Detection of Quantitative Trait Loci in Broilers

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Proefschrift

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Detection of Quantitative Trait Loci in Broilers

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Abstract

In this dissertation methods for the detection of quantitative trait loci (QTLs) were developed and applied to experimental broiler data. QTL analyses were undertaken in a population consisting of ten full sib families of a cross between two broiler lines. Microsatellite genotypes were determined on generation one and two. Phenotypes were collected on generation three animals in two experiments. For initial genome scans, a full sib regression method using interval mapping was developed and applied. QTLs for body weight, growth and feed intake traits were found in a feed efficiency experiment. In a carcass experiment QTLs for carcass percentage and meat colour were detected.

An existing Bayesian method was extended to be able to handle multiple trait data and heterogeneity of variance between sexes, by including scale parameters and a polygenic correlation. Advantages of the Bayesian method in comparison with the regression approach are: accounting for uncertainties, polygenic effects are included and variances can be obtained for all random terms in the model. Furthermore, individual observations are used instead of progeny averages and mate correction is no longer necessary, because all genetic relations are taken into account through relationship matrices. A reduced animal model and Markov Chain Monte Carlo algorithms were applied to obtain solutions. Detailed univariate and bivariate Bayesian analyses were undertaken on chromosomal regions where QTLs were found before, which confirmed previous results.

Stellingen

1. Chromosoom 1 bevat een belangrijk QTL met een effect op voeropname en groei.
   Dit proefschrift.

2. Het onderscheidingsvermogen van QTL-studies moet worden uitgedrukt op genoom niveau als genoomwijze significantie-niveaus worden gebruikt.
   Dit proefschrift.

3. De definities voor genoomwijze significantie van Lander & Kruglyak zijn onpraktisch.
   Lander & Kruglyak (Nature Genetics (1995) 11:241-247).

4. Het verder ontwikkelen van de in dit proefschrift gebruikte MCMC methode verdient hoge prioriteit.
   Dit proefschrift.

5. De Bonferroni correctie voor het uitvoeren van meerdere toetsen in één experiment zou veel algemener moeten worden toegepast.

6. Snellere computers verleiden kwantitatieve genetici tot het gebruik van complexere modellen, maar de toename van de daarvoor benodigde hoeveelheid data om de parameters van deze modellen te kunnen schatten houdt daarmee geen gelijke tred.

7. Het betalen van wachtgelden op centraal universitair niveau is niet bevorderlijk voor het tijdig afronden van promotie-onderzoek.

8. Wageningen-UR zal altijd WUR genoemd blijven worden.

9. Het is wenselijk dat de Europese eenwording ook gaat gelden voor titels.

10. Het niet openbaar zijn van de broncode van computerbesturingssystemen werkt ongewenste monopolies in de hand.

11. Het bruto produkt per inwoner is vaak een betere maat voor de staat van de economie als het Bruto Nationaal Produkt per land.

12. Een belangrijke oorzaak waardoor de Verenigde Staten bij belangrijke ontwikkelingen vaak voorop loopt, is de flexibiliteit van arbeid en kapitaal.

13. Tweebaans rotondes zonder stoplichten leiden tot afsnijden.

Stellingen behorend bij het proefschrift van Jan-Thijs van Kaam
'Detection of Quantitative Trait Loci in Broilers'
Wageningen Universiteit, 5 september 2000

Voorwoord

Het in dit proefschrift beschreven onderzoek is uitgevoerd bij de Leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit. Van buiten Wageningen is hieraan bijgedragen door Euribrid B.V. en Cornell University. Vele mensen hebben een directe of indirecte bijdrage geleverd aan dit proefschrift of hebben bijgedragen aan de plezierige tijd tijdens mijn AIO-schap. Te veel om iedereen op te noemen, maar een aantal personen wil ik met name noemen.

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Contents

Chapter 1

General Introduction

Introduction

The art of animal breeding is aimed at achieving genetic progress. In order to be able to select those animals which are most likely to contribute to this genetic progress animal populations are subjected to genetic evaluations. Until recently genetic evaluations were entirely based on phenotypic data. In the last 15 years techniques have been developed which enable scientists to detect genes. Since then a huge increase in efforts to dissect the genome of many species has taken place. In the detection of genes genetic markers are used. These genetic markers are small pieces of DNA, which can be genotyped easily and which are used to follow the transmission of chromosomal segments from parents to offspring.

Reasons for the interest in genomics are (1) the curiosity about the biological function of genes and the construction of chromosomes (2) the identification of genetic differences between individuals, breeds or species (3) pedigree control (4) finding methods to cure diseases which have in part a genetical background (5) prevention against the occurrence of diseases or genetical defects and (6) finding new or more efficient ways to select for desirable traits or against undesirable traits. This latter reason is the most important reason for animal geneticists working on farm animals to make large efforts for discovering and utilising new genomic information.

The utilisation of the newly available information on genetic markers requires statistical techniques to reconstruct the configuration of the genome and to associate the segregation of chromosomal segments to phenotypes. In order to discover the location of the genetic markers a linkage map is constructed. The obtained linkage map is used in the attempts to discover the position of genes. Animal breeders are mostly interested in traits, which have a quantitative nature. These traits are usually influenced by a number of loci. Such loci are named quantitative trait loci or QTLs. Information about these QTLs makes it possible to select more accurately and more quickly. The accuracy increases because it is possible to improve the genetic evaluation by adding new information. The speed of selection can be improved because it is possible to obtain genetic information on animals of any age, even right after conception.

The complexity of the genome makes it difficult and costly to determine exactly which gene has an effect on a certain trait. Therefore animal breeders usually chose for marker assisted selection in which only a marker interval wherein a QTL is located needs to be

known (Fernando and Grossman, 1989). Once the genes or closely linked markers are known the selection can be based on this information.

In commercial broiler breeding genetic improvements for most of the traits analysed in this study can easily be achieved through traditional selection on breeding values obtained from phenotypic information. Therefore marker assisted selection for these traits is not a logical aim. The traits under study are mainly model traits to develop methodology, which can be applied to traits that are hard to select for in commercial breeding such as disease traits. Such traits are usually difficult or costly to measure hence using phenotypic information is undesirable. Using marker assisted selection or locating the genes of interest can be a more viable alternative.

History of the Wageningen-Euribrid experimental population

In 1990 a collaboration, between Euribrid B.V., then a division of Nutreco N.V., and the Animal Breeding and Genetics Group of Wageningen University with the purpose of mapping QTLs in broilers was started. From 1991 till 1996 Dr. Syne van der Beek worked on his dissertation, funded by Euribrid B.V., which aimed at finding an ideal set-up for a QTL mapping population (Van der Beek, 1996). Using these findings and recommendations, Euribrid B.V. started with producing an experimental population in 1993. In the set-up of the experiment the choice was made to divide the third generation animals over five experiments in which phenotypic information on different groups of traits was collected. In total phenotypic observations on 14,000 animals were collected. This experiment was the first experiment in poultry with a set-up of this size.

Five experiments have been undertaken. A feed efficiency experiment, in which measurements were taken at 23, 48 and 63 days on approximately 2,000 animals, was undertaken. Furthermore two carcass experiments were undertaken, one in which measurements were taken at 48 days and the other in which measurements were taken around 70 days, in both experiments approximately 2,000 animals were observed. Finally, two experiments were carried out on diseases each with approximately 4,000 animals.

At the same time, molecular genetic research focussed on developing microsatellites to cover the entire genome. Our laboratory used the blood samples collected on the first and second generation animals to find microsatellite markers and amplified fragment length polymorphism markers and to determine the genotypes of these animals for these markers. The marker information was used to reconstruct the chicken genome leading to a linkage

map, which included a large number of markers and informative meiosis in the world (Groenen *et al.*, 1998). Through collaboration with other laboratories a consensus linkage map has been created by integrating the mapping information available on the East Lansing and Compton reference populations with that of the Wageningen linkage map (Groenen *et al.*, 2000).

Between 1991 and 1998 Dr. Carolien Ruyter-Spira was working on her dissertation (Ruyter-Spira, 1998) dealing with the mapping of monogenic traits on the chicken genome. She contributed in the development of new microsatellite markers and worked on a candidate gene for the autosomal dwarf locus. Furthermore, Richard Crooijmans was working on his dissertation and contributed to the development of the linkage map and genotyping. The construction of the Wageningen chicken BAC library (Crooijmans *et al.*, 2000) is an essential tool for fine-mapping of the QTL regions. Detailed comparative mapping between chicken and human will enable the identification of candidate genes.

Aim of this dissertation

This dissertation deals with the statistical analysis of data aimed at the localisation of QTLs and the estimation of magnitude of the additive effect of these QTLs. A whole genome analysis has been undertaken using regression analysis and a detailed analysis of chromosomal regions containing QTLs was performed using a Bayesian method modelling an animal model including a QTL. Carcass, growth and feed efficiency traits were studied.

Outline of this dissertation

In Chapter 2 the regression methodology used to analyse the experimental data is explained. Chapter 3 and 4 show the results of the application of the regression methodology for whole genome scans on data obtained in a feed efficiency respectively a carcass experiment. In Chapter 5 the Z-chromosome is analysed using a modification of the regression methodology to enable the analysis of sex chromosomes. Chapter 6 introduces a Bayesian method for more detailed analysis of chromosomal regions in which all parameters are sampled simultaneously accounting for uncertainties. In Chapter 7 the Bayesian method is extended to enable a multiple trait analysis, which is expected to increase the power of QTL detection. Finally, Chapter 8 is a general discussion.

Chapter 2

Whole Genome Scan for Quantitative Trait Loci affecting Body Weight in Chickens using a three Generation Design

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Abstract

An experimental population containing 10 full sib families of a cross between two broiler lines was created. In this population, blood samples from 20 full sib animals in generation 1 and 451 full sib animals in generation 2 were used for marker genotyping. Data on body weight at slaughter age (48 days) collected in a feed efficiency experiment with 2,049 individually housed grandoffspring was analysed. Large differences in mean and variance between male and female body weight were found. To account for these differences, a bivariate analysis treating body weight of males and females as separate traits was used to estimate (co)variance components and breeding values. The model accounted for systematic environmental effects and maternal effects. The estimated heritability of body weight was 0.28 in the males and 0.33 in the females and the genetic correlation between male and female body weight did not significantly deviate from unity. Estimated breeding values, fixed and maternal genetic effects were used to calculate average adjusted progeny trait values for all generation 2 animals adjusted for fixed and maternal genetic effects and for the additive genetic contribution of the other parent. Male and female progeny trait values were combined in one trait value adjusting for sex differences by standardisation for mean and variance. This average adjusted progeny trait value was used for QTL detection.

To study presence of QTLs, an across family weighted regression interval mapping approach was used both in half sib as well as a full sib QTL analysis. Genotypes from 368 markers mapped on 24 autosomal linkage groups were available. The most likely position for a QTL affecting body weight was found on chromosome 1 at 240 cM with a test statistic of 2.32. Significance levels were obtained using the permutation test. The chromosomewise significance level of this QTL was 10%, whereas the genomewise significance level was 41%.

New aspects of this study are: Genomewide QTL analysis in poultry, full sib analysis in an outbred population structure and correction for heterogeneous variances between sexes.

Introduction

For many years, poultry breeding schemes have operated without knowledge of the actual genes underlying the traits under selection. Until recently, the tools to detect the genes responsible for genetic differences between individuals or between populations were not available. Recently a considerable number of DNA markers have been mapped for poultry (Crooijmans *et al.*, 1996). The availability of a genetic linkage map for chickens (Bumstead and Palyga, 1992; Levin *et al.*, 1994; Burt *et al.*, 1995; Crooijmans *et al.*, 1996) facilitates the mapping of genes affecting quantitative traits (QTLs).

Several studies have indicated that knowledge about genetic markers linked to genes affecting quantitative traits can increase the selection response of animal breeding programs, especially for traits that are difficult to improve when using traditional selection (e.g. Kashi *et al.*, 1990; Meuwissen and Van Arendonk, 1992; Van der Beek and Van Arendonk, 1996; Meuwissen and Goddard, 1996). For an outcross poultry breeding population, Van der Beek and Van Arendonk (1996) predicted additional cumulative selection responses, after five generations of selection, of 6 to 13% due to the incorporation of a marked QTL. Besides increased genetic improvement, the detection of QTL is a first step towards cloning genes underlying quantitative traits and studying their physiology. This would greatly advance our understanding of quantitative genetic variation and its physiological background.

In poultry, a few genes with economically important effects have been identified, e.g. the dwarf genes and the naked neck gene (Mérat, 1990). Further, the effects of some protein polymorphisms and blood groups have been studied (Mérat, 1990). Bitgood and Somes (1993) give an overview of identified genes in chickens. More recently, a number of studies have reported associations between random genetic markers and quantitative traits (e.g. Plotsky *et al.*, 1993; Dunnington *et al.*, 1992; Lakshmanan *et al.*, 1994). However, to our knowledge so far no genome wide scans for QTLs have been performed in poultry, i.e. using markers covering the whole or most of the genome.

A number of quantitative genetic studies has been undertaken to determine appropriate experimental designs for association studies in outbred populations (Weller *et al.*, 1990; Van der Beek *et al.*, 1995). To make optimal use of the reproductive capacity of poultry, Van der Beek *et al.* (1995) suggested the use of a three generation full sib-half sib design. In this

design, first generation animals are used to produce full sib families. The second generation animals are mated to individuals from other families to produce half sib grandoffspring. Genotypes are determined for generation one and two animals while phenotypic information is collected on third generation grandoffspring. This design turned out to be very efficient in terms of number of marker genotypes: a full sib design resulted in a doubling of information compared to a half sib design because two instead of one marker contrast per family can be computed. Regression methods to analyse data from association studies have been developed for half sib designs (Knott *et al.*, 1994). However, no regression methods have been described so far for the QTL analysis of a full sib design in outbred populations.

The aim of the present study is to detect and localise QTLs in a three generation design. For this purpose, a regression approach was developed that takes the full sib structure of the marker data into account. The method was applied to body weight. Half sib sire and half sib dam QTL analysis was performed to determine whether a QTL segregates in male or female generation 2 animals only. Fitting a QTL for both parents is compared with fitting a QTL for one parent only. Separate QTL analysis was performed for male and female generation 3 animals in order to determine the extent to which genetic differences in both sexes are controlled by the same QTLs. Unique aspects of this study are: Genomewide QTL study in poultry, full sib analysis in outbred population and correction for heterogeneity between sexes.

Material and methods

Experimental population

The family structure of the population used in the QTL mapping experiment was designed following recommendations of Van der Beek *et al.* (1995). The design was based on what Van der Beek *et al.* (1995) termed a three generation full sib half sib design: parents, full sib offspring and half sib grandoffspring. In this article, parents, offspring and grandoffspring are called generation 1, 2 and 3 animals or G_1, G_2 and G_3 animals, respectively. G_1, G_2 and G_3 were chosen instead of F_1, F_2 and F_3 to avoid confusion with the terminology for inbred lines.

In order to increase the probability of parents being heterozygous for QTLs, and therefore the power, the population was produced by crossing two genetically different outcross broiler dam lines originating from the White Plymouth Rock breed. The maternal line had a relatively high reproductive performance and the paternal line had a relatively high growth performance. The two lines were chosen out of a group of six lines with a genetic distance, calculated as Rogers's distance (Nei, 1987) on 16 microsatellite markers, ranging from 0.15-0.40. For these two lines, Rogers's distance was 0.37. Phenotypic differences in number of eggs and slaughter weight were about 20% respectively 15%. The population structure is given in Table 1. The G_1 animals were mated to produce full sib G_2 families. Each G_2 animal was repeatedly mated with other G_2 animals to generate sufficiently large half sib families. G_2 animals from each full sib family were mated to G_2 animals from different families. Each G_2 male was mated to on average 4.5 G_2 females, and each G_2 female was mated to 2.8 G_2 males to produce the G_3 animals. On average, each G_2 animal was mated to 3.4 mates resulting in 2.7 G_3 full sib animals per mating. In each generation, mating of related individuals was avoided.

Table 1. Population structure with observations and numbers of animals used in the analysis.[a]

Generation[b]	Males	Females	Total	Observations
G_0[c]	14	14	28	
G_1	10	10	20	Genotypes
G_2	172	279	451	Genotypes
G_3	1,012	1,037	2,049	Phenotypes

[a] Numbers exclude outliers and missing values.

[b] G_0 etc. = generation 0 etc.

[c] Male and female G_0 animals are from different lines.

In the three generation design, G_1 and G_2 animals were typed for genetic markers and phenotypic information was collected for G_3 animals. On average, each G_1 full sib family consisted of 45.1 genotyped G_2 animals and on average, each G_2 animal had 8.9 progeny.

Phenotypic observations of the G_3 animals were obtained for several traits in a feed efficiency experiment. In this article, body weight at slaughter age (48 days) was analysed. In total 5 hatches of G_3 animals were produced and phenotyped. All animals within a hatch were born within approximately 24 hours. From 0 to 22 days, the animals were kept in

groups. The animals were housed in individual cages between the age of 22 and 48 days. During their entire life, feed and water were supplied *ad libitum*. The barns were artificially lighted 23 hours a day. Climate was controlled according to normal commercial practice. Phenotypic observations for body weight at 48 days were available for 2,081 G_3 animals. Within each hatch separately, observations deviating more than 3 standard deviations from the mean, were considered to be the result of measurement errors and were therefore excluded from the analysis. These outliers were randomly distributed across families, indicating that no genetic component was involved. In total 32 records (1.5%) were excluded, so data on 2,049 G_3 animals was used for analysis. Not all G_3 animals contributed information to both a G_2 male and a G_2 female, because 8 G_2 males and 6 G_2 females were not genotyped. In total, 1,995 G_3 animals contributed phenotypic information to the G_2 males and 2,021 to the G_2 females.

Marker data

Blood samples from the G_1 and G_2 animals were collected for genotyping. In total, 20 G_1 and 456 G_2 animals were analysed for marker genotypes and 451 G_2 animals had progeny with observations in this experiment. All 368 markers, which were used in this experiment, were microsatellite markers and were informative in some of the families. Marker alleles were recorded in basepair units. The markers were mapped to 24 autosomal linkage groups. When averaging over both sexes the markers cover 3,128 cM using the Haldane (1919) mapping function or 2,712 cM using the Kosambi (1944) mapping function. In total 240 markers were genotyped on all 10 families, and for efficiency reasons, 128 additional markers were genotyped on 4 families. The linkage map was constructed using CRI-MAP (Green *et al.*, 1990) which uses the Kosambi mapping function. Further analyses were performed, using the recombination fractions obtained from CRI-MAP (Green *et al.*, 1990) transformed to Haldane map distances. Because the linkage map is very dense, the influence of the mapping function will be negligible. The average distance between successive markers was 8.5 cM.

Because not all linkage groups have been assigned to chromosomes, the numbering of linkage groups in this paper does not correspond to chromosome numbers. Linkage groups differ in length from a few centimorgans to over 600 cM. The chicken genome consists of 39 chromosome pairs. Bloom (1981) divided the chromosomes in three size groups, 5 are

considered as macrochromosomes, 5 as intermediate chromosomes and the remaining 29 as microchromosomes. Chromosomes, for which no markers were available, were not analysed. These are all microchromosomes or sex chromosomes. Estimates of the length of the chicken genome based on chiasma counts are between 2,800 and 3,300 cM (Rodionov *et al.*, 1992; Bitgood and Shoffner, 1990, discussing data of Pollock and Fechheimer, 1978). Because the sex chromosomes and some microchromosomes were not analysed, the markers covered around 85% of the genome. More information about the marker data is given in Table 2.

Analysis of the phenotypic data

The data was analysed in a two step procedure: first phenotypic data was analysed and combined and secondly QTL analysis was performed using the results of the previous analysis.

In a three generation design, G_3 animal's phenotypes are used to calculate the mean progeny performance of G_2 animals (Van der Beek *et al.*, 1995). Phenotypic observations on G_3 animals might be influenced by a number of systematic environmental effects. In addition, phenotypic observations might be influenced by a maternal genetic effect of the G_2 dam. These effects are expected to result in a less efficient detection of QTL and therefore phenotypic observations need to be adjusted for these effects.

Males and females differed with respect to mean and standard deviation for body weight at 48 days: average for cocks was 2,369 g with a standard deviation of 309 g, and for hens 2,030 g with a standard deviation of 230 g. Because methodology used for QTL detection assumes homogeneous residual variances, it was expected that differences in variances between sexes might influence QTL detection. To account for this, male and female body weight were treated as separate traits and a bivariate variance component and breeding value estimation was performed.

Table 2. Information about the linkage groups. Length in centimorgans, number of markers, percentage of heterozygosity of the markers and the average information content for both sexes is given for all linkage groups.

Linkage group[a]	Length in cM	Number of markers	Marker Heterozygosity	Average information content Males	Females
WAU1	622	78	60.7%	0.75	0.73
WAU2	475	64	57.4%	0.75	0.73
WAU3	343	36	58.3%	0.65	0.68
WAU4	291	33	63.0%	0.71	0.70
WAU5	193	24	61.4%	0.71	0.73
WAU6	123	13	61.9%	0.58	0.61
WAU7	184	14	64.8%	0.66	0.65
WAU8	90	11	61.7%	0.79	0.76
WAU9	89	11	56.0%	0.69	0.81
WAU10	101	8	54.4%	0.66	0.67
WAU11	103	14	59.0%	0.66	0.64
WAU12	57	6	60.4%	0.64	0.74
WAU13	60	6	69.2%	0.78	0.74
WAU14	87	6	66.7%	0.62	0.60
WAU15	50	7	63.8%	0.80	0.79
WAU16	70	6	66.2%	0.69	0.74
WAU17	53	5	60.6%	0.78	0.74
WAU18	21	3	63.2%	0.78	0.81
WAU19	27	5	57.7%	0.79	0.83
WAU20	4	2	49.0%	0.82	0.80
WAU21	3	3	45.7%	0.83	0.80
WAU22	3	2	50.5%	0.78	0.83
WAU23	59	7	69.4%	0.65	0.71
WAU24	21	4	69.8%	0.83	0.80
WAU1-24	3,128	368	60.4%	0.71	0.71

[a] WAU = Wageningen University, Wageningen.

A preliminary analysis using a fixed effect model was performed with Statistical Analysis System (SAS) Proc GLM (SAS institute, 1985) to determine significance of main

effects and interactions. The most important fixed effects were included in the final mixed model for male and female body weight:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & 0\\ 0 & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_1 & 0\\ 0 & \mathbf{Z}_2\end{bmatrix}\begin{bmatrix}\mathbf{u}_1\\ \mathbf{u}_2\end{bmatrix}+\begin{bmatrix}\mathbf{W}_1 & 0\\ 0 & \mathbf{W}_2\end{bmatrix}\begin{bmatrix}\mathbf{d}_1\\ \mathbf{d}_2\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

where:

$\mathbf{y}_i$ = Vector of observations for trait i = 1 (male) or 2 (female), where $y_{i,jklmn}$ is an element representing an observation for trait i on animal m born in hatch j and housed in location l and with dam n born in hatch k $(m = 1,...,2{,}049)$

$\mathbf{b}_i$ = Vector of fixed effects for trait i with elements $b_{i,jkl}$

$\mathbf{u}_i$ = Vector of random direct additive genetic effects for animal m on trait i with elements $u_{i,m}$

$\mathbf{d}_i$ = Vector of random maternal genetic effects for dam n on trait i with elements $d_{i,n}$ $(n = 1,...,285)$

$\mathbf{e}_i$ = Vector of random residual effects for trait i with elements $e_{i,jklmn}$

$\mathbf{X}_i$ = Incidence matrix relating observations for trait i to fixed effects

$\mathbf{Z}_i$ = Incidence matrix relating observations for trait i to direct additive genetic effects

$\mathbf{W}_i$ = Incidence matrix relating observations for trait i to maternal genetic effects

The elements in the vectors of fixed effects included:

μ_i = Overall mean for trait i

$hatch_{i,j} \cdot hatch_{i,k}$ = Interaction term for trait i between the hatch j of animal m and the hatch k of animal m's dam n $(j = 1,...,5; k = 1,...,8)$

$location_{i,l}$ = The effect of location l in the barn where animal m was housed on trait i $(l = 1,...,36)$

Expectations for all random effects were 0 and variances of the model were defined using the additive genetic covariance matrix multiplied with the additive genetic relationship matrix for the genetic components and the identity matrix multiplied with the covariance matrix for residual effects. Covariances between genetic and residual effects were 0.

In the model, the interaction term between the 'hatch of the animal' and the 'hatch of the dam' represented the period of the year and the age of the dam at reproduction. The barn in which the experiment was conducted was divided in 36 locations. Row (1 to 6), part of a row (1 to 3) and levels within a row (1 or 2) determined each location. Variance component and breeding value estimation were performed using the bivariate animal model above as implemented in MTDFREML (Boldman *et al.*, 1995). Pedigree data of all animals from G_0 to G_3 was used.

Adjusted trait values were calculated by correcting the phenotypic observations for the fixed and maternal genetic effects in the model and for the additive genetic contribution of the other parent. For each of the grandoffspring, two adjusted trait values were calculated: one for each of its parents. As correction for the other parent, half the estimated breeding value of this parent was subtracted. The differences between the sexes in mean and standard deviation of 48 day body weight was taken into account by standardising the adjusted trait values before combining them to one average adjusted progeny trait value for each G_2 animal. Standardisation was done by subtracting the average male adjusted trait value from all male adjusted trait values and the female average from all female adjusted trait values. Furthermore the female adjusted trait values were divided by their standard deviation and multiplied with the standard deviation of the male adjusted trait values. This puts the female trait values on the same scale as the male trait values. Subsequently for all G_2 animals, adjusted and standardised trait values were averaged over their G_3 progeny. The average adjusted progeny trait values from the G_2 animals were used as the dependent variable in the QTL analysis.

QTL analysis

To analyse the data of the full sib design a method was developed based on the multi-marker regression method of Knott *et al.* (1994) for outbred populations with a half sib structure.

Marker inheritance and haplotype reconstruction

Haplotypes of all G_1 animals were reconstructed using the marker order and the recombination fractions as obtained from a CRI-MAP (Green *et al.*, 1990) analysis. For each marker allele of every G_2 animal, it was determined whether it was identical by

descent to the first or the second allele of the G_1 sire or G_1 dam. Transmission of marker alleles to the G_2 animals could only be traced back to the parental haplotypes, if the parent was heterozygous and the other parent did not have the same genotype. Subsequently, the most likely haplotypes of the G_1 animals were reconstructed for each linkage group by minimising the number of observed recombinants for each pair of adjacent informative markers (Knott *et al.*, 1994). In cases where both phases were equally likely for a given interval then, one of them was taken at random.

Conditional probabilities of putative QTL inheritance

For each G_2 animal, the probabilities of inheriting a putative QTL allele from one of the parents was calculated at fixed positions throughout all linkage groups. The Haldane mapping function was used. Probabilities were calculated conditional on the marker genotypes of the G_2 animals on the nearest informative marker or marker bracket per parent. These informative markers can be different for the G_1 sire and the G_1 dam within a family. Probabilities depended only on the genotypes for these markers or marker brackets and on the recombination fraction between the putative QTL and these markers. The procedure was restricted to the areas covered by the linkage map and therefore covered the same areas in all families. For some individuals or families, the chosen QTL position was outside the area with flanking informative markers. For this situation, the conditional probabilities depended only on one marker, namely the nearest informative marker.

Information content

For all linkage groups, the information content was calculated. If the inheritance of each cM of DNA would be known with certainty then the distribution of the conditional QTL probabilities would have an expected mean of 0.5 and variance of 0.25: progeny has (1) or has not (0) inherited the QTL allele. The variance reduces when there is uncertainty about the inheritance of a QTL allele. The information content shows the ratio of the actual variance found in the data and the expected variance under full information (Spelman *et al.*, 1996). The information content will be lower when the distance from the nearest informative marker is higher and when markers are less informative. Power of detection of QTLs will be less in regions where the information content is lower.

Full sib QTL analysis

QTL analysis was undertaken with a regression approach to fit a single multi-allelic QTL across all families. Because marker-QTL linkage phase can differ between families, QTL analysis was nested within families. The average adjusted progeny trait values from the G_2 animals were regressed on the probabilities of inheriting the first parental allele of each parent. The family mean was included in the model to account for polygenic genetic differences between families. The across family full sib model to fit a QTL was:

$$y_{ij} = f_i + b_{s,ik}x_{s,ijk} + b_{d,ik}x_{d,ijk} + e_{ijk}$$

where:

y_{ij} = Average adjusted progeny trait value for the j^{th} G_2 animal of family i

f_i = Polygenic effect of family i

$b_{s,ik}$ = Regression coefficient for sire s in family i at chromosomal position k

$x_{s,ijk}$ = Probability that the j^{th} G_2 offspring from sire s in family i received the chromosomal segment at position k from haplotype 1.

$b_{d,ik}$ = Regression coefficient for dam d in family i at chromosomal position k

$x_{d,ijk}$ = Probability that the j^{th} G_2 offspring from dam d in family i received the chromosomal segment at position k from haplotype 1.

e_{ijk} = Random residual

The regression coefficients represent the QTL allele substitution effects per parent (Falconer, 1989). Note that allele substitution effects on average adjusted progeny trait values are about half the size of allele substitution effects on breeding values, because breeding values represent twice the average deviation of the progeny from the mean. A weighting factor was applied to account for differences in number of G_3 animals contributing to G_2 average adjusted progeny trait values. The weighting factor for the full sib design is based on the variance of the average adjusted progeny trait values of the G_2 animals being:

$$\text{Var(average adjusted progeny trait value)} = \frac{\sigma_p^2}{w_{ij}}$$

Assuming, that the phenotypic variance for all observations is equal, the weighting factor was calculated as:

$$w_{ij} = \left(\frac{to}{1-.5h^2+.25h^2\left(to+\left(\frac{fo_1^2+\ldots+fo_t^2}{to}\right)\right)} \right)$$

Where h^2 is the heritability, to is the total number of G_3 offspring for a G_2 animal, t is the number of mates for that G_2 animal and fo_1 till fo_t are the number of full sib G_3 offspring of that G_2 animal with each of its t different mates. Therefore, fo_1 till fo_t are the number of full sibs per combination and the total, $\left(\sum_{i=1}^{t} fo_i\right)$, is equal to the total number of G_3 offspring to. The average of the heritabilities for male and female body weight was used to calculate the weighting factor.

In solving the equations, singularity problems can occur due to equal haplotypes of both parents in a block of adjacent markers. On short linkage groups, containing no other informative markers, this can result in equal probabilities of inheriting an allele from either parent and therefore full singularity occurs. On larger linkage groups, this can result in near-singularity, because markers outside the block will contribute some information. When full singularity occurred, a generalised inverse was used to obtain solutions. In situations with near-singularity, all effects were fitted, but the solutions for the effects and their standard error can be very large.

In order to test for the alternative hypothesis of the presence of QTL effects, versus the null hypothesis of the absence of QTL effects, several test statistics were calculated. The models underlying these test statistics are given in Table 3.

Table 3. Genetic effects, which were fitted in the four models, used in the analysis.

Model	Family mean	QTL effect sire	QTL effect dam
1	+	+	+
2	+	+	
3	+		+
4	+		

For the full sib QTL analysis 3 test statistic were calculated to compare model 1 with model 2, 3 and 4. The test statistics are indicated with the models, which are compared, within brackets, for example test statistic(1:4) compares model 1 as alternative model with model 4 as model under the null hypothesis. All test statistics are a ratio of the explained mean square of the QTL effects under study in the numerator and the residual mean square of the full model in the denominator. These test statistics are similar to an F-statistic but do not follow an F-distribution and therefore are not termed as such (Spelman *et al.*, 1996). The test statistics at position k were calculated as:

$$\text{Test statistic}_k(\mathrm{H_1} : \mathrm{H_0}) = \frac{\left(\dfrac{\mathrm{RSS}_k(\mathrm{H_0}) - \mathrm{RSS}_k(\mathrm{H_1})}{df_{QTL}}\right)}{\left(\dfrac{\mathrm{RSS}_k(\mathrm{H_1})}{df_{total} - df_{family} - df_{QTL}}\right)}$$

where $\mathrm{RSS}_k(\mathrm{H_0})$ is the residual sums of squares from all families of the reduced model. When the null hypothesis is the absence of a QTL (model 4):

$$\mathrm{RSS}_k(\text{model 4}) = \sum_{j=1}^{n} w_{ij}\left(y_{ijk} - f_i\right)^2$$

When the null hypothesis states the presence of QTL effects for either sex (model 2 and 3) then the term for the other sex $\left(b_{s,ik}x_{s,ijk} \text{ or } b_{d,ik}x_{d,ijk}\right)$ was included in the previous equation. $\mathrm{RSS}_k(\text{model under } \mathrm{H_1})$ is the sum of the residual sums of squares from all families of the full model (model 1) at position k:

$$\mathrm{RSS}_k(\text{model 1}) = \sum_{j=1}^{n} w_{ij}\left(y_{ijk} - f_i - b_{s,ik}x_{s,ijk} - b_{d,ik}x_{d,ijk}\right)^2$$

The degrees of freedom were determined by the number of G_2 animals $\left(df_{total}\right)$, the number of QTL effects $\left(df_{QTL}\right)$ fitted and the number of family means fitted $\left(df_{family}\right)$.

These test statistics were calculated at every centimorgan for each linkage group. The position maximising the test statistic is the most likely location for the presence of a QTL on that linkage group. The effect of (near-)singularities on the test statistics was found negligible.

Half sib QTL analysis

Besides a full sib QTL analysis, a half sib QTL analysis was performed in order to compare half sib and full sib QTL analysis. With a limited number of families, different results between half sib sire, half sib dam and full sib analysis can be expected, due to differences in frequency of parental QTL genotypes between sexes. The difference between the full sib and the half sib QTL analysis lies in number of regression coefficients in the regression model. For a half sib sire QTL analysis, the dam term in the model $(b_{d,ik}x_{d,ijk})$ was removed from the QTL model equation. Likewise, for a half sib dam QTL analysis the sire term $(b_{s,ik}x_{s,ijk})$ was removed. For the half sib QTL analysis the same full sib weighting factor was applied because the weighting factor was based on the population structure and the heritabilities and not on the number of QTL effects fitted. Test statistics for the presence of QTL effects were calculated to compare model 2 and 3 with model 4 (Table 3). The calculation of the test statistics was adjusted accordingly.

Significance thresholds

Significance thresholds were calculated using the method of permutation testing (Churchill and Doerge, 1994). This is an empirical method, which accounts for the distribution of the marker and phenotypic data. Through random shuffling of the phenotypic observations and the corresponding weighting factors of these observations, any relation between QTLs and marker genotypes is broken. For each shuffle a test statistic was calculated and stored. The stored test statistics were sorted in descending order and the i^{th} highest value taken for the x % significance level, e.g., the 100th highest value from 10,000 for a 1% significance level.

For each chromosome 10,000 permutations were performed. To obtain genomewise significance thresholds the chromosomewise significance thresholds were corrected for multiple testing along the genome with the Bonferroni correction. Alternatively, permutation on all 24 linkage groups simultaneously was applied to calculate the genomewise significance threshold.

Results

Variance estimations

The estimated phenotypic variance for male and female body weight at 48 days was 59,916, respectively 42,555 and the covariance between them was 15,416. Estimated genotypic variance was 16,742, respectively 13,944 for male and female body weight at 48 days and the covariance between them was 14,820. Maternal genetic effect resulted in a variance of 1,819 respectively 483 on male and female body weight. The heritabilities and the genetic correlations are presented in Table 4.

Table 4. Matrix with heritabilities h^2 and m^2 on the diagonal and genetic correlations r_g as off-diagonal elements.[a]

	u_1	u_2	m_1	m_2
u_1	0.28			
u_2	0.97	0.33		
m_1	-0.03	0.21	0.03	
m_2	-0.52	-0.30	0.85	0.01

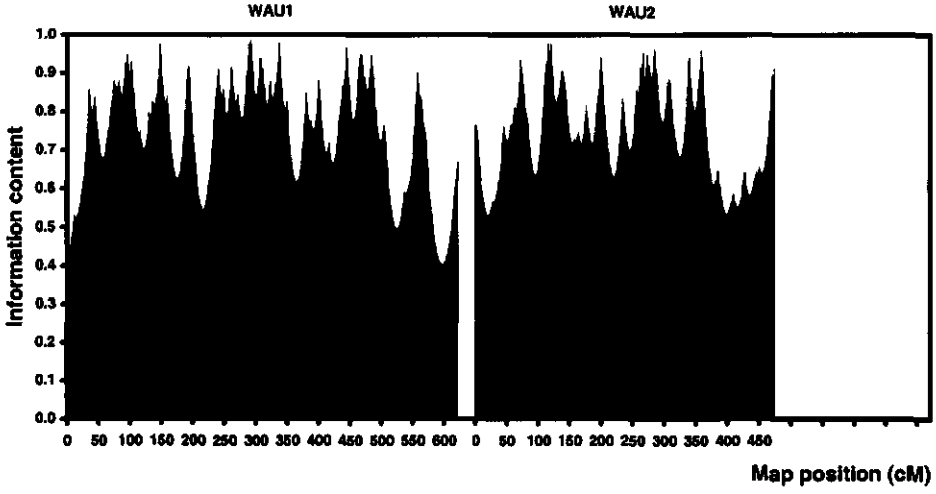
[a] u_1,u_2 = additive genetic effect on male respectively female body weight, m_1,m_2 = maternal genetic effect on male respectively female body weight.

The estimated heritability of body weight was 0.28 for males and 0.33 for females. Thomas *et al.* (1958) reported that several investigations found higher estimates for the heritability of body weight in female than in male broilers. The genetic correlation between male and female body weight was close to unity, indicating that body weight is mainly determined by the same genes in males and females. The size of the effect of the genes for body weight, however, seems to differ between males and females. The maternal effects on male and female body weight were quite small and were highly correlated. The genetic correlations between the additive effects and the maternal genetic effects differed between various runs of MTDFREML (not shown) although the likelihood values were

almost equal. This indicates that the likelihood surface for these parameters was almost flat which results in inaccurate estimates.

Information content

Figure 1 shows the average information content over males and females of all 24 linkage groups. The information content of both sexes was very similar (not shown). The information content is also summarised in Table 2, which gives the average information content of each linkage group. The average information content on the 24 linkage groups was 0.71 for both males and females. The minimum information content was 0.35 for males, 0.34 for females and 0.39 for the combined average. The maximum values were 1.00, 0.99 and 0.98, respectively. The average information content of large and small linkage groups was similar. The average number of different alleles for the markers was 4.3 in this population.



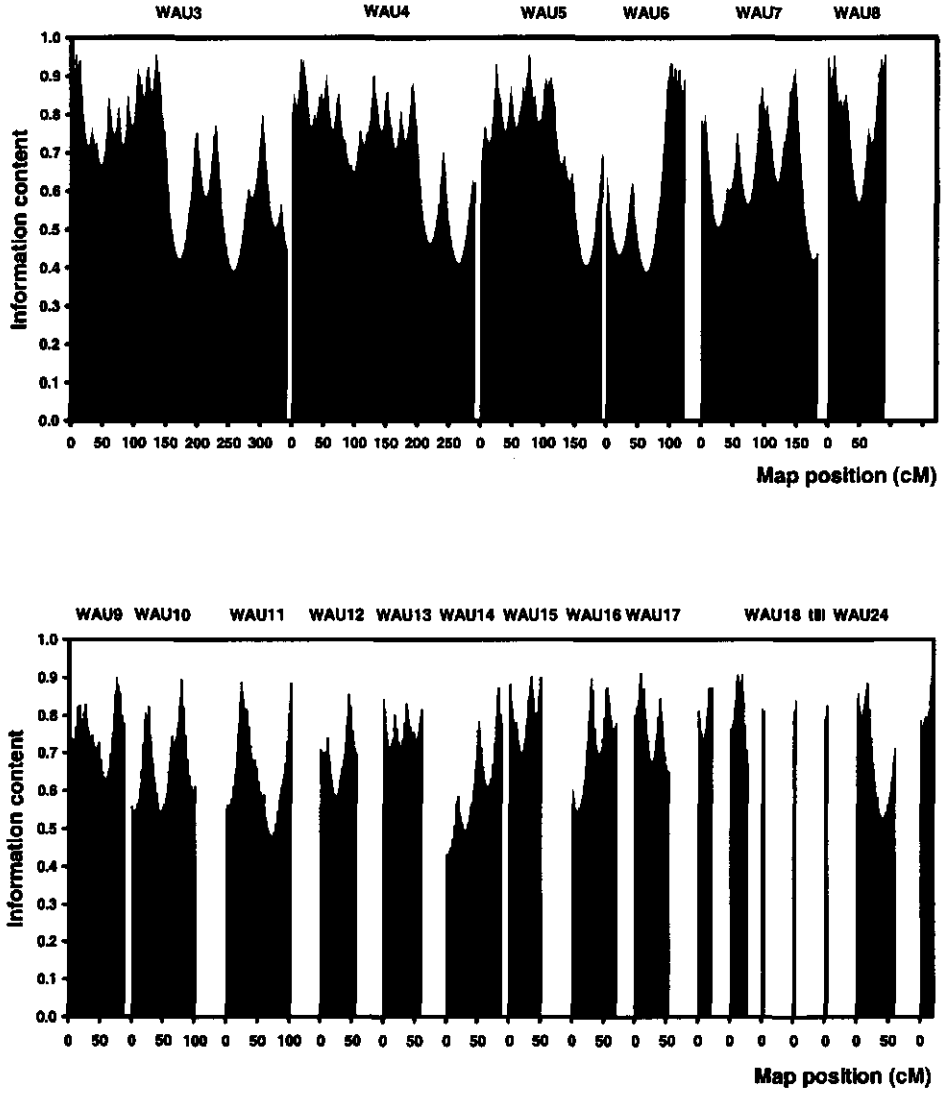


Figure 1. Sex averaged information content on all 24 linkage groups. Map positions are given using Haldane scale. (WAU = Wageningen University, Wageningen).

Significance thresholds

Chromosomewise significance thresholds are presented in Table 5. Table 5 shows that the chromosomewise significance thresholds decrease with a decreasing length of a linkage group. This reflects that a smaller number of independent tests are performed on a shorter linkage group. The 5% genomewise significance threshold was calculated as 2.84. This threshold was calculated through permutation over all 24 linkage groups simultaneously in one analysis. The genomewise significance threshold was also calculated by correcting the chromosomewise thresholds using the Bonferroni correction. This yielded only slightly different thresholds depending on the linkage group used. For linkage group WAU1 till WAU5 the 5% genomewise thresholds were 2.80, 2.80, 2.81, 2.83 and 2.88 respectively. On average, these results are similar to the genomewise threshold obtained by the overall permutation test. The differences observed are likely caused by inaccuracies in the chromosomewise thresholds.

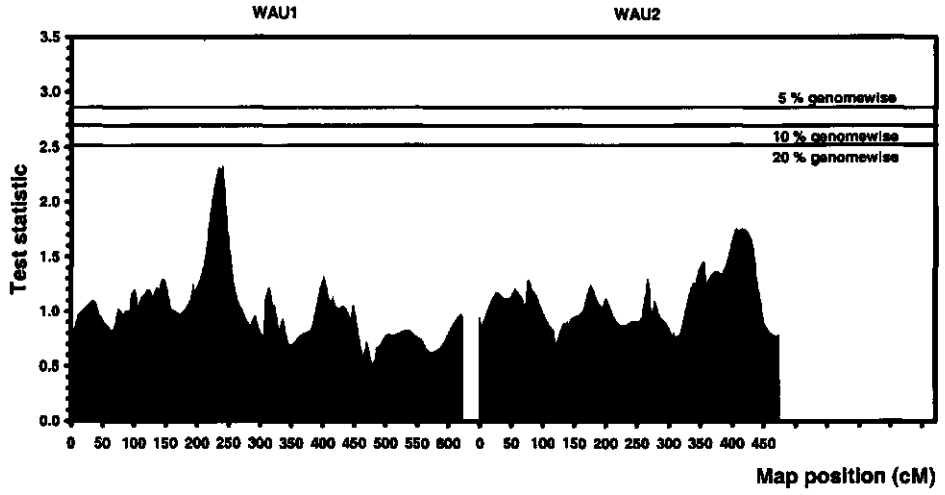
Full sib QTL analysis

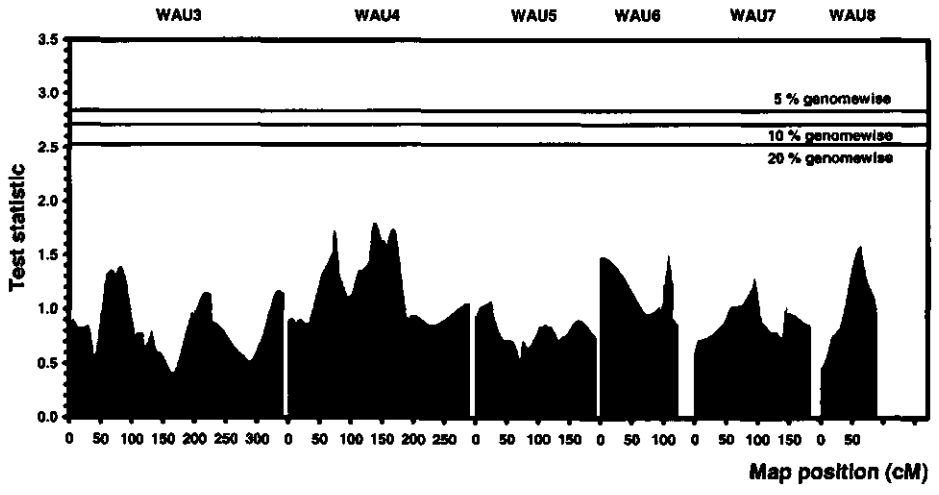
Figure 2 gives the test statistic for the presence of a QTL, comparing model 1 with model 4, at every cM on each linkage group. Table 5 shows the maximum test statistic per linkage group, and the corresponding position. The results show that at no position the 5% chromosomewise significance threshold was exceeded, one position exceeded the 10% chromosomewise significance level. This is an indication for the presence of a QTL located at 240 cM on linkage group WAU1. This linkage group is assigned to chromosome 1. The test statistic at the most likely position was 2.32. The genomewise significance level of this position was 41%. The information content for males and females at this position was 0.90 and 0.92. To look at the origin of this QTL, allelic effects, their standard errors and t-values are given in Table 6 for all families.

Table 5. Results from chromosomewise full sib, half sib sire and half sib dam QTL analysis comparing model 1, 2, and 3 with model 4. The maximum value of the test statistic and the most likely QTL position in cM are presented for all linkage groups. Additionally the 1% and 5% chromosomewise thresholds from the full sib analysis are given.

	Full sib		Half sib sire		Half sib dam		Chromosomewise	
Linkage	Test	QTL	Test	QTL	Test	QTL	threshold	
group[a]	statistic	position	statistic	position	statistic	position	5%	1%
WAU1	2.32	240	1.55	233	3.30	240	2.46	2.81
WAU2	1.76	417	1.76	354	2.04	76	2.39	2.73
WAU3	1.39	81	1.76	59	1.29	95	2.29	2.69
WAU4	1.80	138	2.75	137	2.91	74	2.28	2.66
WAU5	1.07	24	1.17	24	1.36	168	2.16	2.53
WAU6	1.50	108	1.98	108	1.41	24	2.12	2.54
WAU7	1.29	95	1.20	70	1.67	96	2.16	2.57
WAU8	1.58	62	1.84	62	1.46	74	2.02	2.36
WAU9	1.05	7	1.30	88	1.53	7	2.09	2.49
WAU10	1.19	100	1.47	100	0.92	63	2.09	2.50
WAU11	1.87	24	2.00	25	1.71	102	2.05	2.44
WAU12	1.27	36	0.99	0	1.71	36	1.96	2.35
WAU13	1.34	17	1.46	18	1.20	16	1.92	2.31
WAU14	1.32	67	1.24	54	1.51	73	1.94	2.36
WAU15	1.17	49	1.87	0	0.74	49	1.93	2.27
WAU16	1.43	0	2.08	0	1.09	56	1.97	2.36
WAU17	1.56	0	2.10	7	1.17	0	1.93	2.35
WAU18	0.57	0	0.81	0	0.35	0	1.86	2.24
WAU19	1.01	0	1.03	0	1.25	11	1.86	2.25
WAU20	0.72	0	0.71	0	0.76	0	1.88	2.35
WAU21	0.63	3	0.86	3	0.28	3	1.76	2.24
WAU22	1.13	0	0.63	0	1.66	0	1.89	2.37
WAU23	1.14	59	1.71	18	1.02	59	1.93	2.29
WAU24	1.26	20	1.46	20	1.12	19	1.78	2.15

[a] WAU = Wageningen University, Wageningen.


WAU1
WAU2
5 % genomewise
10 % genomewise
20 % genomewise
Test statistic
Map position (cM)


WAU3
WAU4
WAU5
WAU6
WAU7
WAU8
5 % genomewise
10 % genomewise
20 % genomewise
Test statistic
Map position (cM)

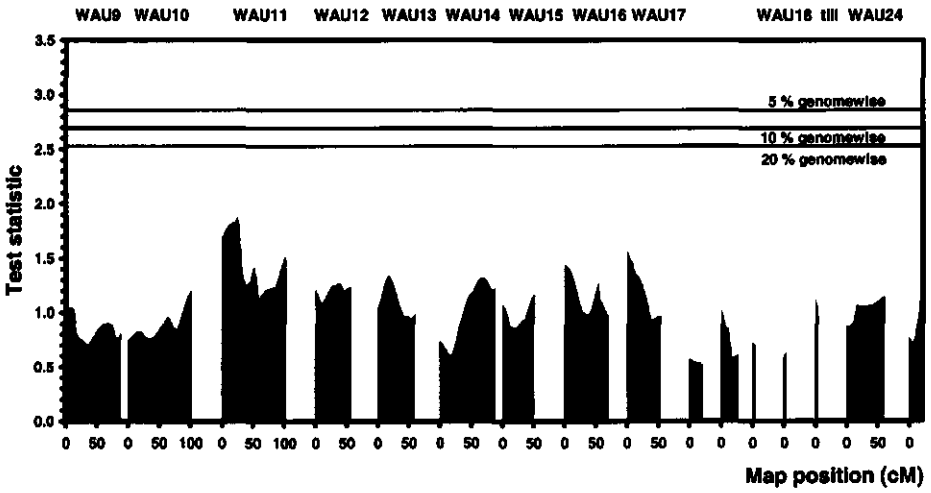


Figure 2. Test statistic values from the full sib QTL analysis on all 24 linkage groups comparing model 1 and 4. 5, 10 and 20% genomewise significance thresholds are included. Map positions are given using Haldane scale. (WAU = Wageningen University, Wageningen).

Table 6. Estimated QTL allele substitution effects with standard errors and *t* -values from the full sib QTL analysis for the QTL fitted at 240 cM on linkage group WAU1 are given for all parents. [a]

	QTL fitted for the sire		QTL fitted for the dam	
Family	Allele substitution effect	*t*-value	Allele substitution effect	*t*-value
1	51 (31)	1.62	37 (31)	1.17
2	44 (33)	1.34	74 (34)	2.18
4	26 (27)	0.95	33 (27)	1.20
5	17 (28)	0.60	46 (38)	1.23
6	42 (24)	1.74	60 (24)	2.55
7	17 (36)	0.48	28 (28)	1.00
9	218 (283)	0.77	161 (261)	0.62
11	22 (24)	0.89	23 (25)	0.92
12	32 (25)	1.29	91 (26)	3.45
13	49 (48)	1.03	71 (39)	1.81

[a] WAU = Wageningen University, Wageningen.

Results suggest the segregation of a QTL effect in 3 dams, in family 2, 6 and 12. In all cases, the sires were likely not contributing to the detected QTL variance. The chance of 3 animals from one sex segregating a QTL out of 20 animals, 10 from each sex, while none of the animals from the other sex has a QTL is 21%. The average allele substitution effect (α) of this QTL in the 3 dams was equal to $1.2\,\sigma_a$ (Georges *et al.*, 1995). In family 9, the matrix was nearly singular at this position, which complicated accurate separation of sire and dam allelic effect, and therefore resulted in extreme estimates with high standard errors.

Table 7 presents the test statistics from full sib, half sib sire and half sib dam QTL analysis at 240 cM on linkage group WAU1. The QTL analysis was performed using average adjusted progeny trait values based on all G_3 animals or on G_3 males and females only. Results show that the QTL effect was most clearly found in the full sib analysis, comparing model 1 and 2, and in the half sib dam analysis. Furthermore, the putative QTL seems to have a more clear effect on G_3 males than on G_3 females.

Table 7. Test statistic values from QTL analysis at 240 cM on linkage group WAU1. Full sib, half sib sire and half sib dam QTL analysis done for average adjusted progeny trait values based on G_3 males, G_3 females and all G_3 animals.[ab]

	Test statistic				
	Half sib QTL analysis		Full sib QTL analysis		
	Sire	Dam	Sire & Dam	Sire	Dam
Observations	Model 2:4	Model 3:4	Model 1:4	Model 1:3	Model 1:2
G_3 males	2.42	3.28	2.98	2.62	3.49
G_3 females	0.64	1.70	1.17	0.66	1.72
All G_3 animals	1.26	3.30	2.32	1.36	3.40

[a] WAU = Wageningen University, Wageningen.

[b] See materials & methods for details on models.

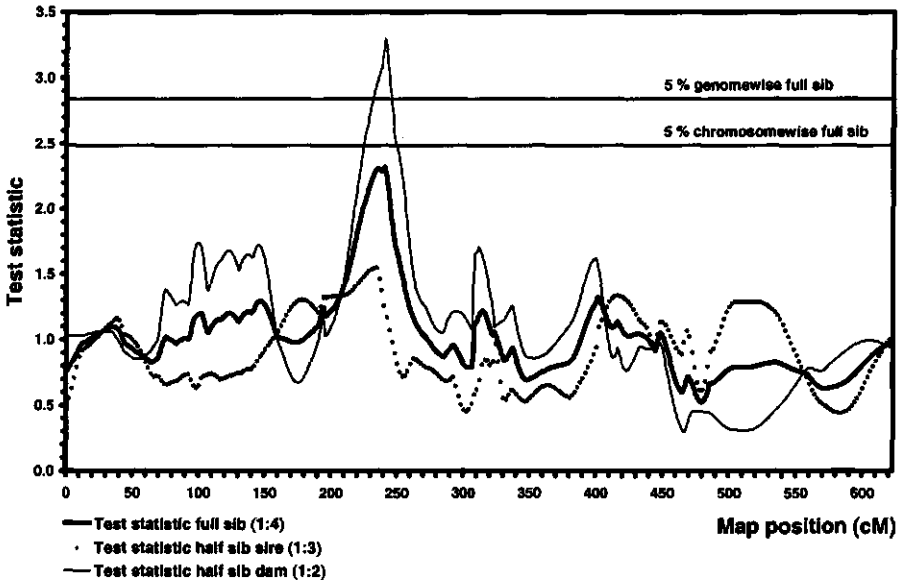


Figure 3. Test statistic values from the full sib, half sib sire and half sib dam QTL analysis on linkage group WAU1. 5% genomewise and chromosomewise significance thresholds are included for the full sib analysis only. The models, which are compared, are indicated within brackets. Map positions are given using Haldane scale. (WAU = Wageningen University, Wageningen).

Half sib QTL analysis

An overview of the results from the half sib QTL analyses is presented in Table 5. Only 3 times a test statistic above the 5% chromosomewise significance threshold was found: one at linkage group WAU1 and 2 times at linkage group WAU4. The location at 240 cM on WAU1 was at the 11% genomewise significance threshold in the half sib dam analysis. The half sib QTL analyses for linkage group WAU1 are presented in Figure 3. For comparison, the test statistic from the full sib QTL analysis is also given in Figure 3. Test statistics for model 4 versus model 2 were very similar to the test statistics for model 3 versus model 1, and were therefore not shown. Similarly, test statistics for model 4 versus 3 were similar to the test statistics for model 2 versus model 1. Figure 3 shows that the analysis based on sires or dams alone produced different results. The QTL, found at 240 cM on linkage group WAU1, was more clearly present in the dams. For the half sib sire QTL analysis, the most likely location on linkage group WAU1 was 233 cM but no clear peak was observed. Test statistic values obtained from the full sib QTL analysis,

comparing model 1 and 4 were always halfway between the half sib sire and half sib dam QTL analysis. The test statistics, comparing model 1 and 2 or 3 were only slightly higher compared to the half sib dam and sire analysis test statistics, respectively. This shows that the effect of correcting for one sex hardly effects the test statistic for the presence of a QTL in the other sex. Furthermore, Figure 4 shows that linkage group WAU4 gave a clear peak in both the half sib sire and half sib dam QTL analysis, but not at the same position. In the half sib sire QTL analysis the most likely position was 137 cM with a test statistic of 2.75 and in the half sib dam QTL analysis the most likely position was 74 cM with a test statistic of 2.91. These results suggest the presence of a QTL effect in 3 sires respectively 3 dams.

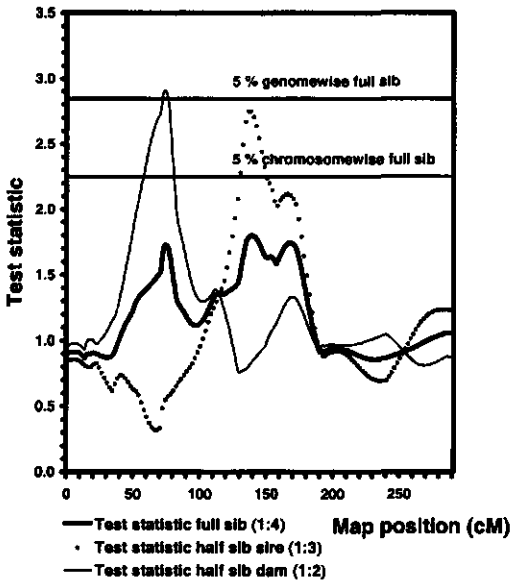


Figure 4. Test statistic values from the full sib, half sib sire and half sib dam QTL analysis on linkage group WAU4. 5% genomewise and chromosomewise significance thresholds are included for the full sib analysis only. The models, which are compared, are indicated within brackets. Map positions are given using Haldane scale. (WAU = Wageningen University, Wageningen).

Discussion

Analysis of the phenotypic data

Corrections on the phenotypic data are performed in order to reduce the influence of systematic effects. This should lead to more reliable results in detection of QTLs The difference in mean and variance between the phenotypic observations on male and female body weight are accounted for by standardisation. Other methods for transformation of the data could have been used. One of these is a log transformation of the phenotypic observations. Log transformation did not lead to homogeneous variance in the current data, which indicates that the heterogeneous variance is caused by more than a scale effect. An interesting point is whether there should be a correction for heterogeneous variances between families or not (Jansen *et al.*, 1998).

Marker inheritance and haplotype reconstruction

A potential weakness in the procedure for estimating a QTL effect is the reconstruction of the parental haplotypes. Only the most likely situation is taken into account and assumed to be true. Given the family size and marker density, linkage phase was known at most positions. Uimari *et al.* (1996) also used the most likely linkage phase, but they used all markers simultaneously, instead of a bracket-wise approach in determining the most likely linkage phase. It would be better to take all possible haplotype constructions into account (Georges *et al.*, 1995), or to take a sample of all possible haplotype constructions. However, for a genomewise study with a lot of markers per linkage group, considering all possibilities, would be to demanding computationally. Furthermore, the linkage map is also assumed to be known without error, because only one linkage map is taken based on the sex-averaged recombination fractions. Using sex-averaged recombination fractions is considered acceptable because the overall difference in map length between both sexes is small in chickens (Groenen *et al.*, 1996).

Information content

The information content is influenced by the amount on heterozygosity in the markers, which in turn is influenced by the number of different alleles from these markers. Information content was not constant over all linkage groups and within each linkage group. Differences in information content might influence the chance to detect a QTL and the position assigned to a QTL. The information content can be improved by increasing the marker density. The ends of linkage groups often have a lower information content. This can be overcome by mapping 1 or 2 highly informative markers at the ends (Spelman *et al.*, 1996). If the QTL position analysed is outside the range of informative markers then position and estimated effect of that QTL can not be separated. To overcome this problem, QTL analysis was restricted to chromosomal regions covered by informative markers in at least one family.

Significance thresholds

Bonferroni correction can be applied to obtain genomewise significance thresholds by correcting chromosomewise significance thresholds to account for multiple testing. As an alternative genomewise permutation thresholds can be calculated. This requires that the test statistic is comparable across all linkage groups and assumes that the differences in information content across all linkage groups are not extreme. Results in this study show that both procedures result in the same genomewise threshold. Spelman *et al.* (1996) also found that marker data had little effect on the significance thresholds. The choice of the critical value is still uncertain. Taking a significance threshold of 5% is arbitrary. The level to be chosen depends on the objective of an experiment and the effect of either utilising false positives or missing real QTLs (Lander and Kruglyak, 1995).

Full sib QTL analysis

Only one QTL with a significant effect on the chromosomewise level was found in the full sib QTL analysis. The chromosomewise significance level of this QTL is 10%, whereas the genomewise significance level is 41%. Although 3 dams have a t-value above 2 for the QTL positioned at 240 cM, this is only an indication that it is probably

segregating in these dams and no proof that it is not segregating in any of the other parents. A two QTL analyses might be interesting, because other positions might become more significant after fitting the first QTL. Finding one QTL on 24 linkage groups suggests that most QTLs influencing this trait might be too small to be detected significantly in this experiment. Maybe fixation of the same QTL alleles has already occurred in these parental lines for the most important QTLs for this trait. This, however, seems not very likely in our case because the experimental population was created by crossing two lines. The power to detect a QTL might be increased by mapping more markers or by collecting observations on more animals. The power of this design to detect a QTL with an effect of $1.2\sigma_a$ is approximately 0.99 with α is 0.05. This power was calculated with the program from Van der Beek *et al.* (1995), assuming a QTL heterozygosity of 0.50 and an average distance between informative markers of 20 cM. The power given here was based on one marker bracket.

Problems with singularity were solved by using a generalised inverse. An alternative might be to use wider marker brackets at locations where a block of markers with equal haplotypes in both parents occurs. A wider bracket could be chosen by using only one marker of this block and omitting the other markers in the block.

Half sib QTL analysis

Results from half sib sire and half sib dam QTL analysis can be quite different. Analysis based on only sires or dams can give different results when the number of families is limited, because the parental QTL genotypes can differ. Another explanation could be imprinting, which can result in expression of a QTL allele only if it is inherited either from the sire or from the dam.

Potential candidate genes

The combined physical and genetic maps in chicken currently contain about 120 identified genes. Given this relatively small number of genes, it is clear that at this stage the chance to identify potential candidate genes is rather small. Nevertheless, several of these genes appear to be on chromosomal segments that seem to be conserved between chicken and man. One rather large region of the chicken genome that appears to be

syntenic between chicken, man and mouse is located on the p arm of chromosome 1 (Klein *et al.*, 1996), and might also even include the centromere and part of the q arm as well. In chicken a histone gene cluster, insulin like growth factor 1 (IGF1), lysozyme, lactate dehydrogenase B (LDHB), high mobility group I-C (HMGI-C) and glyceraldehyde-3-phosphate dehydrogenase (GAPD) (Burt *et al.*, 1995) have all been mapped to this region, and these genes are also syntenic in man on chromosome 12 and in mice on chromosome 10. Interestingly, two of these genes, IGF1 and HMGI-C, directly are involved in the regulation of growth. However, based upon their location they both can be excluded as candidate genes for the QTL found at 240 cM on chromosome 1. The confidence interval for the QTL for growth on chromosome 1, is partially overlapping the conserved syntenic region described above. In human, a gene involved in growth that has been mapped close to GAPD on chromosome 12, is the fibroblast growth factor-6 (FGF6) gene. Another gene mapped in chicken to a region that is overlapping with the confidence interval, is the glucose-6-phosphate dehydrogenase (G6PD) gene. In man, the G6PD gene has been mapped to the X-chromosome, but a G6PD like gene has also been mapped to human chromosome 17. For the potential QTLs found on chromosome 4, so far, no genes have been mapped in chicken to these regions.

In conclusion, although it is tempting to speculate on potential candidate genes at the moment this is not very sensible for two reasons: (1) The QTL has not yet been localised very precisely and (2) the number of genes mapped in chicken so far that have also been mapped in man (or mouse) is to small to be able to precisely align the chicken and human map. Regarding the QTL on chromosome 1, potential syntenic regions could be identified and the information from the human map can now be used to specifically increase the number of genes in this region on the chicken map. This will increase the ability to align this region with the human map, and consequently increase the chance to identify potential candidate genes.

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Chapter 3

Whole Genome Scan in Chickens for Quantitative Trait Loci Affecting Growth and Feed Efficiency

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Abstract

A feed efficiency experiment was conducted in a population consisting of progeny from 10 full sib families of a cross between two broiler lines. Microsatellite genotypes were determined on Generation (G) 1 and 2. On G_3, body weight at 23 and 48 days and feed intake were measured and were used to calculate growth between 23 and 48 days, feed intake adjusted for body weight and feed efficiency. Average adjusted progeny trait values were calculated for G_2 animals by averaging after adjusting phenotypic observations on offspring for fixed effects, covariables, maternal genetic effects, the additive genetic contribution of the mate and heterogeneity between sexes and were used as dependent variable in the quantitative trait loci (QTL) analysis.

A full sib interval mapping approach was applied using genotypes from 420 markers on 27 autosomal linkage groups. Four QTLs exceeded the significance thresholds. The most significant QTL was located on chromosome 1 at 235 cM and had a 4% genomewise significance for feed intake between 23 and 48 days. Furthermore, this QTL exceeded suggestive linkage for growth between 23 and 48 days and body weight at 48 days. A second QTL was located on linkage group WAU26 at 16 cM and showed suggestive linkage for feed intake between 23 and 48 days. On chromosome 4, at 147 cM, a third QTL, which had an effect on both feed intake traits, was found. Finally, a fourth QTL, which affected feed intake adjusted for body weight, was located on chromosome 2 at 41 cM.

Introduction

Knowledge on position and effects of quantitative trait loci (QTLs) is missing for most traits of interest to animal breeders. Such information on QTLs would be useful for marker assisted breeding as well as helpful for improving the understanding of the biological background (i.e. which genes are involved and their effects) of traits. In QTL mapping experiments, genotypes and performance data need to be collected on many animals to achieve sufficient power. In a three generation design, genotypes are collected on first and second generation animals and performance recording is on third generation animals. In

the second generation, full sibs are favourable compared to half sibs, because transmission from both parents can be followed by a marginal increase in marker genotypes (Van der Beek *et al.*, 1995). The high reproductive capacity of hens enables the production of large full sib families. Performance recording on third generation animals reduces the number of genotypes, which are needed to achieve a given power compared to performance recording on second generation animals (Weller *et al.*, 1990). Performance recording on half sibs results in a higher power in comparison with full sibs (Van der Beek *et al.*, 1995). In order to obtain information on QTLs affecting traits of interest in broilers, a large experiment was initiated using a three generation full sib-half sib design.

Recently, a large number of genetic markers has been generated and mapped in this experimental population (Crooijmans *et al.*, 1997; Groenen *et al.*, 1998) to enable QTL analysis. In contrast to other QTL studies in poultry, the analysis in our experimental population was genomewide (Van Kaam *et al.*, 1998). Other studies reporting QTLs in chicken were published by Khatib (1994), who studied juvenile growth rate and, Vallejo *et al.* (1998), who detected QTLs affecting susceptibility to Marek's disease virus induced tumours.

In this paper, the results of a whole genome scan aimed at detection and localisation of QTLs in a feed efficiency experiment are described. For this purpose, the regression interval mapping methodology presented by Van Kaam *et al.* (1998) was applied. In the present study, more traits were analysed and additional marker data was included. Traits analysed were body weight at 23 days (BW23) and at 48 days (BW48), feed intake in a fixed age interval (FIFA) and in a fixed weight interval (FIFW), growth (GAIN) and feed efficiency between 23 and 48 days (FE). These traits are of great interest to the broiler industry, because growth rates and feed efficiency have a big influence on economic results.

Material and Methods

Experimental Population

A three generation population was created for the purpose of QTL detection, following recommendations of Van der Beek *et al.* (1995). Founder animals, parents, offspring and

grandoffspring are indicated as generation 0, 1, 2 and 3 animals or G_0, G_1, G_2 and G_3 animals, respectively. G_0 etc. was chosen instead of F_0 etc. to avoid confusion with the terminology for inbred lines. In the three generation design, G_1 and G_2 chickens were typed for genetic markers and phenotypic observations were collected on G_3 chickens and were used for calculation of average adjusted progeny trait values on the G_2 chickens.

Table 1. Population structure with numbers of animals used in the analysis and types of observations collected.[a]

Generation[b]	Males	Females	Total	Observations
G_0[c]	14	14	28	———
G_1	10	10	20	Genotypes
G_2	172	279	451	Genotypes
G_3	1,063	1,083	2,146	Phenotypes at 23 days
G_3	1,012	1,037	2,049	Phenotypes at 48 days

[a] Numbers exclude outliers and missing values.

[b] G_0 etc. = generation 0 etc.

[c] Male and female G_0 animals are from different lines.

The number of animals and the population structure are presented in Table 1. Two genetically different outcross broiler dam lines from the White Plymouth Rock breed were chosen as the foundation of the experimental population. The two lines had a genetic distance of 0.37, calculated as Rogers's distance (Nei, 1987) on 16 microsatellite markers, and were selected out of a group of six lines with a genetic distance ranging from 0.15 to 0.40. In one line, 14 males and in the other line 14 females were chosen and 14 G_0 couples were created. These 14 couples together produced 10 G_1 males and 10 G_1 females. From these 20 G_1 chickens, 10 couples were created without known relationship, each couple being the base of a family. The G_1 chickens were mated to produce G_2 full sibs. The G_2 chickens were mated with several G_2 chickens from different families to produce G_3 chickens. The G_3 offspring of each G_2 chicken, therefore, are mostly half sibs with a small number of full sibs. Each full sib family consisted of two G_1 parents and on average 45.1 G_2 chickens and each G_2 chicken had on average 9.3 and 8.9 G_3 offspring at 23 and 48 days of age respectively. For more details see Van Kaam *et al.* (1998).

Five hatches of G_3 animals were raised consecutively in the same floor pens up to 22 days and housed individually in another building between the age of 22 and 48 days. Individual cages were used to enable individual measurement of feed intake. During the lifetime of the broilers, feed and water were supplied for *ad libitum* consumption and illumination was 23 hours per day. A commercial broiler feed containing 12,970 kJ/kg was used.

Traits measured were BW23, BW48 and FIFA. Within each hatch, observations deviating more than 3 SD from the mean of that hatch, were considered the result of measurement errors and therefore were excluded from the analysis. These outliers were randomly distributed across families, indicating that no genetic component was involved. In total 38 animals were excluded, 16 at 23 days and an additional 22 animals at 48 days. After removal of the outliers, 2,146 chickens with observations at 23 days and 2,049 chickens with observations at 48 days remained. The difference of 97 chickens contained 75 birds measured at 23 days, which did not reach the age of 48 days.

Marker Data

Genotypes for microsatellite markers were determined using DNA derived from blood samples from 20 G_1 and 451 G_2 animals. Marker alleles were recorded in basepair units. For more details see Groenen *et al.* (1997, 1998). In total 437 informative markers were mapped to 28 linkage groups: 420 markers were mapped on 27 autosomal linkage groups and 17 markers were mapped on the Z-chromosome. Marker data used in this analysis is an extended dataset compared to the marker data used in a previous analysis of BW48 (Van Kaam *et al.*, 1998). Additionally 69 markers were added and 20 existing markers, previously determined on 4 families were now typed on all 10 families. In total 271 mapped markers were now determined on all 10 families and 166 mapped markers were typed on 4 families only. The linkage map used in this study was calculated with CRI-MAP (Green *et al.*, 1990) using the marker genotypes for all these markers and all these families. Compared with the linkage map used by Van Kaam *et al.* (1998), the number of autosomal linkage groups increased from 24 to 27. Marker and linkage map data were nearly identical to those presented by Groenen *et al.* (1998), but 14 additional markers were included. The estimated coverage of this linkage map is between 90% and 95% of the chicken genome (Groenen *et al.*, 1998). Linkage groups WAU1 to WAU7, WAU11

and WAUZ were assigned to chromosomes 1 to 7, 8 and Z respectively (Groenen *et al.*, 1998).

The size of the linkage groups varied between 11 and 625 cM and the number of markers on the linkage groups varied between 2 and 82 markers. Map distances given in this paper are always sex-averaged distances in centimorgans on the Haldane scale. The total linkage map covered 3,566 cM: 3,363 cM on autosomal linkage groups and 203 cM on the Z-chromosome. Because the segregation of the Z-chromosome is different from autosomal chromosomes, the Z-chromosome was not included in the present genome scan. Furthermore, it should be noted that the growth hormone receptor gene, which causes sex-linked dwarfism, was not segregating in this population.

More information about the length of the linkage groups, the number of markers on each linkage group, the average percentage of marker heterozygosity and the average information content is given in Table 2. The information content was calculated as the variance of the probabilities of inheriting the first parental allele, divided by the expected variance of these probabilities under full information, which is 0.25 (Spelman *et al.*, 1996). The information content on a linkage group follows from the number of markers and the marker heterozygosity on the linkage group.

For the first 20 linkage groups, all 20 parents were informative. The number of informative parents was 8 on linkage group WAU21, 16 on WAU22, 19 on WAU23, 18 on WAU24, 7 on WAU25, 16 on WAU26 and 9 on WAU27. For linkage group WAU21, marker data was only collected for 4 families.

Analysis of the Phenotypic Data

The data were analysed using a two step procedure: first average adjusted progeny trait values were calculated by adjusting phenotypic observations for systematic effects, and secondly QTL analysis was performed using the average adjusted progeny trait values as the dependent variable.

Table 2. Information about the analysed linkage groups. Length in centimorgans, number of markers, average percentage of marker heterozygosity in the generation one animals and the average information content for both sexes is given for the analysed linkage groups. Linkage groups without a quantitative trait locus are combined.

	Length	Number of	Marker	Average information content	
Linkage group[a]	in cM	markers	Heterozygosity[b]	Sires	Dams
Chromosome 1	625	82	67.7%	0.76	0.74
Chromosome 2	464	71	64.3%	0.79	0.76
Chromosome 4	282	34	69.1%	0.73	0.72
WAU26	23	3	58.3%	0.68	0.66
Other groups	1,969	230	65.3%	0.69	0.70
Total	3,363	420	66.0%	0.72	0.72

[a] WAU = Wageningen University, Wageningen.

[b] In Generation 1 chickens.

Six traits were analysed: three measured traits and three inferred traits. Measured traits were BW23, BW48 and FIFA. Inferred traits were growth between 23 and 48 days (GAIN), feed intake in a fixed weight interval (FIFW) and percentage feed efficiency (FE). Percentage FE was defined as the ratio between GAIN and FIFA multiplied with 100% and can be seen as gross efficiency. Values for FIFW were obtained from FIFA by using BW23 and BW48 as covariables to adjust for differences in body weight. Bernon and Chambers (1988) and Chambers *et al.* (1994) also adjusted feed intake for initial and final body weight. Feed intake unadjusted for weight differences includes effects due to differences in growth, feed utilisation and size, which affects growth and maintenance requirements, during the experiment. Therefore, an adjustment with initial and final body weight results in an evaluation of feed intake closer to net efficiency (Bernon and Chambers, 1988).

For all traits, observations on male and female G_3 animals were treated as different but correlated traits, using a bivariate approach in order to account for heterogeneity of variance between both sexes (Van Kaam *et al.*, 1998). The following bivariate mixed

model for male and female observations was used:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & 0 \\ 0 & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{W}_1 & 0 \\ 0 & \mathbf{W}_2 \end{bmatrix} \begin{bmatrix} \mathbf{d}_1 \\ \mathbf{d}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix}$$

where:

$\mathbf{y}_i$ = Vector of observations for i = 1 (male) or 2 (female)

$\mathbf{b}_i$ = Vector of fixed effects and covariables for trait i

$\mathbf{u}_i$ = Vector of random direct additive genetic effects on trait i

$\mathbf{d}_i$ = Vector of random maternal genetic effects on trait i

$\mathbf{e}_i$ = Vector of random residual effects for trait i

$\mathbf{X}_i$ = Incidence matrix relating observations for trait i to fixed effects and covariables

$\mathbf{Z}_i$ = Incidence matrix relating observations for trait i to direct additive genetic effects

$\mathbf{W}_i$ = Incidence matrix relating observations for trait i to maternal genetic effects

The elements in the vectors of fixed effects and covariables included as fixed effects the overall mean of the trait, an interaction term between the hatch of the recorded animal and the hatch of the dam, the location of the animal's cage in the building, and as covariables the deviation of BW23 and BW48 from their average. The interaction term between the hatch of the recorded animal and the hatch of the dam represented the period of the year and the age of the dam at reproduction. G_3 chickens were born in five hatches, their dams were born in eight hatches. The building in which the experiment was conducted was divided in 36 locations. The location in the building was not included in the model for BW23, as the chickens were housed in a floor pen up to 22 days. Observations on BW23 and BW48 were included as linear covariables in the model for the analysis of FIFW. Variance components, fixed effects, covariables, breeding values and maternal genetic effects were estimated using MTDFREML (Boldman *et al.*, 1995).

Average adjusted progeny trait values were calculated for G_2 animals by averaging trait values on offspring. These were derived by adjusting phenotypic observations for fixed effects, covariables, maternal genetic effects, the additive genetic contribution of the other parent and heterogeneity between sexes. For more details see Van Kaam *et al.* (1998).

QTL Analysis

Full sib QTL analysis was undertaken using a regression approach (Van Kaam *et al.*, 1998) in which a single multi-allelic QTL was fitted across all families. This method is an extension of the multi-marker regression method of Knott *et al.* (1994) for outbred populations with a half sib family structure. Because marker-QTL linkage phase can differ between families, QTL analysis was nested within families. Average adjusted progeny trait values of G_2 animals were regressed on the probabilities of inheriting the first allele of each G_1 parent. The family mean was included in the model to account for polygenic differences between families. The model to fit a QTL at position k was:

$$y_{ij} = f_i + b_{s,ik}x_{s,ijk} + b_{d,ik}x_{d,ijk} + e_{ijk}$$

where:

y_{ij} = Average adjusted progeny trait value for G_2 animal j of family i

f_i = Polygenic effect of family i

$b_{s,ik}$ = Regression coefficient for the sire (s) of family i at position k

$x_{s,ijk}$ = Probability that G_2 animal j in family i at position k received the chromosomal segment from haplotype 1 from the sire

$b_{d,ik}$ = Regression coefficient for the dam (d) of family i at position k

$x_{d,ijk}$ = Probability that G_2 animal j in family i at position k received the chromosomal segment from haplotype 1 from the dam

e_{ijk} = Random residual

The regression coefficients represent QTL allele substitution effects per parent (Falconer, 1989). A weighting factor was applied to account for differences in number of G_3 chickens contributing to G_2 average adjusted progeny trait values. The weighting factor is based on the variance of the average adjusted progeny trait values of the G_2 chickens (Van Kaam *et al.*, 1998).

Test statistics were calculated at each centimorgan, in order to test for the alternative hypothesis of the presence of QTL effects, versus the null hypothesis of the absence of QTL effects. The test statistic is the ratio of the explained mean square of the QTL effects

under study in the numerator and the residual mean square of the full model in the denominator. The test statistic at position k was calculated as:

$$\text{Test statistic}_k(H_1 : H_0) = \frac{\left(\frac{RSS_k(H_0) - RSS_k(H_1)}{df_{QTL}}\right)}{\left(\frac{RSS_k(H_1)}{df_{total} - df_{family} - df_{QTL}}\right)}$$

where RSS_k is the cumulative residual sums of squares over all families after fitting the full (H_1) or reduced (H_0) model and df are the degrees of freedom for total (df_{total}), number of family means fitted (df_{family}) and number of QTL effects fitted (df_{QTL}), which were taken to be 451, 10 and 20 respectively.

Significance Thresholds

Significance thresholds were determined for each trait separately because differences in the distributions of the average adjusted progeny trait values result in differences in the distributions of the test statistics (Spelman *et al.*, 1996). Comparisonwise and chromosomewise significance thresholds were calculated empirically using the permutation method (Churchill and Doerge, 1994). To obtain genomewise significance thresholds, chromosomewise significance thresholds were adjusted for multiple testing along the genome using the Bonferroni correction. Genomewise significance thresholds were used to calculate two significance levels: significant and suggestive linkage (Lander and Kruglyak, 1995). Significant linkage is defined as a 5% genomewise significance threshold and suggestive linkage is equivalent to an expectation of one false positive result per trait on a whole genome scan. The number of independent tests on a linkage group follows from the percentage chromosomewise significance, which results in the same value of the test statistic as a 1% comparisonwise significance threshold. On the whole genome 3,579 tests were undertaken, which was equivalent to 87.1 independent tests.

The first 20 linkage groups were permuted together because all parents were informative on these linkage groups and consequently the test statistics were comparable (Van Kaam *et al.*, 1998). For each trait, 10,000 permutations were conducted. For the remaining linkage groups, no QTL effect could be fitted for some parents, which were uninformative. These linkage groups were short and therefore a large Bonferroni

correction was necessary. Hence, 100,000 permutations were conducted to obtain reliable thresholds.

In order to determine which parents were segregating for a QTL, permutation was also applied to single families on those locations where a QTL was located in the across family analysis. Per parent, a test comparing a model with a QTL versus a model without a QTL was applied, accounting for the presence or absence of QTL effects in the mate. A 10% comparisonwise threshold was applied and 10,000 permutations were executed. Parents with a test statistic exceeding this threshold were assumed to be segregating for a QTL.

Results

Marker Information

Table 2 provides information on the length, number of markers, the average percentage of marker heterozygosity and the average information content on the analysed linkage groups. The average percentage of marker heterozygosity was calculated as the total number of heterozygous markers on all G_1 chickens, divided by the total number of typed markers on all G_1 chickens, and expressed as a percentage. The average percentage of marker heterozygosity for G_1 chickens varied from 42.3% to 83.3% per linkage group. Information content on single positions varied between 0.24 and 0.99 for sires and between 0.34 and 0.98 for dams. The average information content over all positions per linkage group was between 0.54 and 0.83 for sires and between 0.53 and 0.83 for dams. Average information content over all positions on all analysed linkage groups was 0.72 in both sexes.

Variance Components

Estimated heritabilities, genetic correlations and phenotypic variances are presented in Table 3. For all traits, phenotypic variances of male observations were greater than for female observations. For most traits, estimated heritabilities for males and females were similar. For BW23, the heritability for males (0.67) was higher than for females (0.46).

The opposite was the case for FIFA with 0.25 on males and 0.39 on females. Furthermore, the proportion of variance explained by the maternal genetic effect tended to be larger on male as on female observations. The highest proportion of variance explained by the maternal genetic effect was 0.11 for FIFW on males. Variances of FIFW were considerably lower as variances of FIFA, because variation in feed intake caused by differences in body weight was removed. For all traits correlations between additive genetic effects on male and female observations were at least 0.87 and for maternal genetic effects at least 0.64.

Table 3. Heritabilities, genetic correlations and phenotypic variances.

Trait[a]	h_m^2 [b]	h_f^2	m_m^2	m_f^2	$r_{g,a}$	$r_{g,m}$	$\sigma_{p,m}^2$	$\sigma_{p,f}^2$
BW23	0.67	0.46	0.06	0.00	1.00	0.64	7,366	6,118
GAIN	0.23	0.19	0.01	0.01	0.98	0.86	42,573	27,734
BW48	0.28	0.33	0.03	0.01	0.97	0.85	59,916	42,555
FIFA	0.25	0.39	0.05	0.00	0.95	0.99	145,651	116,630
FIFW	0.36	0.39	0.11	0.03	0.91	0.97	43,046	29,535
FE	0.48	0.54	0.08	0.05	0.87	0.89	14.67	11.25

[a] BW23 = body weight at 23 days; GAIN = growth between 23 and 48 days; BW48 = body weight at 48 days; FE = percentage feed efficiency between 23 and 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval.

[b] h_m^2, h_f^2 = heritability of male, respectively, female observations; m_m^2, m_f^2 = proportion of variance due to maternal genetic effect on male, respectively, female observations; $r_{g,a}$ = correlation between additive genetic effects on male and female observations; $r_{g,m}$ = correlation between maternal genetic effects on male and female observations; $\sigma_{p,m}^2, \sigma_{p,f}^2$ = phenotypic variances based on male respectively female observations measured in grams.

Table 4 shows the correlations between the average adjusted progeny values of the G_2 chickens for all traits. BW48, GAIN and FIFA were highly correlated traits. A strong negative correlation of -0.79 between FIFW and FE was estimated. FIFA and FIFW were only moderately correlated, which shows that the adjustment for body weight had a strong effect. BW23 was not highly correlated with any of the other traits.

Table 4. Correlations between the average adjusted progeny trait values of the Generation 2 chickens.

Trait[a]	BW23	GAIN	BW48	FIFA	FIFW	FE
BW23						
GAIN	0.41					
BW48	0.65	0.95				
FIFA	0.60	0.80	0.86			
FIFW	0.09	-0.01	0.02	0.52		
FE	-0.22	0.43	0.26	-0.18	-0.79	

[a] BW23 = body weight at 23 days; GAIN = growth between 23 and 48 days; BW48 = body weight at 48 days; FE = percentage feed efficiency between 23 and 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval.

QTL Analysis

Four QTLs were found: one QTL showed significant linkage and three QTLs showed suggestive linkage. Four of the six analysed traits showed suggestive linkage at least once. Three QTLs had an effect on FIFA. These results are summarised in Table 5. For BW23 and FE, the test statistic did not reach the suggestive linkage threshold on any linkage group.

Quantitative trait locus 1 was located on chromosome 1 as shown in Figure 1 and exceeded the threshold for significant linkage, reaching 4% genomewise significance for FIFA. A QTL was also detected at very similar positions for BW48 and GAIN showing suggestive linkage. The test statistic for FIFA and GAIN peaked at 235 cM and BW48 peaked at 240 cM. The test statistic for these traits followed a similar pattern, which can be expected given the high correlations between these traits (Table 4). Because these traits were highly correlated and the positions were close, it seems reasonable to assume that the same QTL affected these traits. Eight parents showed significant QTL effects for FIFA, five parents for GAIN and five parents for BW48. The allele substitution effect (α; Falconer, 1989) averaged over these parents was $0.8\,\sigma_a$ for FIFA, $1.0\,\sigma_a$ for GAIN and $1.1\,\sigma_a$ for BW48.

Table 5. Summary of interesting regions per trait. Indicated per trait are the number assigned to the quantitative trait locus (QTL), the linkage group, the most likely location in centimorgans, the markers bracketing this location and the genomewise significance level of the QTL at this location.

Trait[a]	QTL	Linkage group[b]	Location in cM	Markers[c]	Significance[d]
BW23	–	WAU26	22	ADL0262 – MCW0165	95%
GAIN	1	Chromosome 1	235	UMA1.107 – MCW0058	34% *
BW48	1	Chromosome 1	240	MCW0058 – LEI0071	44% *
FIFA	1	Chromosome 1	235	UMA1.107 – MCW0058	4% **
FIFA	3	Chromosome 4	147	MCW0085 – LEI0122	51% *
FIFA	2	WAU26	16	ADL0289 – ADL0262	16% *
FIFW	4	Chromosome 2	41	MCW0082 – MCW0341	57% *
FE	–	Chromosome 2	417	MCW0314 – MCW0245	72%

[a] BW23 = body weight at 23 days; GAIN = growth between 23 and 48 days; BW48 = body weight at 48 days; FE = percentage feed efficiency between 23 and 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval.

[b] WAU = Wageningen University, Wageningen.

[c] ADL = Avian Disease and Oncology Laboratory, Michigan State University, East Lansing; LEI = University of Leicester, Leicester; MCW = Microsatellite chicken Wageningen; UMA = University of Massachusetts, Amherst.

[d] ** = significant linkage; * = suggestive linkage.

Quantitative trait locus 2 (Figure 2) was located on linkage group WAU26 and showed suggestive linkage for FIFA. Furthermore, high test statistics were also found for BW23 and BW48 on this linkage group, but not high enough to reach the suggestive linkage threshold. The peak for FIFA was located at 16 cM and the test statistics for BW23 and BW48 showed their highest value at the end of the linkage group at 22 cM. As these positions are close and the traits were correlated (Table 4), it is assumed that it was the same QTL affecting these traits. Significant QTL effects were found for three parents for FIFA and BW23 and for five parents for BW48. The average allele substitution effect in these parents was $1.5\,\sigma_a$ for FIFA, $0.8\,\sigma_a$ for BW23 and $1.0\,\sigma_a$ for BW48.

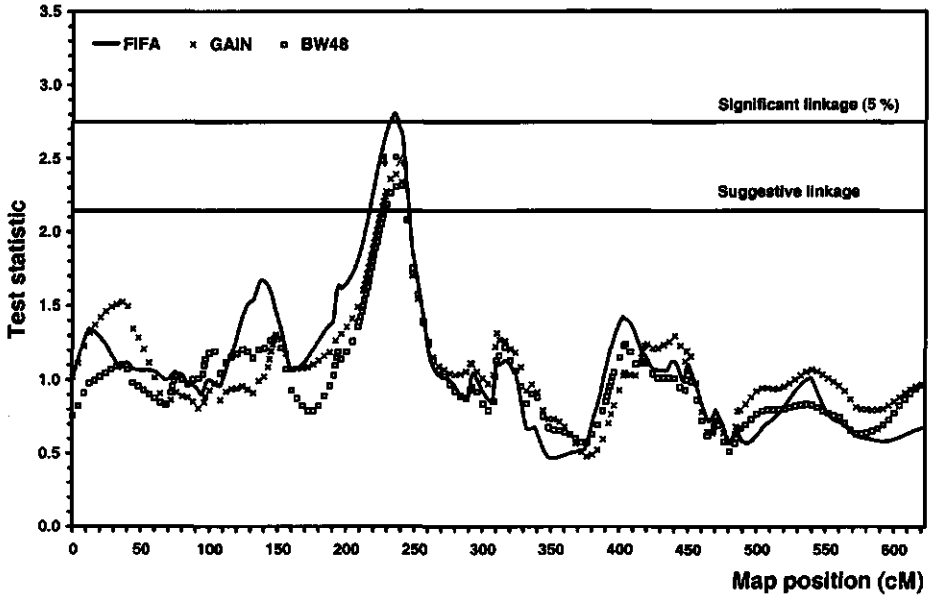

Figure 1. Test statistic values from the analysis of body weight at 48 days (BW48), growth between 23 and 48 days (GAIN) and feed intake between 23 and 48 days (FIFA) for quantitative trait loci on chromosome 1. Significant and suggestive linkage thresholds of FIFA are included. The thresholds for BW48 and GAIN were slightly higher. Map positions are given using the Haldane scale.

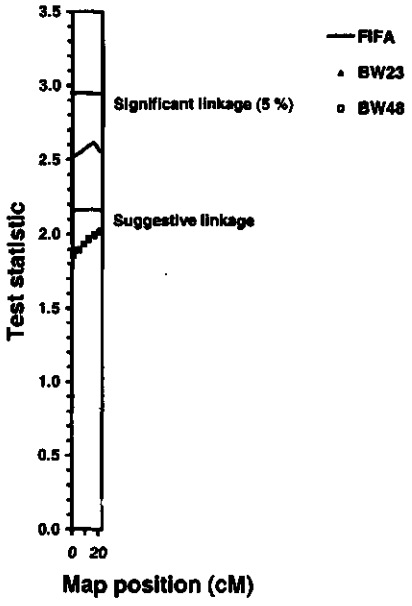

Figure 2. Test statistic values from the analysis of body weight at 23 days (BW23), body weight at 48 days (BW48) and feed intake between 23 and 48 days (FIFA) for quantitative trait loci on linkage group WAU26. Test statistic values of BW23 and BW48 are overlapping. Significant and suggestive linkage thresholds of FIFA are included. The thresholds for BW23 and BW48 were slightly higher. Map positions are given using the Haldane scale.

Quantitative trait locus 3 (Figure 3) showed suggestive linkage on chromosome 4 for FIFA. Furthermore, the test statistic of FIFW also peaked on chromosome 4. The most likely QTL position for FIFA was at 147 cM and for FIFW at 162 cM. For FIFA and FIFW, six parents showed significant effects, five of them being different parents. The estimated average allele substitution effect was $1.0\,\sigma_a$ for both FIFA and FIFW in these parents.

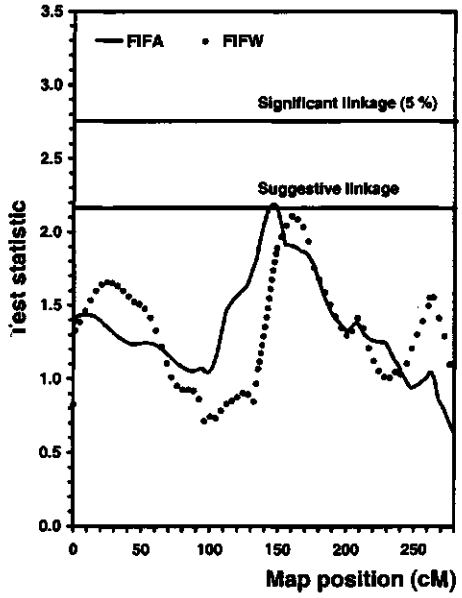

Figure 3. Test statistic values from the analysis of feed intake between 23 and 48 days (FIFA) and feed intake in a fixed weight interval (FIFW) for quantitative trait loci on chromosome 4. Significant and suggestive linkage thresholds of FIFA are included. The thresholds for FIFW were slightly higher. Map positions are given using the Haldane scale.

On chromosome 2 (Figure 4), QTL4 showed suggestive linkage for FIFW. The highest test statistic occurred at 41 cM. None of the other traits showed a clear peak at this location. Four parents showed significant effects for the segregation of a QTL. An average allele substitution effect of 1.4 σ_a in these parents was found.

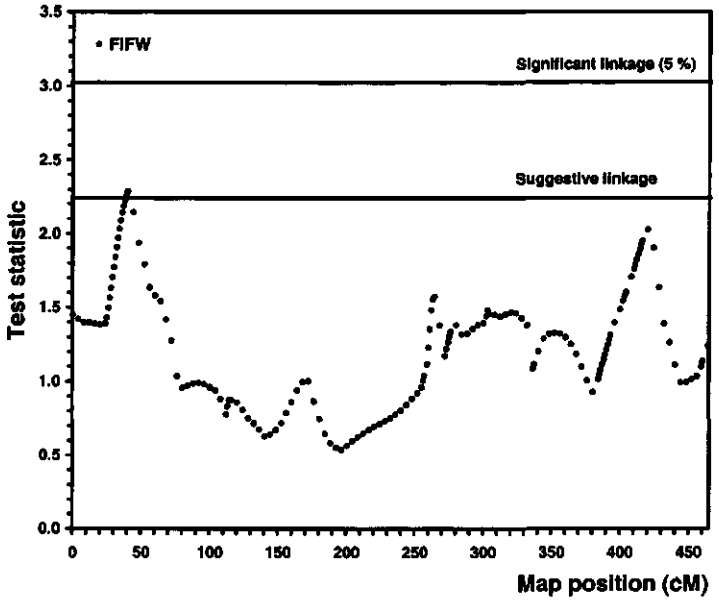

Figure 4. Test statistic values from the analysis of feed intake in a fixed weight interval (FIFW) for quantitative trait loci on chromosome 2. Significant and suggestive linkage thresholds are included. Map positions are given using the Haldane scale.

Discussion

Analysis of the Phenotypic Data

Compared to heritabilities reported by Bernon and Chambers (1988), Wang *et al.* (1991[a]) and Chambers *et al.* (1994) our estimates of heritabilities for BW23 and FE were relatively high. Chambers (1990) indicated that heritabilities of FE are usually in the range of 0.4-0.5, which agrees with results found in our study. Estimated heritabilities for FIFW and FIFA were similar to estimates reported in the literature. Heritability estimates for BW48 and GAIN were below most reported estimates. A large difference between the heritability of BW23 and BW48 was found (Table 3). BW23 and FIFA showed a clear difference in heritability for males and females. Thomas *et al.* (1958) suggested that

divergence in heritabilities based on male and female progeny might be evidence for the importance of sex-linked genes in the expression of the trait involved.

The correlation between the average adjusted progeny trait values between GAIN and BW48 was larger than the correlation between GAIN and BW23 (Table 4), which can be expected because GAIN is a part of BW48. Wang *et al.* (1991[b]) found similar results for genetic correlations. The correlation between the average adjusted progeny trait values can be considered as a lower bound estimate of the genetic correlation. The correlation of 0.52 between FIFA and FIFW clearly indicates that adjustment for initial and final body weight has a large influence on this trait. Bernon and Chambers (1988) present similar genetic correlations of 0.76 and 0.41 for their sire and dam population. FIFW showed a strong negative correlation with FE and approximately zero correlations with body weight, which is similar to the phenotypic correlations reported by Bernon and Chambers (1988). FE showed small correlations with body weight, which agrees with genetic correlations reported by Wang *et al.* (1991[b]).

QTLs Affecting Growth and Feed Efficiency

The most significant results for QTL1, QTL2 and QTL3 were all found for FIFA. Other traits showed lower significance levels. The QTL for BW48 at 240 cM on chromosome 1 found in our previous studies (Groenen *et al.*, 1997; Van Kaam *et al.*, 1998) has been confirmed. The traits FIFA and GAIN also showed significant or suggestive linkage for this position. Because these traits are correlated with BW48, it is possible that a single QTL affected these three traits. The same parents seemed to segregate for the QTL affecting these three traits. Finding similar results for these correlated traits builds more confidence in the presence of a QTL. From a biological point of view it can be expected that a higher feed intake, without changes in efficiency, leads to higher growth and therefore to a higher BW48.

For QTL2, the same parents showed evidence for segregation of this QTL in two traits, FIFA and BW48. In one of these parents, the QTL also seemed to have an effect on BW23. QTL3 showed evidence for segregation in different parents for FIFW and FIFA, with the exception of one dam. Therefore, it is possibly not the same QTL, which affected these two traits.

The correlation of 0.41 between the average adjusted progeny trait values of the G_2 chickens on BW23 and GAIN indicates that different genes might influence growth at different life stages. Cheverud *et al.* (1996) indicated that QTLs affecting early and late growth in mice were generally distinct, which was explained by different physiological mechanisms active at different life stages.

Contrasting to our results so far, Khatib (1994) found seven significant associations with juvenile growth rate, measured as body weight at 14 weeks, out of 21 microsatellite markers. Although our study covers a much larger part of the chicken genome, fewer results were declared significant compared to Khatib (1994). However, Khatib determined significance per marker and significance is therefore on a comparisonwise base, which is less stringent compared to our genomewise significance thresholds. One marker, which was significant in Khatib's study, MCW0004, was also used in our study, but did not show any high test statistics. Furthermore, the gene for Ovalbumine Y (OVY, previously GGY), which was significant in Khatib's study, is located on chromosome 3, were our study did not have significant results.

Acknowledgements

We thank Dr. S.A. Knott for the half sib QTL analysis program and R. Yunis for contributing literature. Furthermore, we acknowledge Euribrid B.V. for their collaboration and financial support.

Chapter 4

Whole Genome Scan in Chickens for Quantitative Trait Loci Affecting Carcass Traits

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Abstract

An experiment was conducted to enable quantitative trait loci (QTLs) mapping for carcass traits. The population consisted of 10 full sib families originating from a cross between male and female founders chosen from two different outcross broiler lines. Founder animals, parents, offspring and grandoffspring are denoted as generation 0, 1, 2 and 3 animals. Microsatellite marker genotypes were collected on generation 1 and 2. Phenotypic observations were collected on generation 3 animals. Recorded traits were BW at 48 days, carcass weight, carcass percentage, breast meat colour and leg score.

Average adjusted progeny trait values were calculated for each generation two animal and for each trait after adjusting phenotypic observations on generation three animals for fixed effects, covariables, the additive genetic contribution of the other parent and differences between sexes. The average adjusted progeny trait values were used as the dependent variable in the QTL analysis.

A QTL analysis was undertaken by modelling the segregation from generation one to generation two, using a full sib across family regression interval mapping approach. In total, 27 autosomal linkage groups covered with 420 markers were analysed. Genomewise significance thresholds were derived using the permutation test and a Bonferroni correction. Two QTLs, affecting two of the five analysed traits, exceeded suggestive linkage. The most significant QTL was located on chromosome 1 at 466 cM and showed an effect on carcass percentage. The other QTL, which affected meat colour, was located on chromosome 2 and gave a peak at 345 and 369 cM.

Introduction

Recently a lot of effort is spent on obtaining knowledge about quantitative trait loci (QTLs) in several species (e.g., Andersson *et al.*, 1994; Georges *et al.*, 1995). Such information on QTLs would be useful for marker assisted breeding as well as for improving the understanding of the biological background (i.e., which genes are involved and their effects) of traits. Usually, information from genetic markers is used for detecting QTLs on chromosomes. Recently, a large number of genetic markers was generated in

chicken (Crooijmans *et al.*, 1996, 1997), which enabled QTL detection. In order to detect QTLs for broilers, an experimental broiler population was set up following recommendations of Van der Beek *et al.* (1995). Marker genotypes were collected in the first two generations of this population and used to construct a linkage map (Groenen *et al.*, 1998). This facilitated a genomewide QTL analysis. Phenotypic observations were collected on third generation animals in different experiments. The first of these experiments was the feed efficiency experiment and the second was the experiment on carcass traits. Results of the feed efficiency experiment have been reported previously (Van Kaam *et al.*, 1998, 1999[b]). In total, four QTLs were detected. One QTL was located on chromosome 1 at 235 cM and had an influence on feed intake and growth between 23 and 48 days and on body weight at 48 days (BW48). A second QTL was located on linkage group WAU26 at 16 cM and showed an effect for feed intake between 23 and 48 days. On chromosome 4 at 147 cM, a third QTL affecting feed intake between 23 and 48 days and feed intake adjusted for BW, was located. Finally, a fourth QTL, which affected feed intake adjusted for BW, was located on chromosome 2 at 41 cM.

In the present paper, the results of a whole genome scan aimed at the detection and localisation of QTLs affecting carcass traits are presented. The traits analysed were BW at 48 days, carcass weight (CW), carcass percentage (CP), meat colour (MC) and leg score (LS). These traits are economically important for the broiler industry (Pollock, 1997, Emmerson, 1997). QTLs for carcass traits are interesting for animal breeders, because most of these traits can not be measured on living animals, which hampers selection. For these traits, utilisation of QTLs through marker assisted selection could be beneficial.

Material and Methods

Experimental Population

A broiler population, consisting of three generations, was created for the purpose of QTL detection. The number of animals and the population structure are given in Table 1.

Table 1. Population structure with numbers of animals used in the analysis and types of observations collected.[a]

Generation[b]	Males	Females	Total	Observations[c]
G_0[d]	14	14	28	———
G_1	10	10	20	Genotypes
G_2	175	274	449	Genotypes
G_3	969	984	1,953	Phenotypes on BW48
G_3	977	999	1,976	Phenotypes on CW
G_3	969	984	1,953	Phenotypes on CP
G_3	960	981	1,941	Phenotypes on MC
G_3	962	983	1,945	Phenotypes on LS

[a] Numbers exclude outliers and missing values.

[b] G_0 etc. = generation 0 etc.

[c] BW48 = body weight at 48 days; CP = carcass percentage; CW = carcass weight; LS = leg score; MC = meat colour.

[d] Male and female G_0 animals are from different lines.

Founder animals, parents, offspring and grandoffspring are denoted as generation 0, 1, 2 and 3 animals or G_0, G_1, G_2 and G_3 animals, respectively. Two genetically different outcross broiler dam lines (G_0) originating from the White Plymouth Rock breed, were chosen as the foundation of the experimental population. In one line, 14 males and in the other line 14 females were chosen and 14 G_0 couples were created. These 14 G_0 couples were mated in order to obtain 20 G_1 animals, 10 of each sex. From these 20 G_1 animals, 10 couples were created without known relationship, each couple being the base of a family. The G_1 couples were mated to produce G_2 full sibs. G_2 animals were mated with G_2 animals from other families to produce G_3 animals. Each full sib family consisted of two G_1 parents and on average 44.9 G_2 animals with marker genotypes and each G_2 animal had on average between 8.6 and 8.8 G_3 offspring with observations per trait. More details are given by Van Kaam *et al.* (1998, 1999[b]).

In this population, G_1 and G_2 animals were typed for microsatellite markers and phenotypic observations were collected on G_3 animals. Phenotypic observations on G_3 animals were used for the calculation of average adjusted progeny trait values on G_2 animals. G_1 and G_2 animals were the same animals as in the previously reported feed

efficiency experiment (Van Kaam *et al.*, 1998, 1999[b]). However, in the experiment on carcass traits, different G_3 animals were used and housing was in floor pens instead of individual cages. Seven G_2 animals had no offspring with observations in this experiment.

G_3 animals were raised in six hatches and housed in a litter system for broilers until the age of 48 days. Animal density was around 20 animals/m^2. The animals were in the same pen starting from day 0, where they received feed and water for *ad libitum* consumption and illumination was 23 hours a day. A commercial broiler feed, consisting of crumbled concentrates containing 12,970 kJ/kg and 21% protein was used. Around day 47, the legs of these G_3 animals were scored on a scale from 1 to 9, by looking at the hock-joints. Straight legs were considered as the optimum and received 9 as score. The lateral deviation of the legs from this optimum was judged. The further away from this optimum the lower the score the animals received. Leg problems were considered as an effect of weak hock ligaments or tendons, which could result in both *varus* (proximal hocks) as well as *valgus* (distal hocks). Therefore, both *varus* and *valgus* animals had a score below the optimum. In practice, the majority of the animals showed *varus*.

At 48 days, BW was measured and animals were slaughtered. After day 48, CW was measured. For one hatch, CW was measured on 2 days. CW was measured on the chilled carcass after removal of feathers, head, lungs, liver, kidneys, gastrointestinal tract, abdominal body fat, subcutaneous leg fat and lower legs and after loss of part of the animals blood due to bleeding. On the same day that CW was measured, measurements of the MC were taken at three spots on the chilled breast fillet, using a fibre optic meat probe (TBL Fibre Optics Ltd., Leeds, LS10 1AT, England). These three measurements were considered as repeated measurements of the same trait. The last hatch of animals was measured on a longer scale due to problems with the fibre optic meat probe. Linear transformation was applied to re-scale these measurements to the same scale as measurements taken on other animals. Transformation was performed by multiplying the deviation of each observation from the mean with a constant and successively adding the mean. In total, 23 G_3 animals had missing data on BW48, 0 for CW, 23 for CP, 46 for MC and 42 for LS.

Outlier detection was applied for BW48, CW, CP and separate fibre optic measurements. Because LS was classified from 1 to 9, outlier detection did not seem useful here, because it would lead to exclusion of the extreme animals (1) or the desired animals (9). Outliers for BW48, CW, CP and fibre optic measurements were detected by

applying the deviation of the observation from the mean divided by the standard deviation as test statistic for a single outlier. In order to be able to detect multiple outliers, the outlier test was applied iteratively, removing only a single outlier after each iteration, until no new outlier was detected. To account for different levels and variances between hatches and between males and females, the detection was applied per sex within each hatch separately. Critical values were those of Grubbs and Beck (1972) for a single outlier in normally distributed data of 0.5% per tail. These critical values depend on the sample size, i.e. with a larger sample size a larger deviation from the mean is still considered as normal. The number of outliers was respectively 8 for BW48, 7 for CW, 2 for CP and 4 for fibre optic scores. The outliers were randomly distributed across families, indicating that there probably was no genetic component involved. All outliers for BW48 and all except one outlier for CW were on the lower tail. All these animals with a low BW48 also had a low CW and vice versa. Because these traits are measured at different moments the observations were probably correct and these animals were most likely suffering from illness. In case BW48, CW or CP was considered as outlier, then all three traits were assigned missing. In total 11 animals obtained missing values for these traits.

An additional check was applied to the fibre optic scores. The availability of three fibre optic measurements for each animal provides a build-in control possibility. Fibre optic measurements, which differed more than three standard deviations from their expectation based on the other two fibre optic measurements on the same animal, were considered as incorrect measurements and were assigned missing. In total for 45 animals, which were randomly distributed over hatches and families, one of the three measurements was assigned missing. These 45 animals had a standard deviation among their remaining two fibre optic measurements of 83% of the standard deviation, which the other animals had over all three measurements. Before removal of the extreme measurements, this was 318%. For all animals, the (remaining) fibre optic measurements were averaged to obtain a single value for MC.

Marker Data

The marker data and linkage map utilised in this study were identical to the information used in our previous study on other traits (Van Kaam *et al.*, 1999[b]). Genotypes for microsatellite markers were determined on 20 G_1 and 456 G_2 animals. In total 265

markers were determined on all 10 families and 155 markers were only typed on 4 families. These 420 informative markers were mapped on 27 autosomal linkage groups, which covered 3,363 cM. Map distances presented in this paper are always sex-averaged distances in centimorgans on the Haldane scale (Haldane, 1919). Because the segregation of the Z-chromosome is different from autosomal chromosomes, the Z-chromosome was not included in the present genome scan. Linkage groups WAU1 to WAU7 were assigned to chromosome 1 to 7 and WAU11 to chromosome 8 (Groenen *et al.*, 1998). On the first 20 linkage groups, all 20 parents were informative. The number of informative parents was 8 for linkage group WAU21, 16 for WAU22, 19 for WAU23, 18 for WAU24, 7 for WAU25, 16 for WAU26 and 9 for WAU27. All markers on linkage group WAU21 were only typed on 4 families.

Analysis of the Phenotypic Data

A two step procedure was applied for analysis of the data: first average adjusted progeny trait values were calculated by adjusting phenotypic observations for systematic effects, and secondly a QTL analysis was undertaken using the average adjusted progeny trait values as the dependent variable.

Five traits were analysed: four measured traits and one inferred trait. Measured traits were BW48, CW, MC and LS. The inferred trait was CP, which was defined as the ratio between CW and BW48 multiplied with 100%. For MC, the average of the fibre optic measurements per animal was taken. For MC, an analysis was done without and with adjustment for BW48. These analyses are labelled with MC1 and MC2 respectively, when necessary. The reason for adjustment for BW48 is the phenotypic correlation between BW48 and MC, which was 0.29 in males and 0.15 in females. This correlation could be caused by differences in muscle composition (water content) or post-mortal transition to meat (pH change and drip loss) (Schreurs, 1999) and might have a genetic component. Because the distribution of LS had a skewness of -0.37, a second analysis was applied in which the scores were replaced with new values. With these new values, the distribution mimicked an underlying normal distribution with a mean of five, a standard deviation of two and a skewness of zero. This transformation was applied because normality is assumed in the estimation of variance components. A third analysis was applied in which the transformed values were used and an adjustment for BW48 was included. The analyses

of LS are labelled with LS1, LS2 and LS3 respectively. The phenotypic correlation between BW48 and LS1 was -0.01 for males and -0.07 for females.

Two of the five traits showed a difference between standard deviation in males and females of more than 50%. These traits were BW48 and CW. In order to account for the heterogeneity of variance between sexes these traits were analysed with a bivariate approach, i.e. treating observations on male and female G_3 animals as different but correlated traits. Although CP had a low difference in standard deviation between both sexes, it was analysed in the same manner as BW48 and CW, because it was derived from these traits. The following bivariate mixed model for male and female observations was applied:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & 0 \\ 0 & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix}$$

where:

$\mathbf{y}_i$ = Vector of observations for i = 1 (male) or 2 (female)

$\mathbf{b}_i$ = Vector of fixed effects and covariables for trait i

$\mathbf{u}_i$ = Vector of random direct additive genetic effects on trait i

$\mathbf{e}_i$ = Vector of random residual effects for trait i

$\mathbf{X}_i$ = Incidence matrix relating observations for trait i to fixed effects and covariables

$\mathbf{Z}_i$ = Incidence matrix relating observations for trait i to direct additive genetic effects

Elements in the vectors of fixed effects included for each trait the overall mean of the trait. Furthermore, for BW48 an interaction term between the hatch of the recorded animal and the hatch of the dam was included and for CW and CP an interaction term between the hatch of the recorded animal, the hatch of the dam and the day of measuring CW was included. The interaction term between the hatch of the recorded animal and the hatch of the dam represented the period of the year and the age of the dam at reproduction. G_3 animals were born in six different hatches, their dams were born in eight hatches. Because CW was measured on 2 days in one hatch, dehydration can have an influence on the measurement within hatch, and therefore the day of measuring CW was included in the interaction term.

The difference in standard deviation between males and females for MC was smaller than 1% and for LS1 and LS2/LS3 it was 9% and 2%. Therefore, for LS and MC no

adjustment for heterogeneity of variance between the sexes was necessary. A univariate approach, with an equivalent model was applied. For MC, the overall mean of the trait, the sex and an interaction term between the hatch of the recorded animal, the hatch of the dam and the day of measuring MC were included as fixed effects. BW48 was included as linear covariable for MC2 only. For LS, the overall mean of the trait, the sex and an interaction term between the hatch of the recorded animal and the hatch of the dam were included as fixed effects. For LS3, BW48 was included as a linear covariable. Variance components, fixed effects, covariables and breeding values were estimated using MTDFREML (Boldman *et al.*, 1995).

After adjusting the phenotypic observations for fixed effects and covariables, adjustment was for the additive genetic contribution of the male or the female parent, which resulted in two adjusted trait values for each G_3 animal. In the bivariate approach, all adjusted trait values were standardised to a mean of zero and to the variance of the male G_3 adjusted trait values. Subsequently, adjusted trait values were combined to average adjusted progeny trait values for G_2 animals by averaging over all their G_3 progeny.

QTL Analysis

The multi-marker regression method for outbred populations with a half sib structure (Knott *et al.*, 1994) was extended to enable analysis of the full sib design (Van Kaam *et al.*, 1998, 1999[b]). The analysis is an across family weighted full sib regression analysis, which is nested within families in order to account for differences in marker-QTL linkage phase. Average adjusted progeny trait values of G_2 animals were regressed on the probabilities of inheriting the first allele of each G_1 parent. In order to account for polygenic differences between families, the family mean was included in the model. Differences in number of G_3 animals contributing to G_2 average adjusted progeny trait values were taken into account by applying a weighting factor, which is based on the variance of the average adjusted progeny trait values. At each centimorgan, test statistics were calculated to test for the presence of QTL effects vs the absence of QTL effects. The test statistic was the ratio of the explained mean square of the QTL effects in the numerator and the residual mean square of the full model in the denominator. A constant number of degrees of freedom was applied across all linkage groups.

Significance Thresholds

For each trait, significance thresholds were calculated empirically using the chromosomewise permutation method (Churchill and Doerge, 1994). To obtain genomewise significance thresholds, chromosomewise significance thresholds were adjusted for multiple testing along the genome using the Bonferroni correction. Using the genomewise significance thresholds, two types of significance thresholds were derived: significant and suggestive linkage (Lander and Kruglyak, 1995). Significant linkage is defined as a 5% genomewise significance threshold and suggestive linkage is equivalent to one expected false positive result per trait in a whole genome scan. Because all parents were informative on the first 20 linkage groups, the test statistics on these linkage groups were comparable (Van Kaam *et al.*, 1998). Therefore these linkage groups were permuted together and common thresholds were applied. For each trait, 10,000 permutations were performed. For the other linkage groups, some parents were uninformative. Hence no QTL effect could be fitted for these parents and test statistics on these linkage groups are not comparable with other linkage groups. For each of these linkage groups, 100,000 permutations were executed, because a larger Bonferroni correction was necessary to obtain reliable genomewise significance thresholds.

Permutation was also applied to determine which parents were segregating for a QTL on those locations where a QTL was detected in the across family analysis. Per parent, a test comparing a model with a QTL vs a model without a QTL was applied, accounting for the presence or absence of QTL effects in the mate. A 10% comparisonwise threshold was obtained from 10,000 permutations. Parents with a test statistic above this threshold were assumed to be segregating for the QTL.

Results

Variance Components

In Table 2 estimated heritabilities, genetic correlations and phenotypic variances are presented. Three traits, BW48, CW and CP have separate variance estimates per sex, because these traits were analysed using a bivariate approach. Estimated heritabilities based on males and females differed at most 0.12. The genetic correlation between male and female observations was close to unity for all three traits. Estimated heritabilities for BW48 and CW were similar to those mentioned by Bernon and Chambers (1988) and Wang *et al.* (1991[a]). LS1, LS2 and LS3 had a low heritability of 0.13, which might in part be due to the subjective scoring.

Table 3 shows the correlations between the average adjusted progeny trait values of the G_2 animals for all traits. BW48 and CW showed a very high correlation of 0.97 and therefore similar results were expected in the QTL analysis. This high correlation can be expected because CW is a large part of BW48. MC1 and MC2 showed a correlation close to unity. The same holds for LS1, LS2 and LS3. These high correlations indicate that the effect of the differences in the analyses were small. A moderate correlation was found between CW and CP. All other combinations of traits showed a correlation close to zero.

QTL Analysis

Two QTLs were detected: both QTLs showed suggestive linkage. However, no QTL showed significant linkage. Two of the five analysed traits showed QTLs reaching suggestive linkage. CP showed suggestive linkage once and MC showed suggestive linkage twice in both analyses. In Table 4, the most interesting regions are presented for each trait. For BW48, CW, LS1, LS2 and LS3 the test statistic did not reach the suggestive linkage threshold on any linkage group, although LS3 came very close to it.

QTL1 (Figure 1) was located on chromosome 1 and showed suggestive linkage for CP. The peak of the test statistic was located at 466 cM. Five sires and three dams showed significant QTL effects. The average allele substitution effect (α; Falconer, 1989) in these parents was $0.7\,\sigma_a$.

Table 2. Heritabilities, genetic correlations and phenotypic variances.

Trait[a]	h_m^2 [b]	h_f^2	r_g	$\sigma_{p,m}^2$ [c]	$\sigma_{p,f}^2$
BW48	0.36	0.48	0.92	60,725	38,351
CW	0.36	0.47	0.93	30,055	19,628
CP	0.43	0.52	1.00	2.07	2.62
	h^2			σ_p^2	
MC1	0.37			16.53	
MC2	0.38			16.30	
LS1	0.13			3.49	
LS2	0.13			3.57	
LS3	0.13			3.51	

[a] BW48 = body weight at 48 days; CP = carcass percentage; CW = carcass weight; LS1 = original leg score; LS2 = transformed leg score; LS3 = transformed leg score adjusted for BW48; MC1 = meat colour unadjusted for BW48; MC2 = meat colour adjusted for BW48.

[b] h^2, h_m^2, h_f^2 = heritability of all observations respectively only male or female observations; r_g = correlation between additive genetic effects on male and female observations; $\sigma_p^2, \sigma_{p,m}^2, \sigma_{p,f}^2$ = phenotypic variances based on all, male or female observations.

[c] Weights were measured in grams.

Table 3. Correlations between the average adjusted progeny trait values of the G_2 animals.

Trait[a]	BW48	CW	CP	MC1	MC2	LS1	LS2	LS3
BW48								
CW	0.97							
CP	0.16	0.37						
MC1[b]	0.19	0.17	-0.07					
MC2[b]	0.14	0.11	-0.08	1.00				
LS1	0.14	0.13	0.00	0.05	0.04			
LS2	0.14	0.13	-0.00	0.05	0.04	0.99		
LS3	0.06	0.05	-0.02	0.03	0.03	0.99	1.00	

[a] BW48 = body weight at 48 days; CP = carcass percentage; CW = carcass weight; LS1 = original leg score; LS2 = transformed leg score; LS3 = transformed leg score adjusted for BW48; MC1 = meat colour unadjusted for BW48; MC2 = meat colour adjusted for BW48.

[b] Higher values represent darker meat.

Table 4. Summary of interesting regions per trait. Indicated per trait are the number assigned to the quantitative trait locus (QTL), the linkage group, the most likely location in centimorgans, the markers bracketing this location and the genomewise significance level of the QTL at this location.

Trait[a]	QTL	Chromosome	Location	Markers[b]	Significance[c]
BW48	–	3	366	LEI0166 – MCW0148/MCW0116	85%
CW	–	3	365	LEI0166 – MCW0148/MCW0116	72%
CP	1	1	466	ADL0183 – LEI0079	17% *
MC1	2	2	345	MCW0185 – MCW0234	38% *
MC2	2	2	344	MCW0185 – MCW0234	42% *
MC1	2	2	369	MCW0264 – ADL0164	41% *
MC2	2	2	369	MCW0264 – ADL0164	48% *
LS1	–	2	269	MCW0065 – ADL0212	93%
LS2	–	2	268	MCW0065 – ADL0212	80%
LS3	–	1	565	ADL0238 – UMA1.003	64%

[a] BW48 = body weight at 48 days; CP = carcass percentage; CW = carcass weight; LS1 = original leg score; LS2 = transformed leg score; LS3 = transformed leg score adjusted for BW48; LS3 = transformed leg score adjusted for BW48; MC1 = meat colour unadjusted for BW48; MC2 = meat colour adjusted for BW48.

[b] ADL = Avian Disease and Oncology Laboratory, Michigan State University, East Lansing; LEI = University of Leicester, Leicester; MCW = Microsatellite chicken Wageningen; UMA = University of Massachusetts, Amherst; WAU = Wageningen University, Wageningen.

[c] * = Suggestive linkage.

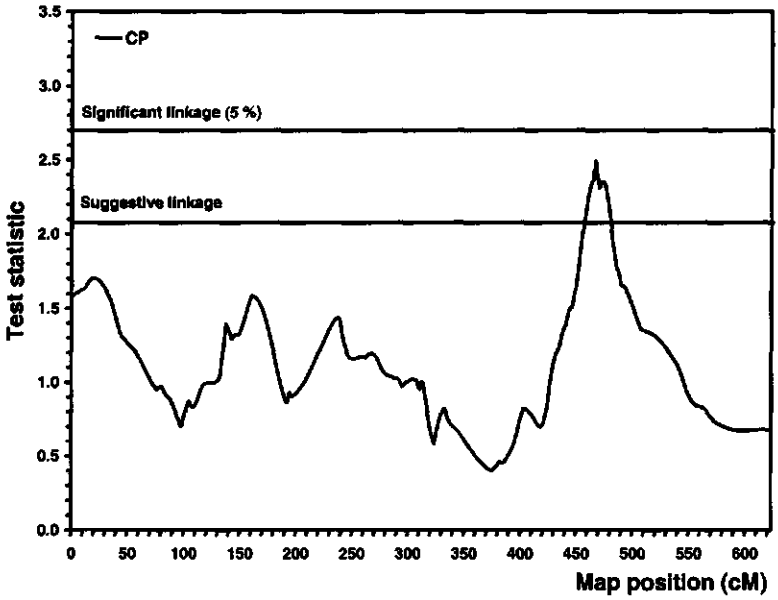

Figure 1. Test statistic values from the analysis of carcass percentage (CP) for quantitative trait loci on chromosome 1. Significant and suggestive linkage thresholds of CP are included. Map positions are given using the Haldane scale.

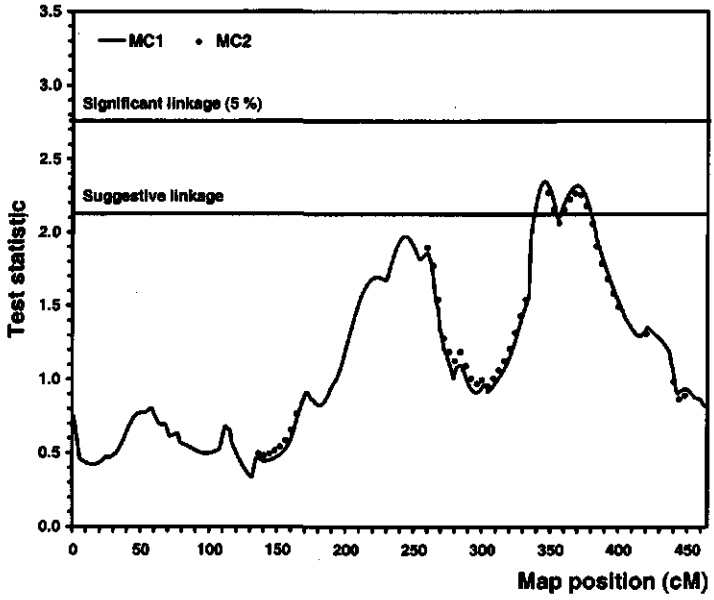

Figure 2. Test statistic values from the analysis of meat colour unadjusted for BW48 (MC1) for quantitative trait loci on chromosome 2. Significant and suggestive linkage thresholds of MC1 are included. Locations were meat colour adjusted for BW48 (MC2) differed from MC1 are indicated with dots. Map positions are given using the Haldane scale.

QTL2 (Figure 2) was detected on chromosome 2. Two peaks for this QTL showed suggestive linkage for MC1 and MC2. The highest test statistic for QTL2 was found at 345 cM for MC1 and at 344 cM for MC2. In both analyses, a slightly lower test statistic was found at 369 cM between markers MCW0264 and ADL0164. Although the possibility of presence of more than one QTL cannot be excluded, the present data set does not provide enough evidence to conclude that more than one QTL is segregating and therefore one QTL is assumed. One sire and two dams showed significant QTL effects for a QTL at the first peak. In both analyses, the estimated average allele substitution effect was $1.0\,\sigma_a$ in these parents. For MC1, an additional sire and two additional dams gave significance for the segregation of a QTL at the second peak. For MC2, one of these additional dams was not significant. The estimated average allele substitution effect was $1.2\,\sigma_a$ in these

five respectively six parents. Because the same parents tend to show the largest effect, it seems most likely that only one QTL is segregating in this region.

On chromosome 1, at 565 cM suggestive linkage was almost reached for LS3 (Figure 3). Two sires and three dams showed significant QTL effects. The average allele substitution effect in these parents was $1.4\,\sigma_a$. The two most likely locations of LS1 and LS2 swapped in order of likelihood for LS3.

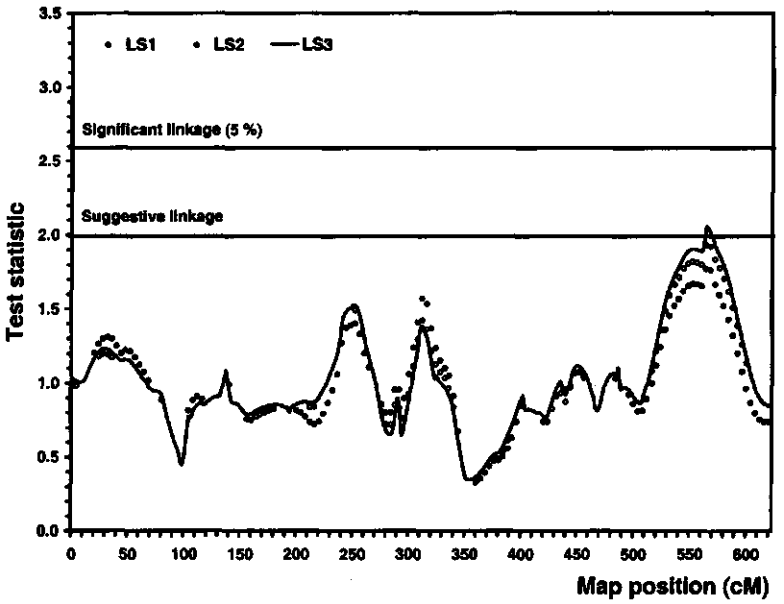

Figure 3. Test statistic values from the analysis of transformed leg score adjusted for BW48 (LS3) for quantitative trait loci on chromosome 1. Significant and suggestive linkage thresholds of LS3 are included. Locations were original leg score (LS1) and transformed leg score (LS2) differed from LS3 are indicated with dots and circles respectively. Map positions are given using the Haldane scale.

Discussion

Carcass Traits

Five carcass traits have been analysed. Both BW48 and CW are related to the growth rate. A higher growth rate is important for farmers, because it enables them to increase the production per pen. For processors, CW is a more useful measure than BW48, however in practice BW48 is easier to measure. In order to increase the efficiency of growth, CP could be increased.

MC is important as a quality trait for processors, retailers and consumers. Relations between MC and several other quality traits have been reported. Lighter meat is associated with a lower pH, lower water binding capacity, lower total pigment, myoglobin and iron concentrations and higher cooking loss (Allen *et al.*, 1997; Boulianne and King, 1995; Barbut, 1993, 1997). Darker meat is related to a higher pH, a higher susceptibility to bacterial spoilage and loss of a fresh odour and a shorter shelf life (Allen *et al.*, 1997; Fletcher, 1995). A protein, which might be related to MC, is myosin. The light polypeptide of the myosin gene (MYLL1) is located about 25 cM left of QTL2 at 320 cM.

Leg problems are of increasing importance for the poultry industry and can affect growth performance, efficiency and mortality (Emmerson *et al.*, 1991). Kestin *et al.* (1992) report up to 90% gait abnormalities in broilers. Furthermore, they signal an increase in gait abnormalities with increasing BW. Given the differences in prevalence of gait abnormalities between the breeds in their study, a genetic base is assumed. With increasing BW there is a tendency towards an increase of the proportion breast muscle and a decrease of the proportion leg muscle (Emmerson *et al.*, 1991; Pollock, 1997), which might increase leg problems. LS was scored by looking at the lateral deviation of the legs. The lateral deviation was previously scored in turkeys by Nestor (1984).

Comparison with Previous Results

Because BW48 was also analysed in the feed efficiency experiment (Van Kaam *et al.*, 1998, 1999[b]), it is interesting to compare the results. In the feed efficiency experiment, a QTL was located at 240 cM on chromosome 1. Furthermore, high test statistic values were

found on linkage group WAU26, although not significant for BW48. In the experiment on carcass traits, however, no evidence for the presence of QTLs at these locations was found. The test statistic was below one on both locations. These differing results can be explained by the low correlation between the average adjusted progeny trait values of the G_2 animals (0.25) for BW48 in both experiments. The genetic correlation between BW48 in both experiments was 0.60. Apparently, the performance of chickens is quite different under different housing conditions, free housing vs individual housing, despite the same genetic background, availability of feed and water and commercial broiler feed and a 23 hours a day light scheme. The mortality rate from 22 until 48 days was 4% for both husbandry systems. It is possible that the QTL has an effect on BW48 under certain conditions and hardly any effect under other conditions i.e. genotype × environment interaction. Stress can be a factor causing differences between free and individual housing. In free housing there could be more competition between chickens. On the other hand, chickens housed individually can be stressed due to their limited freedom and due to change in housing at 22 days, when they were switched to individual housing. Some chickens might show a temporary growth stop when switched over to individual housing, whereas other chickens seem unaffected. The low correlation between the average adjusted progeny trait values of the G_2 animals affects the power for detecting the same QTL. Tolon and Yalcin (1997) concluded that husbandry system by sex interaction significantly affected 7 week BW in broilers. Other reasons for different results can be that the previously reported result is a false positive result or that a QTL is segregating, but is not detected.

Acknowledgements

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Chapter 5

Detection of Genes on the Z-chromosome Affecting Growth and Feathering in Broilers

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Abstract

Detection of genes located on the Z-chromosome has some differences as compared to the detection of genes located on autosomal chromosomes. In the present study, the chicken Z-chromosome is scanned for genes affecting growth traits and feathering. For this purpose, data from a three generation full sib-half sib design was available: parents, full sib offspring and half sib grandoffspring. The parents and full sib offspring were genotyped for 17 markers on the Z-chromosome. Phenotypic data was only available on grandoffspring. Only the segregation of male chromosomes provides information on the presence of genes and therefore a half sib interval mapping approach was used. The feathering gene was detected significantly and located between markers ADL0022 and MCW0331. No significant indications were found for the presence of QTLs affecting growth traits on the Z-chromosome.

Introduction

Selection for quantitative traits has been performed effectively for many years without knowledge on the action of individual genes. The development of an abundance of molecular genetic markers, such as microsatellites, has provided the opportunity to resolve quantitative genetic variation into individual loci and to understand the basis of genetic variation. Recently a considerable number of DNA markers has been mapped for poultry (Crooijmans *et al.*, 1996). The availability of a genetic linkage map for chickens facilitates the mapping of genes affecting quantitative traits (QTLs).

Genetic mapping of a trait comes down to finding those chromosomal regions that tend to be shared among good performing relatives and tend to differ between good performing and poor performing relatives. Conceptually, this amounts to the following steps: scan the entire genome with a dense collection of genetic markers; calculate an appropriate linkage statistic at each position along the genome; and identify the regions in which the statistic shows a significant deviation from what would be expected under independent assortment (Lander and Kruglyak, 1995).

In previous studies, several QTLs were found in broilers. Van Kaam *et al.* (1999[b]) reported four QTLs that were identified in a feed efficiency experiment and Van Kaam *et al.* (1999[a]) reported two suggestive QTLs in a carcass experiment. Only autosomal chromosomes have been scanned for QTLs in broilers so far but there might also be QTLs located on the Z-chromosome. These QTLs might explain the observed difference in mean and variance of growth between males and females. Furthermore, Tixier-Boichard *et al.* (1995) found a genetic correlation between adult male and female body weight of 0.71. Hagger (1994) reported a genetic correlation of 0.84 between adult male and female body weight. This suggests that male and female body weight are genetically different traits which can be due to genes located on the sex chromosomes. In mice a large single QTL was detected affecting body weight (Rance *et al.*, 1997[a,b]). The estimated effect of the QTL was approximately 20% of mean body weight in males and females at 10 weeks. In pigs, QTLs were detected affecting backfat thickness and intramusculair fat content (Harlizius *et al.*, 2000). These indications suggest that it is worthwhile to search for the presence of genes affecting growth traits on the sex chromosomes.

Besides information on quantitative traits, information on feathering was available. Feathering (early or late) is of interest because it can be used to distinguish day-old males and females. The feathering locus is known to be located on the Z-chromosome (Nicholas, 1987). Feathering is a so-called single gene trait as opposed to the quantitative traits, which are influenced by several genes and by the environment. It is therefore expected that if the methodology used is appropriate then it should be possible to detect and localise the feathering gene.

The aim of the present study is to detect and localise QTLs for growth- and feed efficiency traits on the Z-chromosome in a three generation design. In addition, the feathering gene will be localised. For this purpose, the theoretical backgrounds for the detection of QTLs on the Z-chromosome for a three generation design will be developed.

Material and Methods

Experimental population

For this QTL detection study, a three generation population was created, as described by Van der Beek *et al.* (1995). This design was termed a three generation full sib-half sib design: parents, full sib offspring and half sib grandoffspring. In this article, the parents will be referred to as G_1 animals (Generation 1), the full sib offspring as G_2 animals and the half sib grandoffspring as G_3 animals. In order to increase the probability of parents being heterozygous for QTLs the population of G_1 animals was produced by crossing two genetically different outcross broiler dam lines originating from the White Plymouth Rock breed. The maternal line had a relatively high reproductive performance and the paternal line had a relatively high growth performance. The two lines had a genetic distance, calculated as Rogers' distance (Nei, 1987) based on 16 microsatellite markers, of 0.37. Phenotypic differences between the two lines in number of eggs was 20% and in slaughter weight 15%.

The population structure with observations and numbers of animals used in the analysis is given in Table 1. The G_1 animals were mated to produce full sib G_2 families. G_2 animals from one full sib family were mated to G_2 animals from other families in order to produce G_3 animals. Each G_2 animal was repeatedly mated with other G_2 animals to generate sufficiently large half sib families. Each G_2 male was mated to on average 4.5 G_2 females, and each G_2 female was mated to on average 2.8 males. Over all, each G_2 animal was mated to 3.4 mates resulting in 2.7 G_3 full sib animals per mating. In each generation, mating of related individuals was avoided. As shown in Table 1, more female than male G_2 animals were available but the number of G_3 males and G_3 females were almost equal.

Table 1. Number of animals used in the analysis.

Generation	Males	Females	Total	Observations
G_0	14	14	28	
G_1	10	10	20	Genotypes
G_2	172	279	451	Genotypes
G_3	1,063	1,083	2,146	Phenotypes body weight at 23 days[a]
G_3	1,012	1,037	2,049	Phenotypes body weight at 48 days (2) and growth between 23 and 48 days[a]
G_3	977	999	1,976	Phenotypes carcass weight[b]
G_3	969	984	1,953	Phenotypes body weight at 48 days (1) and carcass percentage[b]
G_3	2,054	2,099	4,153	Phenotypes for feathering[ab]

[a] Recorded in the feed efficiency experiment.
[b] Recorded in the carcass experiment.

In the three generation design, G_1 and G_2 animals were typed for genetic markers and phenotypic information was collected for G_3 animals. On average, each G_1 full sib family consisted of 45.1 genotyped G_2 animals and on average, each G_2 animal had 8.9 progeny. Different experiments were performed using the same G_1 and G_2 animals, but different G_3 animals. In the feed efficiency experiment, in total five hatches of G_3 animals were produced and phenotyped for several traits. From 0 to 22 days, the animals were kept in groups. The animals were housed in individual cages between the age of 22 and 48 days. Feed and water were supplied *ad libitum* at all times. The barns were artificially illuminated 23 hours a day. Climate was controlled according to normal commercial practice. In the carcass experiment, the G_3 animals were raised in six hatches. The broilers were housed in a litter system until an age of 48 days. Animal density was around 20 animals per square meter. Illumination was 23 hours a day and animals received feed and water for *ad libitum* consumption. For more details on the experiments we refer to Van Kaam *et al.* (1999[a,b]).

Marker genotypes

Genotypes for microsatellite markers were determined using DNA derived from blood samples from 20 G_1 and 451 G_2 animals. Marker alleles were recorded in base pair units.

For more details see Groenen *et al.* (1997, 1998). In total 437 informative markers were mapped to 28 linkage groups: in the present study information from 17 markers mapped to the Z-chromosome were used. The Haldane mapping function (Haldane, 1919) was used in this paper. The linkage map was constructed using CRI-MAP (Green *et al.*, 1990).

Analysis of the phenotypic data

For analysis of the phenotypic data a two step procedure was applied: first average adjusted progeny trait values were calculated by adjusting phenotypic observations for systematic environmental effects, and secondly a QTL analysis was undertaken using average adjusted progeny trait values as dependent variables. The carcass and the feed efficiency experiments each consisted of approximately 2,000 G_3 animals. The present study focuses on the traits body weight at 48 days, carcass weight and carcass percentage obtained in the carcass experiment and body weight at 23 days, body weight at 48 days and growth between 23 to 48 days obtained in the feed efficiency experiment. Reason for focussing the study on these traits is that especially for these traits differences exist in mean and standard deviation between both sexes. This might be due to genes located on the Z-chromosome.

The data on the quantitative traits is used to calculate average adjusted progeny trait values. Observations on male and female G_3 animals were treated as different but correlated traits, using a bivariate approach in order to account for heterogeneity of variance between both sexes (Van Kaam *et al*, 1998). More details on the model can be found in Van Kaam *et al.* (1997;1998). Average adjusted progeny trait values were calculated for G_2 animals after the data was adjusted for a number of systematic environmental effects.

Besides the quantitative traits, data on feathering is available. In total, 4,153 G_3 animals have been scored for feathering, i.e. both on animals in the carcass as well as in the feed efficiency experiment. Two alleles are segregating at the feathering locus: K (late) and k (early). Late feathering animals were scored as 1 and early feathering animals were scored as 2. Similar as for the quantitative traits, progeny means of G_2 animals are calculated for feathering and these are used in the QTL analysis.

QTL analysis of the Z-chromosome

Figure 1 gives a schematic representation of the three generation design in which males and females are distinguished. In this design, the segregation of G_1 alleles is followed and genes can be detected if G_1 animals are heterozygous. For autosomal chromosomes, both male and female alleles can be traced (full sib design). The analysis of such a design has been described by Van Kaam *et al.* (1998). For the analysis of the Z-chromosome only the segregation of G_1 males can be used: G_1 females have one Z- and one W-chromosome. Therefore, a half sib analysis was applied to the data. Figure 1 distinguishes between male and female G_2 offspring of cocks heterozygous for a QTL (Z^1Z^2). For the G_2 animals, average adjusted progeny trait values can be calculated either based on male or female G_3 progeny. This leads to four different groups of animals: G_2 males for which the average adjusted progeny trait values are based on G_3 male animals (MM), G_2 male - G_3 female (MF), G_2 female - G_3 male (FM) and G_2 female - G_3 female (FF). Because G_2 animals can have inherited either the Z^1 or the Z^2 sire allele, in total 8 different means can be calculated (Figure 1). Additive gene effects in the males and in the females are distinguished in order to make it possible to account for different gene actions in males and females (e.g. due to dosage compensation). Furthermore, allele frequencies in males and females are distinguished. If allele frequency differences for loci located on the Z-chromosome exist between the two lines (G_0), then the population is expected to be in disequilibrium for a number of generations (Falconer, 1989). The contrast between the Z^1 and the Z^2 allele for the MM group is $0.5[a_m + (q_f - p_f)d]$, i.e. similar to the contrast for an autosomal gene in a three generation design. For the FM group the contrast is $[a_m + (q_m - p_m)d]$, in case frequencies in males and females are identical this is twice the contrast of the MM group. For the MF group, the contrast is a_f and for the FF group, the contrast between Z^1 and Z^2 is 0.

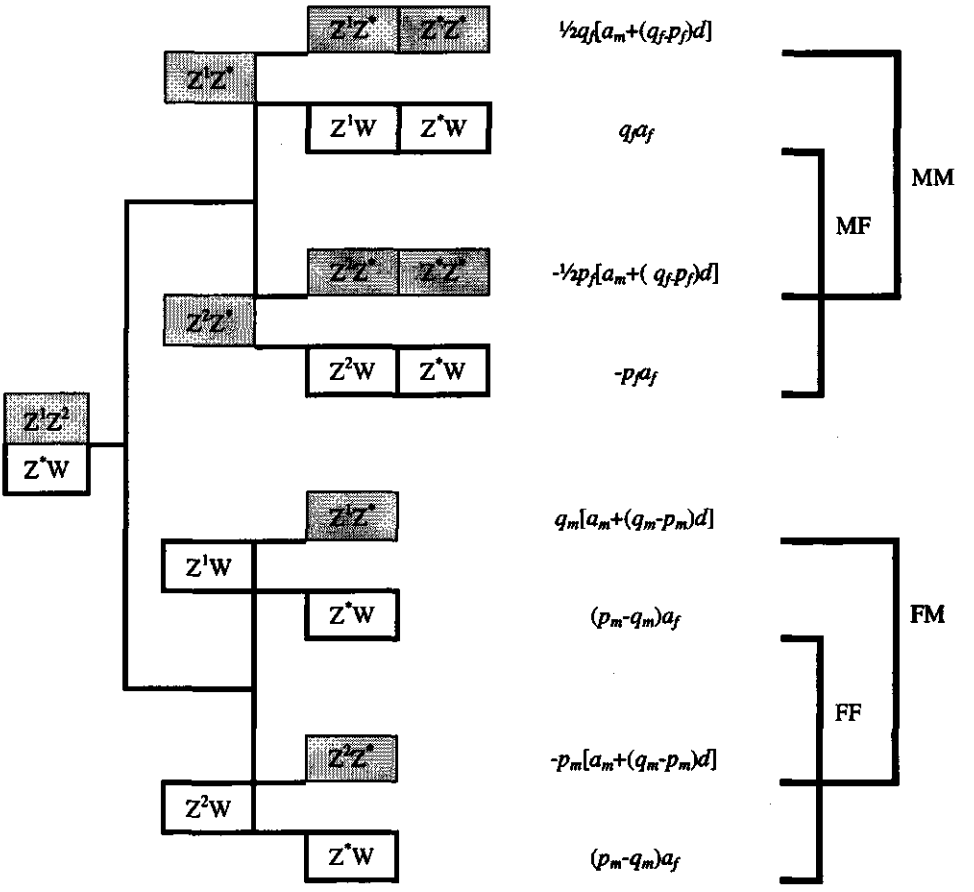


Figure 1. Three generation full-sib half-sib design were males and females are distinguished.
[a] p = frequencies of Z^1 allele, q = frequency of Z^2 allele, subscripts m and f for p and q indicate frequencies in males or females. a_m, a_f: additive genetic effects for males and females, d: dominance effect, Z^* is arbitrary allele, MM = male G_2 - male G_3, MF = male G_2 - female G_3, FM = female G_2 - male G_3, FF = female G_2 - female G_3.

QTL analyses were performed using the multi-marker regression method for outbred populations with a half sib structure (Knott *et al.*, 1994). Using the full-sib QTL analysis

like Van Kaam *et al.* (1998) is not possible, because only the segregation of the G_1 male alleles can be followed. Because marker-QTL linkage phase can differ between families, QTL analysis was nested within families. Average adjusted progeny trait values of G_2 animals were regressed on the probabilities of inheriting the first allele of the male G_1 parent. A family mean was included in the model to account for polygenic differences between families. G_2 female average adjusted progeny trait values based on G_3 females were excluded from the analyses, as they do not provide information on the presence of a QTL. In a first analysis the information of the remaining three groups, MM, MF and FM, were combined in order to have maximum power of detecting QTLs while realising that the interpretation of the regression coefficients is not straightforward. In case significant effects were detected, analyses were performed separately for the MM, MF and FM groups. Regression coefficients obtained in these analyses can be interpreted using the theoretically expected contrasts (Figure 1).

The model to fit a QTL at position k was:

$$y_{ij} = f_i + b_{ik}x_{ijk} + e_{ijk}$$

where:

y_{ij} = Average adjusted progeny trait value for G_2 animal j of family i

f_i = Polygenic effect of family i

b_{ik} = Regression coefficient for sire i at position k

x_{ijk} = Probability that G_2 animal j in family i at position k received the chromosomal segment from haplotype 1 from the sire

e_{ijk} = Random residual of animal j in family i at position k

A weighting factor was applied to account for differences in number of G_3 animals contributing to G_2 average adjusted progeny trait values. The weighting factor is based on the variance of the average adjusted progeny trait values of the G_2 animals (Van Kaam *et al.*, 1998). Note that although half sib analyses are performed the population does consist of full sib G_2 animals hence the weighting factor should be calculated as in the full sib analysis.

In order to test for the alternative hypothesis of the presence of QTL effects, versus the null hypothesis of the absence of QTL effects, a test statistic was calculated at each centimorgan. The test statistic is the ratio of the explained mean square of the QTL effects under study in the numerator and the residual mean square of the full model in the denominator. The test statistic at position k is calculated as:

$$\text{Test statistic}_k(\mathrm{H}_1 : \mathrm{H}_0) = \frac{\left(\frac{RSS_k(\mathrm{H}_0) - RSS_k(\mathrm{H}_1)}{df_{QTL}}\right)}{\left(\frac{RSS_k(\mathrm{H}_1)}{df_{total} - df_{family} - df_{QTL}}\right)}$$

Where RSS_k is the residual sums of squares across families after fitting the full (H_1) or the reduced model (H_0) and df are the total degrees of freedom (df_{total}), number of family means fitted (df_{family}) and number of QTL effects fitted (df_{QTL}).

Information content

If the inheritance of each cM of DNA would be known with certainty then the distribution of the conditional QTL probabilities would have an expected mean of 0.5 and variance of 0.25: the G_2 progeny has (1) or has not (0) inherited the QTL allele. The variance reduces when there is uncertainty about the inheritance of a QTL allele. The information content shows the ratio between the actual variance found in the data and the expected variance under full information (Spelman *et al.*, 1996). The information content will be lower when the distance from the nearest informative marker is larger and when markers are less informative. Power of detection of QTLs will be less in regions where the information content is lower. Table 2 shows the relative location of the 17 markers on the Z-chromosome used in the present experiment.

Table 2. Linkage map of the Z-chromosome including marker names and marker positions in centimorgans.

Marker	Location in cM
ADL0022	0
MCW0331	24
MCW0055	35
MCW0258	49
ROS0072	50
ADL0273	101
ADL0201	116
ADL0250/MCW0241/MCW0246	121
MCW0154	122
MCW0294/ MCW0292	129
ROS0017	135
LEI0121	166
MCW0128	197
LEI0075	203

Significance thresholds

Significance thresholds were calculated using the permutation test (Churchill and Doerge, 1994). To obtain genomewise significance thresholds the chromosomewise significance thresholds were corrected for multiple testing along the genome with the Bonferroni correction (Van Kaam *et al.*, 1998). Besides significant genomewise thresholds, suggestive linkage thresholds were calculated (Lander and Kruglyak, 1995). Significant linkage was declared if the 5% genomewise significance threshold was exceeded. Suggestive linkage is equivalent to an expectation of one false positive result per trait on a whole genome scan. Significance thresholds were determined for each trait separately because differences in the distributions of the average adjusted progeny trait values result in differences in the distribution of the test statistics (Spelman *et al.*, 1996).

Results

Information content

Figure 2 shows the information content on the Z-chromosome. The information content varies between 0.37 and 0.87. The information content is highest in regions where several informative markers are available. When the distance to the nearest informative marker is larger, the information content is reduced.

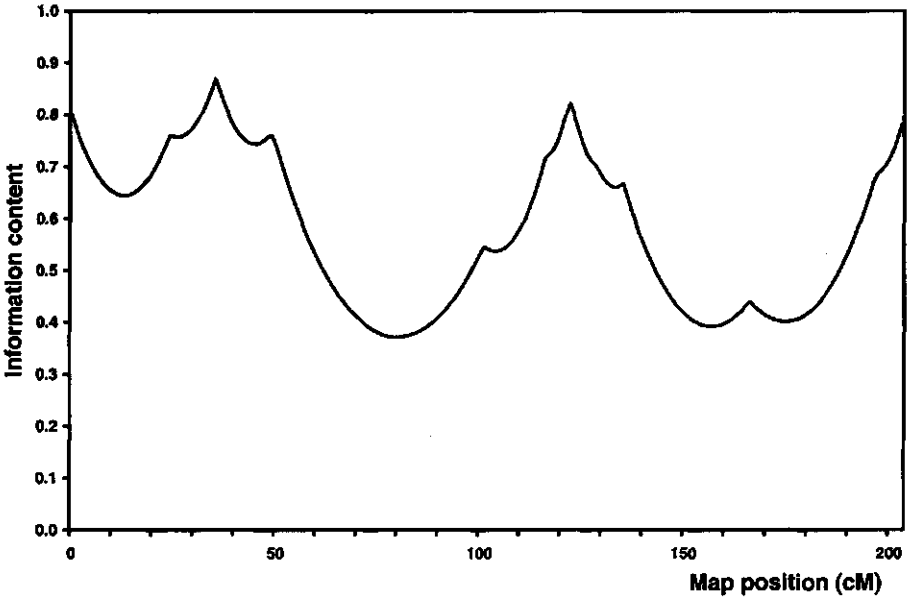


Figure 2. Information content on the Z-chromosome.

Feathering

Figure 3 shows the profile of the test statistic for feathering. In this analysis, the data of the MM, MF and FM groups are combined. Data from the FF group is excluded. The profile shows a clear peak at 18 cM, i.e. between markers ADL0022 (0 cM) and MCW0331 (24 cM). At the maximum the test statistic reaches a value of 29.9 and exceeds

the 5% genomewise significance threshold of 3.4. Subsequently, the FM, MF and MM groups were analysed separately. Regression coefficients for the 10 sires that were obtained in these analyses are shown in Table 3. The average regression coefficients are 0.25 for FM, 0.55 for MF and 0.29 for the MM group.

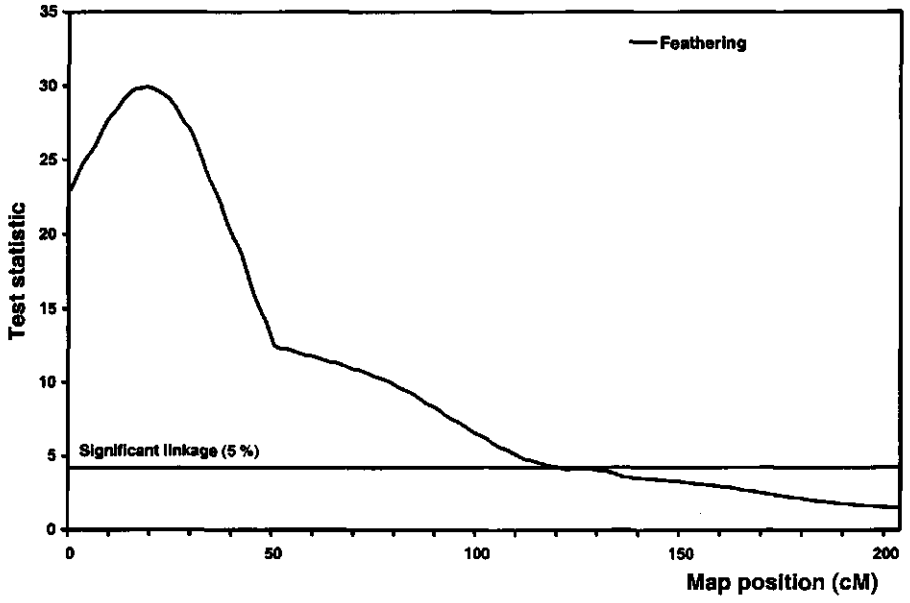


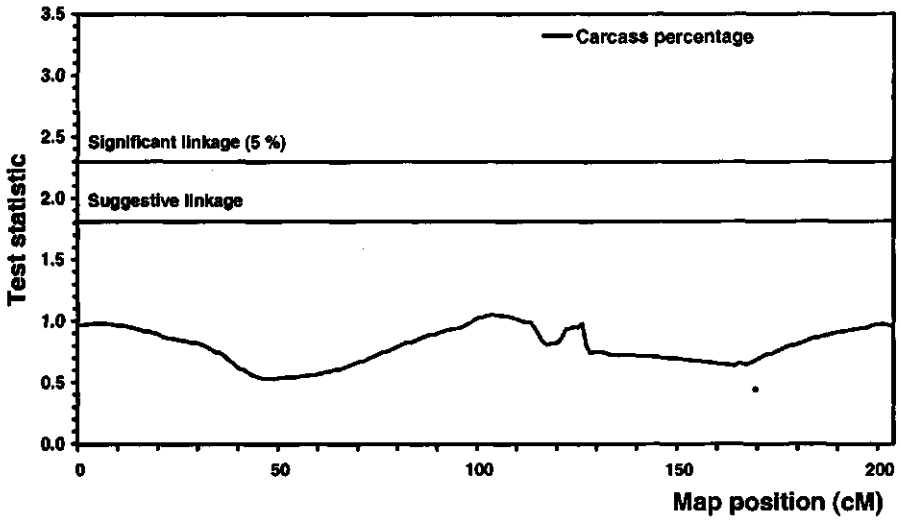
Figure 3. Test statistic profile resulting from the QTL analysis of the Z-chromosome for feathering including 5% genomewise significance threshold.

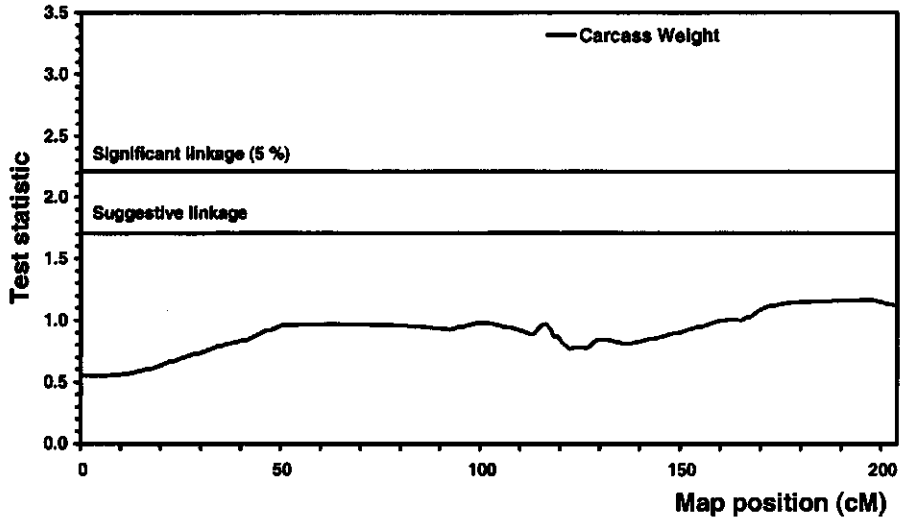
Growth traits

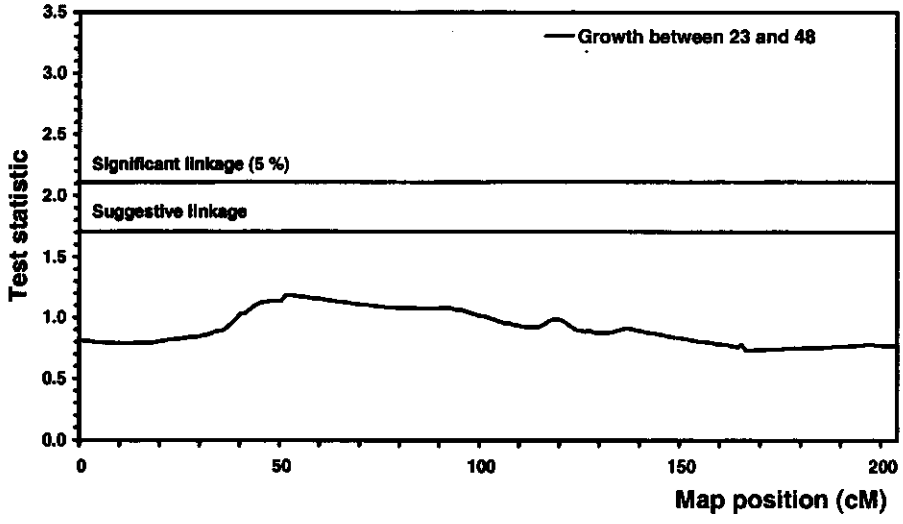
Figure 4 shows the results of the analyses that were performed for carcass percentage, carcass weight, growth between 23 and 48 days, body weight at 23 days and body weight at 48 days. Body weight at 48 days was measured both in the carcass experiment as well as in the feed efficiency experiment and was analysed separately. None of the growth traits exceeded the suggestive linkage threshold. Further analysis only revealed suggestive linkage for body weight at 48 days as measured in the feed efficiency experiment when including only G_2 male data (MM and MF groups) in the analysis (results not shown).

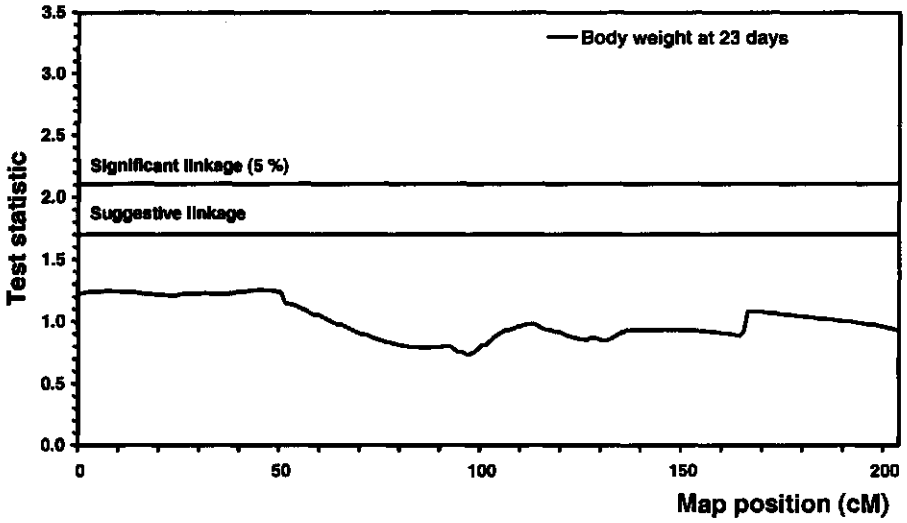
Table 3. Estimated regression coefficients from the half sib QTL analysis for the feathering gene evaluated at 18 cM on the Z-chromosome.

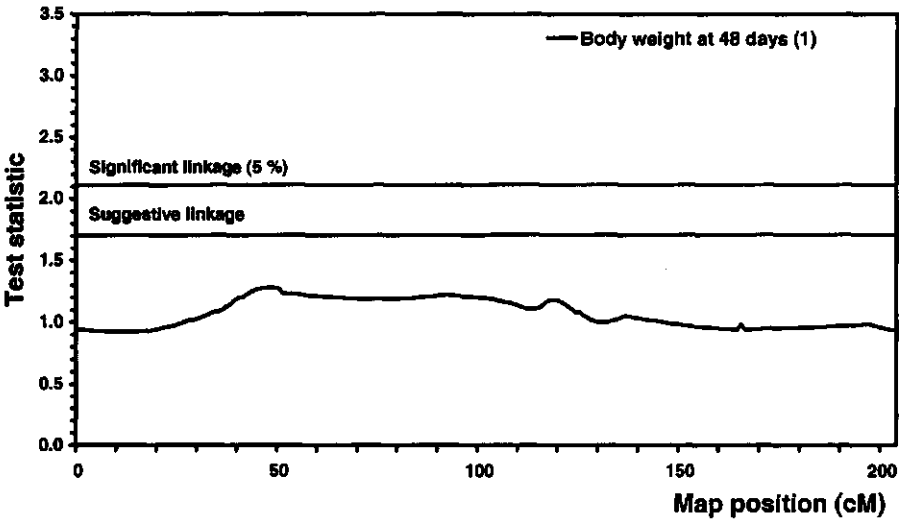
	FM	MF	MM
Family	QTL effect (std err)	QTL effect (std err)	QTL effect (std err)
1	0.31 (0.09)	0.54 (0.16)	0.32 (0.15)
2	0.26 (0.09)	0.94 (0.19)	0.19 (0.18)
4	0.20 (0.06)	0.08 (0.16)	0.20 (0.15)
5	0.20 (0.07)	0.41 (0.33)	0.38 (0.40)
6	0.33 (0.06)	0.46 (0.11)	0.25 (0.11)
7	0.29 (0.10)	0.53 (0.19)	0.20 (0.19)
9	0.07 (0.08)	0.20 (0.16)	0.23 (0.16)
11	0.32 (0.06)	1.14 (0.26)	0.77 (0.26)
12	0.34 (0.06)	0.58 (0.13)	0.18 (0.13)
13	0.15 (0.06)	0.59 (0.36)	0.13 (0.36)
Average	0.25 (0.07)	0.55 (0.21)	0.29 (0.21)




Carcass Weight
Significant linkage (5 %)
Suggestive linkage
Test statistic
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Map position (cM)


Growth between 23 and 48
Significant linkage (5 %)
Suggestive linkage
Test statistic
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Map position (cM)


Body weight at 23 days
Significant linkage (5 %)
Suggestive linkage
Test statistic
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Map position (cM)


Body weight at 48 days (1)
Significant linkage (5 %)
Suggestive linkage
Test statistic
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Map position (cM)

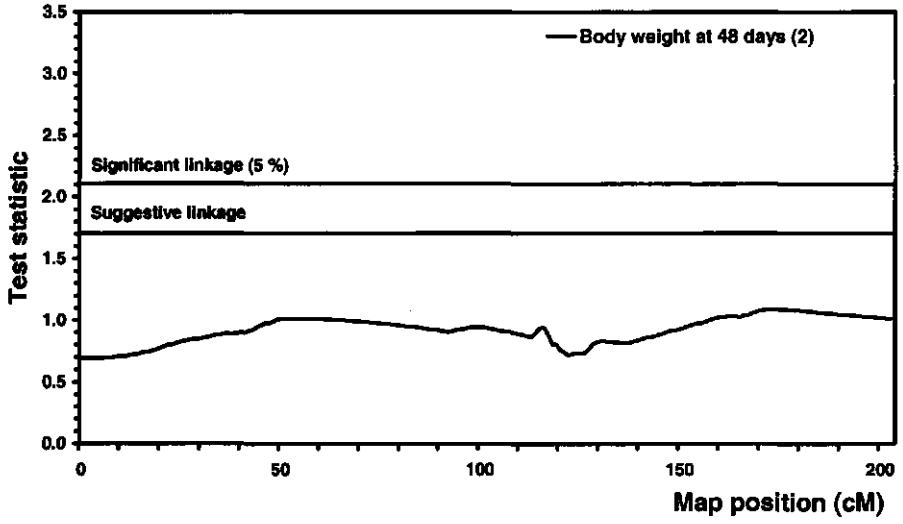


Figure 4. Test statistics profiles from the QTL analyses of the Z-chromosome for carcass percentage, carcass weight, growth between 23 and 48 days and body weight at 23 and 48 days. Genomewise 5% significant linkage thresholds and suggestive linkage thresholds are included. Body weight at 48 days (1) and (2) represents body weight recorded in the feed efficiency and the carcass experiment, respectively.

Discussion

Van Kaam *et al.* (1999[a,b]) estimated heritabilities, genetic correlations and phenotypic variances of the growth related traits. Bivariate analysis treating male and female growth as different traits resulted in genetic correlations between male and female growth traits for animals included in the present experiment. These correlations were close to 1.00 except for body weight at 48 days and carcass weight. For body weight at 48 days, the correlation was 0.97 in the feed efficiency experiment and 0.92 in the carcass experiment. The estimated genetic correlation between male and female carcass weight was 0.93. A low genetic correlation suggests that male and female body weight are genetically different traits which might be due to genes located on the sex chromosome. Genetic correlations found by Van Kaam *et al.* (1999[a,b]) in the present data were not as extreme as those reported by Tixier-Boichard *et al.* (1995) and Hagger (1994). This suggests that in

the analysed population the effect of possible QTLs located on the sex chromosome are relatively small and might be a reason why no sex-linked QTLs affecting growth traits have been detected in the population studied. However, large differences exist between male and female phenotypic variance: the phenotypic variance for body weight at 48 days in the carcass experiment is 60.7 for males and 38.4 for females (Van Kaam *et al.*, 1999[a]). Also for other traits considerable differences in male and female phenotypic variances were found (Van Kaam *et al.*, 1999[a,b]). These differences might be due to the interaction between genes located on the sex chromosome and genes on autosomal chromosomes. Effects due to interactions were not included in the present study.

In this study, no evidence was found for the presence of QTLs for growth traits on the Z-chromosome. This might be due to the statistical power of the experiment. For the experimental design used in this study, Van Kaam *et al.* (1998) reported a power of 99% for a QTL with an effect of $1.2\sigma_a$ (h^2=0.30, heterozygosity of the QTL is 50%, average informative bracket size is 20 cM, single bracket Type I error = 5%). However, this power applies to an autosomal QTL. For the Z-chromosome, no full sib analysis can be applied because the female G_1 individuals are not informative. Further, the FF group does not add any information about the presence of a QTL because the expected marker contrast is zero. It is expected that these two factors reduce the power for a QTL with an effect of $1.2\sigma_a$ from 99% to approximately 89%. For autosomal loci the expected contrast based on G_3 progeny is half an allele substitution effect: $0.5[a+(q-p)d]$, i.e. $0.5a$ when $d=0$. In the absence of dominance contrasts for a QTL located on the Z-chromosome are $0.5a_m$ for the MM group, a_m for the FM group and a_f for the MF group. If $a_m = a_f$ then contrasts for QTLs located on the Z-chromosome are larger than for QTLs located on autosomal chromosomes. This will increase the power of detecting QTLs on the Z-chromosome. Based on approximate power calculations, it is expected that QTLs located on the Z-chromosome with an effect greater than $0.7\sigma_a$ have a reasonable probability (power >70%) of being detected in this experiment.

Chambers *et al.* (1993) review influences of k and K alleles on traits related to growth, egg production and fitness as well as physiological measures. Chambers *et al.* (1993) found inconclusive results on effects of sex-linked feathering alleles on growth, but it was concluded that any comparison of k and K most probably will be confounded by the presence or absence of the *ev21* provirus as well as background genome. The proviral

ev21 locus is very closely linked to the k locus. In spite of the close linkage, evidence for recombination has been reported (Boulliou *et al.*, 1992). In our study, no evidence was found for the presence of a QTL affecting growth traits. From this, it can be concluded that the feathering locus (or the closely linked *ev21* provirus) probably does not have a large effect on growth.

For the present experiment, late feathering KK males from one line were mated to early feathering kW females from the other line (G_0 animals). As a result all G_1 males are heterozygous for the feathering locus (Kk) and G_1 females are all KW. G_1 animals are mated among each other to produce G_2 full sib families. Consequently, 50% of the G_2 females are late feathering (KW) and 50% are early feathering (kW). All G_2 males are late feathering but 50% is homozygous (KK) and the other 50% is heterozygous (Kk). Expected marker contrasts are derived for G_1 males. As in this set-up all G_1 males are heterozygous, we expect to find the contrast in each of the ten G_1 paternal sib families. Theoretical expected contrast for the MM groups is $0.5[a_m + (q_f - p_f)d]$, for the MF group it is a_f and for the FM group $[a_m + (q_m - p_m)d]$. When assuming that the frequency of the k allele is p and the frequency of the K allele is q and given that late feathering was assigned a trait value of 1 and early feathering a value of 2, $a_m = a_f = 0.5$ and $d = -0.5$. Expected contrast for MM now is $0.5p_f$, for MF, it is 0.5 and for the FM group, the contrast is p_m. Contrasts are based on G_3 individuals inheriting alternative paternal alleles and therefore p_f and p_m relate to frequencies of the k allele in G_2 individuals. G_2 males are Kk or KK and thus $p_m = 0.25$. Females are KW or kW, which makes $p_f = 0.25$. The theoretically expected contrast for the MM group therefore is $0.5p_f = 0.25$ which is close to the observed average regression coefficient of 0.29 (Table 3). For the MF group the expected contrast is 0.5 and the average observed contrast is 0.55 and for the FM group the expected marker contrast is $p_m = 0.25$ whereas the average observed contrast is also 0.25. This illustrates that theoretically expected and observed contrasts agree. At first sight, contrasts for the MM ($0.5p_f$) and for the FM group (p_m) seem to differ, however due to differences in allele frequencies between males and females expected contrasts turn out to be equal.

In the present study, the location of the feathering gene was estimated using a regression analysis approach. Alternatively, the location of the feathering locus could have been estimated based on a linkage analysis. In such an approach genotypes of G_2 individuals for feathering need to be inferred based on feathering scores of G_3 animals. The inferred genotypes were used in a CRI-MAP analysis (Green *et al.*, 1990). Analysis revealed significant linkage between the feathering locus and ADL0022 (lod-score = 19.00), MCW0331 (lod-score = 18.28), MCW0055 (lod-score = 11.19) and MCW0258 (lod-score = 6.59). The most likely location of the feathering locus was at 13 cM on the Kosambi mapping function (Kosambi, 1944), i.e. between markers ADL0022 and MCW0331. This shows that regression and CRI-MAP analysis point towards the same marker bracket. The most likely location within that bracket differs slightly between the two methods. This is partly due to a difference between the Haldane and Kosambi mapping function. The remaining 3 cM difference might be due to the fact, that inconsistent G_3 genotypes are ignored in the CRI-MAP analysis.

Chapter 6

Scaling to account for heterogeneous Variances in a Bayesian Analysis of Broiler Quantitative Trait Loci

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Submitted for publication

Abstract

A Bayesian method for QTL analysis, which is capable of accounting for heterogeneity of variance between sexes is introduced. The Bayesian method utilises a parsimonious model which includes scaling parameters for polygenic and QTL allelic effects per sex. Furthermore the method employs a reduced animal model in order to increase computational efficiency. Markov Chain Monte Carlo techniques were applied to obtain estimates of genetic parameters. In comparison with previous regression analyses, the Bayesian method (1) estimates dispersion parameters and polygenic effects and (2) utilises individual observations instead of offspring averages (3) estimates fixed effect levels and covariates and heterogeneity of variance between sexes simultaneously with other parameters, taking uncertainties fully into account. Broiler data collected in a feed efficiency and a carcass experiment was used to illustrate QTL analysis based on the Bayesian method. The experiments were conducted in a population consisting of 10 full sib families of a cross between two broiler lines. Microsatellite genotypes were determined on generation one and two animals and phenotypes were collected on third generation offspring from mating members from different families. Chromosomal regions, which appeared to contain a QTL in previous regression analyses and showed heterogeneity of variance, were chosen. Analysed traits in the feed efficiency experiment were body weight at 48 days and growth, feed intake and feed intake corrected for body weight all three between 23 and 48 days. In the carcass experiment, carcass percentage was analysed. The Bayesian method was successful in finding QTLs in all regions previously detected.

Introduction

In recent years, the availability of genetic markers for most farm animals has increased rapidly (Rohrer *et al.*, 1996; Groenen *et al.*, 2000). The usage of these markers makes it possible to reconstruct the transmission of chromosomal segments from parents to offspring. Several statistical methods have been developed for mapping quantitative trait loci (QTL) (e.g. regression, (restricted) maximum likelihood, Markov Chain Monte Carlo

(MCMC), for review see Bovenhuis *et al.*, 1997; Hoeschele *et al.*, 1997). The methods differ in their computational requirements, the underlying genetic model and/or the ability to handle different population structures. Computationally inexpensive methods like regression interval mapping are very suitable for initial genomewide analyses, providing results quickly. However, a standard regression analysis only considers the most likely haplotype configuration, requires pre-adjustment of data for environmental factors and heterogeneity of variance and does not take genetic relations for the polygenic effects into account. Furthermore, standard regression analysis is limited for usage in complex populations because only genotypes from two generations are utilised.

Bayesian analysis, facilitated by sampling from conditional parameter distributions via MCMC, is computationally expensive but can take fully account of the uncertainty associated with all the unknown parameters in the QTL analysis (Wang, 1998). When applied to an animal model including polygenic and QTL effects with relationship matrices, a Bayesian analysis is not limited to a specific pedigree structure and can accommodate partly missing marker genotypes (Bink and Van Arendonk, 1999).

In previous studies Van Kaam *et al.* (1998, 1999[a], 1999[b]) performed whole genome scans and identified QTL affecting growth, feed efficiency and carcass traits in broilers using regression interval mapping. This approach required pre-adjustment of offspring observations for fixed effects, heterogeneity of variance between sexes and parental mate contributions. In a pre-adjustment for heterogeneity of variance, it is not possible to distinguish polygenic, QTL and environmental variance. The aim of the present study was to develop a method that simultaneously handles fixed effects, heterogeneity of variance between sexes and polygenic and QTL effects while accounting for uncertainties. This method is applied to chromosomal regions where QTLs were previously found using regression analysis. It is expected that the current method will give a better representation of reality and results in estimates of QTL variance and position that are closer to their true values.

Material and Methods

Bayesian animal model

Fernando and Grossman (1989) extended the animal model by including normally distributed QTL effects in addition to a polygenic effect. In a Bayesian setting their model can be represented as:

$$\mathbf{y} \mid \mathbf{b}, \mathbf{u}, \mathbf{v} \sim N\left(\mathbf{Xb} + \mathbf{Zu} + \mathbf{Wv}, \mathbf{I}\sigma_e^2\right)$$

with

$$\mathbf{b}' = \begin{bmatrix} b_1 & b_2 & \cdots & b_p \end{bmatrix} \quad b_j \sim U\left[b_{min}, b_{max}\right]$$

$$\begin{bmatrix} \mathbf{u} \\ \mathbf{v} \end{bmatrix} \sim N\left(0, \begin{bmatrix} \mathbf{A}\sigma_u^2 & 0 \\ 0 & \mathbf{G}_k\sigma_v^2 \end{bmatrix}\right)$$

where $\mathbf{y}$ is a n-vector of phenotypes, $\mathbf{X}$ is a $n \times p$ incidence matrix relating fixed effect levels and covariates to phenotypes, $\mathbf{b}$ is a p-vector of fixed effect levels and covariates, $\mathbf{Z}$ is a $n \times q$ incidence matrix relating individuals to phenotypes, $\mathbf{u}$ is a q-vector of random additive polygenic effects, $\mathbf{W}$ is a $n \times 2q$ incidence matrix relating QTL alleles to phenotypes, $\mathbf{v}$ is a $2q$-vector of random additive QTL allelic effects, $\mathbf{A}$ is the additive genetic relationship matrix, σ_u^2 is the polygenic variance excluding the QTL, $\mathbf{G}_k$ is the gametic relationship matrix for the QTL and depends on the QTL position k and the marker information, and σ_v^2 is the additive variance of the QTL allelic effects. The same error variance, σ_e^2, is applied for all observations, hence error terms are assumed to be uncorrelated with homogeneous variance.

In the present experiment, heterogeneity of variance between sexes occurs e.g. body weight related traits (Van Kaam *et al.*, 1998). Van Kaam *et al.* (1999[a], 1999[b]) previously reported additive genetic correlations between sexes ranging from 0.87 to 1. Therefore, we assume that the same genes are responsible for these traits in both sexes and we postulate that the genetic part of the heterogeneity is due to differences in the magnitude of allelic effects in both sexes. Hence, heterogeneity can be either due to different polygenic effects, QTL effects and/or otherwise fixed or random environmental effects.

For the genetic effects, heterogeneity is modelled with the introduction of scale parameters (Quaas *et al.*, 1989). Separate scale parameters per sex are used for polygenic and QTL allelic effects. Furthermore, separate fixed effects and error variances are modelled per sex. This leads to the scaled model:

$$\mathbf{y}_s \mid \mathbf{b}_s, \mathbf{u}, \mathbf{v} \sim N\left(\mathbf{X}_s\mathbf{b}_s + c_s\mathbf{Z}_s\mathbf{u} + d_s\mathbf{W}_s\mathbf{v}, \mathbf{I}\sigma^2_{e_s}\right) \text{ for } s = m, f$$

with

$$b_{s_j} \sim U[b_{min}, b_{max}]$$

$$\begin{bmatrix}\mathbf{u}\\ \mathbf{v}\end{bmatrix} \sim N\left(0, \begin{bmatrix}\mathbf{A} & 0\\ 0 & \mathbf{G}_k\end{bmatrix}\right)$$

where c_s and d_s represent scale parameters for the polygenic respectively QTL allelic effects and subscript s indicates sex: male (m) or female (f). In the scale model the variances of the random genetic effects are fixed, because otherwise the scale parameters and these variance components would both be measuring the same dispersion and not both be identifiable. The solution, taken here, is to fix σ^2_u and σ^2_v to one and hence $\mathbf{u}$ and $\mathbf{v}$ have a standard normal distribution. Rather than a single polygenic variance σ^2_u as in the homoskedastic case, we now have c^2_m and c^2_f depending on the sex in which genes are expressed. Likewise we have d^2_m and d^2_f for variances of QTL allelic effects. The total additive genetic variance equals the polygenic variance and twice the QTL allelic variance. A scale parameter can be interpreted as a standard deviation but in the model equation, it is a regression coefficient. Regression coefficients typically have normal conjugate priors like other mean effects. Here a left-truncated normal prior is used to assure non-negativity for the scale parameters:

$$c_s \sim TN\left(\mu_{c_s}, \sigma^2_{c_s}\right) \text{ with } c_s \geq 0$$

$$d_s \sim TN\left(\mu_{d_s}, \sigma^2_{d_s}\right) \text{ with } d_s \geq 0$$

New candidate values are sampled using a normal distributed candidate generating density.

In the present case, uncorrelated error terms with homogeneous variance within sex are assumed. Inverted gamma distributions with pre-defined hyperparameters α and λ are used to represent prior knowledge on these error variances as $\sigma^2_{e_s} \sim IG(\alpha, \lambda_s)$.

MCMC algorithm

The solutions of the model are obtained using MCMC techniques, which enable sampling from the posterior distribution of parameters. A reduced animal model (RAM) was used to obtain solutions more efficiently because polygenic effects for non-parents and QTL allelic effects for ungenotyped non-parents do not have to be sampled (Bink *et al.*, 1998[a], Cantet and Smith, 1991). In QTL mapping, only the genetic effects of parents are of direct interest, because it is their allelic segregation, which is providing information on the presence of a QTL. In Appendix 1, the full conditional distributions of the fixed and random effects and the dispersion parameters in the scaled RAM are presented. Fixed effect levels and covariates, random polygenic and QTL allelic effects and haplotypes are sampled using Gibbs sampling. With a RAM residuals of non-parents consist of an error term and the Mendelian parts of the additive genetic variance depending on the RAM category, therefore the conditional distributions of the dispersion parameters do not have a simple form to facilitate Gibbs sampling. Hence, Metropolis-Hastings is used to sample scale parameters and error variances. The likelihood of the RAM, which is evaluated in the Metropolis-Hastings algorithm, is as follows:

$$\prod_{i=1}^{4}\prod_{s=M,F}\left[\tau_{is}^{-0.5n_{is}}\times\exp\left\{-0.5\frac{\sum_{j=1}^{n_{sj}}e_{isj}^{2}}{\tau_{is}}\right\}\right]$$

where $e_{isj}=y_{isj}-x'_{isj}b-c_s z'_{isj}u-d_s w'_{isj}v$ is the error term for the observed animal j with sex s in the RAM category i with residual variance $\tau_{is}=\sigma^2_{e_s}+\omega_i\left(c_s^2+2d_s^2\right)$ and ω_i reflects the total amount of additive genetic variance present in τ_{is}. There is one RAM category for parents $(\omega_1=0)$ and three for non-parents: both parents known $(\omega_2=0.5)$, one parent known $(\omega_3=0.75)$ and both parents unknown $(\omega_4=1.0)$. For parents, there are "ones" in z'_{isj} and w'_{isj} corresponding to the individuals' own genetic effects and for non-parents these are "halves" corresponding to the genetic effects of the identified parent(s) and "zeros" corresponding to unidentified parent(s). Note that if parents have no phenotypic observations the model reduces to a sire-dam model. The vectors **u** and **v** only

contain parental genetic effects and the relationship matrices in their priors only contain parental contributions, hence $\mathbf{u} \sim N(0, \mathbf{A}_P)$ and $\mathbf{v}| \, m,k \sim N(0, \mathbf{G}_{kP})$.

The QTL position k is modelled by including information from flanking markers m in the computation of the inverse of the gametic relationship matrix $\mathbf{G}_{kP}^{-1}$. Marker information is described in terms of the allelic constitution of the chromosomal homologues of the founders and identity by descent values for all non-founders (Jansen *et al.*, 1998; Bink and Van Arendonk, 1999).

Experimental population

A three generation population was created for the purpose of QTL detection, following recommendations of Van der Beek *et al.* (1995). Founder animals, parents, offspring and grandoffspring are indicated as generation 0, 1, 2 and 3 animals or G_0, G_1, G_2 and G_3 animals, respectively. In the three generation design, G_1 and G_2 animals were typed for genetic markers and phenotypic observations were collected on different hatches of G_3 animals distributed over a feed efficiency and a carcass experiment.

Table 1. Population structure with numbers of animals used in the analyses and types of observations collected.[a]

Generation[b]	Males	Females	Total	Observations[d]
G_0[c]	14	14	28	———
G_1	10	10	20	Genotypes
G_2	172	279	451	Genotypes
G_3	1,012	1,037	2,049	Phenotypes BW48, FIFA, FIFW, GAIN
G_3	969	984	1,953	Phenotypes CP

[a] Numbers exclude outliers and missing values.

[b] G_0 etc. = Generation 0 etc.

[c] Male and female G_0 animals are from different lines, G_0 animals were not included in the analyses because marker genotypes were unknown.

[d] BW48 = body weight at 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval; GAIN = growth between 23 and 48 days; CP = carcass percentage.

The number of animals and the population structure are presented in Table 1. Two genetically different outcross broiler dam lines from the White Plymouth Rock breed were

chosen as the founders of the experimental population. In one line, 14 males and in the other line 14 females were chosen and 14 G_0 couples were created. These 14 couples together produced 10 G_1 males and 10 G_1 females. From these 20 G_1 animals, 10 couples were created, which on average produced 45.1 G_2 full sibs. G_2 animals were mated with several G_2 animals from different families to produce nine G_3 animals on average. For more details see Van Kaam *et al.* (1998; 1999[a]; 1999[b]). In the analyses G_1, G_2, and G_3 animals were included and G_0 animals were omitted, because the Bayesian method requires known marker genotypes for base animals.

Traits

Traits from the feed efficiency experiment analysed in this study were body weight at 48 days (BW48) and growth (GAIN), feed intake (FIFA) and feed intake adjusted for body weight (FIFW) all three measured between 23 and 48 days. In total 2,049 animals with phenotypic observations were included in the analysis (Van Kaam *et al.*, 1999[b]).
The only trait from the carcass experiment analysed in this paper was carcass percentage (CP). In total 1,953 animals with observations on CP were analysed (Van Kaam *et al.*, 1999[a]).

Fixed effects for BW48, GAIN, FIFA and FIFW were the location of the animal's cage within the building and an interaction between the hatch of the dam and the hatch of the offspring. For FIFW, BW23 and BW48 were used as covariates. For CP, an interaction between hatch of the dam, hatch of the offspring and the day of measuring carcass weight was included as fixed effect. Because carcass weight was measured on 2 days in one hatch, dehydration might have an influence on the measurement within hatch, and therefore the day of measuring was included in the interaction term.

Marker data

Genotypes for microsatellite markers were determined using DNA derived from blood samples from all 20 G_1 and 451 G_2 animals. Marker alleles were recorded in basepair units. Marker data used in this analysis is a subset of the marker data used for creating the linkage map (Groenen *et al.*, 1998). Only seven chromosomal regions, which showed heterogeneity of variance between sexes and suggestive significance for the presence of a QTL in previous analyses (Van Kaam *et al.*, 1999[a]; 1999[b]) were selected for further

analysis. Marker alleles were determined in all 10 families for most of the markers in these regions. Genotypes for some markers however were only collected in 4 families. More details on the regions analysed are given in Table 2. A minimum marker spacing of about 2 cM was aimed at except for the most lateral markers, which were used to increase informativity at the ends of the map. On all analysed regions, all 20 parents were informative, except on linkage group WAU26 were 4 parents were uninformative. The genotypes of two markers with the same location, MCW0023 and ADL0183, are combined. All analysed marker brackets are indicated in Table 3.

Table 2. Chromosomal regions analysed per trait. Indicated per chromosomal region are the trait, the chromosome or linkage group, the names of the markers flanking the analysed region, the length of the region in Haldane scale between the left and the right marker, and the number of markers in this region.

Trait[a]	Chromosome	Left marker	Right marker	Length of region in cM	Number of markers
GAIN	1	ADL0150	ADL0314	96.2	11
BW48	1	ADL0150	ADL0314	96.2	11
FIFA	1	ADL0150	ADL0314	96.2	11
FIFA	4	ADL0288	MCW0284	91.7	11
FIFA	WAU26	ADL0289	MCW0165	22.8	3
FIFW	2	ADL0228	MCW0247	78.2	13
CP	1	LEI0169	ADL0328	88.5	13

[a] BW48 = body weight at 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval; GAIN = growth between 23 and 48 days; CP = carcass percentage.

MCMC and prior distribution settings

For all chromosomal regions of interest, several independent QTL analyses were each based on a single chain of 2,000,000 cycles after 1,000 cycles burn-in time. A single run required 4 hours on a 450 Mhz Pentium II. In each analysis, the QTL position was fixed in the middle of a marker bracket. Parameters, which are known to converge more slowly, were sampled more often than other parameters as was suggested by Uimari *et al.* (1996). Dispersion parameters were sampled in each cycle, fixed effects, polygenic effects and QTL allelic effects were sampled in every 5th cycle and haplotypes were sampled every 50th cycle, because they are very time demanding to sample.

Table 3. Marker brackets analysed per trait. Indicated per chromosomal region are the trait, the chromosome or linkage group and the names of the markers flanking the marker brackets.

Trait[a]	Chromosome	Flanking markers of analysed marker brackets
GAIN	1	LEI0174-UMA1.107-MCW0058-LEI0071-MCW0101-LEI0101
BW48	1	LEI0174-UMA1.107-MCW0058-LEI0071-MCW0101-LEI101
FIFA	1	LEI0174-UMA1.107-MCW0058-LEI0071-MCW0101-LEI0101
FIFA	4	ADL0246-ADL1094-MCW0085-LEI0122-ADL0266-LEI0144
FIFA	WAU26	ADL0289-ADL0262-MCW0165
FIFW	2	ADL0343-MCW0082-MCW0341-MCW0071-ADL0270-MCW0184
CP	1	LEI0106-MCW0023[b]-LEI0079-MCW0177-MCW0255-LEI0168

[a] BW48 = body weight at 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval; GAIN = growth between 23 and 48 days; CP = carcass percentage.

[b] Information of MCW0023 and ADL0183 was combined.

Priors for dispersion parameters were chosen assuming that: (1) The residual variance is 40% of the observed variance without adjustment for fixed effects, resulting in λ, (2) The heritability is 0.3, (3) The expected variance explained by the putative QTL is 20% of the additive genetic variance with the mode of the QTL scale parameters at zero, hence $\mu_{d_s} = 0$, (4) The variance on the polygenic scale parameter, $\sigma^2_{c_s}$, is 0.09 × the expected polygenic variance, and (5) There is no heterogeneity of variance between sexes, i.e. the same priors were used for males and females. Using the first two assumptions, the additive genetic variance can be calculated. With the third assumption, the additive genetic variance can be divided over the polygenic and QTL variance. Then $\sigma^2_{d_s}$ follows from the expected QTL variance and μ_{c_s} is obtained from a small simulation. The α hyperparameter of the inverted gamma prior for the error variances was 2.000001 in all cases. The settings for the prior distributions for the scale parameters as well as the λ hyperparameter of the inverted gamma prior for the error variances are shown in Table 4.

Table 4. Values of the left-truncated normal priors for scale parameters and inverted gamma residual priors for the error variances. Indicated per trait are the prior values for left-truncated normal priors of the polygenic scale parameters and the QTL allelic scale parameters and inverted gamma priors of the error variances. The α hyperparameter of the inverted gamma prior was 2.000001 in all cases. For both sexes, the same prior values were used.

Model including QTL					
Trait[a]	μ_c	σ_c^2	μ_d	σ_d^2	λ
GAIN	94	876	0	1,217	28,400
BW48	113	1,271	0	1,766	41,200
FIFA	165	2,703	0	3,754	87,600
FIFW	79	617	0	857	20,000
CP	0.64	0.04	0.00	0.06	1.3
Model without QTL					
Trait[a]	μ_c	σ_c^2	μ_d	σ_d^2	λ
GAIN	105	1,093	0	0	28,400
BW48	127	1,606	0	0	41,200
FIFA	185	3,398	0	0	87,600
FIFW	88	766	0	0	20,000
CP	0.72	0.05	0.00	0.00	1.3

[a] BW48 = body weight at 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval; GAIN = growth between 23 and 48 days; CP = carcass percentage.

Results

Heterogeneity of variance between sexes

Table 5 shows the posterior means for the estimated heritabilities including the QTL, the QTL proportion of the total genetic variance and the phenotypic variance. Results are shown for the most likely marker bracket containing a QTL, i.e. the marker bracket with the largest QTL effect. Differences in phenotypic variances between males and females were found for all traits. For most of the traits, the phenotypic variance in males is larger than in females except for carcass percentage where female phenotypic variance is larger.

The heterogeneity of variance is most pronounced for GAIN where male phenotypic variance is 1.5 times the female phenotypic variance. The estimated male and female heritabilities are for most traits in the same order suggesting that this heterogeneity is to the same extent due to differences in environmental as well as additive genetic variances. For FIFA, additive genetic variances are similar in males and females.

The polygenic variance, which can be derived from Table 5, shows heterogeneity most clearly for GAIN, FIFW and CP. The QTL variance, which also follows from Table 5, shows heterogeneity for the QTL in the region MCW0058-MCW0101 affecting GAIN, BW48 and FIFA and for the QTL in the interval ADL0343-MCW0082 affecting FIFW. The QTLs affecting FIFA in the intervals ADL0194-MCW0085 and ADL0262-MCW0165 and CP in the interval LEI0079-MCW0177 appear to have a similar effect on both sexes.

Presence of QTL

The QTL analyses show evidence for the presence of QTL in each of the nine regions where a QTL was found in the previous regression analyses. A QTL is assumed present if a value of zero is not in the 95% Highest Posterior Density (HPD95) region for the QTL scale parameter i.e. the QTL variance differs significantly from zero. In eight out of 32 marker brackets a QTL was found that affected observations in only one sex and in 18 marker brackets both sexes were affected. The most likely marker bracket which was reported by Van Kaam *et al.* (1998, 1999[a], 1999[b]) always contained a significant QTL except for GAIN expressed in males and BW48 expressed in females. In several cases, a QTL seemed present in one or two of the flanking marker brackets.

Scale parameters

In Figure 1, an example of the marginal posterior densities of male QTL scale parameters is given. These densities are the result of the analysis of BW48. In Figure 2, the marginal posterior densities of female QTL scale parameters obtained in the same analyses are given. The pattern of the densities shows that the closer to marker bracket LEI0071-MCW0101 the further the densities shift away from zero. This provides clear evidence that the most likely marker bracket for the location of a QTL is the bracket LEI0071-MCW0101. The pattern for this trait is similar for the male and female QTL

scale parameters. The densities of the male QTL scale parameters however are further away from zero and hence the QTL effect tends to be larger in males than in females.

Table 5. Posterior means of the heritability, proportion QTL variance of the total genetic variance and phenotypic variances in the most likely marker bracket using a model with a QTL and with a model without QTL. Indicated per trait are two analyses one showing the most likely marker bracket with a model containing a QTL and one with a model without a QTL. For each analysis, the polygenic, QTL and error variances in males respectively females are shown.

Trait[a]	Interval	$h_m^{2\,b}$	h_f^2	$\gamma_m{}^c$	γ_f	$\sigma^2_{p_m}$	$\sigma^2_{p_f}$
GAIN	LEI0071-MCW0101	0.25	0.23	0.46	0.21	42,738	27,960
GAIN	No QTL	0.21	0.21	0.00	0.00	42,388	27,729
BW48	LEI0071-MCW0101	0.30	0.28	0.26	0.14	59,734	42,318
BW48	No QTL	0.27	0.27	0.00	0.00	59,266	42,087
FIFA	MCW0058-LEI0071	0.30	0.33	0.40	0.13	144,050	114,879
FIFA	ADL0194-MCW0085	0.27	0.34	0.21	0.21	143,706	114,871
FIFA	ADL0262-MCW0165	0.27	0.36	0.23	0.21	144,087	115,813
FIFA	No QTL	0.25	0.32	0.00	0.00	142,789	114,078
FIFW	ADL0343-MCW0082	0.40	0.39	0.33	0.26	41,377	28,517
FIFW	No QTL	0.36	0.36	0.00	0.00	40,795	28,173
CP	LEI0079-MCW0177	0.34	0.37	0.16	0.26	2.01	2.46
CP	No QTL	0.31	0.36	0.00	0.00	1.98	2.46

[a] BW48 = body weight at 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval; GAIN = growth between 23 and 48 days; CP = carcass percentage.

[b] Male and female heritabilities are calculated as $h_m^2 = \left(c_m^2 + 2d_m^2\right) / \left(c_m^2 + 2d_m^2 + \sigma_{e_m}^2\right)$ and $h_f^2 = \left(c_f^2 + 2d_f^2\right) / \left(c_f^2 + 2d_f^2 + \sigma_{e_f}^2\right)$.

[c] Male and female proportions QTL variance of the total genetic variance are calculated as $\gamma_m = 2d_m^2 / \left(c_m^2 + 2d_m^2\right)$ and $\gamma_f = 2d_f^2 / \left(c_f^2 + 2d_f^2\right)$.

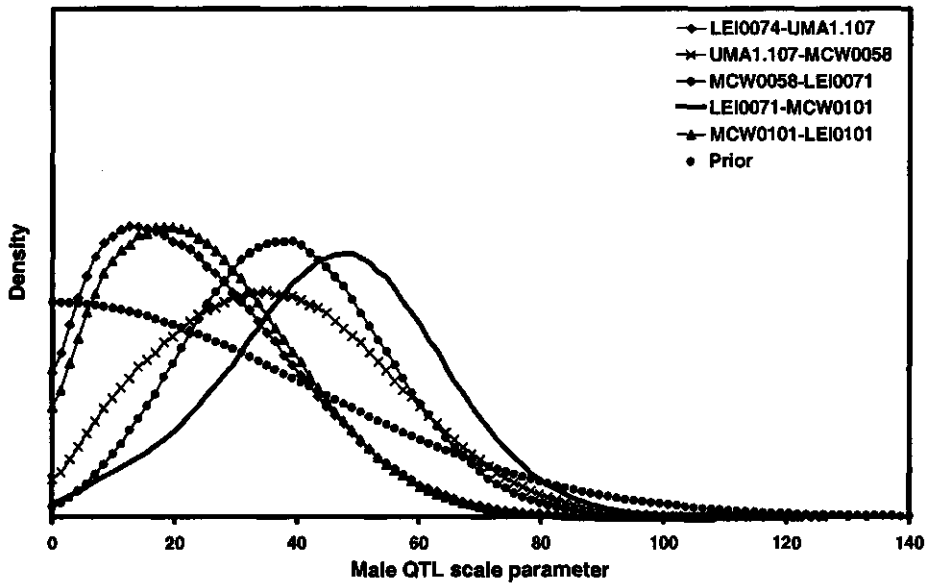

Figure 1. Marginal posterior densities of the male QTL scale parameters obtained in five analyses of BW48 in consecutive marker brackets on chromosome 1.

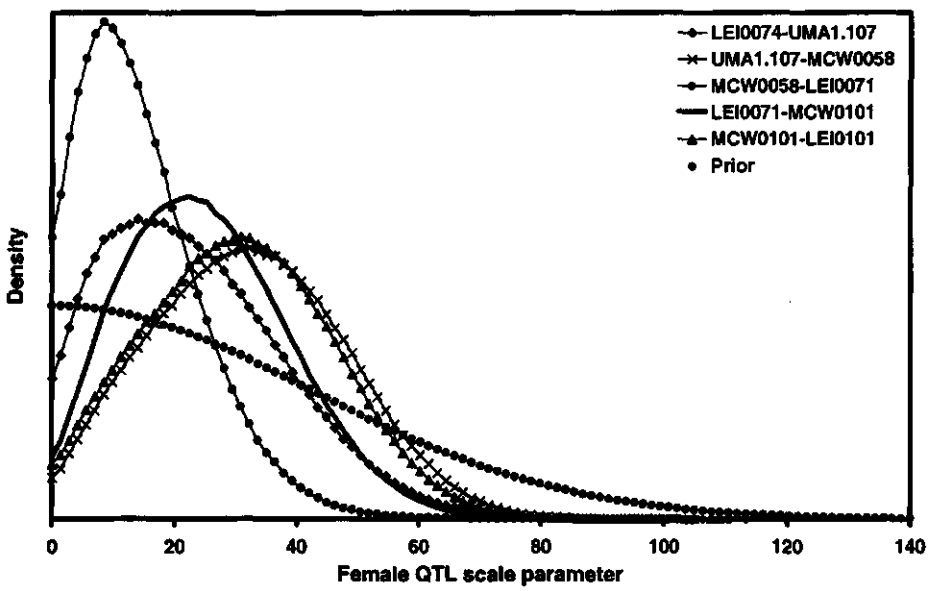

Figure 2. Marginal posterior densities of the female QTL scale parameters obtained in five analyses of BW48 in consecutive marker brackets on chromosome 1.

Influence of priors

In order to obtain an idea of the influence of the settings of the prior for the QTL scale parameters an additional analysis is done using different settings. In these settings, a QTL explaining 10% instead of 20% of the additive genetic variance is assumed. These settings were $TN(120,1434)$ for the polygenic scale parameters and $TN(0,833)$ for the QTL scale parameters. A comparison of the densities of the male QTL scale parameter is shown in Figure 3, which gives the prior distributions reflecting a proportion QTL variance of 10% and 20% and the posterior distributions obtained using these two priors. With a prior of 10% the QTL variance diminished with 31% compared to a prior of 20%. The proportion of the additive genetic variance explained by the QTL diminished from 26% to 19%.

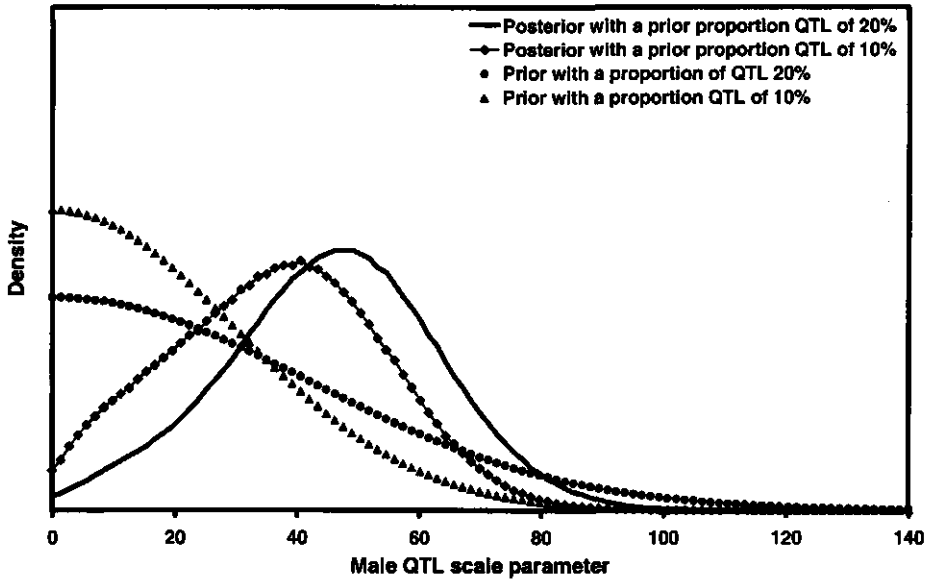

Figure 3. Marginal posterior densities of the male QTL scale parameters of BW48 obtained in three analyses in marker bracket LEI0071-MCW0101 on chromosome 1 using different settings for the priors of the scale parameters.

Discussion

Method of analysis

Advantages of the Bayesian method as compared to regression analysis are that: (1) All parameters except recombination rates are estimated simultaneously taking uncertainty into account. (2) An animal model, which included fixed and polygenic effects and polygenic and gametic relationships matrices, is used. (3) Heterogeneity of variances between sexes is accounted for simultaneously by scaling, and (4) Dispersion parameters are estimated for all random terms in the model. Because fixed effect levels and covariates and heterogeneity of variance can be handled by the Bayesian method, individual observations instead of offspring averages can be used. A polygenic component is part of the model instead of the family effect as in the regression analysis. For the analysis with the regression interval mapping procedure, approximations were needed in the adjustment for contributions of the parental mates to phenotypes. The usage of an animal model in a Bayesian analysis offers the opportunity to exploit all relationships through relationship matrices, which abandons the need for this adjustment. The advantage of accounting for heterogeneity by scaling is that it hardly increases the computational needs (Quaas *et al.*, 1989) because the number of parameters increases only by using fixed effects per sex and by adding two dispersion parameters for genetic effects and one for the error variance.

The current Bayesian method requires marker genotypes for all base parents. In the experimental population, no genotypes were collected on G_0 animals and therefore this generation was excluded from the analyses.

Scaled model

Biologically there is just one genetic constitution per animal and one genetic variation in a population, only the expression of the genes in both sexes differs. The scaled model is similar by assuming one genetic variation, one polygenic effect per animal and one effect per allele and scaling the gene effects with respect to the sex of the animal in which the gene is expressed. The scaled model is similar to a normal bivariate model in which the genetic correlation between sexes is restricted to one. The scaled model however has the advantage that only one polygenic and two QTL allelic effects per animal are required. A

bivariate approach would require two polygenic and four QTL allelic effects per animal. Hence, the scaled model is more parsimonious than a bivariate model, which improves estimability and reduces computational requirements. A disadvantage, however, is that the scaled model can only handle correlations of one, whereas a bivariate approach would allow any correlation. Assuming that the same genes influence a trait in both sexes it seems justified having genetic correlations of unity if the direction of the effect is the same in both sexes. Especially for a QTL which is assumed to be a single gene the correlation between the effects in both sexes should be one. Hence using one polygenic and two QTL allelic effects is sensible.

Ignoring heterogeneity of variance between sexes by assuming homogeneous variances would result in a more emphasis on the variance in males and less in females. In the present analysis, the possibility of opposite genetic effects between sexes was omitted. The scaled model however can accommodate this possibility by using normally distributed priors for the scale parameters instead of left-truncated normals.

The scale parameters are expressed relative to the fixed variance of $\mathbf{u}$ and $\mathbf{v}$. This was done because it is not practical to express the effect in one sex as a ratio of the effect in the other sex, such a ratio would lead to problems in case the effect in the sex, which is in the denominator of the ratio, would be zero.

Estimates

In comparison with our previously published values (Van Kaam *et al.*, 1999[a]; 1999[b]) total phenotypic variances agree closely with maximum differences of 6%. Heritabilities however are different from those obtained previously. In the current study, heritabilities in males and females are more similar than in previous results. The main difference between both analyses is the absence of a maternal genetic effect in the current model, whereas the previous study did not contain a QTL in the model. The heritabilities for CP (0.31 and 0.36) were substantially lower compared with our previous results (0.43 and 0.52). This can be caused by the prior assumption for heritability of 30%. It is also possible that the previous maximum likelihood estimates were not in the global maximum. Estimated QTL variances are between 13% and 46% of the total genetic variance, which seems quite large. An analysis of the influence of the priors shows that the settings chosen for the priors of the scale parameters have a substantial influence on the amount of genetic variance assigned to the QTL. Furthermore, large QTL variances can possibly be caused

by using a normal distribution for QTL allelic effects instead of having just one fixed effect per allele.

Acknowledgements

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APPENDIX 1

Full conditional distributions for the scaled reduced animal model

In scalar notation the distribution of the observations in the scaled RAM is as follows:

$$y_{isj} \mid \mathbf{b},\mathbf{u},\mathbf{v},c_s,d_s,\sigma^2_{e_s} \sim N\left(x'_{isj}\, b + c_s z'_{isj} u + d_s w'_{isj} v, \tau_{is}\right)$$

and in vector notation the distribution is:

$$\mathbf{y} \mid \mathbf{b},\mathbf{u},\mathbf{v},\mathbf{c},\mathbf{d},\sigma^2_{e_s} \sim N\left(\mathbf{Xb} + \tilde{\mathbf{Z}}\mathbf{u} + \tilde{\mathbf{W}}\mathbf{v}, \mathbf{T}\right)$$

where $\tilde{\mathbf{Z}}$ and $\tilde{\mathbf{W}}$ are the matrices formed by concatenation of $c_s z'_{isj}$ and $d_s w'_{isj}$, respectively.

For example, depending on an animal's sex the nonzero elements of an animal's row of $\tilde{\mathbf{W}}$ will be d_m or d_f for parents and $0.5d_m$ or $0.5d_f$ for non-parents. Finally, $\mathbf{T}$ is a diagonal matrix of the residual variances (including Mendelian sampling terms) corresponding the observations i.e. $\tau_{ij} = \sigma^2_{e_s} + \omega_i\left(c_s^2 + 2d_s^2\right)$ for y_{isj}.

The following notations will be used: $[\mathbf{M}]_i$ denotes the i^{th} column of matrix $\mathbf{M}$, $[\mathbf{M}]_{-i}$ denotes matrix $\mathbf{M}$ with the i^{th} column deleted and $\mathbf{m}_{-i}$ denotes vector $\mathbf{m}$ with the i^{th} element deleted.

The full conditional distribution of the fixed effects is as follows:

$$b_i \mid \mathbf{b}_{-i},\mathbf{u},\mathbf{v},\mathbf{y},\mathbf{c},\mathbf{d},\mathbf{T} \sim$$

$$N\left(\frac{\left(\left[\tilde{\mathbf{X}}\right]'\mathbf{T}^{-1}\left(\mathbf{y}-\left[\tilde{\mathbf{X}}\right]_{-i}\mathbf{b}_{-i}-\tilde{\mathbf{Z}}\mathbf{u}-\tilde{\mathbf{W}}\mathbf{v}\right)\right)}{\left(\left[\tilde{\mathbf{X}}\right]'\mathbf{T}^{-1}\left[\tilde{\mathbf{X}}\right]\right)},\left(\left[\tilde{\mathbf{X}}\right]'\mathbf{T}^{-1}\left[\tilde{\mathbf{X}}\right]\right)^{-1}\right)$$

The full conditional distribution of the polygenic effects is as follows:

$$u_i \mid \mathbf{b},\mathbf{u}_{-i},\mathbf{v},\mathbf{y},\mathbf{c},\mathbf{d},\mathbf{T} \sim$$

$$N\left(\frac{\left(\left[\tilde{\mathbf{Z}}\right]'\mathbf{T}^{-1}\left(\mathbf{y}-\tilde{\mathbf{X}}\mathbf{b}-\left[\tilde{\mathbf{Z}}\right]_{-i}\mathbf{u}_{-i}-\tilde{\mathbf{W}}\mathbf{v}\right)-\sum_{j\neq i}a^{ij}u_j\right)}{\left(\left[\tilde{\mathbf{Z}}\right]'\mathbf{T}^{-1}\left[\tilde{\mathbf{Z}}\right]+a^{ii}\right)},\left(\left[\tilde{\mathbf{Z}}\right]'\mathbf{T}^{-1}\left[\tilde{\mathbf{Z}}\right]+a^{ii}\right)^{-1}\right)$$

The full conditional distribution of the QTL allelic effects is as follows:

$$v_i \mid \mathbf{b},\mathbf{u},\mathbf{v}_{-i},\mathbf{y},\mathbf{c},\mathbf{d},\mathbf{T} \sim$$

$$N\left(\frac{\left(\left[\tilde{\mathbf{W}}\right]'\mathbf{T}^{-1}\left(\mathbf{y}-\tilde{\mathbf{X}}\mathbf{b}-\tilde{\mathbf{Z}}\mathbf{u}-\left[\tilde{\mathbf{W}}\right]_{-i}\mathbf{v}_{-i}\right)-\sum_{j\neq i}g_k^{ij}v_j\right)}{\left(\left[\tilde{\mathbf{W}}\right]'\mathbf{T}^{-1}\left[\tilde{\mathbf{W}}\right]+g_k^{ii}\right)},\left(\left[\tilde{\mathbf{W}}\right]'\mathbf{T}^{-1}\left[\tilde{\mathbf{W}}\right]+g_k^{ii}\right)^{-1}\right)$$

If the model would be a scaled animal model then the scale parameters and error variances would have normal and inverted gamma conjugate priors, respectively, thus facilitating Gibbs sampling. With the scaled RAM, however, the full conditionals for these parameters are not standard distributions because the scale parameters appear in both the means and variance of $p\left(y_{isj}\middle|\mathbf{b},\mathbf{u},\mathbf{v},c_s,d_s,\sigma^2_{e_s}\right)$. Thus, a Metropolis-Hastings update is used for these parameters.

Let the conditional residual for y_{isj} be $e_{isj}=y_{isj}-x'_{isj}b-c_sz'_{isj}u-d_sw'_{isj}v$ and the contribution to the likelihood for sex s is $L_s=\prod_{i=1}^{4}\prod_{j=1}^{n_{is}}\left[\tau_{is}^{-0.5}\times\exp\left\{-0.5\frac{e_{isj}^2}{\tau_{is}}\right\}\right]$ then:

$$p\left(c_s\middle|\mu_{c_s},\sigma^2_{c_s},\sigma^2_{e_s},d_s,\mathbf{b},\mathbf{u},\mathbf{v},\mathbf{y}\right)\propto L_s\times\left(\sigma^2_{c_s}\right)^{-0.5}\exp\left\{\frac{-0.5\left(c_s-\mu_{c_s}\right)^2}{\sigma^2_{c_s}}\right\}\text{ with }c_s\geq 0$$

$$p\left(d_s|\mu_{d_s},\sigma^2_{d_s},\sigma^2_{e_s},c_s,\mathbf{b},\mathbf{u},\mathbf{v},\mathbf{y}\right)\propto L_s\times\left(\sigma^2_{d_s}\right)^{-0.5}\exp\left\{\frac{-0.5\left(d_s-\mu_{d_s}\right)^2}{\sigma^2_{d_s}}\right\}\text{ with } d_s\geq 0$$

$$p\left(\sigma^2_{e_s}\,|\,\alpha_s,\lambda_s,c_s,d_s,\mathbf{b},\mathbf{u},\mathbf{v},\mathbf{y}\right)\propto L_s\times\left(\sigma^2_{e_s}\right)^{-\alpha_s-1}\exp\left\{-\frac{\lambda_s}{\sigma^2_{e_s}}\right\}\text{ with }\sigma^2_{e_s}\geq 0$$

These conditional distributions need to be evaluated multiple times with fixed **b**, **u** and **v** in the Metropolis-Hastings updates. This is facilitated by computing the individual sums of squares and crossproducts in the exponential term of each L_s, e.g., $\sum_j\left(y_{isj}-x'_{isj}b\right)\left(z'_{isj}u\right)$ and $\sum_j\left(w'_{isj}v\right)^2$.

Chapter 7

Multiple Trait Bayesian Analysis of Broiler Quantitative Trait Loci using Scaling

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Abstract

Combining data obtained in several experiments is expected to improve the QTL detection power and estimation accuracy. An existing Bayesian method was extended to be able to handle multiple trait data including heterogeneity of variance between sexes. The method employs a scaled reduced animal model. Markov Chain Monte Carlo (MCMC) algorithms were applied to obtain marginal posterior densities.

Broiler body weight measured at 48 days in two experiments was used to illustrate the method. The two experiments, a feed efficiency and a carcass experiment, were conducted in a population consisting of 10 full sib families of a cross between two broiler lines. Microsatellite genotypes were determined on generation one and two and phenotypes were collected on different groups of generation three animals. The model included a polygenic correlation, which had a posterior mean around 0.72 in the analysis. A QTL was found present in marker bracket LEI0071-MCW0101 accounting for 38% of the genetic variation in males and 26% in females in the feed efficiency experiment. In the carcass experiment the QTL was located in the region UMA1.107-LEI0071 and accounted for up to 19% of the genetic variation.

Introduction

Several statistical methods have been developed for mapping quantitative trait loci (QTL) (for review see Bovenhuis *et al.*, 1997; Hoeschele *et al.*, 1997). Most of these methods are limited to analyses of one trait at a time. In many QTL experiments, however, information on multiple traits is collected on the same or on related animals. In such experiments a joint analysis of multiple traits can increase the statistical power of detecting QTLs and the precision of parameter estimates (Korol *et al.*, 1998). Korol *et al.* (1995) and Jiang and Zeng (1995) introduced likelihood-based methods for multiple trait QTL mapping to test biologically interesting hypotheses regarding the nature of genetic correlations between different traits. The application of these methods, however, is restricted to populations originating from inbred lines. Weller *et al.* (1996) suggested to derive a set of uncorrelated traits by application of canonical transformation to handle

multiple traits in an outbred population. This procedure was applied to the analysis of milk production traits in Israeli dairy cattle. The procedure is based on the phenotypic covariance structure and, consequently, assumes that the genetic and environmental correlations between two traits are equal. In addition, it does not make a distinction between the genetic correlation for polygenes (i.e. the average pleiotropic effects of all QTL) and the genetic correlation for the QTL under study. Solutions for the original traits could be obtained by reverse transformation.

In the present paper, the single trait Bayesian method presented by Van Kaam *et al.* (2000) is extended to analyse multiple traits jointly. A multiple trait Bayesian method for QTL detection is new. The method employs an animal model with a single QTL affecting all traits and can account for different magnitudes of polygenic and QTL effects in males and females, through scale parameters. Furthermore a polygenic correlation is included. The correlation between QTL allelic effects on different sex-trait combinations is supposed to be one, assuming that the QTL is a single gene and the effect on all sex-trait combinations is in the same direction. Marginal posterior densities are obtained via sampling using Markov Chain Monte Carlo (MCMC) techniques (Gilks *et al.*, 1996).

Material and Methods

Single trait Bayesian animal model

Van Kaam *et al.* (2000) presented an extension to the Fernando and Grossman (1989) model by including scale parameters to account for heterogeneity of variance between sexes in single trait analysis. It was assumed that the magnitude of the effect of some polygenes as well as QTLs may be larger in one sex compared to the other and hence it seems appropriate to consider effects as scaled (Quaas *et al.*, 1989). The Bayesian representation of the scaled model was:

$$\mathbf{y}_s \mid \mathbf{b}_s, \mathbf{u}, \mathbf{v} \sim N\left(\mathbf{X}_s\mathbf{b}_s + c_s\mathbf{Z}_s\mathbf{u} + d_s\mathbf{W}_s\mathbf{v}, \mathbf{I}\sigma^2_{e_s}\right) \text{ for } s = m, f$$

with the prior assumptions:

$$\mathbf{b}' = [b_1 \quad b_2 \quad \ldots \quad b_p] \quad b_{s_j} \sim U[b_{min}, b_{max}]$$

$$\begin{bmatrix}\mathbf{u}\\ \mathbf{v}\end{bmatrix} \sim N\left(0, \begin{bmatrix}\mathbf{A} & 0\\ 0 & \mathbf{G}_k\end{bmatrix}\right)$$

where $\mathbf{y}$ is a n-vector of phenotypes, $\mathbf{X}$ is a $n \times p$ incidence matrix relating fixed effect levels and covariates to phenotypes, $\mathbf{b}$ is a p-vector of fixed effect levels and covariates, c_s are polygenic scale parameters, where subscripts s indicates sex: male (m) or female (f), $\mathbf{Z}$ is a $n \times q$ incidence matrix relating individuals to phenotypes, $\mathbf{u}$ is a q-vector of random additive polygenic effects on the standard normal scale, d_s are QTL scale parameters, $\mathbf{W}$ is a $n \times 2q$ incidence matrix relating each individual's two QTL alleles to phenotypes, $\mathbf{v}$ is a $2q$-vector of random additive QTL allelic effects on the standard normal scale, $\mathbf{A}$ is the additive genetic relationship matrix, $\mathbf{G}_k$ is the gametic relationship matrix for the QTL and depends on QTL position k and the marker information. In the above representation, uncorrelated error terms with homogeneous variance within sex, $\sigma^2_{e_s}$, are assumed. Inverted gamma distributions with pre-defined hyperparameters α and λ are used to represent prior knowledge on these error variances as $\sigma^2_{e_s} \sim IG(\alpha, \lambda_s)$. Each sex has a scaling parameter for the polygenic and QTL allelic effects. The variances of the random genetic effects are fixed to one and hence standard normally distributed $\mathbf{u}$ and $\mathbf{v}$ are used (Van Kaam *et al.*, 2000). Scale parameters can be considered as genetic standard deviations, hence genetic variances can be obtained by squaring scale parameters. The prior distributions for the scale parameters are normal distributions left-truncated at zero:

$$c_s \sim TN(\mu_{c_s}, \sigma^2_{c_s}) \text{ with } c_s \geq 0$$

$$d_s \sim TN(\mu_{d_s}, \sigma^2_{d_s}) \text{ with } d_s \geq 0$$

Because these prior distributions are left-truncated, the prior expectations are bigger than the means μ_{c_s} and μ_{d_s} and the prior variances are smaller than the variances $\sigma^2_{c_s}$ and $\sigma^2_{d_s}$ of untruncated normal distributions, but can be obtained by integration or simulation. New candidate values are sampled using a normal distributed candidate generating density.

Multiple trait Bayesian animal model

The scaled model is now extended to account for multiple traits, here the case of two traits is considered. Each animal has two polygenic effects, one for each trait, where the traits of interest might have a polygenic correlation, ρ, that varies from –1 to +1. However, for the QTL, each animal has only two additive allelic effects (paternal and maternal), assuming unity-correlation. In other words, the correlation between the effects of the QTL alleles is one for all sex-trait combinations, assuming that the direction of the effect in all sex-trait combinations is the same, only the magnitude of the effect differs. This leads to the following extended scaled model in a two-trait situation with heterogeneity of variance between sexes:

$$\mathbf{y}_{ts} \mid \mathbf{b}_{ts}, \mathbf{u}_t, \mathbf{v} \sim N\left(\mathbf{X}_{ts}\mathbf{b}_{ts} + c_{ts}\mathbf{Z}_{ts}\mathbf{u}_t + d_{ts}\mathbf{W}_{ts}\mathbf{v}, \mathbf{I}\sigma^2_{e_{ts}}\right) \text{ for } t = 1,2 \text{ and } s = m, f$$

Here the observations are divided into four sets defined by trait indicated with subscript t and sex indicated with subscript s. Each combination of trait and sex has its own scaling parameter for the polygenic and QTL allelic effects. Therefore the QTL can have an effect on some of the sex-trait combinations and not on others. Hence we now have the following prior assumptions:

$$\begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \\ \mathbf{v} \end{bmatrix} \sim N\left(0, \begin{bmatrix} \mathbf{A} & \mathbf{A}\rho & 0 \\ \mathbf{A}\rho & \mathbf{A} & 0 \\ 0 & 0 & \mathbf{G}_k \end{bmatrix}\right)$$

$$c_{ts} \sim TN\left(\mu_{c_{ts}}, \sigma^2_{c_{ts}}\right) \text{ with } c_{ts} \geq 0$$

$$d_{ts} \sim TN\left(\mu_{d_{ts}}, \sigma^2_{d_{ts}}\right) \text{ with } d_{ts} \geq 0$$

$$\rho \sim U\left[\rho_{min}, \rho_{max}\right]$$

A diffuse uniform prior was chosen for the polygenic correlation in which the boundaries ρ_{min} and ρ_{max} are determined by the requirement of positive definiteness of the correlation matrix. In this case with one polygenic correlation the boundaries are plus and minus one. New candidate values for ρ are sampled using a uniform candidate generating density. If all observations have no environmental correlations because no animals are measured for more than one trait and common environmental effects are negligible, as assumed here, then the error variance, $\sigma^2_{e_{ts}}$, within each sex-trait

combination is homogeneous and an inverted gamma prior distribution on the error variances is specified as $\sigma^2_{e_{ts}} \sim IG(\alpha, \lambda_{ts})$.

Marker information is described in terms of the allelic constitution of the chromosomal homologues of the founders and identity by descent values for all non-founders (Jansen *et al.*, 1998) and is needed to calculate the inverse of $\mathbf{G}_k$ similar to Bink and Van Arendonk (1999).

Bayesian inferences were based on the joint posterior distribution of missing data and parameters, given the observed marker and phenotypic data. The solutions of the Bayesian model are obtained using MCMC algorithms, which enable sampling from the conditional posterior distribution of parameters. A reduced animal model (RAM), similar to Bink *et al.* (1998[b]), was used to obtain solutions more efficiently because polygenic effects of the non-parents and QTL allelic effects of the ungenotyped non-parents do not have to be sampled. Haplotypes, fixed effect levels and covariates, random polygenic and QTL allelic effects are sampled using Gibbs sampling. The use of a RAM means that the residual of the non-parents consists of the error and the Mendelian part of the additive polygenic and QTL variance. Therefore a Metropolis-Hastings algorithm is used to sample the scale parameters, polygenic correlation and error variances. The full conditional distributions of the dispersion parameters are given in Appendix 1.

Experimental data

An experimental population, consisting of founder animals, parents, offspring and grandoffspring, indicated as generation 0, 1, 2 and 3 animals or G_0, G_1, G_2 and G_3 animals, was analysed. In this design, G_1 and G_2 animals were typed for genetic markers and phenotypic observations were collected on G_3 animals. The number of animals and the population structure are presented in Table 1. A more detailed description of the population was given by Van Kaam *et al.* (1998, 1999[a], 1999[b]). In the anályses G_0 animals are omitted, because the method requires known marker genotypes on all base animals.

Two traits were analysed in this study. These traits were body weight at 48 days (BW48) measured in a feed efficiency experiment and measured in a carcass experiment. Different hatches of G_3 animals were used in each experiment. After removal of outliers 2,049 observations remained in the feed efficiency experiment and 1,953 observations remained in the carcass experiment.

Fixed effects for BW48 in the feed efficiency experiment were the location of the animal's cage within the building and an interaction between the hatch of the dam and the hatch of the offspring. The only fixed effect for BW48 in the carcass experiment was an interaction between the hatch of the dam and the hatch of the offspring.

Table 1. Population structure with numbers of animals used in the analysis and types of observations collected.[a]

Generation[b]	Males	Females	Total	Observations[d]
G_0[c]	14	14	28	———
G_1	10	10	20	Genotypes
G_2	172	279	451	Genotypes
G_3	1,012	1,037	2,049	Phenotypes $BW48_{FE}$
G_3	969	984	1,953	Phenotypes $BW48_{CA}$

[a] Numbers exclude outliers and missing values.

[b] G_0 etc. = Generation 0 etc.

[c] Male and female G_0 animals are from different lines, G_0 animals were not included in the analysis because marker genotypes were unknown.

[d] $BW48_{FE}$ = body weight at 48 days as observed in a feed efficiency experiment, $BW48_{CA}$ = body weight at 48 days as observed in a carcass experiment.

Marker data

Genotypes for microsatellite markers were determined using DNA derived from blood samples from all 20 G_1 and 451 G_2 animals. Marker alleles were recorded in basepair units. Only one chromosomal region showed suggestive significance in the previous QTL analysis of the feed efficiency experiment (Van Kaam *et al.*, 1999[b]), however in this region no evidence was found in the carcass experiment (Van Kaam *et al.*, 1999[a]). This region was located on chromosome 1 and contained eleven markers over 96.2 cM starting with marker ADL0150 and ending with marker ADL0314 (Groenen *et al.*, 1998). A minimum marker spacing of about 2 cM was aimed at except for the most lateral markers, which were used to increase marker informativity at the ends of the map. Marker alleles were determined in all ten families for seven of the markers in this region. Genotypes for four markers however were only collected in four families. All 20 parents were informative in this region.

MCMC and prior distribution settings

Multiple QTL positions are investigated by keeping the QTL position fixed in the middle of a number of consecutive marker brackets. For all marker brackets of interest, a separate analysis based on a single chain of 3,000,000 cycles plus 1,000 cycles burn-in time was undertaken. A single run required 620 minutes on a 450 Mhz Pentium II. For comparison reasons, a model without a QTL was studied as well. This required 310 minutes for the same number of cycles. Parameters that were suspected to converge relatively slowly were sampled more often than other parameters as was suggested by Uimari *et al.* (1996). The polygenic correlation was sampled 5 times each cycle, scale parameters and error variances were sampled once each cycle, fixed effects, polygenic effects and QTL allelic effects were sampled in every 5^{th} cycle and haplotypes were sampled every 50^{th} cycle, because they are very time demanding to sample.

The α hyperparameter of the inverted gamma prior distribution of the error variances was set to 2.000001 for all sex-trait combinations. The other hyperparameters of the priors for the dispersion parameters were set based on the following assumptions: (1) The residual variances are 40% of the variance in the observations unadjusted for fixed effects, resulting in λ, (2) Heritabilities of both traits equal 0.30, (3) The putative QTL accounts for 20% of the additive genetic variance with a mode at zero, hence $\mu_{d_{ts}} = 0$, (4) The variance of the polygenic scale parameter, $\sigma^2_{c_{ts}}$, is 0.09 × the expected polygenic variance, and (5) There is no heterogeneity of variance between sexes, i.e. the same priors were used for males and females. Based on assumption 1 and 5, the λ hyperparameter was set to 41,200 for the error variances in the feed efficiency experiment and to 44,000 in the carcass experiment. Using the first two assumptions the additive genetic variance can be calculated. With the third assumption the additive genetic variance can be divided over the polygenic and QTL variance. Then $\sigma^2_{d_{ts}}$ follows from the expected QTL variance and $\mu_{c_{ts}}$ is obtained from a small simulation. The priors for body weight in the feed efficiency experiment were $TN(113,1271)$ for the polygenic scale parameters and $TN(0,1766)$ for the QTL scale parameters, both were left-truncated at zero. The priors for body weight in the carcass experiment were $TN(117,1358)$ and $TN(0,1886)$ respectively. In the model without QTL the polygenic scale priors were respectively $TN(127,1606)$ for the feed efficiency

experiment and $TN(131,1702)$ for the carcass experiment. These latter priors were chosen in order to have the same expected additive genetic variance and the same coefficient of variation for the polygenic scale parameters in the model with and the model without QTL.

Results

Model without QTL

In the absence of a QTL, the phenotypic variance in body weight of males was 59,172 in the feed efficiency experiment and 58,470 in the carcass experiment (Table 2). The variance in females was 29% respectively 36% lower. The estimates of the heritability of body weight were very similar for females and males in both experiments (0.23 to 0.26) with the exception of females in the carcass experiment for which a higher heritability (0.38) was found. These heritability estimates are below those obtained by using restricted maximum likelihood (Van Kaam *et al.*, 1999[a]; 1999[b]).

The polygenic correlation between body weight measured in the two environments was moderately high (0.73). This estimate is slightly higher than the rough estimate of 0.60 obtained by Van Kaam *et al.* (1999[a]).

Models containing a QTL

Analyses with a model containing a polygenic and QTL effect were performed for five marker brackets on chromosome 1. The posterior means for the phenotypic variance for a given sex and experiment were very similar for the different marker brackets (Table 2). The difference with estimates obtained under the model without QTL ranged from 0% to +2%.

Table 2. Posterior means of phenotypic variances in the multiple trait analyses of body weight at 48 days in two experiments using a model with a QTL fixed in different marker brackets or a model without QTL. Indicated per analysis are the marker bracket and the variance associated with a QTL expressed in males (*m*) respectively females (*f*) for body weight at 48 days in two experiments. Also results of a model without QTL are shown.

	Feed eff. exp.		Carcass exp.	
Marker interval	$\sigma^2_{p_m}$	$\sigma^2_{p_f}$	$\sigma^2_{p_m}$	$\sigma^2_{p_f}$
LEI0174-UMA1.107	59,567	42,306	59,249	37,739
UMA1.107-MCW0058	59,436	42,103	59,182	37,750
MCW0058-LEI0071[a]	59,987	42,136	59,058	37,762
LEI0071-MCW0101	60,299	42,345	58,875	37,680
MCW0101-LEI0101	59,539	42,127	58,957	37,639
No QTL	59,172	41,790	58,470	37,373

[a] Interval which contained a QTL in previous regression analysis (Van Kaam *et al.*, 1998; 1999[b]).

The estimates of the polygenic correlation were slightly reduced by including the QTL in the model (Table 3). This reduction is likely caused by the fact that a genetic correlation of one was assumed for the QTL.

Figure 1 shows the marginal posterior density of the polygenic correlation obtained in analysis of BW48 in the five consecutive marker brackets. The similarity between the densities indicates that the convergence was good. The lag 1 serial correlation of the polygenic correlation was 0.990. The polygenic correlation had the highest serial correlation of all parameters. The trace of the polygenic correlation (not shown) however reflects proper mixing of the Markov chain.

Table 3. Posterior means of the polygenic correlation and heritabilities in the multiple trait analyses of body weight at 48 days in two experiments using a model with a QTL fixed in different marker brackets or a model without QTL. Indicated per analysis are the marker bracket, the polygenic correlation and the heritabilities in males (m) respectively females (f) for body weight at 48 days in two experiments. Also results of a model without QTL are shown.

	Polygenic	Feed eff. exp.		Carcass exp.	
Marker interval	correlation	h_m^2 [b]	h_f^2	h_m^2	h_f^2
LEI0174-UMA1.107	0.71	0.28	0.28	0.27	0.40
UMA1.107-MCW0058	0.71	0.27	0.26	0.26	0.40
MCW0058-LEI0071[a]	0.71	0.30	0.27	0.26	0.40
LEI0071-MCW0101	0.72	0.31	0.28	0.25	0.40
MCW0101-LEI0101	0.73	0.27	0.27	0.25	0.39
No QTL	0.73	0.26	0.25	0.23	0.38

[a] Interval which contained a QTL in previous regression analysis (Van Kaam *et al.*, 1998; 1999[b]).

[b] Male and female heritabilities are calculated as $\left(c_m^2 + 2d_m^2\right) / \left(c_m^2 + 2d_m^2 + \sigma_{e_m}^2\right)$ respectively $\left(c_f^2 + 2d_f^2\right) / \left(c_f^2 + 2d_f^2 + \sigma_{e_f}^2\right)$.

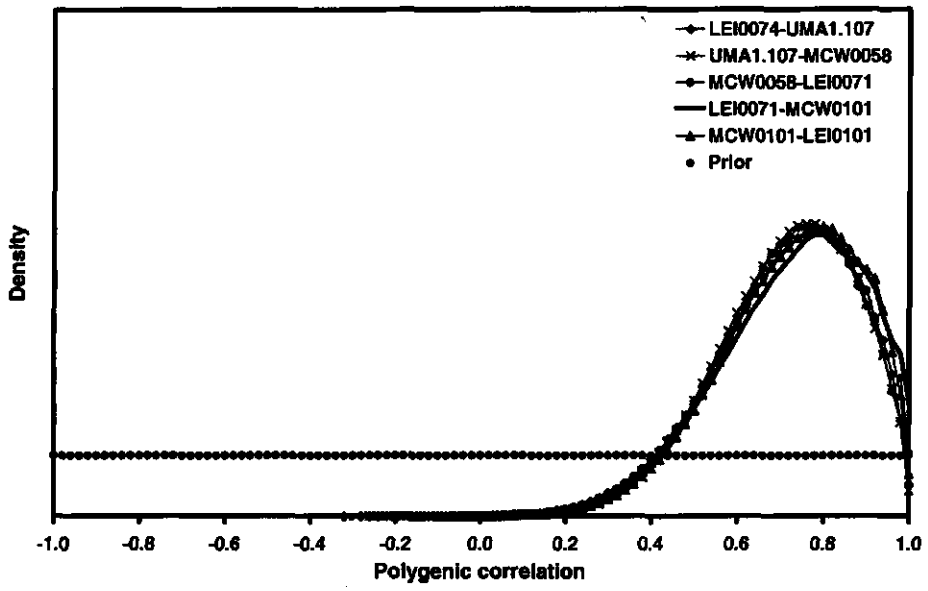

Figure 1. Marginal posterior densities of the polygenic correlation between body weight at 48 days in a feed efficiency and a carcass experiment obtained in five multiple trait analyses of consecutive marker brackets on chromosome 1 using observations in a feed efficiency and a carcass experiment.

The posterior means of the polygenic correlation and the heritability, which measures the polygenic and QTL variance as fraction of the phenotypic variance, for the five marker brackets are shown in Table 3. Slightly higher heritability estimates were found after including the QTL in the model. The differences between the five marker brackets, however, were negligible.

The posterior means for the proportion QTL, which measures the variance explained by the QTL as proportion of the total additive genetic variance, are shown in Table 4. For the feed efficiency experiment, marker interval LEI0071-MCW0101 resulted in the highest estimates for proportion QTL, namely 0.38 in males and 0.26 in females. For the flanking brackets, the estimates ranging from 0.13 to 0.23, were substantially lower. The patterns observed in estimates for the feed efficiency experiment were very similar for males and females. In the carcass experiment, however, the proportion QTL varied less than in the feed efficiency experiment. The marker interval UMA1.107-MCW0058 gave the highest estimated proportion in males (0.19) and the interval MCW0058-LEI0071 gave the highest estimated proportion in females (0.19) in the carcass experiment.

Table 4. Posterior means of the proportion of the additive genetic variance associated with a QTL in the multiple trait analyses of body weight at 48 days in two experiments using a model with a QTL fixed in different marker brackets or a model without QTL. Indicated per analysis are the marker bracket, length of the marker bracket in the Haldane scale and the proportion of the additive genetic variance associated with a QTL expressed in males respectively females for body weight measured at 48 days in a feed efficiency and a carcass experiment. Also results of a model without QTL are shown.

	Length of	Feed eff. exp.		Carcass exp.	
Marker interval	region in cM	Male	Female	Male	Female
LEI0174-UMA1.107	38.8	0.11	0.16	0.17	0.13
UMA1.107-MCW0058	4.2	0.10	0.13	0.19	0.14
MCW0058-LEI0071[a]	2.0	0.23	0.15	0.10	0.19
LEI0071-MCW0101	7.5	0.38	0.26	0.10	0.13
MCW0101-LEI0101	13.8	0.14	0.13	0.10	0.12
No QTL		0.00	0.00	0.00	0.00

[a] Interval which appeared to contain a QTL in previous regression analysis (Van Kaam *et al.*, 1998; 1999[b]).

Figure 2 shows the QTL scale parameters in males in both experiments at different cycles of the MCMC chain for marker bracket LEI0071-MCW0101. This figure demonstrates that the QTL scale parameters for both experiments vary independently from each other during the Markov chain. This reflects the ability of the method to estimate QTL variances for different traits independently. Lag 1 serial correlations of the polygenic scale parameters were around 0.97 and around 0.95 for the QTL scale parameters.

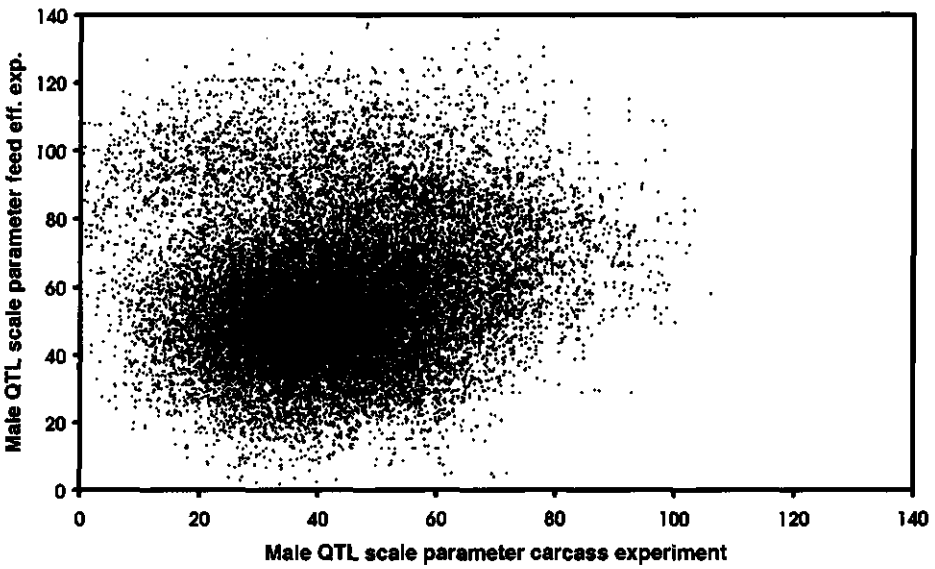

Figure 2. Scatter plot of the male QTL scale parameters for body weight at 48 days in a feed efficiency versus in a carcass experiment obtained in the multiple trait analysis of marker bracket LEI0071-MCW0101 on chromosome 1.

The marginal posterior densities of the QTL scale parameters in the feed efficiency experiment are given for the five marker brackets in Figures 3 and 4. These Figures reveal strong evidence for the presence of a QTL that affects males and females located in marker bracket LEI0071-MCW0101. The density at zero is very small for this bracket. The densities for the flanking marker brackets are more similar to the prior distribution. These densities suggest that there is only a single QTL in this region.

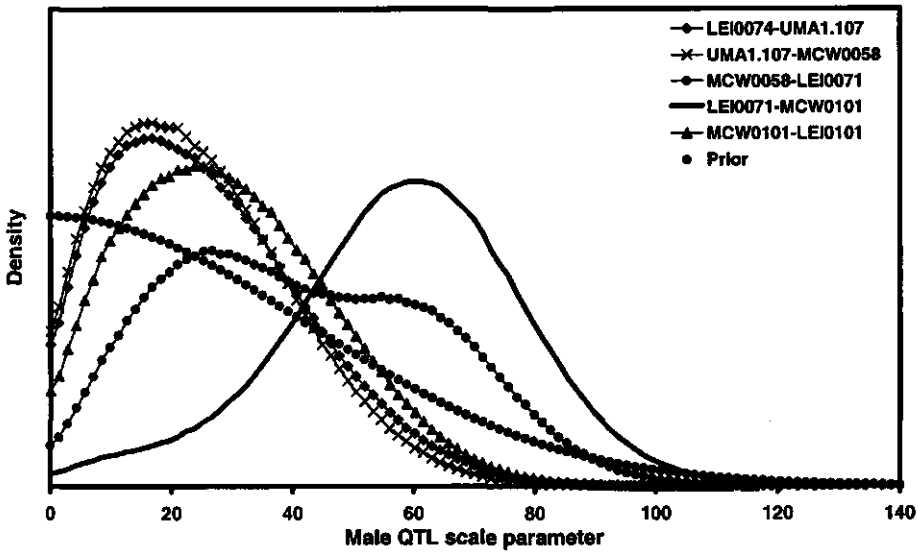

Figure 3. Marginal posterior densities of the male QTL scale parameters modelling dispersion of body weight at 48 days in a feed efficiency experiment obtained in five multiple trait analyses of consecutive marker brackets on chromosome 1 using observations in a feed efficiency and a carcass experiment.

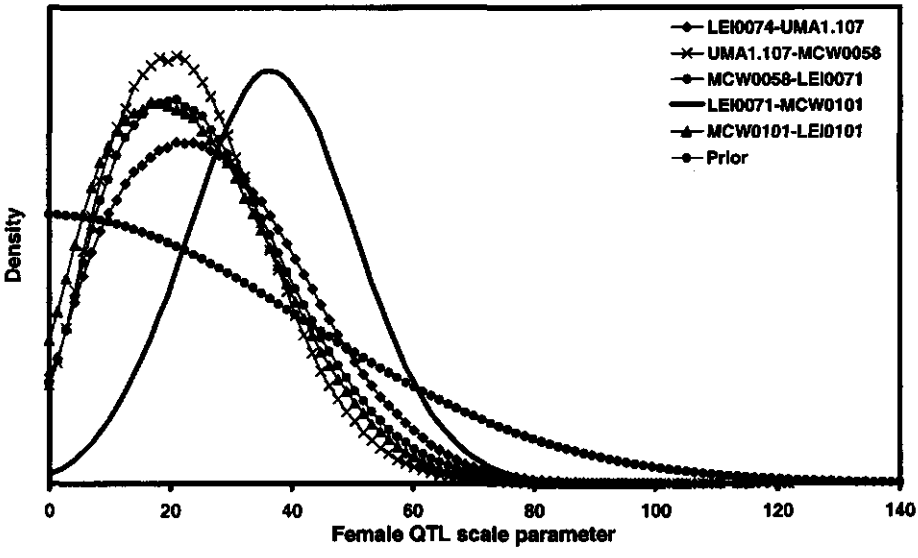

Figure 4. Marginal posterior densities of the female QTL scale parameters modelling dispersion of body weight at 48 days in a feed efficiency experiment obtained in five multiple trait analyses of consecutive marker brackets on chromosome 1 using observations in a feed efficiency and a carcass experiment.

The marginal posterior densities of the QTL scale parameters for the carcass experiment are shown in Figures 5 and 6. The densities for the male QTL scale parameter in all marker brackets have a high density around zero, only the density obtained for marker bracket UMA1.107-MCW0058 shows slight significant evidence for the presence of a QTL. The densities for the female QTL scale parameter, however, show clear evidence for the presence of a QTL in marker bracket MCW0058-LEI0071.

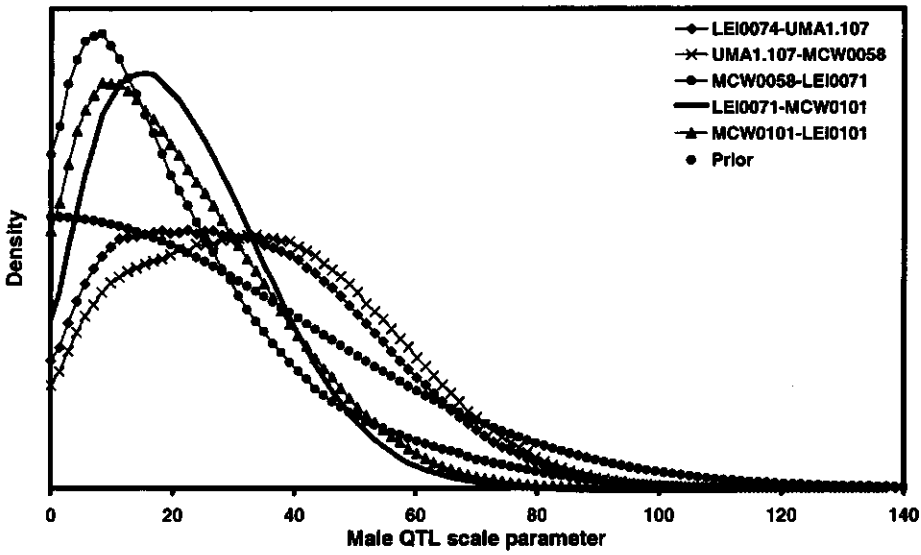

Figure 5. Marginal posterior densities of the male QTL scale parameters modelling dispersion of body weight at 48 days in a carcass experiment obtained in five multiple trait analyses of consecutive marker brackets on chromosome 1 using observations in a feed efficiency and a carcass experiment.

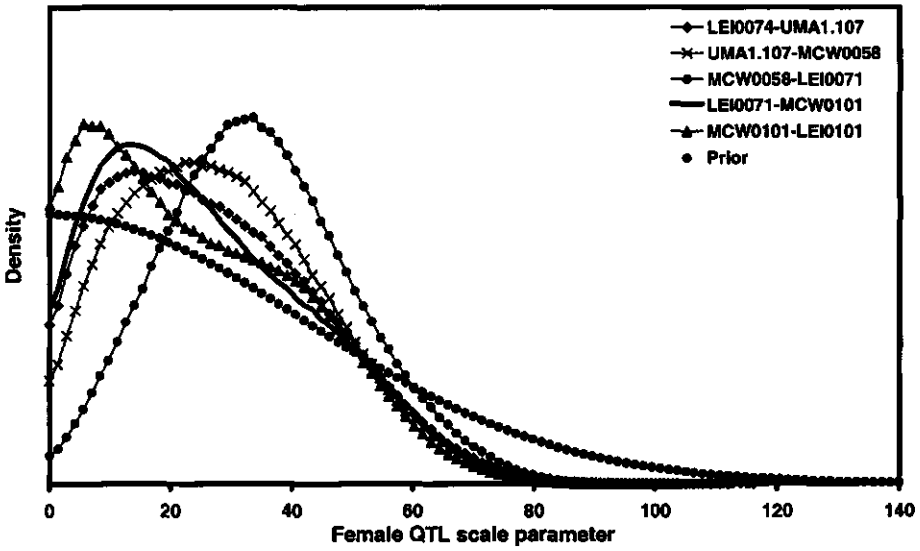

Figure 6. Marginal posterior densities of the female QTL scale parameters modelling dispersion of body weight at 48 days in a carcass experiment obtained in five multiple trait analyses of consecutive marker brackets on chromosome 1 using observations in a feed efficiency and a carcass experiment.

Discussion

Multiple trait scaled model

A parsimonious model specification was chosen on purpose to limit the number of parameters, which needs to be estimated. Especially in small populations like the experimental populations used for QTL detection, it is important to consider the estimability of parameters hence over-parameterisation should be avoided.

In the present analysis the polygenic correlation for body weight measured in both sexes was assumed to be one. This is very much in line with the genetic correlations of BW48 between sexes of 0.97 and 0.92 obtained in earlier analysis of the data (Van Kaam *et al.*, 1999[a]; 1999[b]). Furthermore, the QTL allelic effects were assumed to have a genetic correlation of one for all sex-trait combinations. The scaled model, however, can accommodate the possibility of opposite genetic effects by using normally distributed

scale parameters instead of left-truncated normal distributions. Allowing scale parameters to be negative means that the direction of the effects is handled entirely by the scale parameters and the polygenic correlation(s) would have to be limited to be non-negative.

Results for body weight

In this study, body weight measured in two different experiments was treated as two different traits. The housing system applied in both experiments differed considerably, in the feed efficiency experiment animals were housed individually in cages and the carcass experiment animals were housed in groups in floor pens. The difference in environment results in a genotype × environment interaction as reflected by the polygenic correlation of 0.73. The phenotypic variances in both experiments were very similar. As demonstrated in Figure 2, the method can model the presence of a QTL for each sex-trait combination independently. Summarising the evidence obtained in these analyses we conclude that a single QTL affecting body weight at 48 days is most likely located in the region from marker MCW0058 to MCW0101. Alternatively two QTLs can be present one affecting body weight in individual cages located in marker bracket LEI0071-MCW0101 and one affecting body weight in floor pens located in the region UMA1.107-LEI0071. Possibly a two QTL model would be capable of dissecting the inheritance more clearly.

Multiple trait analysis versus single trait analysis

In single trait regression analysis of BW48, a QTL was found in the feed efficiency experiment (Van Kaam *et al.*, 1999[b]) but not in the carcass experiment (Van Kaam *et al.*, 1999[a]). This difference in results can be caused by a variety of reasons: (1) The low correlation between body weight under different circumstances, (2) lack of significance due to a power below one or (3) a false positive result in the feed efficiency experiment. The multiple trait analysis of body weight should increase the power of detection and hence increase the significance of a QTL if the QTL is not a false positive result. Because a QTL was found for both experiments with the Bayesian method, it proves that a multiple trait analysis with this method is more powerful than a single trait regression analysis. Also a multiple trait analysis with the Bayesian method is more powerful than a single trait analysis with the Bayesian method, because in the multiple trait analysis a QTL was

found in all four sex-trait combinations and in the single trait analysis (not shown) a QTL was found in three sex-trait combinations.

The multiple trait approach offers the opportunity to dissect the genetic covariance among traits. It provides the opportunity to test whether the genetic correlation is due to pleiotropy or linkage for certain regions of the genome (Jiang and Zeng, 1995). In order to address this important biological question, our approach could be extended by removing the restriction of a genetic correlation of one at the QTL. This however would require data sets of sufficient size to be able to estimate such a correlation.

Acknowledgements

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Appendix 1

Full conditional distributions of the scale parameters, error variances and polygenic correlation in the scaled reduced animal model

With the scaled RAM the full conditionals for scale parameters and error variances are not standard distributions because the scale parameters appear in both the means and variance of $p\left(y_{itsj}\middle|\mathbf{b},\mathbf{u},\mathbf{v},c_{ts},d_{ts},\sigma^2_{e_{ts}}\right)$ where i represents the RAM category and j is the animal. Therefore a Metropolis-Hastings update is used for these parameters.

Let the error term be $e_{itsj} = y_{itsj} - x'_{itsj}b - c_{ts}z'_{itsj}u - d_{ts}w'_{itsj}v$ and the residual variance is $\tau_{its} = \sigma^2_{e_{ts}} + \omega_i\left(c^2_{ts} + 2d^2_{ts}\right)$ where ω_i reflects the total amount of additive genetic variance present in τ_{its}. There is one RAM category for parents $(\omega_1 = 0)$ and three for non-parents with both parents known $(\omega_2 = 0.5)$, one parent known $(\omega_3 = 0.75)$ and both parents

unknown $(\omega_4 = 1.0)$. Then the RAM likelihood for a sex-trait combination is:

$$L_{ts} = \prod_{i=1}^{4}\prod_{j=1}^{n_{its}}\left[\tau_{its}^{-0.5} \times \exp\left\{-0.5\frac{e_{itsj}^2}{\tau_{its}}\right\}\right]$$

and the full conditional distributions of the scale parameters and error variances are:

$$p\left(c_{ts}|\mu_{c_{ts}},\sigma^2_{c_{ts}},\sigma^2_{e_{ts}},d_{ts},\mathbf{b},\mathbf{u},\mathbf{v},\mathbf{y}\right) \propto L_{ts} \times \left(\sigma^2_{c_{ts}}\right)^{-0.5} \exp\left\{\frac{-0.5\left(c_{ts}-\mu_{c_{ts}}\right)^2}{\sigma^2_{c_{ts}}}\right\} \text{ with } c_{ts} \geq 0$$

$$p\left(d_{ts}|\mu_{d_{ts}},\sigma^2_{d_{ts}},\sigma^2_{e_{ts}},c_{ts},\mathbf{b},\mathbf{u},\mathbf{v},\mathbf{y}\right) \propto L_{ts} \times \left(\sigma^2_{d_{ts}}\right)^{-0.5} \exp\left\{\frac{-0.5\left(d_{ts}-\mu_{d_{ts}}\right)^2}{\sigma^2_{d_{ts}}}\right\} \text{ with } d_{ts} \geq 0$$

$$p\left(\sigma^2_{e_{ts}}|\alpha_{ts},\lambda_{ts},c_{ts},d_{ts},\mathbf{b},\mathbf{u},\mathbf{v},\mathbf{y}\right) \propto L_{ts} \times \left(\sigma^2_{e_{ts}}\right)^{-\alpha_{ts}-1} \exp\left\{-\frac{\lambda_{ts}}{\sigma^2_{e_{ts}}}\right\} \text{ with } \sigma^2_{e_{ts}} \geq 0$$

The full conditional distribution of the polygenic correlation is:

$$p\left(\rho|\mathbf{b},\mathbf{u},\mathbf{v},\mathbf{y},\mathbf{c},\mathbf{d}\right) \propto -0.5q \times \log\left(1-\rho^2\right)^{-0.5q} \exp\left\{-0.5\mathbf{u}'\begin{bmatrix}\mathbf{A} & \mathbf{A}\rho\\ \mathbf{A}\rho & \mathbf{A}\end{bmatrix}^{-1}\mathbf{u}\right\}$$

Chapter 8

General Discussion

Introduction

This thesis focussed on the methodology and results of QTL analyses in an experimental broiler population. This general discussion begins with summarising the detected QTLs and results of analyses fitting two QTL. Then some comments on the power of the design are given. Furthermore, the regression and Bayesian methodology are discussed and some extensions are presented. Finally a guideline for future QTL analyses is given.

Number of QTLs found

In the single QTL genome analysis using full sib regression, six suggestive and one significant QTL were detected. Jansen (1993) suggested using multiple QTL analysis to increase the power of detecting QTL. Therefore, additional two QTL regression analyses were undertaken by fitting two QTLs affecting the same trait simultaneously. The most likely two QTL models were found by using a two dimensional genome scan on the first 20 linkage groups. On the other linkage groups, not all parents were informative and hence they are not comparable to the first 20 linkage groups. The two QTLs were fitted independently of each other on different linkage groups or on the same linkage group with a minimum distance of 40 cM between both QTLs. Significance levels were obtained by two dimensional permutation to test for the presence of two QTLs versus no QTLs. The significance levels were adjusted to genomewise levels using the same Bonferroni adjustment as applied in the single QTL genome scan. The results of the most likely two QTL models per trait are presented in Table 1. The two QTL regression analyses of the first 20 linkage groups revealed 15 QTL of which 5 were found in the single QTL analysis as well and an extra 10 QTL were added.

Table 1. Most likely two QTL models per trait over first 20 linkage groups.[a]

Trait[b]	Linkage group	Marker bracket	Significance	Level[c]
BW23	12	ADL0290-MCW0219	75.37%	
	17	ROS0020-ADL0149		
GAIN	1	UMA1.107-MCW0058	47.59%	S
	4	LEI0122-MCW0085		
$BW48_{FE}$	1	MCW0058-LEI0071	55.09%	S
	4	LEI0122-MCW0085		
FIFA	1	UMA1.107-MCW0058	2.27%	G
	4	LEI0122-MCW0085		
FIFW	1	UMA1.100-ADL0319/MCW0019	31.67%	S
	2	MCW0082-MCW0341		
FE	1	UMA1.100-ADL0319/MCW0019	46.35%	S
	2	MCW0314-MCW0245		
$BW48_{CA}$	3	MCW0148/MCW0116-LEI0166	61.88%	S
	14	MCW0123-LEI0066		
CW	2	LEI0147-MCW0096	41.41%	S
	3	MCW0148/MCW0116-LEI0166		
CP	1	MCW0023/ADL0183-LEI0079	25.30%	S
	20	MCW0328-MCW0076		
MC	2	MCW0039-ADL0226	23.37%	S
	2	MCW0185-MCW0234		
LS	1	ADL0238-UMA1.003	64.26%	
	2	MCW0065-ADL0212		

[a] For each trait the most likely two QTL model is shown. For each model 2 marker brackets containing QTL and the significance level of the whole model are shown.

[b] BW23 = body weight at 23 days; $BW48_{CA}$ = body weight at 48 days in the carcass experiment; $BW48_{FE}$ = body weight at 48 days in the feed efficiency experiment; CP = carcass percentage; CW = carcass weight; FE = percentage feed efficiency between 23 and 48 days; FIFA = feed intake in a fixed age interval; FIFW = feed intake in a fixed weight interval; GAIN = growth between 23 and 48 days; LS = leg score; MC = meat colour.

[c] G = Genomewise significance 5%, S = Suggestive linkage 63.424%.

Because this is the first whole genome study in broilers, it is difficult to make a good comparison with other studies in literature. Intuitively one might conclude that the number of QTLs found per trait in the single QTL whole genome scans is not large. Because most of the analysed traits are correlated, some QTLs are detected for multiple traits. Furthermore, besides the number of QTL their size is relevant as well. Most QTL found in this research accounted for quite a large part of the additive genetic variance. In the Bayesian analyses the QTLs accounted for up to 46% of the additive genetic variance. Hence not many QTL were found but the ones, which were found, had a large effect. This might seem surprising because broilers have been selected for many generations on growth traits (Dunnington and Siegel, 1996) and one might expect that the largest QTLs would be fixated. Georges *et al.* (1995) found QTL accounting for up to 60% of the additive genetic variance in dairy cattle, but selection in dairy cattle has been over fewer generations as in broilers.

Power of the QTL analysis

Several reasons can be given to provide an explanation for the limited number of QTLs found per trait:

a) Power of the design is not as high as desired for a whole genome analysis. Since the time that power calculations were performed (Van der Beek *et al.*, 1995) more stringent criteria for the significance of QTL have been proposed (Lander and Kruglyak, 1995). Their criteria for suggestive and significant QTL require adjustments of the significance thresholds to account for multiple testing across the whole genome. As a consequence of this adjustment of significance thresholds, the power for detecting QTL is lower. In retrospect, the power of a design for QTL analysis should be calculated using the significance criteria applied in the analysis. If a genomewise analysis is undertaken and genomewise significance thresholds are used then the power should be calculated in agreement with genomewise thresholds by adjusting the Type I error rate. In Table 2 power calculations, adjusted to genomewise level, for five different designs are presented. Alternative A shows a design similar to the actual experimental design used in this thesis. The alternatives B, C, D and E each show the effect of a change in one parameter compared to alternative A. In alternative B the number of families was doubled. In alternative C, the number of offspring per family was doubled. In alternative D, the number of grandoffspring per offspring was doubled

and in alternative E the average distance between informative markers was halved hence, the number of markers was doubled. A heritability of 0.30 and a QTL heterozygosity of 0.50 are assumed. Powers are shown for each design with a QTL of $1\sigma_a$ (i.e. $055\sigma_p$) and with a QTL of $0.75\sigma_a$ (i.e. $0.41\sigma_p$). The Type I error rate was 0.05 for genomewise significant linkage and 0.63 for genomewise suggestive linkage. The results for alternative A show that the power of the design is only substantial for QTL with a large effect. The design is not powerful enough to find the small QTL. Comparing the alternatives shows that the most effective way to improve the power of the design is to increase the number of offspring or the number of grandoffspring per family. Doubling the number of markers hardly improves the power of the design. Hence, we can conclude that the amount of phenotypic information is limiting the power much more than the amount of marker genotypes.

b) The genetic difference between the lines used to set up the experimental population might have been to low. A larger difference between lines can increase the difference in allele frequencies at the QTL. The lines are outcross and mated in such a way that the probability of heterozygosity is increased. It could be a better idea to use a cross between a broiler line and a wild type chicken or a layer line. The reason, however, for choosing the lines used in this study was that these lines are closer to commercial broiler lines and therefore the chance of finding QTLs, which are relevant in the current breeding practice, is higher. Because outcross lines are used, a full sib analysis is undertaken in which two different QTL alleles per parent are modelled. Some studies apply a line cross analysis in which only one additive and one dominance allele effect per line are modelled. The line cross analysis is more powerful when the assumption of fixation holds (De Koning *et al.*, 1999). Line cross analysis can also be applied when the QTL allele frequency differs between the lines but will no longer be more powerful. A line cross analysis therefore seemed more appropriate for more extreme lines than for the current population.

c) The lines used to set up the experimental population have been under selection and therefore the genes with the largest effect might be fixated. In that case, the genetic variation in chickens would be largely due to polygenes, epistasis or imprinting and not so much due to single genes with large effects.

d) Only a limited number of traits is analysed. Furthermore, these traits are often correlated. Consequently only a small effective number of traits has been analysed and by chance these might have given a limited number of QTLs.

Table 2. Power of five alternative experimental chicken designs adjusted with Bonferroni to 5% genomewise levels assuming only one QTL of sufficient size.

	Alternative				
Settings	**A**	**B**	**C**	**D**	**E**
Number of families	10	**20**	10	10	10
Average number of offspring/family	45.1	45.1	**90.2**	45.1	45.1
Average number of grandoffspring/family	4.5	4.5	4.5	**9.1**	4.5
Distance between informative markers	20 cM	20 cM	20 cM	20 cM	**10 cM**
Power with a QTL of size $a = 1\sigma_a$ and $d = 0$					
Significant linkage	0.56	0.90	0.96	0.93	0.66
Suggestive linkage	0.79	0.97	0.99	0.98	0.84
Power with a QTL of size $a = 0.75\sigma_a$ and $d = 0$					
Significant linkage	0.18	0.44	0.66	0.59	0.24
Suggestive linkage	0.41	0.71	0.85	0.81	0.48

Bayesian versus regression method

First a full-sib regression method was developed and used for an initial whole genome scan. Because regression uses only the most likely parental haplotype configuration, only one analysis at each location is required for each model of interest. This computational efficiency enables a whole genome scan and also enables the use of permutation tests to obtain significance levels and bootstrapping to obtain confidence intervals for QTL position.

Disadvantages of the applied regression method are: (1) Prior adjustment of observations was necessary, hence some uncertainty is not accounted for (2) Only a simple population structure can be handled (3) No polygenic effect is included (4) Only the most likely parental haplotype configuration is considered. Considering only the most likely parental haplotype configuration is only a problem if two configurations have very

similar probabilities, which is the case if the number of recombinants is nearly equal to the number of non-recombinants in a certain marker bracket. This is most likely to occur in large marker brackets and with a low number of marker alleles.

Secondly, a Bayesian method has been developed to enable analysis of bivariate data including heterogeneity of variance between sexes. The advantages of the Bayesian method are: (1) Inclusion of a polygenic effect. (2) Prior adjustment of observations is no longer necessary because all parameters except recombination rates are sampled in one analysis. (3) Simultaneous analysis using sampling accounts for the uncertainty in other parameters. (4) Complex population structures can be handled due to application of an animal model including relationship matrices. Bink and Van Arendonk (1999) clearly demonstrated the benefit of incorporating additional relations. In this study based on an experimental population, the scope to include additional relationships was very limited. (5) All haplotype configurations are considered. (6) Dispersion parameters are obtained. (7) Densities of parameters are obtained.

Although the genetic model applied in the Bayesian analyses seems more realistic than that of the regression methodology, it is important to realise the negative aspects of Bayesian analysis as well. Disadvantages of the Bayesian method are: (1) Computational requirements are higher, hence more time is needed. (2) More skills, time and experience of the researcher are required. (3) More sensitive to settings e.g. priors. (4) A normally distributed QTL is assumed, whereas a limited number of alleles might be more realistic. The number of alleles at the QTL and their frequencies, however, are unknown. Furthermore, the current method has the disadvantages that sampling of QTL position was unsuccessful.

In conclusion, the Bayesian methodology seems more powerful but also more demanding. In complex populations, regression analysis is too limited, hence an animal model including a QTL is preferable. For a quick analysis or for an initial whole genome scan the regression method is preferable.

Extensions on the regression methodology

Some additional regression analyses have been undertaken. In a regression analysis, the use of permutation to obtain significance thresholds is computationally the most demanding task. Especially if a multiple QTL, hence multidimensional, scan would be undertaken. It therefore might be an efficient approach to reduce the number of

permutations to say 10% and use curve fitting to obtain a proper distribution of the test statistic under the hypothesis of absence of QTL.

For further fine-mapping of the QTL or for application of marker assisted selection it is useful to have a confidence interval for the position of a QTL. Confidence intervals for QTL position in regression analysis can be obtained using bootstrapping (Visscher *et al.*, 1996). In a bootstrapping analysis, a random sample of *n* animals is taken from a family of *n* individuals. Some individuals can be sampled more than once others might not be sampled. In this manner other populations, which could have been obtained from the same parents, are mimicked. In each population, the usual regression analysis is done and the most likely QTL position is stored. This is repeated a lot of times to obtain a density for the most likely QTL position. Figure 1 shows the results of a bootstrap analysis for the QTL affecting feed intake, growth and body weight at chromosome 1. There is a 78% chance that the QTL is located in a confidence interval of only 8 cM.

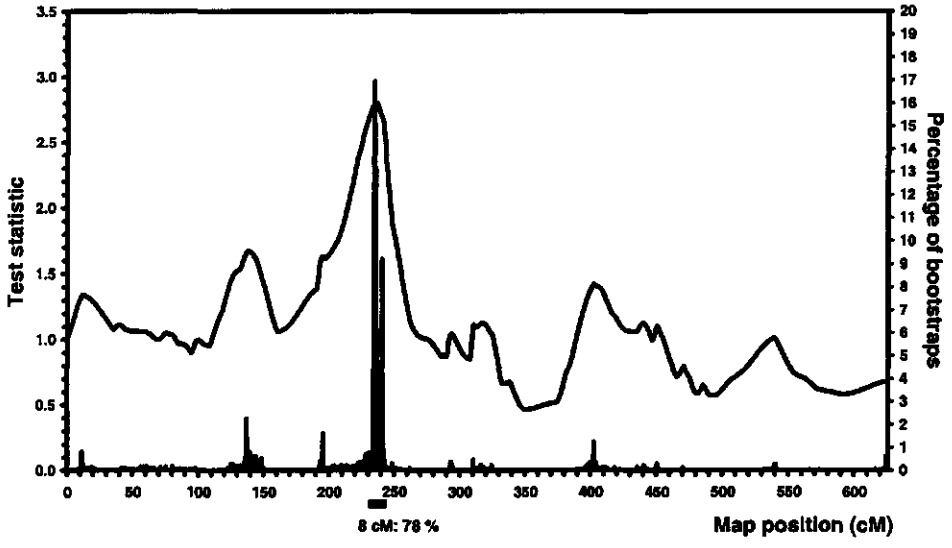

Figure 1. Bootstrapping confidence interval for QTL position of the QTL affecting Feed intake at chromosome 1.

It is important to realise that with the application of bootstrapping the same assumptions are made as with the regression QTL analysis. Only the most likely parental

Also all prior adjustments are included without taking uncertainties into account. It therefore seems that bootstrapping confidence intervals are estimated to optimistic in case doubt about the haplotype configuration or prior adjustments exists.

Extensions for the Bayesian methodology

There are several areas in which the Bayesian method, present here, can be improved or extended. The most important extensions are:

a) The most obvious extension is the inclusion of sampling of the QTL position. The difficulty in sampling QTL position is in switching between different marker brackets, because in each marker bracket the IBD pattern of the flanking markers differs substantially. Bink *et al.* (2000) implemented simulated tempering as an algorithm to enable the sampling of QTL position. Simulated tempering, however, is a method which requires a lot of tuning to obtain settings, which work properly and has high computational requirements. Recently Bink (personal communication) proposed a joint sampling algorithm, which samples QTL position and QTL allelic effects jointly. This algorithm seems to solve the difficulties in switching between marker brackets with limited computational requirements. Furthermore, Bink (personal communication) included a reversible jump algorithm, which enables the sampler to switch between a model containing one QTL and a model without a QTL. Attempts to sample QTL position in the current study, either with simulated tempering or with joint sampling, failed.

b) A further extension, to enhance the scope of the Bayesian method, is the ability to handle multiple traits measured on the same individuals. This can be accounted for by enabling the method to handle correlated error terms.

c) In order to achieve higher power and prevent against ghost QTLs it seems worthwhile to fit multiple QTLs simultaneously.

d) Another extension could be the ability to sample a correlation between QTL effects instead of fixing this to one. In this way, closely linked QTLs can be accounted for, but estimability might be problematic with data sets of current size.

e) Recent evidence for imprinting (De Koning *et al.*, 2000) makes it interesting to extend the Bayesian analysis with the possibility to analyse imprinted QTLs. The underlying Fernando and Grossman model distinguishes paternal and maternal QTL effects, but they have not been estimated separately. The QTL variance was set equal to the sum

of the paternally and maternally inherited QTL effect. The scaled RAM can be extended in the following two manners to include genetic imprinting:

1) Proportion-imprinting model. In this model the elements in the first column of matrix **W** equal the proportion of the QTL variance caused by the paternal alleles and the elements in the second column of **W** equal the proportion of the QTL variance caused by the maternal alleles. Only one of these proportions has to be sampled. In this model, the proportion in column 1 would be near 0 in case of paternal imprinting (maternal expression only) or near 1 in case of maternal imprinting. In case of absence of imprinting, the proportions in both columns would be 0.5.
2) Indicator-imprinting model. In this model, the elements in the first column of **W** are $indicator_{paternal}$ and the elements in the second column of **W** are $indicator_{maternal}$. The indicator-imprinting model assumes that a QTL allele is either completely expressed or not expressed at all. In the indicator model the sampler would sample between 4 options: $indicator_{paternal}, indicator_{maternal} = 0,0 \text{ or } 0,1 \text{ or } 1,1 \text{ or } 1,0$. Implementation can possibly be done with a reversible jump step as in Bink (personal communication), where the sampler can switch between a model including or excluding a QTL. Notice here that the option of absence of QTL is specified with $indicator_{paternal}, indicator_{maternal} = 0,0$. If an indicator for maternal or paternal expression is zero then the maternal or paternal QTL allelic effect is zero. If imprinting leads to no expression of an allele then this model is biologically more correct than the proportion-imprinting model. However, steps between the four options would be larger than in the proportion-imprinting model.

Guideline for future QTL analyses

In my opinion it is very important to realise what you can estimate with a reasonable accuracy on a data set of a certain size and population structure. Over-parameterisation is very tempting. Therefore the extensions for QTL models, suggested above, should only be considered if the data set is of sufficient size.

Summary

This dissertation deals with the development and application of methods for the detection of genes with a substantial influence on quantitative traits, so called quantitative trait loci (QTLs) in broilers. For the purpose of detection of QTLs, an experiment was initiated. A three generation full sib-half sib experimental population consisting of 10 full sib families originating from a cross between two broiler dam lines was set up. Genotypes of up to 437 microsatellite markers on 28 linkage groups were determined on all 20 generation one and 451 generation two full sib animals. Generation three half sib animals were divided in batches and phenotypic observations on several traits were collected in different experiments. Data from a feed efficiency and a carcass experiment were used in the QTL analyses. In both experiments approximately 2,000 phenotypic observations were collected per trait.

In Chapter 2, a detailed description of the experimental population is given for the feed efficiency experiment, the approach for the QTL analysis is described and results for body weight at 48 days are presented. The data were analysed using a two step procedure: first average adjusted progeny trait values were calculated, and secondly QTL analysis was performed using the average adjusted progeny trait values as the dependent variable. Large differences in mean and variance of male and female body weight were found. Prior adjustment of these differences is necessary to ensure that each observation has a similar effect within the QTL analysis. Therefore, a bivariate analysis was used to estimate variances, fixed and genetic effects. These estimated effects were used to calculate average adjusted progeny trait values for all generation two animals by averaging progeny observations, which were standardised after adjusting for fixed and maternal genetic effects and for the additive genetic contribution of the other parent. A full sib regression interval mapping approach was applied, because it enables a quick initial scan of the entire genome and simultaneously includes the segregation of alleles from both generation one parents. The QTL analyses were across family and average adjusted progeny trait values were weighted to account for the number of third generation observations included. In total, 24 autosomal linkage groups were analysed in this chapter. The most likely QTL position was found between markers MCW0058 and LEI0071on chromosome 1.

In Chapter 3, the approach described in Chapter 2 was applied on all traits in the feed efficiency experiment. These traits were body weight at 23 and 48 days, growth between

23 and 48 days, feed intake between 23 and 48 days, the same feed intake adjusted for body weight, and feed efficiency. In total 27 autosomal linkage groups were analysed and four QTLs for body weight, growth and feed intake traits were found. The most significant QTL was located between markers UMA1.107 and MCW0058 on chromosome 1 and had a 4% genomewise significance for feed intake between 23 and 48 days. Furthermore, this QTL exceeded suggestive linkage for growth between 23 and 48 days and body weight at 48 days. The other QTLs showed suggestive linkage. The second QTL, affecting feed intake between 23 and 48 days, was located between markers ADL0289 and ADL0262 on linkage group WAU26. On chromosome 4, between markers MCW0085 and LEI0122, a third QTL was found, which had an effect on both feed intake traits. Finally, a fourth QTL, which affected feed intake adjusted for body weight, was located between markers MCW0082 and MCW0341 on chromosome 2.

In a similar way, Chapter 4 describes the analysis of all traits in a carcass experiment. These traits were body weight at 48 days, carcass weight, carcass percentage, breast meat colour unadjusted and adjusted for body weight, original leg scores, transformed leg scores and transformed leg scores adjusted for body weight. The same approach used before in Chapter 2 and 3 was applied to undertake a genome scan on all autosomal linkage groups. Two suggestive QTLs for carcass percentage and meat colour were detected. The QTL affecting carcass percentage was located between markers ADL0183 and LEI0079 on chromosome 1. The QTL for meat colour was located on chromosome 2 and gave a peak between markers MCW0185 and MCW0234 and between markers MCW0264 and ADL0164.

In Chapter 2, 3 and 4, the sex chromosomes were omitted from the genome scans. In Chapter 5, the Z chromosome was analysed for growth and carcass traits. Additionally, feathering was analysed. For the Z chromosome, only the segregation of male chromosomes provides information on the presence of genes and therefore a half sib interval mapping approach was used. No QTLs were found which affected growth or carcass traits. For feathering, however, a huge QTL effect was found. The feathering gene was located between markers ADL0022 and MCW0331.

In Chapter 6, an existing Bayesian method is extended to enable the analysis of the experimental broiler data accounting for the heterogeneity of variance between sexes. Heterogeneity is accounted for by including separate scale parameters for the polygenic and QTL allelic effects per sex and by separate error variances per sex. A detailed Bayesian analysis is undertaken on chromosomal regions where QTLs were found with

the initial regression analyses. Advantages of the Bayesian method in comparison with the regression analysis are that normally distributed random polygenic and QTL effects are modelled and dispersion parameters are estimated for all random terms in the model. Furthermore, individual observations are used instead of offspring averages and mate correction is no longer necessary, because all genetic relations are taken into account through relationship matrices. By simultaneous sampling of all model parameters, uncertainties are taken into account. The use of a reduced animal model enables the analysis of complex populations. Markov Chain Monte Carlo algorithms were applied to obtain solutions. The Bayesian method was successful in finding QTLs in all regions previously detected.

In Chapter 7, the Bayesian method is extended even further to enable a bivariate analysis of body weight data obtained in both experiments. Combining data from both experiments is expected to improve the QTL detection power and estimation accuracy. For each sex-trait combination separate error variances and separate scale parameters for the polygenic and QTL allelic effects were included. Furthermore, a polygenic correlation was included. Broiler body weight data measured at 48 days was used to illustrate the method. The QTL on chromosome 1 found previously in the feed efficiency experiment but not in the carcass experiment, was now detected in both experiments demonstrating that the QTL detection power indeed increased. The most likely QTL location, however, was in a different marker bracket for both experiments.

Finally, in Chapter 8, the number of QTLs and the power of the design is discussed. Differences between the regression and the Bayesian method are mentioned and potential extensions on both methods are discussed. With the regression method, a two QTL analysis was applied to increase the power and bootstrapping was used to provide confidence intervals of the QTL position. For the Bayesian method, the most important extensions to be implemented are the sampling of the QTL position, the inclusion of correlated residuals, which would enable bivariate analysis of traits measured on the same individuals, and the ability to handle imprinting.

Samenvatting

Dit proefschrift gaat over de ontwikkeling en toepassing van methoden voor de detectie van genen met een substantiële invloed op kwantitatieve kenmerken, zogenoemde quantitative trait loci (QTLs) in vleeskuikens. Een experiment was opgestart voor het doel van detectie van QTLs. Een 3 generatie experimentele populatie bestaande uit 10 families afstammend van een kruising tussen twee vleeskuiken moederlijnen was opgezet. De eerste twee generaties dieren bestaat uit volle broers en zussen de derde generatie bestaat uit halfbroers en halfzussen. Genotypes van maximaal 437 microsatellite merkers op 28 koppelingsgroepen zijn bepaald voor alle 20 generatie 1 en 451 generatie 2 dieren. Generatie 3 dieren zijn verdeeld in groepen die deel namen aan experimenten. Data van een voorefficiëntie en een karkas experiment werden gebruikt in de QTL analyses. In beide experimenten werden ongeveer 2000 waarnemingen per kenmerk geregistreerd.

In hoofdstuk 2 is een gedetailleerde beschrijving van de experimentele populatie gegeven voor het voorefficiëntie experiment, de aanpak van de QTL analyse is beschreven en resultaten voor lichaamsgewicht op 48 dagen worden gepresenteerd. De data zijn geanalyseerd volgens een twee stappen procedure: eerst zijn gecorrigeerde kenmerkwaarden gemiddeld over nakomelingen berekend, en vervolgens is een QTL analyse uitgevoerd waarbij deze waarden als afhankelijke variabelen zijn gebruikt. Grote verschillen in gemiddelde en variantie van lichaamsgewicht van hennen en hanen zijn gevonden. Correctie vooraf van deze verschillen is noodzakelijk om er voor te zorgen dat alle observaties een vergelijkbaar effect hebben in de QTL analyse. Daarom is een bivariate analyse gebruikt om varianties, vaste en genetische effecten te schatten. Deze geschatte effecten zijn gebruikt om gecorrigeerde kenmerkwaarden voor generatie 2 dieren te berekenen door kenmerkwaarden van nakomelingen te middelen, welke zijn gestandaardiseerd, na correctie voor vaste en maternale genetische effecten en de additief genetische bijdrage van de andere ouder. Een regressie interval mapping benadering is toegepast, omdat dit een snelle genoom scan mogelijk maakt en waarbij de segregatie van allelen van beide generatie 1 ouders tegelijkertijd wordt gevolgd. De QTL analyses zijn over alle families tegelijk uitgevoerd en de kenmerkwaarden zijn gewogen om rekening te houden met het aantal derde generatie observaties waaruit deze zijn berekend. In totaal zijn 24 autosomale koppelingsgroepen geanalyseerd in dit hoofdstuk. De meest

waarschijnlijke QTL positie is gevonden tussen merkers MCW0058 en LEI0071 op chromosoom 1.

In hoofdstuk 3 is de benadering van hoofdstuk 2 toegepast op alle kenmerken die geanalyseerd zijn in het voerefficiëntie experiment. Deze kenmerken zijn lichaamsgewicht op 23 en 48 dagen, groei tussen 23 en 48 dagen, voeropname tussen 23 en 48 dagen al dan niet gecorrigeerd voor lichaamsgewicht en voerefficiëntie. In totaal 27 autosomale koppelingsgroepen zijn geanalyseerd en 4 QTLs voor lichaamsgewicht, groei en voeropname kenmerken zijn gevonden. Het meest significante QTL is gelokaliseerd tussen de merkers UMA1.107 and MCW0058 op chromosoom 1 en had een 4% genoomwijze significantie voor voeropname tussen 23 en 48 dagen. Verder overschrijdt dit QTL het suggestieve significantieniveau voor groei tussen 23 en 48 dagen en lichaamsgewicht op 48 dagen. De andere QTLs zijn suggestief. Het tweede QTL heeft een invloed op voeropname tussen 23 en 48 dagen en was gelokaliseerd tussen de merkers ADL0289 en ADL0262 op koppelingsgroep WAU26. Op chromosoom 4 tussen de merkers MCW0085 en LEI0122 is een derde QTL gevonden, welke een effect vertoonde op beide voeropname kenmerken. Tenslotte is een vierde QTL, met een effect op voeropname gecorrigeerd voor lichaamsgewicht, gelokaliseerd tussen de merkers MCW0082 en MCW0341 op chromosoom 2.

Op soortgelijke manier beschrijft hoofdstuk 4 de analyse van alle kenmerken in een karkas experiment. Deze kenmerken zijn lichaamsgewicht op 48 dagen, karkas gewicht, karkas percentage, borstvleeskleur al dan niet gecorrigeerd voor lichaamsgewicht en pootscores, getransformeerde pootscores en getransformeerde pootscores gecorrigeerd voor lichaamsgewicht. Dezelfde benadering zoals gebruikt in hoofdstuk 2 en 3 is toegepast om een genoom scan op alle autosomale koppelingsgroepen te doen. Twee suggestieve QTLs voor karkas percentage en borstvleeskleur zijn gevonden. Het QTL met een effect op karkas percentage was gelokaliseerd tussen de merkers ADL0183 en LEI0079 op chromosoom 1. Het QTL voor borstvleeskleur is gelokaliseerd op chromosoom 2 met een piek tussen de merkers MCW0185 en MCW0234 en de merkers MCW0264 en ADL0164.

In hoofdstuk 2, 3 en 4 zijn de seks chromosomen niet meegenomen in de genoom scans. In hoofdstuk 5 is het Z chromosoom geanalyseerd voor groei en karkas kenmerken. Verder is ook de bevederingssnelheid geanalyseerd. Voor het Z chromosoom geeft alleen de segregatie van mannelijke chromosomen informatie over de aanwezigheid van genen

en daarom is een halfzus/halfbroer interval QTL analyse gebruikt. Geen QTLs met een effect op groei of karkas kenmerken zijn gevonden. Voor bevederingssnelheid is echter een groot QTL effect gevonden. Het bevederingsgen is gelokaliseerd tussen de merkers ADL0022 en MCW0331.

In hoofdstuk 6, is een bestaande Bayesiaanse methode uitgebreid om de analyse van de experimentele vleeskuiken gegevens mogelijk te maken, rekening houdende met de verschillen in variantie tussen beide geslachten. Met deze heterogeniteit van variantie wordt rekening gehouden door de opname van afzonderlijke schaal parameters voor de polygene en QTL allelische effecten per geslacht en afzonderlijke residuele varianties per geslacht. Een gedetailleerde Bayesiaanse analyse is ondernomen op stukken chromosoom waarop QTLs zijn gevonden met de eerdere regressie analyse. Voordelen van de Bayesiaanse methode in vergelijking met de regressie methode zijn dat normaal verdeelde random polygene en QTL effecten zijn gemodelleerd en variantie parameters voor alle random termen in het model worden geschat. Verder worden individuele waarnemingen gebruikt in plaats van nakomeling gemiddelden en correctie voor de partner is niet langer nodig, omdat alle genetische relaties worden meegenomen via relatie matrixen. Door het simultaan trekken van alle model parameters worden onzekerheden meegenomen. Het gebruik van een gereduceerd diermodel maakt een analyse van complexe populaties mogelijk. Markov keten Monte Carlo algoritmes zijn toegepast om oplossingen te verkrijgen. De Bayesiaanse methode was succesvol in het vinden van QTLs in alle regio's die eerder zijn gevonden.

In hoofdstuk 7 is de Bayesiaanse methode nog verder uitgebreid om een bivariate analyse van lichaamsgewicht gemeten in beide experimenten mogelijk te maken. Het combineren van gegevens van beide experimenten zou het onderscheidingsvermogen om QTLs op te sporen en de schattingsnauwkeurigheid moeten verhogen. Voor elke geslacht-kenmerk combinatie zijn afzonderlijke residuele varianties en afzonderlijke schaal parameters voor polygene en QTL allelische effecten opgenomen. Verder is een polygene correlatie opgenomen. De lichaamsgewichten gemeten op 48 dagen zijn gebruikt om de methode te illustreren. Het QTL op chromosoom 1 voorheen gevonden in het voerefficiëntie experiment, maar niet in het karkas experiment, wordt nu in beide experimenten gevonden, hetgeen demonstreert dat het onderscheidingsvermogen inderdaad verhoogd is. De meest waarschijnlijke QTL locatie was echter in een verschillend merker interval voor beide experimenten.

Tenslotte is het aantal QTLs en het onderscheidingsvermogen van de experimentele opzet bediscussieerd in hoofdstuk 8. Verschillen tussen de regressie en Bayesiaanse methode worden genoemd en potentiële uitbreidingen voor beide methoden worden bediscussieerd. Met de regressie methode is een twee QTL analyse uitgevoerd om het onderscheidingsvermogen te vergroten en bootstrapping is gebruikt om betrouwbaarheidsintervallen voor de QTL positie te schatten. Voor de Bayesiaanse methode zijn de belangrijkste uitbreidingen om te implementeren het trekken van de QTL positie, het meenemen van gecorreleerde rest termen, hetgeen de bivariate analyse van kenmerken gemeten aan dezelfde individuen mogelijk maakt, en de mogelijkheid om imprinting mee te nemen.

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Abbreviation Key

ADL	Avian Disease and Oncology Laboratory, Michigan State University, East Lansing, United States of America
BW23	body weight at 23 days
BW48	body weight at 48 days
$BW48_{CA}$	body weight at 48 days in the carcass experiment
$BW48_{FE}$	body weight at 48 days in the feed efficiency experiment
CP	carcass percentage
CW	carcass weight
FE	percentage feed efficiency between 23 and 48 days
FF	G_2 females for which the average adjusted progeny trait values are based on G_3 female animals
FM	G_2 females for which the average adjusted progeny trait values are based on G_3 male animals
FIFA	feed intake in a fixed age interval
FIFW	feed intake in a fixed weight interval
G_0 etc.	Generation 0 etc.
GAIN	growth between 23 and 48 days
LEI	University of Leicester, Leicester, United Kingdom
LS	leg score
LS1	original leg score
LS2	transformed leg score
LS3	transformed leg score adjusted for BW48
MC	meat colour
MC1	meat colour unadjusted for BW48
MC2	meat colour adjusted for BW
MCW	Microsatellite chicken Wageningen
MF	G_2 males for which the average adjusted progeny trait values are based on G_3 female animals
MM	G_2 males for which the average adjusted progeny trait values are based on G_3 male animals
QTL	Quantitative Trait Locus

Abbreviation Key

UMA	University of Massachusetts, Amherst, United States of America
WAU	Wageningen University, Wageningen, The Netherlands

Curriculum Vitae

Johannes Baptist Cornelis Henricus Maria (Jan-Thijs) van Kaam werd op 11 oktober 1969 geboren te Breda en groeide op in Bavel. In 1988 behaalde hij het VWO diploma aan het Onze Lieve Vrouwe Lyceum en Havo te Breda. In 1992 behaalde hij het diploma Nederlandse Landbouw met als specialisatie Rundveehouderij aan de Agrarische Hogeschool van de K.N.B.T.B. te 's Hertogenbosch. In hetzelfde jaar begon hij met het doorstroomprogramma Zoötechniek met als studierichting Veefokkerij aan de toenmalige Landbouwuniversiteit te Wageningen. Vanaf 1993 begon hij ook met het doorstroomprogramma Economie van Landbouw en Milieu met als studierichting Agrarische Bedrijfseconomie aan de Landbouwuniversiteit. In augustus 1995 werden beide studies afgerond, de studie Zoötechniek met lof en scriptieprijs. Vanaf september 1995 was hij aangesteld als assistent in opleiding (AIO) bij de Vakgroep Veefokkerij (thans Leerstoelgroep Fokkerij en Genetica). Sinds 24 Juli 2000 is hij werkzaam als systeemontwikkelaar bij Coöperatieve Rundveeverbetering Delta (CR-Delta) in Arnhem.