. Furthermore, F_{Mend} and F_{red} are expected to give conflicting answers, because F_{red} assumes complete imprinting while F_{Mend} does not.

The effect of the null hypothesis: The H_0 of F_{Mend} is that of a Mendelian QTL whereas the H_0 of F_{red} is that of an imprinted QTL. The results of the simulation study indicate a relationship between the power of the design to detect QTL and the power to discriminate between Mendelian and imprinted QTL. When the power to detect QTL reduces, both F_{Mend} and F_{red} favor the acceptance of their respective H_0, leading to different conclusions, depending on the H_0 of the test. Malécot (1999) demonstrated that the choice of the null hypothesis is never objective, but a result of experiences and ideas of a researcher or a group of researchers. When testing for imprinting, the H_0 of the test clearly affects the conclusion. The null hypothesis that genes, and hence QTL, show Mendelian expression may be the most reasonable H_0 when one is the first researcher to study a new genetic phenomenon. It could, however, be argued that this is partly because most, if not all, genetical research of the 20th century was based on Mendelian principles. The Mendelian principles provide no explanation for reciprocal differences that are observed in crossbreeding and that may be attributable to genomic imprinting. Genomic imprinting has been studied only during the last decade and on the basis of recent findings (*e.g.*, de Koning *et al.* 2000) it should no longer be considered a rare phenomenon.

Furthermore, it could be argued whether the inference of the mode of expression of a QTL should be tested with the same stringent criteria as the existence of that QTL. In other words, is spurious inference of imprinting for a Mendelian QTL (or vice versa) just as serious as spurious detection of a QTL? The discrepancies between the tests as a result of different H_0's make it unlikely that the issue of testing the mode of expression of a QTL can be solved in a classical testing framework. An appealing alternative is to adopt a Bayesian approach (Malécot 1999), where QTL are assigned prior probabilities to show Mendelian or uniparental expression on the basis of knowledge about the proportion of imprinted genes among identified genes.

As science progresses and new observations accumulate, the effect of the subjective parts (*i.e.*, the assumptions and H_0) is expected to diminish (Malécot 1999). With regard to the detection of imprinted QTL, the new information should come not only from independent replicates of QTL studies, but especially from expression studies that can provide proof for imprinting at the molecular level.

Implications: The simulation study showed that, compared to detecting Mendelian QTL, the successful detection and inference on mode of inheritance of a QTL put more demands on the design of the experiment as well as the interpretation of the results. Because the possibility to test for imprinting effects in QTL experiments was only recently described by Knott *et al.* (1998), most QTL mapping experiments to date are not optimized to detect imprinted QTL. It is recommended that researchers include tests for imprinting whenever possible, but critically reflect upon their results with regard to the design of the experiment and the probability of segregation of QTL alleles within founder lines. This holds not only for F_2 crosses between outbred species but also for making strategic backcrosses to test for imprinting effects following Clapcott *et al.* (2000). This strategy relies on finding a QTL in a certain backcross and not in the reciprocal backcross. This is no problem when using completely inbred mice strains, but when it is not completely sure that all F_1 individuals will be heterozygous for the QTL, the design must be

optimized to minimize the spurious detection of imprinted QTL.

Sara Knott is gratefully acknowledged for the source code of the simulation program. Jack Dekkers and Piter Bijma are acknowledged for stimulating discussions and useful comments on the manuscript. This research was supported financially by the Netherlands Technology Foundation (STW). Additional financial support was provided by the Dutch Product Board for Livestock, Meat, and Eggs and the Dutch pig breeding organizations Hypor BV, Dumeco Breeding BV, and Topigs.

LITERATURE CITED

Alfonso, L., and C. S. Haley, 1998 Power of different F2 schemes for QTL detection in livestock. Anim. Sci. **66:** 1–8.

Alleman, M., and J. Doctor, 2000 Genomic imprinting in plants: observations and evolutionary implications. Plant Mol. Biol. **43:** 147–161.

Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

Clapcott, S. J., A. J. Teale and S. J. Kemp, 2000 Evidence for genomic imprinting of the major QTL controlling susceptibility to trypanosomiasis in mice. Parasite Immunol. **22:** 259–263.

de Koning, D.-J., A. P. Rattink, B. Harlizius, J. A. M. van Arendonk, E. W. Brascamp *et al.*, 2000 Genome-wide scan for body composition in pigs reveals important role of imprinting. Proc. Natl. Acad. Sci. USA **97:** 7947–7950.

de Koning, D.-J., A. P. Rattink, B. Harlizius, M. A. M. Groenen, E. W. Brascamp *et al.*, 2001 Detection and characterization of quantitative trait loci for growth and reproduction in pigs. Livest. Prod. Sci. **72:** 185–198.

Falconer, D. S., and T. F. C. Mackay, 1996 *An Introduction to Quantitative Genetics*, Ed. 4. Longman Group, Essex, UK.

Hoeschele, I., P. Uimari, F. E. Grignola, Q. Zhang and K. M. Gage, 1997 Advances in statistical methods to map quantitative trait loci in outbred populations. Genetics **137:** 1445–1457.

Haley, C. S., S. A. Knott and J. M. Elsen, 1994 Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics **136:** 1195–1207.

Jeon, J. T., O. Carlborg, A. Tornsten, E. Giuffra, V. Amarger *et al.*, 1999 A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. Nat. Genet. **21:** 157–158.

Knott, S. A., L. Marklund, C. S. Haley, K. Andersson, W. Davies *et al.*, 1998 Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs. Genetics **149:** 1069–1080.

Lee, H. K., J. C. M. Dekkers, R. L. Fernando and M. F. Rothschild, 2001 Statistical models and tests for detecting imprinted genes in QTL scans. Proceedings of the Annual Meeting of Midwestern Section of ADSA/ASAS, Des Moines, IA, pp. 5–6 (available at http://www.asas.org).

Lloyd, V. K., D. A. Sinclair and T. A. Grigliatti, 1999 Genomic imprinting and position-effect variegation in *Drosophila melanogaster*. Genetics **151:** 1503–1516.

Malécot, G., 1999 Statistical methods and the subjective basis of knowledge. Genet. Sel. Evol. **31:** 269–298.

Morison, I. M., C. J. Paton and S. D. Cleverley, 2001 The imprinted gene and parent-of-origin effect database. Nucleic Acids Res. **29:** 275–276.

Nezer, C., L. Moreau, B. Brouwers, W. Coppieters, J. Detilleux *et al.*, 1999 An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. Nat. Genet. **21:** 155–156.

Sillanpää, M. J., and E. Arjas, 1999 Bayesian mapping of multiple quantitative trait loci from incomplete outbred offspring data. Genetics **151:** 1605–1619.

Communicating editor: J. B. Walsh