QUANTITATIVE TRAIT LOCI MAPPING OF SEXUAL MATURITY TRAITS APPLIED TO CHICKEN BREEDING

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DEDICATION

I dedicate this thesis to my beloved children

DECLARATION

I declare that this thesis is my own composition and is an account of analyses performed by me whilst studying for the degree of Doctor of Philosophy at the University of Edinburgh.

Baitsi K. Podisi

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PEER REVIEWED PUBLICATIONS

Podisi B.K., Knott S.A, Dunn I.C., Law A.S., Burt D.W. & Hocking P.M. (2011) **Overlap of quantitative trait loci for early growth rate, and for body weight and age at onset of sexual maturity in chickens.** *Reproduction* **141,** 381–389. **(**Appendix**).**

ABSTRACT

Many phenotypes are controlled by factors which include the genes, the environment, interactions between genes and interaction between the genotypes and the environment. Great strides have been made to understand how these various factors affect traits of agricultural, medical and environmental importance. The chicken is regarded as a model organism whose study would not only assist efforts towards increased agricultural productivity but also provide insight into the genetic determination of traits with potential application in understanding human health and disease. Detection of genomic regions or loci responsible for controlling quantitative traits (QTL) in poultry has focussed mainly on growth and production traits with limited information on reproductive traits. Most of the reported results have used additive-dominance models which are easy to implement because they ignore epistatic gene action despite indications that it may be important for traits with low heritability and high heterosis. The thesis presents results on the detection of loci and genetic mechanisms involved in sexual maturity traits through modelling both additive-dominance gene actions and epistasis. The study was conducted on an F_2 broiler x White Leghorn layer cross for QTL detection for age, weight, abdominal fat, ovary weight, oviduct weight, comb weight, number of ovarian yellow follicles, a score for the persistence of the right oviduct and bone density. In addition, body weight QTL at 3, 6, 12, 24, 48 and 72 weeks of age, QTL for growth rate between the successive ages and QTL for the parameters of the growth curve were also detected. Most of the QTL for traits at sexual maturity acted additively. A few of the QTL explained a modest proportion of the phenotypic variation with most of the QTL explaining a small component of the cumulative proportion of the variation

explained by the QTL. Body weight QTL were critical in determining the attainment of puberty. The broiler allele had positive effects on weight at first egg and negative effects on age at first egg. Most QTL affecting weight at first egg overlapped with QTL for age at first egg and for early growth rate (6-9 weeks) suggesting that growth rate QTL are intimately related to the onset of puberty. Specific QTL for early and adult growth were detected but most QTL had varying influence on growth throughout life. Chromosome 4 harboured most of QTL for the assessed traits which explained the highest proportion of the phenotypic variation in the traits confirming its critical role in influencing traits of economic importance. There was no evidence for epistasis for almost all the studied traits. Evidence for role of epistasis was significant for ovary weight and suggestive for both growth rate and abdominal fat.

CHAPTER ONE

General Introduction

1.1 Background

Detection of Quantitative Trait Loci (QTL) is important due to their potential use in marker-assisted breeding programmes to speed up genetic progress and for selecting traits that are difficult or expensive to measure. Identifying loci with large effects on economic traits and animals bearing those alleles would assist in concentrating the frequency of the desired alleles in marker assisted selection (MAS) lines (Ikeobi *et al.* 2002; Abasht *et al.* 2006a). Complex sex limited traits like egg production and egg quality could benefit from the implementation of marker assisted selection (Schreiweis *et al.* 2006). The efficiency of MAS depends on both the efficiency of the detection of the QTL and the marker-based selection (Liu *et al.* 2003).

The application of marker assisted selection in livestock (which was primarily based on microsatellite markers) has been limited compared to the high expectations raised since the inception of the idea (Lande & Thompson 1990; Meuwissen & Goddard 1996; Dekkers 2004; Goddard & Hayes 2009). This concept has evolved from selection based on single markers or isolated genome regions to using information on the whole genome based on the use of dense single nucleotide polymorphisms (SNP) markers in what is now termed genomic selection (Meuwissen *et al.* 2001; Habier *et al.* 2009). This matter is explored in later sections of this thesis.

Although QTL mapping has been very successful in domestic animals, the identification of the Quantitative Trait Mutations (QTM) has been difficult

(Andersson 2009). QTL linkage mapping gives resolution only up to intervals of about 10 cM (~ 3 million base pairs in chickens) and association mapping has been proposed as a better alternative (Dodgson 2007). The availability of SNP markers which offer dense map coverage is leading to a shift towards genome-wide association studies as a powerful method for high resolution mapping (Andersson 2009). However, QTL linkage mapping remains useful in studying the biology of traits such as the critical mode of gene action involved in the inheritance and possible correlated responses to selection of traits (Andersson 2001, 2009).

1.1.1 QTL for key traits in poultry

QTL affecting body weight, muscling, fatness, disease resistance, behaviour, egg production, and bone traits have been reported in poultry (Hocking 2005; Sharman *et al.* 2007). Chromosome 4 has a critical region associated with a number of different traits (Schreiweis *et al.* 2006). More QTL for growth were reported than for other traits and different independent studies show some general consensus on QTL location for similar traits (Abasht *et al.* 2006). However, these authors noted that some wide confidence intervals (>20cM) were reported for QTL positions of most traits. Therefore, they recommended fine mapping to be done using higher marker density and advanced designs as well as tests for candidate genes.

1.1.2 Role of epistasis in quantitative traits

The detection of QTL and major genes controlling traits assumes marginal additive and dominance effects of the individual loci (Carlborg *et al.* 2004a; Alvarez-Castro & Carlborg 2007). However, there is increasing interest to understand the role of how non-additivity contributes to the expression of traits through interactions between loci typically known as epistasis (Carlborg & Haley 2004; Carlborg *et al.* 2004a; Alvarez-Castro & Carlborg 2007). Variation of a phenotype of a particular allele across different genetic backgrounds is suggestive of epistasis (Wade 2001). Epistasis and gene-by-environment interaction makes the process of finding and mapping genes complicated. Therefore, understanding aspects of epistasis can facilitate finding the genes behind complex traits.

The need for more studies to evaluate how much epistasis is segregating within natural populations has been highlighted (Carlborg *et al.* 2004a). Using large datasets of high quality phenotypic measurements, appropriate family structures and highly informative markers would provide high power to detect epistasis (Carlborg *et al.* 2004a). Earlier analysis of the Roslin broiler x layer cross examined growth, health and skeletal traits (Ikeobi *et al.* 2002; Sewalem *et al.* 2002; Ikeobi *et al.* 2004; Navarro *et al.* 2005b; Sharman *et al.* 2007).

Hocking (2005) identified a future need to conduct multi-trait analysis of broiler and egg production traits to explore genetic correlations between growth and reproductive traits and possibly the estimation of epistatic effects from combined and

therefore larger data sets. The current study is designed to address these particular needs.

This project conducts an analysis on a larger data set from different populations to those used in earlier studies (Sewalem *et al.* 2002; Carlborg *et al.* 2004a) by searching for QTL for adult growth and reproductive traits where epistasis is expected to occur because the heterosis expressed by reproductive traits is believed to be a result of non-additive action particularly epistasis (Williams *et al.* 2002).

1.1.3 Overview of production and genetic trends in the poultry industry

The most significant improvements in the poultry industry have been in the areas of: i) Environment control ii) Nutrition iii) Poultry health and iv) Genetics (McKay 2009). The Food and Agriculture Organisation (FAOSTAT, 2010) statistics show that chicken meat production has increased 10 fold since the 1960s up to the mid-2000s with egg production per chicken and carcase increases of about 30% each (Thornton 2010). The highest rates of genetic gain in the livestock industry have been achieved for chickens and pigs under industrialised production systems. It has also been forecasted that future improvements in breeding, nutrition and animal health will contribute further towards increasing future production and further efficiency and genetic gains under more challenging constraints imposed by environmental and animal welfare legislation (Thornton 2010).

The increasing demand for food due to the increasing human population and competition for land makes poultry production an important land intensive sector in producing protein for human consumption to meet future meat demand (Rothschild & Plastow 2008; Thornton 2010). Future livestock production is likely to get additional benefits from molecular genetics technologies such as genomic selection where it has been predicted that it would at least double the rates of genetic gain in dairy cattle (Hayes *et al.* 2009). The use of SNP chips in chicken is already under exploration however, the costs have been prohibitive for the uptake of this technology and further technology advances are anticipated to lower the costs of genotyping in the near future and make genomic selection profitable in poultry breeding (Andreescu *et al.* 2007; Gonzalez-Recio *et al.* 2009; Preisinger 2010). Feed and growth efficiency will become important as the cost of feed is expected to increase relative to the price of meat. The identification of genes responsible for the expression of traits of economic importance through linkage studies and other emerging technologies such as association studies is useful in identifying causal mutations affecting the phenotype of interest. Linkage mapping also contributes to the understanding of the architecture of traits that informs the design and implications of innovative breeding strategies such as the increasingly embraced genomic selection which will be combined with existing and other emerging technological tools to achieve production efficiency and optimal welfare objectives (Cheng 2010; Thornton 2010).

Impressive genetic progress has been achieved in poultry breeding through genetic selection to increase production and efficiency, but this progress has also resulted in negative consequences on other important traits (Decuypere *et al.* 2010). The classic

case is the intense selection for broiler traits that has led to the undesirable decline in correlated traits associated with fitness such as reproductive performance (Muir & Aggrey 2003; Decuypere *et al.* 2010). Similarly, selection for layers for high egg production has created birds which are susceptible to osteoporosis (Webster 2004). The existence of these marked differences between the specialized chicken breeds presents an opportunity to study the genetic mechanisms affecting the traits using crosses between broiler and layer lines (Andersson & Georges 2004; Andersson 2009).

1.1.4 Specific objectives

This research mainly focussed on the analysis of traits expressed at the critical physiological phase of the attainment of sexual maturity by addressing the following objectives:

To detect QTL and investigate the role of epistasis for the following traits:

- Body weight, growth rate and growth curve parameter estimates (Chapter 4)
- Body weight and age at first egg (Chapter 5).
- Reproductive traits (ovarian traits and comb weight) (Chapter 6).
- Bone mineral density at sexual maturity and at slaughter age (Chapter 7)

The following sections in Chapter 2 give a brief overview of the concepts and issues highlighted in the literature on QTL analysis (with a bias towards poultry). This is followed by a generic description of methods that are repeated across the experiments and the resource populations in Chapter 3. The thesis presents findings

of data analysis of QTL for age at first egg (AFE) and weight at first egg (WFE), ovary weight (ORW), oviduct weight (OVW), number of normal yellow follicles (NYF), comb weight (CBW), score for the persistence of the right oviduct (ROS), abdominal fat weight at first egg (AFW), bone mineral density (BMD) at first egg, BMD at 72 weeks of age, and body weight at: 3, 6, 12, 24, 48 and 72 weeks of age. QTL for the growth rate at the respective age intervals of 3-6, 6-12, 12-24, 24-48 and 48-72 weeks of age, and parameters of the growth curve are also included. The above traits are conveniently organised into four chapters as named above. Finally, brief chapter summaries and a general discussion of main findings plus cross cutting issues as well research gaps are presented in Chapter 8.

CHAPTER TWO

Overview of QTL detection methodologies

2.1 QTL mapping challenges

Mapping QTL would help to identify genes controlling specific traits, contribute towards understanding of the architecture of quantitative traits and how QTL results can best be utilised in breeding programs (Abasht *et al.* 2006a). However, the lack of ability to determine which genes are responsible for the variation in complex quantitative traits has been a major constraint due to limitations in technology and the associated costs. Furthermore, for those subsets of genes that were identified, they only explained a limited proportion of the observed variation associated with the trait (Doerge 2002).

Issues related to sample size, statistical design and modelling, multiple testing as well as statistical significance are among the statistical challenges hampering QTL mapping (Doerge 2002). Complex traits are mostly controlled by many genes (Wade 2001; Hill *et al.* 2008b) and sample size limits the sensitivity of methods to detect genes affecting polygenic traits. As a result QTL analysis methods favour detecting factors of large rather than small effect (Wade 2001).

Liu *et al.* (2003) advised that MAS should target QTL with small effects to be able to surpass traditional breeding, cautioned against the use of small population sizes, which tend to overestimate QTL effects, and called for the use of stringent criteria in order to reduce the detection of false positives. Using small population sizes of about

100 progeny provides poor detection of QTL with small effects and inflates the estimated effects, a phenomenon referred to as the Beavis effect (Xu 2003).

2.2 QTL detection approaches

There has been a rapid increase in the development of QTL mapping methodologies since Lander & Botstein (1989) published their pioneering work on interval mapping using the maximum likelihood approach to map a QTL lying within a chromosome region surrounded by two markers (Ma *et al.* 2002; 2006). Haley and Knott (1992) developed a regression approach which approximated the interval mapping method but with far less computational demand (DiPetrillo & Zou 2009). Interval mapping was improved by Jansen & Stam (1994) and Zeng (1994) to have higher QTL detection power by using markers from other intervals as covariates to minimise the residual error and this technique was re-named composite interval mapping (Ma *et al.* 2002). Kao *et al.*(1999) proposed the use of multiple marker intervals simultaneously to map epistatic QTL.

Zhang *et al.,* (2008) categorized QTL detection approaches into three main groups i.e. i) maximum likelihood (ML) methods (Lander & Botstein 1989; Zeng 1994; Kao *et al.* 1999), ii) regression (Haley & Knott 1992; Martínez & Curnow 1992; Haley *et al.* 1994; Feenstra *et al.* 2006) and iii) Bayesian modelling (Sen & Churchill 2001; Yi *et al.* 2005; Yi *et al.* 2006; Yandell *et al.* 2007). After comparing maximum likelihood and regression interval mapping Kao *et al*., (1999) concluded that regression methods are faster in computation especially when a large number of QTL

in the model is being considered. In general there is little difference in the outcome of using either ML or regression methods (Haley & Knott 1992; Kao 2000). DiPetrillo & Zou (2009) observed that Bayesian methods are also popular for multiple QTL mapping due to its flexibility in handling a large number of QTL, missing data and prior information but have the disadvantages in computing efficiency and repeatability of mapping results (Yi & Shriner 2007; DiPetrillo & Zou 2009; Wei *et al.* 2010b).

2.3 QTL experimental designs

Two general approaches are used in the identification and mapping of QTL: those based on crosses between lines that differ for the trait of interest, and approaches that are based on segregating populations (Falconer and MacKay, 1996). For microsatellite-based QTL studies, the key aspect is to be able to track markers from the parent to the progeny, usually in large half or full-sib families. Lines crosses, F_2, etc are methods aimed at increasing the probabilities of segregating QTL. The following description mainly highlights the popular designs used in poultry QTL studies.

The F_2, backcross (BC) and F_1 (i.e. half-sib and full-sib) experimental designs have been used in chicken QTL detection studies (Abasht *et al.* 2006a). Divergent populations are usually crossed to produce the first generation. One of the F_1 is then back-crossed to the parental lines in the second generation in the BC design, while in the F_2 design the F_1 are intercrossed and phenotypic information from the second

generation is used for QTL mapping. A third generation may be produced from intercrossing the second generation in an F_2-F_3 design and the progeny in the third generation are assessed for phenotypic traits. For species with long generation intervals it can be expensive and time consuming to develop experimental crosses such as the BC and F_2 and alternatively F_1 designs such as half-sib and full-sib designs can be used (de Koning *et al.* 2003a). The use of the half-sib design is particularly popular in dairy cattle where half-sib data is readily available due to the extensive use of artificial insemination (A.I.) bulls. In a half-sib design genotype data is collected from the grandsires and the half-sib offspring and phenotype is collected from the half-sibs themselves or from progeny of the half-sibs (de Koning *et al.* 2003a). In the full-sib design genotype data is needed for both parents and their full-sib offspring. The F_2 is the most popular design used in chicken QTL studies (Abasht *et al.* 2006a) where typically the F_2 is generated from a cross between two lines to produce 250 to 700 birds (Hocking 2005).

The above methods have the advantage of generating maximum linkage disequilibrium (LD) enabling the use of relatively few markers to detect QTL but give low resolution in the position of the QTL. Therefore, the advanced backcross (AB) and advanced intercross line (AIL) strategies are used for high resolution mapping (Abasht *et al.* 2006a). In the AB design, individuals that are carriers of recombinant chromosomes are identified in the BC population and are progeny tested. In the advanced AIL design, the intercrossing of the F_2 generation is done for several generations to accumulate recombinations leading to greater precision in

linkage mapping (Song *et al.* 1999). It requires the use of inbred lines to start it otherwise all generations will have to be genotyped.

2.4 Detection of epistatic QTL

In QTL analyses the modelling of additive/dominance effects is frequently used and recently, methods for mapping epistatic effects have been proposed (Carlborg *et al.* 2003). A significant role of epistasis with the largest impact on early growth (before 6 weeks of age) has been reported in chickens (Carlborg *et al.* 2004a). Neglecting the role of epistasis in MAS programmes when it is present leads to considerable loss in genetic gain (Liu *et al.* 2003). Existing data and theory generally show that additive genetic variance explains much of the variation in complex traits partly because some the epistatic variance could appear as additive genetic variance Hill *et al* (2008a).

Modelling epistasis is challenging (Carlborg & Haley 2004; Ankra-Badu *et al.* 2010) and Wei *et al.*, (2010b) categorised the research issues related to modelling epistasis into the following fives themes: i) statistical modelling, ii) search algorithm, iii) model parameterization, iv) multiple testing and v) computing efficiency. Some of the available analysis packages have deficiencies which may contribute to the detection of false positive results (Hill *et al.* 2008a; Wei *et al.* 2010b). Hill *et al*., (2008a) observed that reported evidence for epistasis in many QTL studies tend to be over-estimated due to failure to account for multiple testing.

The availability of large numbers of single nucleotide polymorphisms (SNP), sequence data, new bioinformatics tools and the use of an integrated approach of all fields of genomics (quantitative genetics, whole genome sequencing, functional genomics, transcriptomics and proteomics) also presents an opportunity to overcome limitations in the methodologies of detecting epistasis and QTL in general (Doerge 2002; Carlborg & Haley 2004; Abasht *et al.* 2006a). The rapid advances in both computing and DNA technologies has generated significant effort towards developing methods and software tools for detecting epistasis (Chase *et al.* 1997; Kao *et al.* 1999; Carlborg *et al.* 2000; Jannink & Jansen 2001; Broman *et al.* 2003; Carlborg & Haley 2004; Wu *et al.* 2004; Hanlon & Lorenz 2005; Malmberg & Mauricio 2005; Zeng *et al.* 2005; Alvarez-Castro & Carlborg 2007; Yang *et al.* 2007). New methods and technologies for improving the chance of detecting epistatic QTL have been proposed that employ simultaneous scans and randomization tests to detect QTL that do not have individual effects (Carlborg *et al.* 2003; Carlborg & Haley 2004).

Analysis of the commonly used F_2 population design is unable to detect epistasis among closely linked QTL because of its limited mapping resolution (Abasht *et al.* 2006a). Carlborg & Haley (2004) recommended the use of good quality data from a minimum of 500 F_2 individuals to be able to detect epistasis. It has been demonstrated that a large F_2 population of over 1000 individuals has enough power to detect weak epistasis signals (Wei *et al.* 2010a; Wei *et al.* 2010b).

Large data sets are required to study epistasis to accommodate the complexity of the models and the associated multiple tests (Carlborg & Haley 2004; Phillips 2008; Wei *et al.* 2010a). Fitting complex models with additional terms such as for high order interactions creates a dimensionality problem due to the resulting large search space that requires efficient search algorithms (Carlborg *et al.* 2000; Ljungberg *et al.* 2004; Yi & Shriner 2007) and computing power which can now be realized through grid computing (Seaton *et al.* 2006). An epistasis module which uses the regression interval mapping approach (Haley & Knott 1992) and an efficient search algorithm has been developed and runs on a grid of computers for efficient computation. This GridQTL epistasis module uses a nested test framework to control false positive rates and uses the regression approach that to enable easy replication and interpretation of the results (Seaton *et al.* 2006; Wei *et al.* 2010b). The GridQTL epistasis module was adopted in this study. This module conducts automated detection of QTL with significant marginal effects and it makes use of the pre-identified QTL in the detection of epistatic QTL pairs. The models and methodology of the GridQTL epistasis module are briefly explained in Chapter 3.

CHAPTER THREE

DESCRIPTION OF RESOURCE POPULATIONS AND DATA COLLECTION

3.1 Introduction

The F_2 resource populations used in this study were created by crossing a layer line with a small body size and a sire line of broiler parent stock with a large body size as described in detail by Sewalem *et al.,* (2002) and summarised below.

3.2 Resource Population

3.2.1 Parent lines for GM7 and GM9 populations

To represent the parental lines, a total of 25 male and female chicks per line were kept under similar conditions to those described below for the F_2 flock. One-day-old female chicks were obtained from the Ross 308 male line broiler (Aviagen, Newbridge, UK) and a White Leghorn egg laying line maintained at the Roslin Institute. The chicks were brooded and reared under conventional husbandry practices in floor pens. At 12 weeks of age, 12 birds from each line were randomly allocated to individual cages to record phenotypic data on the age and weight at the onset of lay as described below. However for the broilers, only 10 birds that survived were used.

3.2.2 The production of the F_2 population

The GM7 and GM9 F_2 populations used in the study were each produced from the mating of a commercial broiler male line to a White Leghorn egg layer line (in the grandparental generation) to produce the F_1 generation as described previously

(Sewalem et al. 2002). This was done by mating in balanced manner 2 males and 2 females from each line to the 2 males and 2 females from the other line to create the 4 F_1 families. Eight males and 32 females of the F_1 generation were then randomly selected and mated in a balanced mating scheme to produce F_2 birds (Sewalem et al. 2002). One female died and was replaced making a total of 33 full sib families.

3.3 Management of animals

The female birds were reared in floor pens and moved to individual cages that measured 40 cm wide x 45 cm deep x 80 cm high at 12 weeks of age. The birds were fed *ad libitum* on a conventional poultry diet. The birds were exposed to a constant photoperiod of 14 hours per day from hatch to the end of the experiment.

3.4 Data Collection

3.4.1 Phenotypic data

A batch of about 500 female chicks from 9 hatches representing 32 families (herein referred to as GM7 F_2 population) was raised from hatching up to 72 weeks of age. The individual birds' body weight was recorded at 3, 6, 12, 24, 48 and 72 weeks of age (when they were slaughtered). Bone density, comb weight and ovarian traits were recorded at 72 weeks of age.

A second flock (GM9) of about 500 F_2 birds produced from the same GM7families but from entirely different hatches (11) representing 32 families were only raised to

the onset of lay when half of them were killed. Onset of lay was defined as the day of first recorded oviposition. Similarly, body weights at 3, 6 and 12 weeks of age were recorded in the GM9 birds. Bone mineral density and ovarian traits (reproductive traits) were measured as detailed below and in Chapter 6 respectively.

3.4.1.1 Data collection for bone density

The bone mineral density was measured as described by Hocking *et al*., (2003). The right wing was thawed and the humerus was dissected from adhering tissue including tendons and ligaments. The bone was radiographed on a lateral plane alongside a calibration aluminium step-wedge. The density of the image of the whole bone was then compared to the density of the wedge to determine the bone density defined as the density equivalent of a specified depth (mm) of aluminium using NIH-image analysis software (http://rsb.info.nih.gov/nih-image/).

3.4.2 Genotyping of GM7 & GM9 populations

Fresh blood samples were collected for DNA extraction and genotyping by superficial venipuncture of each bird's wing vein at 12 weeks of age after caging. DNA was extracted from the sample and genotyped using a Robot (Pakard) and the Quiagen PCR Mix Kit (p/n 206145) to standardize procedures and minimize pipetting errors as described in the Roslin Institute SOP IGF 136.00 version 1 of 04 Nov.10 (summarized below). Up to 143 microsatellites markers covering 25 to 26

autosomal linkage groups and the Z sex chromosome (Table 3.1) were genotyped in a total reaction volume of 8µl per well.

Using the Packard Robot (ARK Genomics, Lab 620) program (David 384/David 96 to 384), a volume of 3.0µl of 5.0ng/µl stock DNA (template) was added to each well of a 384 well plate (Abgene p/n AB-1111). When four kits of Primers were being used, sample 1 of the DNA was added to wells A1, B1, A2, B2 and the second sample 2 would be added to wells C1, D1, C2, D2 etc. The plates were dried down either overnight at room temperature or on a PCR block at 60^{o}C (until dry). The plate was stored for up to one week in a fridge at 4^{o}C prior to making a Master Mix step. For wet DNA the plates were used immediately (i.e. not dried down).

To make a solution for 1 x 8 µl reaction a volume of 3.75 µl Quiagen PCR mix, 0.75 primer mix and 3.50µl or 0.5µl for wet DNA were mixed together. The appropriate Master Mix was added to the DNA plate using the Packard Robot using the program setting: David 384 /David Master Mix. The plate was sealed with adhesive plastic film (Abgene AB-0558) and spun down at 1000 rpm for 10 seconds. DNA that had been dried down was re-suspended by shaking it vigorously for 30 minutes on a plate shaker and the wet DNA was shaken for 5 minutes. This was followed by spinning down the sample at 1000 rpm for 10 seconds. The PCR was done using GeneMap/QIA35CYC program. PCR plates were stored at –20^{o}C until required.

Table 3.1 The number of microsatellite markers, first and last marker and map length on each linkage chromosome in the QTL analysis of body weight at different ages, age at first egg, growth rate, Gompertz parameters and bone density (GM7 only).

Chromosome	Number of markers	First marker	Last marker	Map length (cM)
1	17	ROS0008	MCW0107	548
2	13	LEI0163	MCW0157	473
3	15	MCW0169	MCW0037	286
4	4	ADL0317	MCW0180	195
5	5	ROS0013	ADL0298	119
6	4	ADL0323	ADL0142	113
7	3	LEI0064	ADL0180	109
8	9	ROS0021	ROS0075	92
9	4	ROS0078	MCW0134	132
10	1	ADL0209.2	ADL0209	-
11	5	LEI0110	ROS00112	71
12	2	ADL0240	ADL0044	34
13	2	MCW0340	ADL0225	68
14	1	MCW0123	MCW0123	-
15	2	LEI0083	MCW0080	49
16	1	LEI0258	LEI0258	-
17	1	ADL0199	ADL0199	-
18	2	ROS0022	ROS0027	24
19	1	MCW0094	MCW0094	-
22	1	ROS0073	ROS0073	-
23	1	MCW0249	MCW0249	-
26	2	ADL0285	LEI0074	-
27	1	ROS0071	ROS0071	-
28	3	ROS0095	ADL0299	39
z	6	ROS0072	LEI0075	127
Total	**106**			**2479**

Compatible sets of 4 to 10 markers were organized based on the fragment size and dye colour of the PCR product. Fluorescent microsatellite detection was performed on Applied Biosystems 3730xl genetic analyzer (Applied Biosystems/Hitachi, Applera, USA) and the Foundation Data Collection v3.0 was used to collect the data generated. Genemapper Software v3.5 (Applied Biosystems, Applera, USA) was used to estimate fragment sizes by comparing them to the LIZ500 as an internal size standard.

3.5 Data management preparation and cleaning

All pedigree, marker genotypes and recorded traits were stored in the resSpecies database (Law & Archibald 2000). The data was exported from resSpecies database and prepared for QTL analysis by creating 3 analysis files named the genotype, map and phenotype files.

The map files were created through CRIMAP program (Green *et al.* 1990) and confirmed by comparing it to other maps created by other researchers who used the same resource population (Navarro *et al.* 2005b). When the files were uploaded into the QTL analysis tool (QTL Express or GridQTL) some warning or in some instances fatal errors due to genotyping errors in the data were reported. The errors were investigated and corrected using a Roslin Institute developed tool called RTools (personal communication, Dr Ricardo Pong-Wong, 2007).

3.5.1 Genotype reconstruction using RTools

To investigate any errors on the genotype files through RTools software required the creation of two files: a pedigree file and a marker file. The program would look through the pedigree to verify the genotypes of individuals given the genotypes of the parents and other relatives. Finally any suspicious genotype would be highlighted and for those with missing genotypes where there was complete certainty imputation was done based on the existing information on close relatives. In two cases, where the genotype could not be resolved the implicated alleles was set to missing. The

reconstructed files were then used for QTL analyses. To resolve any QTL analysis warnings such as for lack of corresponding genotype or phenotype, some individuals having no corresponding data points in the genotype or phenotype files or vice versa were deleted from the file.

3.6 Creating linkage maps using CRIMAP program

The Flips command option of the CRIMAP program (Green *et al.* 1990) was used to determine the marker order and the Fixed command was used to estimate the Kosambi genetic distances between the markers in centi-Morgans (cM). The 2005 consensus genetic linkage map in ArkDB (Hu et al. 2001) was used to verify the maps generated through the CRIMAP program (Green *et al.* 1990).

3.7 Execution of the epistastic QTL analysis on GridQTL

The interval mapping method (Haley *et al.* 1994) for QTL analysis was conducted using a newly developed module for epistasis analysis in GridQTL (Seaton *et al.* 2006) to detect significant QTL with an additive/dominance model and to detect epistatic QTL pairs with an epistasis model (Wei *et al*.(2009) (described below). The programme initially conducts the standard processes of QTL searching, testing, permutation and bootstrapping for a single-QTL F_2 analysis.

Mapping and significance testing for epistatic QTL was conducted by the interval mapping method (Haley *et al.* 1994) for QTL analysis which has been adapted for

epistasis detection in the epistasis analysis module in GridQTL (Seaton *et al.* 2006; Wei et al. 2009). The mapping procedure detailed in Wei et al. (2009) comprised four key elements summarised below.

3.7.1 Identification of main effect QTL under an additive-dominance model

The first element involved the standard processes of conducting QTL searching, testing, permutation and bootstrapping for a single-QTL F_2 analysis by fitting an additive-dominance model following Haley et al. (1994). Whole genome scans were conducted iteratively using forward selection of significant QTL for each trait (Carlborg *et al.* 2004a). The probabilities of the parent of origin of each gamete based on the marker genotypes were calculated at 1 cM intervals throughout the genome. Under the assumption that the QTL were fixed for alternate alleles in the broiler and layer lines, coefficients of additive and dominance components for putative QTL at each position were calculated from the conditional probabilities given the marker genotypes. The trait data were then regressed against the coefficients and an F-test to determine association was conducted at 1 cM intervals (Haley et al. 1994; Jacobsson et al. 2005). Exhaustive QTL searches performed at 1 cM intervals with an updated model were implemented by fitting the suggestive and significant QTL as co-factors (Jansen 1994; Zeng 1994) until no additional significant QTL were detected.

3.7.2 Determination of significance thresholds under the additive – dominance model

Significance thresholds for detection of single QTL with significant marginal effects were determined through 5000 permutations (Churchill & Doerge 1994) and 5000 bootstraps were used to generate 95% confidence intervals for the QTL positions (Visscher *et al.* 1996). An F value greater than the P≤0.05 and P≤0.01 experiment-wide threshold values respectively were used to identify a significant and highly significant QTL (Kruglyak & Lander 1995). Alternatively QTL that achieved an F ratio exceeding the P≤0.05 chromosome-wide threshold were considered to be suggestive. The genome-wide level thresholds of highly significant, significant and suggestive mean that there is a probability to make 0.01, 0.05 and 1 false positive(s) respectively per genome scan (Lander & Kruglyak 1995).

3.7.3 Using two approaches to identify epistatic QTL

The second element entails using two approaches. In the first search approach, one dimensional (1D) exhaustive genome scans were conducted to search for interactions between the pre-identified QTL with significant marginal effects and all other genomic positions at 1cM intervals. In the second approach, two dimensional (2D) exhaustive genome searches were carried out for all combinations of two locations across the genome regardless of any pre-identified marginal effect QTL.

3.7.4 Modified nested test framework to detect epistasis

Significance testing for epistatic pairs in both the 1D and 2D search approaches used F ratio tests for model comparisons in a nested test framework. Following Wei *et al.* (2009) the detection of epistasis in an F_2 population was based on pairwise interactions (Model 1) unlike in the previous sections under the additive-dominance model (Model 2) where epistasis was ignored. The explanations of the tests are detailed in Wei *et al. (2010a; 2010b)* and are based on comparisons of four models.

Model 1 (With epistasis): Trait = μ + Locus A + Locus B + Locus A*Locus B + *e*

Model 2 (No epistasis): Trait = μ + Locus A + Locus B + *e*

Model 3 (Single locus model): Trait = μ + Locus A + *e*

Model 4 (Null model): Trait = μ + *e*

where trait is the phenotype of interest, μ is the model constant, and *e* is the random error.

An overall F test termed F_{all} compares Model 1 to Model 4 and QTL pairs that pass the F_{all} are then subjected to an interaction test denoted as F_{int} in a comparison of Model 1 vs Model 2. To ensure that the aggregate effect of a pair of loci which involved a marginal-effect QTL explained significantly more of the phenotypic variance than the marginal QTL alone, an overall test was conducted by comparing Model 1 vs Model 3.

The additive (a) and dominance (d) genetic effects in the interaction term (i.e. Locus A * Locus B in Model 1) was partitioned into four components (additive x additive, dominance x dominance, additive x dominance, dominance x additive) following

Jana (1971). A significant outcome in the overall test of the marginal effects and all two-way interactions between the additive and dominance effects at two loci, and of this test compared to the two-locus model with no inter-locus interactions, is indicative of epistasis.

3.7.5 Deriving genome wide thresholds under epistasis

Genome-wide thresholds were derived in advance for both the 1D and 2D scans through permutations based on 1000 replications. The DIviding RECTangle (DIRECT) algorithm (Ljungberg *et al.* 2004) was used to perform fast two dimensional genome scans in permutations to derive genome-wide thresholds for the 2D search (Wei *et al.* 2009). In the 1D scan, exhaustive genome scans were performed on permuted data to derive thresholds for each pre-identified marginal effect QTL. The derived 5% genome-wide thresholds were corrected for multiple testing associated with the presence of any additional marginal-effect QTL. For example, the epistatic pairs detected in 1D scan at 5 % genome-wide threshold were only regarded as genome-wide significant if they exceeded corrected thresholds otherwise they were treated as suggestive.

3.7.6 Fitted model effects

Different models with additive, dominance and parent-of-origin genetic effects with family and pen as fixed effects (hatch was confounded with pen) were evaluated in a preliminary analysis. There was no evidence for a parent-of-origin effect (detected as

a difference between the alternative heterozygous genotypes that differ in which allele was inherited from each parent) (Knott *et al.* 1998), which was not considered in subsequent analyses in this study. Additive genetic effects were calculated as half the difference between the broiler and layer homozygotes and dominance effects as the difference between the heterozygote and the mean of the two homozygotes (Falconer & Mackay 1996). A positive additive effect indicates that the QTL allele originating from the broiler line increased the trait value relative to that from the layer line. The Z chromosome was analysed with an additive genetic effects model for the detection of QTL with significant marginal effects. The epistasis analysis did not include the Z chromosome.

3.7.7 Proportion of phenotypic variance explained by QTL

Under the additive-dominance model the proportion of the F_2 phenotypic variance explained by the QTL was calculated as: percentage variance = ((RRMS – FRMS)/RRMS) x 100 where RRMS is the residual mean square from the reduced model in which all the effects including background QTL effects are fitted but the QTL is omitted. The FRMS is the residual mean square from the full model in which all the effects and QTL are fitted.

The proportion of the phenotypic variance of a trait explained by a QTL or an epistatic pair under each model was calculated by contrasting the residual variance when the QTL or an epistatic pair was included in the model compared with the model without the QTL or epistatic pair when other factors including the covariates

were fitted as cofactors (Wei et al. 2010a). The proportion of variance explained by the epistatic QTL pair was calculated as (Residual Mean Squares (RMS) for Model 4 – RMS for Model 1)/Total-variance where the co-factors and covariates are fitted. Similarly, the interaction component of a QTL pair was calculated as (Model 2 RMS – Model 1RMS)/total-variance (Wei et al. 2010a)

CHAPTER 4

GROWTH QTL

4.1 Introduction

4.1.1 Importance of growth curves

A longitudinal trait refers to a quantitative trait whose phenotypic value changes over time (Yang *et al.* 2006). One of the ways in which these traits can be analysed to identify QTL is to fit a growth curve to the phenotypic values across the different points and analyse the fitted parameters of the growth trajectory as the input phenotype (Wu *et al.* 2002; Wu *et al.* 2004; Yang *et al.* 2006). Numerous equations have been developed to describe the sigmoid curve depicting the relationship between growth and time (Grossman & Bohren 1985; Aggrey 2002a; Roush *et al.* 2006). The need to understand growth patterns in chickens is important because it can allow manipulating the variables of the curves such that animals with optimum growth are selected in breeding programmes (Grossman & Bohren 1985; Barbato 1991; Mignon-Grasteau 1999). The poor reproductive performance and obesity resulting from genetic manipulation of early growth could be addressed through breeding by modifying the shape of the growth curve to achieve optimal early growth and protein accretion while restricting later growth and fat deposition (Barbato 1991).

4.1.2 Properties of growth curve equations

The growth curve in chickens has some prominent features namely: an accelerating growth phase from hatching, a point of inflection reflecting a point where the rate of

growth reaches its maximum, a stage where growth rate is declining, and an asymptote where the maximum mature weight is reached (Roush and Branton, 2005).

Table 4.1 Some of the growth functions used for modelling growth in chickens

Equation/Model	Formula[1]	Source[3]
1. Logistic	$W = \frac{a}{1 + \exp(-b(t-c))}$	1
2. Gompertz	W = a *exp(-exp(-b(t-c)))	1
3. Laird-Gompertz	$W_t = W_0 \exp[(L/K)(1 - \exp - Kt)]$	2
4. Richards	$W = a(1 + (b - 1)\exp(-c(t-d)))^{1/(1-b)}$	1
5. Von Bertalanffy	$W = a\left(1 - \frac{1}{3}exp(-b(t-c))\right)^3$	1
6. Weibull	$W = a - (a - b)\exp\left(-\frac{c-1}{c}\left(\frac{t}{d}\right)^c\right)$	1
7. Narushin-Takma†	$W = (at^3 + bt^2 + ct + d)/(t^2 - et + f)$	1
8. Morgan-Mecer-Flodin	$W = a - (a - b)\exp\left(1 + \frac{c-1}{c+1}\left(\frac{t}{d}\right)^c\right)^{-1}$	1
9. Lopez (Morgan)§	$W = \frac{W_0 K^n + W_f t^n}{K^n + t^n}$	3

[1] In equations 1-8, a, b, c, d, e and f are coefficients of proportionality, t = time

†Time in weeks for equation 7

§ K and n are dimensionless and positive

[4] References: 1= (Narushin & Takma 2003), 2= (Aggrey 2002a), 3 = (Lopez *et al.* 2000).

The Gompertz and logistic curves have similar properties and are used to model growth (Winsor 1932) (Table 4.1). The growth curves fall into two groups depending on the number of parameters used in the equation (Narushin & Takma 2003). The most popular group of growth equations have three parameters (like the logistic, Gompertz and von Bertalanffy curves) while the second group has four parameters (like the Richards, Weibull and Morgan-Mercer-Flodin) (Table 4.1). The Morgan

function or Morgan-Mecer-Flodin function is also referred to as the Lopez equation because Lopez *et al* (2000) generalized the Michaelis-Menten equation to estimate growth but Morgan *et al., (1975)* proposed earlier to use the Michaelis-Menten equation as a response function (Darmani Kuhi *et al.* 2010). Improvements to the above equations have been proposed by adding or reducing coefficients and Narushin & Takma (2003) also deduced a model consisting of six coefficients of proportionality. Alternatively, growth functions can be classified into three categories; namely (i) sigmoid curves with a fixed point of inflection (e.g., Gompertz, logistic) or (ii) sigmoid curves with a flexible point of inflection (e.g., von Bertalanffy, Richards, Lopez/Morgan, Weibull) and lastly, (iii) curves that exhibit diminishing returns behaviour (e.g., monomolecular, exponential with a cut-off) (Darmani Kuhi *et al.* 2010).

4.1.3 Suitability and accuracy of growth equations

From the analysis of data from a flock of Shaver White layer breed, Narushin & Takma (2003) ranked these models in the following descending order in terms of accuracy. For describing growth the ordered list include; the Weibull model, the Gompertz, the von Bertalanffy, the Morgan-Mercer-Flodin, the logistic and Richards functions. However, Darmani Kuhi et al. (2010) concluded that the Lopez equation is appropriate for describing growth versus age in poultry and pigs because as a four parameter equation it has a flexible inflection point and therefore fits growth data better compared to three parameter equations like the Gompertz and logistic which are limited by having a fixed point of inflection.

Based on the coefficient of determination and the final loss function (defined as the observed minus the predicted in the second power), the Narushin-Takma model gave the most accurate prediction of growth compared to the traditional models (Narushin & Takma 2003). The Narushin-Takma model fits marginally better than the other curves because it also has more parameters. Darmani Kuhi (2003) recommended the Richards equation over the Gompertz curve for fitting growth data especially for female chickens based on the residual sum of squares. However, based on the r^2 the differences between the models are negligible. A later review of growth functions in poultry by Darmni Kuhi *et al.,* (2010) reached similar conclusions that a fixed point of inflection can be a limitation with equations such the Gompertz and logistic suggested a preference for four parameter equations. However, a case by case consideration was advised because such four parameter equations such as the Richards can have optimization problems.

Despite the highlighted deficiency of the Gompertz equation, the gains from using alternative equations appear to be minor, and it is widely used on chickens and other species (Barbato 1991; Knizetova *et al.* 1991; Mignon-Grasteau 1999; Aggrey 2002a; Wang & Zuidhof 2004; Norris *et al.* 2007; Koncagul & Cadirci 2010). The Laird form of the Gompertz curve has been suggested as the model of choice in fitting growth curves to chicken data (Table 4.1) (Aggrey 2002b; Norris *et al.* 2007; Koncagul & Cadirci 2010). The Laird-Gompertz equation is a function of initial body weight and inflection point compared to the original Gompertz which is a function of the mature body weight (Koncagul & Cadirci 2010). It has been

suggested that imposing constraints on the initial weight can improve the performance of the Laird-Gompertz function (Mignon-Grasteau 1999). Broiler data analyzed with and without restricting the initial body weight showed that the use of the Laird-Gompertz model without any restriction on initial body weight will avoid parameter overestimation (Koncagul & Cadirci 2010). The same study also observed that the curve parameters are affected by environmental factors (Koncagul & Cadirci 2010). The Gompertz also reasonably describes the increase in embryo mass through hatching or birth (Ricklefs 2010).

4.1.4 Detection of QTL influencing growth curve parameters

Combining growth models with QTL mapping facilitates the understanding of the genetics underlying physiological aspects of quantitative traits (Wu *et al.* 2002). Detection of QTL influencing the parameters of the growth curve has been done for a number of species including mice (Long *et al.* 2006), sheep (Hadjipavlou & Bishop 2009), dairy cattle (Rodriguez-Zas *et al.* 2002; Lund *et al.* 2008) and pigs (Varona *et al.* 2005). Using different growth models gives similar QTL detection results provided the chosen growth functions fit the data satisfactorily (Wu *et al.* 2002). Interactions between QTL affecting Gompertz growth curve parameters (epistasis) has also been reported in chickens (Le Rouzic *et al.* 2008). Understanding the biology of the model parameters and their relationships assists in developing a breeding strategy to modify the shape of the curve. It has been demonstrated that parameters of the growth curve are heritable and that the curve can be modified through selection on bodyweight at different ages (Grossman & Bohren 1985;

Mignon-Grasteau 1999; Mignon-Grasteau *et al.* 2001). N'Dri *et al*., (2006) suggested that growth curve parameters and abdominal fatness can be used to indirectly select for feed conversion.

Most body weight QTL mapping studies concentrated on early growth (Sewalem *et al.* 2002; Carlborg *et al.* 2004a; Ambo *et al.* 2009; Terčič *et al.* 2009; Uemoto *et al.* 2009) and few reported on older ages beyond 40 weeks of age (Gao *et al.* 2006; Le Rouzic *et al.* 2008). Several studies have estimated chicken growth curve parameters but there is scant information on QTL for the growth parameter estimates. A study was therefore conducted to detect growth QTL and QTL influencing parameters of the growth curve for chickens aged 3 - 72 weeks of age.

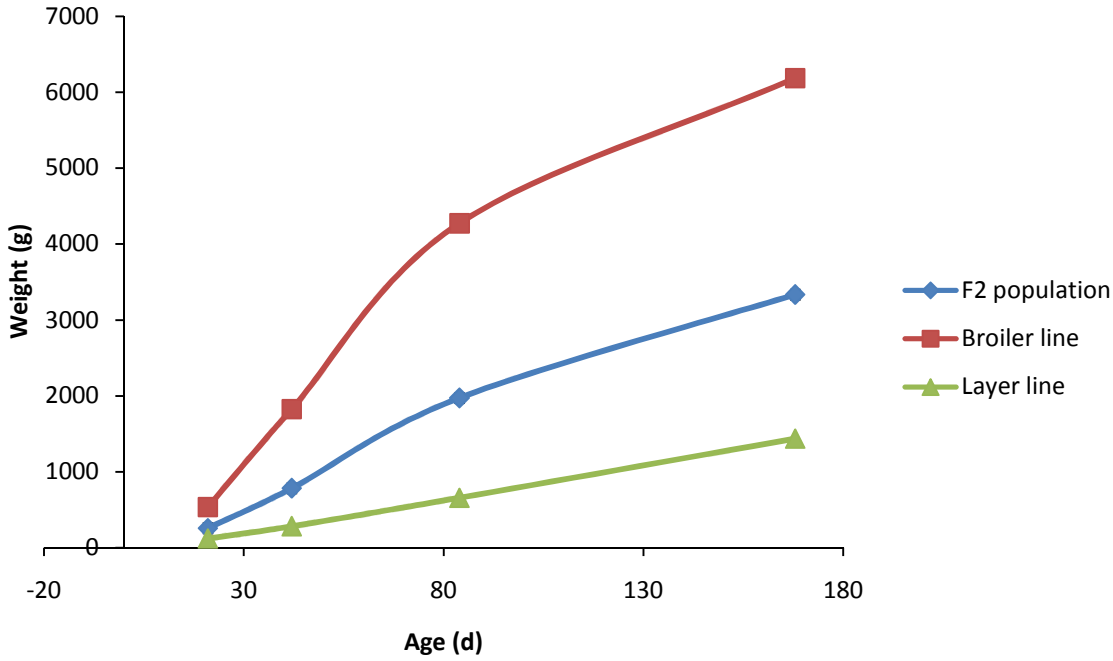
4.2 Materials and Methods

An F_2 study population used in the study had a total of about 500 female individuals from 32 families. The live weights of the birds were recorded at 3, 6, 12, 24, 48, 72 weeks of age and at first egg (17 weeks) (Table 4.2). The population used in the study has been described in detail in Chapter 3. Figure 1 shows the difference in growth performance of the study populations. The phenotypic traits measured in the F_2 population are summarised in Table 4.2.

Table 4.2 Mean, standard deviation and range of body weight at different ages and estimates of parameters of the growth curve of an F_2 broiler-layer cross population

Trait	N	Mean ± (Std Dev)	Range
Body weight at different ages			
3 week weight, g	462	256 (7)	112 - 409
6 week weight, g	459	781 (144)	2420 - 1163
12 week weight, g	462	1971 (278)	1168 - 3034
24 week weight, g	429	3332 (434)	2202 - 4705
48 week weight, g	434	3919 (534)	26340 - 5760
72 week weight, g	414	3924 (566)	2042 - 5800
Weight at first egg, g	432	2879 (400)	1356 - 4236
Age at first egg, d	407	121(11)	99 - 171
Growth curve parameter estimates			
Mature body weight, W_A, g	453	3807 (462)	2550 - 5276
Age at inflection point, $T_{i,}$ d	453	64 (7)	48 - 87
Rate of exponential decay, K, g/d	453	0.02 (0.003)	0.02 - 0.03
Instantaneous growth rate, L , g/d	453	0.1 (0.02)	0.06 - 0.2
Hatching weight,W_0, g	453	50.2 (16.5)	10 - 98

Figure 4.1 Graphs of the mean female body weight versus age for the grandparental lines and the F_2 individual at different ages of an F_2 broiler-layer cross population



4.2.1 DNA analysis and map construction

Genotyping was performed using a total of 106 microsatellite markers covering 25 to 26 autosomal linkage groups including the Z chromosome as described in Section 3.4 for (Table 3.1, Chapter 3). DNA preparation was previously described in Chapter 3. Genetic linkage maps were constructed using the CRIMAP program's prepare, flips and fixed options (Green *et al.* 1990).

4.2.2 Model fitting

The Laird form of the Gompertz curve equation as described by Aggrey *et al*. (2002a), was fitted to data for 453 individuals that had a minimum of 4 data points after removing extreme outliers. The equations for deriving the parameters of the Laird form of the Gompertz curve were defined following Aggrey (2002a) as follows:

$$W_t = W_0 * \exp^{[(L/K)(1 - \exp -Kt)]}$$

where W_t is the body weight of a bird at time *t*, where W_0 is the estimated initial hatching weight, L is the instantaneous rate of growth (per day), K is the rate of exponential decay of the initial specific growth rate a measure of the rate of decline in grow rate. Other derived parameters include:

age at the point of inflection, T_i; where $T_i = (1/K)\log(L/K)$

body weight at the point of inflection, W_i where $W_i = W_0 \exp((L/K)^{-1})$

the bird's asymptotic or mature body weight, W_A; where $W_A = W_0 \exp(L/K)$.

The growth curves were fitted using the Genstat program (Payne 2007) and the parameter estimates of the growth curves were extracted for each individual bird. The model converged for all the data points analyzed.

The estimated parameters were not normally distributed therefore to approximate normality the natural logarithm (ln) of the Laird form of the Gompertz curve was adopted. The predicted growth and the actual growth were both plotted for both equations to check how well the models fitted the data. The natural log transformed Gompertz (lnGompertz) curve was thus chosen and used to estimate the curve parameters which were defined in the QTL analysis as phenotypes for each bird.

4.2.3 QTL Analysis

The body weight at specific ages, growth rate at the different age intervals and curve parameter estimates for each individual were treated as phenotypes or traits. An individual bird's growth rate at a given growth interval was derived by dividing the body weight gained within the specific age interval by its respective time interval (in days). The growth rates at the respective intervals were denoted as Gr36, Gr12, Gr1224, Gr2448, Gr4872 for growth rate from 3 to 6, 6-12, 12-24 , 24 - 48, 48 -72 weeks of age respectively. Regression analysis was used to explore variables that affected the traits that could be included in the QTL analysis models. Family and pen were fitted as fixed effects in the additive-dominance and epistasis models. The interval mapping method for QTL analysis where the founder lines were assumed to be fixed for alternative alleles at the QTL in the parental populations (Haley *et al.*

1994) and, the search for epistatic QTL pairs (Wei *et al.* 2009) was conducted as described earlier in Chapter 3.

4.3 Results

The parental lines used differed in body weight and the F_2 individuals were midway in terms of body weight (Figure 1). The F_2 chickens achieved a mean weight of 2.9 kg at sexual maturity (Table 4.2). There was about 1 kg difference in mean weight between the lnGompertz curve estimated maximum growth weight and the weight at first egg (3.8 vs 2.9 kg) respectively indicating that the chickens reached their highest body weight after sexual maturity. There was no evidence for epistatic QTL pairs for all the growth-related traits analyzed except a suggestive QTL pair involving chromosome 2 and 3 detected for growth rate between 24 to 48 weeks of age (Results not included).

4.3.1 Body weight QTL

All suggestive and significant QTL for both the body weight and the Gompertz parameters are reported in Table 4.3.1 up to Table 4.4.2. A total of 18 body weight QTL segregating at 1 % significance level were detected on chromosomes 1, 2, 4, 8, 11, 27 and Z across the different ages. Thirteen body weight QTL segregating on chromosomes 2, 3, 4, 6 and Z were also identified for body weight at the different ages. A further 25 suggestive QTL were identified to be segregating at 5% significance level chromosome wide for body weight at different ages. Significant

body weight QTL were detected for each growth stage but more body weight QTL were detected for the early growth stages (3 – 12 weeks of age) before sexual maturity than for mature growth (after 24 weeks of age) (Table 4.3.1 up to Table 4.3.6).

4.3.2 Body weight QTL at 3 and 6 weeks of age

The significant QTL for body weight at 3 weeks of age that were identified to be segregating on chromosomes 2, 4, 6 and 11 were detected for body weight at 6 weeks of age (Table 4.3.1 and Table 4.3.2). Similarly, the body weight QTL at 3 weeks of age that were suggestively indentified to be segregating on chromosomes 1, 8 and 13 were represented in the QTL detected segregating on the same chromosomes at 6 weeks of age. However, more body weight QTL were detected segregating at 6 weeks of age than at 3 weeks of age where additional second QTL were detected on chromosomes 2, 3, and 4 as well as one QTL on chromosome Z at 6 weeks than at 3 weeks of age.

4.3.3 Body weight QTL at 12 and 24 weeks of age

Most of the segregating body weight QTL detected at 6 weeks of age were identified at 12 weeks of age with a new suggestive segregating QTL detected on chromosome 9. However, the number of detected QTL were fewer than at 6 weeks of age with no second QTL identified on chromosome 2 and 3 and (Table 4.3.2 and Table 4.3.3).

Only three detected QTL were segregating at 1 % level of significance on chromosomes 4, 8 and Z at 24 weeks. The rest of the detected QTL were

suggestively segregating on chromosome 1, 2, 8, 13 and 27. At 24 weeks of age there were fewer QTL detected at this age compared to the preceding age. New QTL were detected at the age include a second QTL segregating at 1% level of significance on chromosome 8 and a suggestively segregating QTL on chromosome 27. The remainder of the identified QTL segregating on chromosome 1, 2, 4 13 and Z are similar to those detected at 12 weeks of age.

4.3.4 Body weight QTL at 48 and 72 weeks of age

Two significant identified QTL for body weight at 48 weeks of age were segregating on chromosome 3 which were not detected in the preceding age. Furthermore, other significant QTL were identified as segregating for body weight at 48 weeks on chromosomes 2, 4 and 27. Interestingly, a new suggestive segregating body weight QTL at 48 weeks of age which was not detected at any age was found on chromosome 15. The least number of body weight QTL were identified as segregating at 72 weeks of age in this analysis. Highly significant segregating body weight QTL at 72 weeks were identified on chromosome 4 and 27. A significant 72 week body weight QTL was also detected on chromosome 3 affecting body weight at 48 and 72 weeks of age. A suggestive QTL for body weight at 72 weeks was found on chromosome 8 and a unique suggestive QTL was detected on chromosome 28 for the same trait.

Table 4.3.1 Body weight QTL position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL at 3 weeks of age

Trait / Chromosome	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP (%)
3-weeks weight, g							
1	131	6.9†	65-604	LEI0068 - LEI0146	10.1 (2.8)	-2.0(4.0)	2.3
1	505	4.6†	74-615	ROS0081 - LEI0079	13.6 (4.8)	10.4 (12.8)	1.4
2	298	16.1**	43-367	ADL0114 - MCW0056	15.9 (3.2)	16.4 (5.5)	6.0
4	148	8.8*	12-183	ADL0241 - MCW0180	27.8 (6.7)	-6.5 (23.8)	3.1
6	30	9.6*	0-42	ROS0003 - ADL0142	11.1 (3.1)	-11.1(4.9)	2.6
8	63	7.6†	1-87	MCW0100- ROS0075	13.7(4.1)	15.4(8.5)	2.1
11	0	12.5**	0-10	LEI0110 - MCW0097	13.0 (2.7)	7.3 (3.9)	1.4
13	49	5.6†	9-71	LEI0083 - MCW0080	14.2 (4.8)	-19.7 (11.8)	1.8
Z	127	6.9†	0-127	LEI0111 - LEI0075	14.8 (4.0)	2.3 (4.0)	2.3

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.3.2 Six weeks body weight QTL position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL

Trait /Chromosome	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP (%)
6 weeks weight, g							
1	130	16.0**	76-219	LEI0068 - LEI0146	42.4 (7.5)	-2.8 (10.9)	5.1
1	508	4.8†	0-606	ROS0081 - LEI0079	36.0 (12.8)	35.8 (34.4)	5.1
2	148	5.1†	34-370	ADL0176 - ADL0196	45.1 (14.7)	-34.9 (42.4)	1.4
2	286	13.3**	0-400	ROS0074 - ADL0114	39.2 (8.1)	23.7 (12.6)	4.2
3	47	10.4*	14-219	MCW0083-HUJ0006	45.9 (10.2)	11.8 (18.4)	3.2
3	235	5.5†	12-266	MCW0040-LEI0166	19.5 (8.2)	31.0 (13.0)	1.5
4	0	8.3*	0-69	ADL0317 - MCW0295	30.1 (7.4)	-1.7 (10.9)	2.5
4	161	21.5**	140-183	ADL0241-MCW0180	95.5 (14.6)	5.8 (40.7)	6.9
6	8	8.1*	0-43	ROS0062-ROS0003	27.0 (9.0)	-37.4 (15.2)	2.4
8	67	7.4†	0-87	MCW0100-ROS0075	41.5 (11.5)	25.3 (23.0)	2.4
11	0	11.1**	0-57	LEI0110-MCW0097	34.1 (7.4)	13.4 (10.6)	3.4
13	42	5.7†	12-71	MCW0340-ADL0225	47.2 (14.0)	-6.8 (37.7)	1.6
Z	119	9.8**	14-127	LEI0111-LEI0075	52.7 (12.2)	19.8 (13.2)	3.0

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.3.3 Body weight QTL position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL at 12 weeks of age.

Trait / Chromosome	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP (%)
12-weeks weight, g							
1	137	12.2**	67-227	LEI0146-ADL0319	84.5(17.0)	-5.5(26.3)	3.7
1	525	10.2**	103-601	ROS0081-LEI0079	71.1 (21.2)	129.9 (46.2)	3.1
2	281	8.2*	49-290	ROS0074-ADL0114	87.0 (17.1)	23.0 (27.4)	4.0
3	39	10.1*	155-183	MCW0083-HUJ0006	91.1 (20.2)	-5.7(36.2)	3.0
4	0	8.2*	0-177	ADL0317-MCW0295	62.0 (15.2)	-4.0 (22.6)	2.4
4	177	44.4**	155-183	ADL0241-MCW0180	207.7 (22.0)	15.6(44.8)	14.5
6	30	6.2†	0-38	ROS0003-ADL0142	33.7(17.2)	-5.5(26.3)	1.7
8	61	11.2**	12-75	MCW0100-ROS0075	72.9 (23.1)	155.7 (46.0)	1.4
9	90	4.9†	0-120	MCW0135-ROS0030	26.2(22.2)	-127.2 (45.1)	1.3
13	7	5.2†	0-71	MCW0340-ADL0225	48.8 (18.4)	54.0 (32.5)	1.4
Z	117	9.1*	8-127	LEI0111-LEI0075	110.0 (25.9)	32.2 (28.3)	2.7

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.3.4 Body weight QTL position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL at 24 weeks of age.

Trait / Chromosome	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP (%)
24-weeks weight, g							
1	131	6.5†	109-543	LEI0068-LEI0146	91.6(25.9)	26.2(37.1)	2.3
1	560	5.0†	96-598	ADL0183-MCW0107	92.8(33.6)	91.0 (68.7)	1.7
2	276	5.8†	0-297	ADL0236-ROS0074	94.1(27.7)	-6.0(45.8)	2.0
4	142	17.0**	19-169	ADL0241-MCW0180	379.6(65.6)	-186.3(255.0)	6.7
8	14	11.6**	0-86	MCW0305-ADL0258	107.3(26.4)	107.5(38.2)	4.4
8	87	6.1†	14-87	MCW0100-ROS0075	108.2 (31.9)	-72.6 (49.1)	2.2
13	70	7.1†	2.0-71.0	MCW0340-ADL0255	63.2 (30.6)	-156.5 (48.0)	2.6
27	0	9.5†	-	ROS0071	113.1(25.9)	-2.5(35.7)	2.6
Z	127	9.1**	0-127	LEI0111-LEI0075	137.7(37.6)	109.4 (38.8)	3.4

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.3.5 Body weight QTL position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL at 48 weeks of age.

Trait / Chromosome	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP (%)
48-weeks weight, g							
2	286	9.7*	59-297	ROS0074-ADL0114	141.7 (34.0)	-69.6 (52.7)	3.2
3	40	8.8*	1-199	MCW0083-HUJ0006	139.8 (40.3)	162.0 (74.1)	2.8
3	216	8.7*	2-226	ADL0306-ADL0237	106.1 (33.9)	-190.9 (52.5)	3.5
4	153	32.7**	137-183	ADL0241-MCW0180	550.4 (68.0)	-72.8 (224.8)	11.5
6	9	8.2†	0-35	ROS0062-ROS0003	151.5 (37.3)	15.8 (62.6)	2.6
8	24	7.8†	0-87	ADL0258-ADL0179	100.7(30.6)	116.1 (47.5)	2.5
9	81	8.2†	23-103	MCW0135-ROS0030	135.1 (44.7)	-194.7 (87.1)	1.7
15	10	5.7†	0-49	LEI0083-MCW0080	115.8 (39.5)	117.1(70.2)	1.7
27	0	15.2**	-	ROS0071	173.0 (31.4)	-20.3 (43.7)	5.2

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.3.6 Body weight QTL position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL at 72 weeks of age

Trait / Chromosome	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP (%)
72-weeks weight, g							
3	45	9.0*	0-215	MCW0083-HUJ0006	199.2(49.8)	118.3(92.3)	3.5
4	183	24.7**	154-183	ADL0241-MCW0180	324.7(46.7)	-42.2(87.2)	10.3
8	12	5.7†	0-87	ROS0021-ROS0026	120.9(36.9)	29.6(54.2)	2.0
27	0	16.5**	-	ROS0071	213.4(37.1)	36.9(51.6)	6.7
28	24	5.4†	0-42	ROS0085-ADL0299	128.6(48.9)	-194.6(93.4)	1.9

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.4.1 Log-Gompertz mature weight QTL position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL for the F_2 broiler layer cross.

Trait / Chromosome	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP (%)
Asymptotic / mature body weight, W_A, g							
2	283	12.0**	58 -295	ROS0074-ADL0114	150.3 (31.0)	-18.9 (49.0)	4.1
3	46	6.5†	1 - 205	MCW0083-HUJ0006	125.9 (37.0)	71.5 (69.2)	2.0
3	205	5.4†	2 - 236	ADL0306-ADL0237	106.1 (33.9)	-67.9 (60.8)	1.6
4	166	26.7**	143 -148	ADL0241- MCW0180	352.6 (48.7)	-137.4 (125.2)	9.5
7	46	4.4†	0 - 93	LEI0064-ROS0019	65.37 (58.8)	486.2 (175.3)	1.3
8	13	8.6*	0 - 86	ROS0026-MCW0305	107.4 (28.1)	74.3 (40.9)	1.8
9	46	5.4†	21-115	ROS0078-MCW0135	83.3 (39.1)	-177.6 (74.0)	1.6
15	10	5.8†	0 - 45	LEI0083-MCW0080	108.7 (34.4)	81.6 (62.6)	1.8
27	0	18.7**	-	ROS0071	166.6 (28.0)	46.7 (39.2)	6.6

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.4.2 lnGompertz parameter estimates QTL position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL for the F_2 broiler layer cross.

Trait / Chromosome	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP (%)
Age at inflection point, T_i, (d)							
2	32	6.2†	8 - 376	ADL0343-ADL0176	-3.0 (0.9)	0.1 (2.2)	2.5
4	22	5.7†	0 -166	ADL0317-MCW0295	-2.1 (0.6)	-0.6 (1.4)	2.3
11	7	8.2*	0 - 63	MCW0097-ROS0111	-1.8 (0.5)	-1.4 (0.8)	3.5
Rate of exponential decay, K, (g/d)							
4	17	5.6†	0 -169	ADL0317-MCW0295	8.0 E-4 (2.4E-4)	3.0 E-4 (4.8E-4)	2.3
7	93	5.43†	27 - 93	ROS0019-ADL0180	6.0 E-4 (1.8E-4)	-3 E-4 (2.7E-4)	2.2
28	0	5.0†	0 - 42	ROS0095-ROS0085	-3.0 E-4 (1.8E-4)	7.0 E-4 (2.6E-4)	2.0
Instantaneous growth rate, L (g/d)							
1	541	4.0†	0 - 600	ROS0081-LEI0079	0.002 (0.001)	0.003 (0.002)	1.5
4	144	4.9†	0 - 183	ADL0241-MCW0180	0.01 (0.0)	0.01 (0.01)	1.9
Hatching weight, W_0, (g)							
8	78	6.2†	7 - 87	MCW0100-ROS0075	5.0 (1.5)	-4.2 (2.7)	2.6
13	62	4.9†	7 - 71	MCW0340-ADL0225	4.3 (1.4)	2.3 (2.7)	1.9

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

QTL for growths were segregating on chromosomes 3, 4 and 8 throughout the growth cycle. QTL involved mainly in early growth (3 – 6 weeks) were detected on chromosome 11 and QTL on chromosome 1, 13 and Z were involved up to 24 week age which is around sexual maturity (about 17 weeks for this population, Table 4.2). Other QTL on chromosomes 15, 27, and 28 appear to be mainly for older growth from 24 week and later. Few of the detected QTL were segregating at older ages (48 – 72 weeks) compared to earlier growth stages.

4.3.5 Body weight QTL effects and phenotypic variation explained

Most QTL affecting body weight at different ages and also explaining the highest proportion of the phenotypic variance were segregating on chromosome 4. The highest proportion of the phenotypic variation explained by a QTL was 14.5% for the 12 week body weight at 177 cM on chromosome 4 (Table 4.3.3). The contribution of most QTL's varied across stages e.g. the chromosome 4 QTL contribution peaked just before sexual maturity.

Most of the significant segregating QTL had significant positive additive affects implying that the alleles from broiler individuals tended to cause an increase on the phenotype of the respective traits. Dominance effects were generally not significant and in cases where they were significant, they had negative values. This meant that the effect of heterozygous genotypes tended to lower the trait phenotype compared to the average effect of the two homozygous genotypes. A QTL for body weight at 48

weeks of age segregating on chromosome 4 at 153 cM had the highest additive effects (550.4 ± 68.0) g where the QTL explained 11.5% of phenotypic variation. The largest dominance effect (-190.9 ±52.5) was for a QTL on chromosome 3 at locus 216 cM and the QTL accounted for 3.5 % of the phenotypic variation of weight at 48 weeks of age.

Table 4.5.1 Growth rate QTL, position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL at 3 - 6 weeks of age for the F_2 broiler- layer cross

Trait/ Chromosome	Position (cM)	F ratio[1]	CI[2]	Flanking markers	Additive effects± SE	Dominance effects± SE	VP (%)
Growth rate at 3-6 weeks of age, (g/d)							
1	130	14.1**	95 – 237	LEI0068-LEI0146	1.48 (0.28)	-0.06(0.40)	4.7
2	282	10.2**	15 – 382	ROS0074-ADL0114	1.34(0.30)	0.59(0.49)	3.3
3	49	17.2**	23 – 128	MCW0083-HUJ0006	2.11(0.36)	0.52(0.66)	5.9
3	237	7.1†	13 – 266	MCW0040-LEI0166	0.61(0.30)	1.51(0.46)	2.2
4	0	11.2**	0 - 37	ADL0317-MCW0295	1.29(0.27)	-0.27(0.40)	3.7
4	163	21.0**	140 - 183	ADL0241-MCW0180	3.33(0.51)	0.83(1.38)	7.2
6	11	7.5†	0 – 45	ROS0062-ROS0003	0.54(0.33)	-1.82(0.54)	2.4
11	0	7.1†	0 – 67	LEI0110-MCW0097	1.02(0.27)	0.37(0.39)	2.2
13	57	4.5†	19 – 71	MCW0340-ADL0225	1.31(0.42)	-0.57(0.88)	1.4

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.5.2 Growth rate QTL, position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL at 6-12 weeks of age for the F_2 broiler- layer cross

Trait/ Chromosome	Position (cM)	F ratio[1]	CI[2]	Flanking markers	Additive effects± SE	Dominance effects± SE	VP (%)
Growth rate at 6 -12 weeks of age, (g/d)							
1	578	7.5*	65 - 601	ADL0183-MCW0107	1.63(0.51)	2.96(1.44)	2.6
3	13	9.8*	0 – 158	MCW0169-MCW0083	1.48(0.36)	-1.16(0.65)	3.9
4	168	27.0**	146 – 183	ADL0241-MCW0180	3.49(0.48)	0.01(1.16)	2.6
9	97	5.6†	0 – 121	MCW0135-ROS0030	0.38(0.37)	-2.24(0.72)	1.9
27	0	10.7**	-	ROS0071	1.18(0.28)	0.62(0.39)	3.9
Growth rate at 12-24 weeks of age, (g/d)							
8	15	9.7**	7- 87	ROS0026-MCW0305	0.98(0.26)	0.93(0.38)	4.6

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

Table 4.5.3 Growth rate QTL, position, F-value, effects and proportion of phenotypic variance (VP) explained by QTL at the 24 - 48 and 48 – 72 weeks of age interval for the F_2 broiler- layer cross

Trait/ Chromosome	Position (cM)	F ratio[1]	CI[2]	Flanking markers	Additive effects± SE	Dominance effects± SE	VP (%)[3]
Growth rate at 24 -48 weeks of age, (g/d)							
3	2	8.8*	1 – 266	MCW0169-MCW0083	0.70(0.18)	-0.45(0.26)	4.0
3	221	8.7*	3 – 236	ADL0237-MCW0040	0.35(0.16)	-0.91(0.24)	4.0
4	183	6.6†	29 – 183	ADL0241-MCW0180	0.63(0.19)	0.46(0.36)	2.9
28	42	6.1†	0 - 42	ROS0085-ADL0299	0.48(0.16)	-0.40(0.24)	2.7
Growth rate at 48 -72 weeks of age, (g/d)							
2	365	7.8†	138 – 383	MCW0056-MCW0157	-2.74(13.35)	8.06(4.33)	3.7
4	12	6.0†	0 – 183	ADL0317-MCW0295	-0.04(22.41)	7.45(11.85)	2.7
7	92	7.6†	0 – 93	LEI0064-ROS0019	-7.75(26.28)	3.21(18.35)	3.6

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

4.3.6 Gompertz curve parameter QTL

The QTL results for the lnGompertz curve parameter estimates are reported in Table 4.4.1 and Table 4.4.2. Except for the mature weight, most QTL for growth curve parameter estimates detected were suggestive. Most of the QTL detected were for the asymptotic body weight parameter estimate, W_A and most of them appear to be the same detected QTL segregating across different chromosomes (2, 3, 4, 8, 15 and 27) for body weight at different ages. The suggestive QTL segregating on chromosome 4 at 144 cM for L (the growth curve's growth rate parameter estimate) is close to the location of the QTL detected for asymptotic body weight, W_A at 166 cM. Therefore, this appears to be same identified QTL segregating across different ages on the same chromosome at 142 - 183 cM. The instantaneous growth rate, L QTL at 541 cM on chromosome 1 is located in the same region flanked by markers ROS0081-LEI0079 as the body weight QTL detected for

(3-12 weeks of age) (Table 4.4.2).

A QTL for age at maximum growth, T_i was detected to be segregating on chromosome 11. The same QTL was also detected for body weight at 3 and 6 weeks of age and suggestive T_i QTL were detected at 22 and 32 cM respectively on chromosomes 4 and 2 (Table 4.4.2). Suggestive hatching weights, (W0) QTL, are similar to the QTL for body weight from early growth to sexual maturity on chromosome 8 and 13.

4.4 Growth rate QTL

The detected QTL for growth rate at different stages are presented in Table 4.6. Two very significant QTL for growth rate between 3-6 weeks of age were detected on chromosome 4 and other significant single QTL were found on chromosomes 1, 2 and 3. Suggestive QTL for the same trait were also detected on chromosomes 3, 6, 11 and 13.

Significant QTL for the 6-12 weeks of age growth rate were detected on chromosomes 1, 3, 4 and 27 including a single suggestive QTL on chromosome 9. Only one highly significant QTL was detected for growth rate at 12-24 weeks of age. At the 24-48 weeks growth interval two QTL were detected on chromosome 3 and suggestive QTL on chromosomes 4 and 8. Only suggestive QTL were detected for growth rate at 48-72 weeks of age.

The second QTL for 3-6 weeks of age growth rate on chromosome 4 explained the highest proportion of the phenotypic variation (7.2 %) among all the QTL detected for growth rate. The two QTL for the 3-6 weeks of age growth rate jointly explained 10.9 % of the phenotypic variation for this trait. Most the QTL acted additively but some significant dominance effects were found for QTL on chromosomes 1, 8 and 3 for growth rate at 6-12, 12 - 24 and 24 – 48 weeks of age respectively.

4.4.1 Comparison of the three QTL detection approaches

A comparison of QTL detected for body weight and the Gompertz parameters is presented in Table 4.6.

Table 4.6 Comparison of body weight QTL at specific ages versus QTL for lnGompertz curve parameter estimates (significant QTL are underlined)

Chromosome	Age specific weight QTL Position (cM)						lnGompertz Parameter Estimates QTL Position (cM)				
	3wkwt	6wkwt	12wkwt	24wkwt	48wkwt	72wkwt	W_A	T_i	K	L	W_o
1	131, 505	**130**, 508	**137, 525**	131, 560	-	-	-	-	-	541	-
2	**298**	148, **286**	**281**	276	**286**	-	**283**	32	-	-	-
3	-	**47**, 235	**39**	-	**40, 216**	**45**	46, 205	-	-	-	-
4	**148**	**0, 161**	**0, 177**	142	**153**	**183**	**166**	22	17	144	-
6	**30**	**8**	30	-	9	-	-	-	-	-	-

W_A = asymptotic/mature body weight, i.e W_o*exp (L/K), g.

T_i= age at point of inflection, where Ti = (1/K)log(L/K), d.

K= B = rate of exponential decay, g/d.

L= instantaneous growth rate per day, g/d.

W_0= hatching weight, (y –intercept), g

Table 4.6 cont'd Comparison of body weight QTL at specific ages versus QTL for lnGompertz curve parameter estimates (significant QTL are underlined)

Chr[1]	Age specific weight QTL Position (cM)						lnGompertz Parameter Estimates QTL Position (cM)				
	3wkwt	6wkwt	12wkwt	24wkwt	48wkwt	72wkwt	W_A	T_i	K	L	W_o
7	-	-	-	-	-	-	46	-	93	-	-
8	63	67	**61**	**14**, 87	24	12	**13**	-	-	-	78
9	-	-	90	-	**81**	-	46	-	-	-	-
11	**0**	**0**	-	-	-	-	-	**7**	-	-	-
13	49	42	7	70	-	-	-	-	-	-	62
15	-	-	-	0	10	-	10	-	-	-	-
27	-	-	-	-	**0**	**0**	**0**	-	-	-	-
28	-	-	-	-	-	24	-	-	0	-	-
Z	127	**119**	**117**	**127**	-	-	-	-	-	-	-

W_A = asymptotic/mature body weight, i.e W_o*exp (L/K), g.

T_i= age at point of inflection, where Ti = (1/K)log(L/K), d.

K= B = rate of exponential decay, g/d.

L= instantaneous growth rate per day, g/d.

W_0= hatching weight, (y –intercept), g

The detected growth rate QTL co-locate with most of the bodyweight at QTL at the respective ages. However, the number of detected QTL for growth rate is far fewer than those detected for body weight at the respective age intervals (Table 4.3.1 - 4.3.6 and Table 4.5.1 – 4.5.3). A QTL on chromosome 8 affects body weight and mature weight and growth rate between 12 to 24 weeks of age. This QTL was the only significant QTL for growth rate during the period (12-24) which spans sexual maturity. Chromosome 4, and to a lesser extent chromosome 3, QTL were detected for growth rate across the different growth phases except at 12-24 weeks of age.

Generally, QTL detected for growth rate interval were the same as those identified for body weight at the corresponding ages (Table 4.5.1 - 4.5.3 and Table 4.6.1 – 4.6.2). Both the body weight and growth rate approaches identified more significant QTL than the Gompertz curve approach but all methods were able to identify the significant QTL for adult body weight, W_A on chromosomes 2, 4, and 8. All the approaches identified a QTL on chromosome 4 flanked by markers ADL240 and MCW0180 affecting instantaneous growth rate (Table 4.5.2 and Table 4.6), although it was just suggestive under the Gompertz curve approach.

4.5 Discussion

4.5.1 Bodyweight QTL at Specific Ages

Body weight QTL at 3 weeks in our study at chromosomes 1, 4, 13 and Z (Table 4.5.1, Table 5.5.2 and Table 4.5.3) were similar to those detected earlier in a similar population raised as boilers to maximum growth (Sewalem *et al.* 2002). Chromosomes 1, 2, 3, 6, and 11 which harbour significant QTL for body weight at 46 days (Le Rouzic *et al.* 2008) also appear in the list of significant and suggestive QTL for weight at 6 weeks of age in our study (Table 4.3.1 and Table 4.3.2). The QTL for 6 weeks weight on chromosomes 3 at 235 cM, and at 62 and 161 cM on chromosome 4 in our study are similar to the QTL respectively at 252, 0, 149 cM reported by Jacobsson *et al.* (2005). Most of the QTL for this trait are similar to those identified on chromosomes, 1, 2, 4, 6, 8, 13 and Z in the Roslin broiler – layer study (Sewalem *et al.* 2002).

Sewalem *et al.,* (2002) also detected QTL for body weight at 9 weeks on chromosomes, 1, 2, 4, 6, 8, and 13 which resemble those in our study at 12 weeks of age. Positions for the QTL on chromosome 4 and 13 were respectively at 177 and 15 cM in the earlier study and at 177 and 7 cM in ours. This is possibly the same QTL because of high weight correlation at both ages.

The significant 12 week body weight QTL on chromosome 4 at 177 cM also coincides with the carcass weight QTL at 147 cM on the same chromosome (Navarro

et al. 2005b). The same case holds for the QTL on chromosomes 27 and 1 for these two traits which makes sense in that heavy animals would yield heavier carcasses.

More QTL were detected for earlier growth stages before sexual maturity than for later growth reflect the importance of growth function at the early part of each organism (Table 4.6). The contribution of growth QTL also peaks around the critical stage preceding and at sexual maturity. Age specific body weight QTL were detected for each growth stage which supports earlier suggestions and observations that there are different genes and gene actions involved during growth and developmental stages (Wu *et al.* 2002; Carlborg *et al.* 2004a; Wu *et al.* 2004; Gao *et al.* 2006; Long *et al.* 2006). Chromosomes 3, 4 and 8 had QTL involved with growth throughout lifetime. Chromosome 11 harboured QTL involved mainly in very early growth and those on chromosome 1, 13 and Z are involved up to 24 week age which is around sexual maturity. Other chromosomes 15, 27, and 28 appear to be mainly for mature growth from 24 weeks and later. Few QTL were detected at older ages (48 – 72 weeks) compared to earlier growth stages signifying as expected the less importance of growth at this stage in the life.

4.5.2 Common QTL for body weight and Gompertz Parameters

The Gompertz parameter estimates for the female F_2 population (Table 4.2) fall within the range of figures (W_A: 2483 – 5698g, L: 0.0908 – 0.141 g/d, K: 0.0224 – 0.031, T_i: 42.2 – 63 d, W_0: 39.8 – 64g) reported in the literature (Mignon-Grasteau 1999; Aggrey 2002a; Darmani Kuhi *et al.* 2003; N'Dri *et al.* 2006). Common significant and suggestive QTL for body weight and Gompertz parameters (Table 4.6) confirms assertions made earlier that these parameters are genetically determined and can be exploited to improve traits through selection (Wu *et al.* 2002; Gao *et al.* 2006).

Significant QTL for mature body weight, W_A were detected on chromosomes 2, 4, 8 and 27 while suggestive QTL were detected on chromosomes 3, 7, 9 and 15. Similarly, significant asymptotic or mature body weight QTL were reported on chromosomes 2 and 27 by Le Rouzic *et al*., (Le Rouzic *et al.* 2008) however, they also reported other QTL not detected in our study on chromosome 1, 6 and 11. The same QTL for age at the point of inflection was detected on chromosome 11 by Le Rouzic *et al.,* (2008) and our study but our study detected suggestive QTL for this trait on chromosomes 2 and 4 while Le Rouzic *et al.,* (2008) detected significant QTL on chromosomes 1, 12 and 20.

4.5.3 Gompertz curve parameter estimates QTL effects

In our study, chromosome 4 had a QTL for mature body weight at 166 cM with the highest additive effect and explained the highest proportion (9.5%) of the variation. This chromosome is similar to what has been observed in other studies (Schreiweis *et al.* 2005) and has consistently a large effect across studies. The results generally confirm earlier observation about the critical role of chromosome 4 in controlling growth and other traits of economic importance (Sewalem *et al.* 2002; Tuiskula-Haavisto *et al.* 2002; Schreiweis *et al.* 2005). Body weight and growth rate parameter QTL appear to be controlled by similar loci on chromosome 4 which suggests that selecting animals on the basis of the QTL on chromosome 4 would improve the growth rate and also modify the shape of the growth curve.

4.5.4 Growth rate QTL

The growth rate QTL at 3 – 6 weeks detected in this study on chromosomes 1, 2, 3, 4 and 13 were similar at corresponding ages to those reported by Carlborg *et al*., (2004a) and Zhou *et al*., (2006) at chromosome 1 and 2 for 2-4 weeks. Similar QTL for growth rate at 6-12 weeks of age detected on chromosomes 1 and 3 were also reported by Carlborg *et al* (2004a). The detected growth rate QTL were fewer than those for body weight possibly due to the wide intervals used for deriving the growth rates especially at the older ages which may have made the measure insensitive to

short-term growth rate changes and any physiological changes along the growth trajectory.

4.5.5 No evidence for epistasis

Among all the assessed traits there was little evidence for epistasis. Only one suggestive epistatic pair was found which affected growth rate between 24-48 weeks. Failure to detect significant epistasis for the evaluated traits was quite a surprise given that other studies working on similar traits detected epistatic QTL pairs for body weight at early growth stages and for Gompertz curve parameters (Carlborg *et al.* 2003; Carlborg *et al.* 2004a; Le Rouzic *et al.* 2008; Ankra-Badu *et al.* 2010). Most approaches aiming to detect epistasis have a limitation of achieving a high level of false positive results (Wei *et al.* 2010b) and the only suggestive epistatic QTL pair detected could be due to the stricter thresholds enforced in this analysis compared to those reported by other studies.

4.6 Conclusions

Several significant QTL for body weight at specific ages were detected and most of the identified QTL were also detected in the nearest preceding and/or subsequent growth stages due to the high correlation between body weights at nearby growth stages. Most of the detected QTL were reported in other studies and the results confirmed age specific QTL. QTL influencing Gompertz parameters were detected and these QTL also overlapped with loci affecting growth and carcass traits reported

by other studies. The overlap of body weight, growth rate and QTL Gompertz parameters shows that Gompertz parameters can be used in selection to manipulate these traits. Age specific growth QTL show that there are specific genes and gene actions which orchestrate developmental process during the different stages of growth. Some loci featured predominantly in early growth to the attainment of sexual maturity. However, there was limited evidence for epistasis with only one epistatic QTL pair for growth rate between 24-48 weeks of age detected. Chromosome 4 prominently explained much of the observed growth variation across the different ages and also harboured most of the detected QTL for Gompertz parameters confirming its importance in controlling growth as a trait of economic importance.

CHAPTER 5

Detection of QTL for age, weight and abdominal fat weight at first egg

5.1 Introduction

Understanding the genetic mechanism between growth rate and the onset of puberty is of significant biological and agricultural interest. Identification of quantitative trait loci (QTL) and their related genetic relationships is important in understanding the genetic factors controlling reproductive traits associated with sexual maturity, such as age and weight at puberty. Several studies across species have shown that the attainment of puberty (sexual maturity) is dependent on a number of factors like age, minimum weight, body composition (Frisch 1994; Yannakopoulos *et al.* 1995; Eitan & Soller 2001).

In humans the attainment of a critical body weight of 48 kg and a fat percentage of 22% for the onset of puberty was proposed in the early 1970s (Frisch & Revelle 1970). Recent interest in this issue has been reignited by the observed early puberty in humans associated with increasing levels of obesity (Kaplowitz 2008; Aksglaede *et al.* 2009). Similarly, agricultural species that have been intensely selected for early growth such as broiler chickens, have also become heavier and fatter, with negative effects on their reproductive performance as adults (Brody *et al.* 1984; Hocking *et al.* 2002b; Brewer & Balen 2010). Early maturity has also been associated with reproductive problems such as abnormal ovarian hierarchies in chickens (Lacassagne & Jacquet 1965; Hocking *et al.* 1987; Hocking 2004), and understanding the genetic mechanisms influencing these conditions could shed light on reproductive dysfunction in other species (Onagbesan *et al.* 2009).

The existence of a threshold level of weight or fatness that is critical for menarche has been disputed (Garn *et al.* 1983). The hypothesis that puberty depends on a critical amount of body fat is has been rejected repeatedly by experimentalists (Bronson 2001). The linkage between body fat and the reproductive axis in girls is thought to be the result of an evolutionary mechanism in mammals for ensuring that pregnancy will not occur unless there are adequate fat stores to sustain both the mother and the growing foetus (Kaplowitz 2008). According to Kaplowitz (2008), published evidence suggests that obesity may be causally related to earlier puberty in girls. Rodent and human studies suggest that leptin is the critical link between body fat and early puberty but the question of whether earlier puberty is the cause or the result of increased body fat has not been resolved (Kaplowitz 2008).

An alternative view based on chicken studies is that the fat deposition is a result of processes associated with steroidogenesis driven by the development of the ovary (Hocking & Robertson 2000). Some authors have suggested that there is a minimum fat requirement and that body weight was not a limiting factor for achievement of sexual maturity (Robinson *et al.* 2001). While some data points to a possible minimum fat requirement to attain sexual maturity, a cautious approach is needed to identify the actual mechanisms involved (Chen *et al.* 2007) and that there is possible distortion due to effects of selection (Reddish *et al.* 2003).

The chicken (*Gallus gallus domesticus*) is a model organism used in genetic studies with implications for agriculture and biology (Griffin & Goddard 1994; Burt 2007). The relative ease of using chickens to generate DNA based genetic data and the

similarities in the sexual maturity phenomena across species makes the chicken a relevant model to gain more understanding of the genetic relationship between growth rate and the onset of sexual maturity across species. Interactions of several loci have been reported to influence early growth in chickens for example (Carlborg *et al.* 2003), but genetic mechanisms involved in the interplay of other factors impacted by early growth remain to be elucidated.

For female chickens where the critical day length threshold for photoperiod has been provided, the onset of lay is dependent on the attainment of minimum weight and age thresholds (Eitan & Soller 2001). Understanding the genetic mechanisms controlling reproductive traits associated with sexual maturity, such as age and weight at the point of lay, is important in the effort to improve poultry productivity by optimizing early maturity while maximizing weight in broiler chickens. Selecting animals that are early maturing contributes to the minimization of production costs to the onset of lay (Ãlvarez & Hocking 2009). This is very important particularly of broilers that have been intensely selected for growth and have become heavier and fatter, traits that may negatively affect their reproductive performance as adults (Reddish *et al.* 2003).

Identification of quantitative trait loci (QTL) in chickens (*Gallus gallus domesticus*) has focussed mainly on broiler traits and a few health related factors and there are relatively few reported QTL for reproductive traits (Hocking 2005; Abasht *et al.* 2006a). A number of chicken studies have identified QTL for age at first egg (AFE) on chromosome Z (Tuiskula-Haavisto *et al.* 2002; Sasaki 2004; Schreiweis *et al.*

2006). However, few studies have reported specifically on weight at first egg (WFE) but instead have reported on QTL for body weight at earlier ages (Tatsuda & Fujinaka 2001; Sewalem *et al.* 2002; Ruy *et al.* 2005) or included later growth stages than in this study (Tuiskula-Haavisto *et al.* 2002; Kerje 2003; Sasaki 2004).

This study adopted a QTL approach to address four issues. Firstly, to investigate the relationship between growth rate and sexual maturity by identifying the QTL for age at first egg (AFE) and weight at first egg (WFE) in the Roslin broiler-layer cross in which the White Leghorn layer was much later maturing than the male broiler line. Secondly, to identify the relative importance of age and body weight for the attainment of puberty in chickens. Thirdly, reproductive traits are known to exhibit high heterosis (Williams *et al.* 2002) which may be explained partly by epistasis, the non-additive interaction of genes with one another (Williams *et al.* 2002; Carlborg *et al.* 2004a; Melchinger *et al.* 2007). For this reason the effects of epistasis on AFE and WFE were investigated using recently developed software (Wei *et al.* 2009). Lastly, we investigated the effect of fatness on puberty by using abdominal fat weight (ABF) as a covariate due to its high correlation with WFE and total body fat.

5.2 Materials and methods

5.2.1 Resource populations and QTL analysis

The description of the development of all the resource populations (8 F_0 grandparents, 41 F_1 individuals and 912 F_2 offspring), genotyping and recording of phenotypes as well as procedures for QTL detection used in analysis is provided in

Chapter 3. WFE and AFE were recorded at the onset of lay which was defined as the day of first recorded oviposition. In a subset of the birds (about half of the hatches), the birds were killed and the weight of abdominal fat was recorded. AFE was transformed to natural logarithms of (AFE – 94 days) to normalise residual errors. The 94 days was chosen as the lowest AFE in the unedited data. The QTL effects on the original scale were calculated as: back-transformed effect = $e^{(\text{transformed trait mean} + \text{transformed effect estimate})} - e^{(\text{transformed trait mean})}$.

Genotyping was conducted using 106 microsatellite markers covering 25 autosomal linkage groups and the Z sex chromosome (Table 3.1 Chapter 3) on the 8 F_0 grandparents, 41 F_1 (8males plus 33 females) and 912 F_2 offspring with data on age and weight at first egg. The 2005 consensus genetic linkage map (ArkDB, 2007) was used to modify an adopted map (Navarro *et al.* 2005a) based on the same population used in the analysis. The total map length was 2479 cM (Table 3.1).

5.2.2 Fitting covariates

For each trait, models with and without a covariate were fitted. Covariates were included in the model of analysis to detect differences in the assessed trait at a fixed level of the covariate trait (Kerje *et al.* 2003; Park *et al.* 2006). A regression analysis on lnAFE94 and WFE showed that lnAFE94 explained 29 % of the variation in WFE where pen and family were included as fixed effects. Conversely, fitting the same effects, WFE explained a moderately high proportion of the variation (64 %) in lnAFE94. In the QTL analysis for WFE, lnAFE94 was added in the model as a

covariate. Additionally for the subset of the individuals with ABF recorded, another model was run for WFE with lnABF as a covariate. Similarly, WFE was fitted as a covariate in the model for lnAFE94.

5.3 RESULTS

5.3.1 Broiler and layer phenotypes

The mean AFE and WFE of the broiler male line and the White Leghorn layer line are presented in Table 5.1 At first egg, the broiler line was heavier (5.4 kg vs 1.5 kg) and earlier maturing than the layer (130 d vs 177 d).

Table 5.1 Means and standard deviations (SD) for age, abdominal fat and weight at the onset of lay in male line broiler and White Leghorn layer chicken females.

Trait	Male-line broiler (n=10)		White Leghorn Layer (n=12)	
	Mean	SD	Mean	SD
Weight at first egg, WFE (g)	5400	600	1500	100
Age at first egg, AFE (d)	130	8	177	10
Abdominal Fat, ABF (g)	255	71	44	9

5.3.2 F_2 phenotypes

Trait means, standard deviations, ranges and phenotypic correlations between the phenotypic traits in the F_2 population are given in Table 5.2. AFE for the F_2 population ranged from 99 to 226 days. The mean AFE for the broiler male line stock and F_2 were similar (130 and 134 d respectively) and lower than that of the White Leghorn layer line (177 d). The Pearson correlation between WFE and the natural logarithm of age at first egg minus 94 days (lnAFE94) in the F_2 population was low (0.31) (Table 5.2). The transformation of age at first egg was done to normalise residual errors.

Table 5.2 Number of records (N), means, standard deviations (SD), range and phenotypic correlations for age, weight, natural logarithm of age-94 days and abdominal fat at the onset of lay in an F_2 broiler - layer chicken population.

Variable	N	Mean	SD	Min	Max	Correlation	
						AFE	WFE
Weight at first egg, WFE (g)	912	2900	400	1400	4200	0.32	-
Age at first egg, AFE (d)	912	134.2	21.1	99	226.0	-	0.32
ln(AFE94), ln(days)	912	3.56	0.54	1.61	4.88	-	0.31
Abdominal Fat, ABF (g)	455	134.5	48.0	17.4	409.2	0.27	0.71
lnABF, ln(g)	455	4.84	0.37	2.86	6.01	0.18	0.69

Table 5.3 Chromosome, F-ratio, QTL position, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation explained for weight at first egg (WFE) with and without adjustment for covariates in a broiler-layer F_2 chicken population.

Chromosome	F-ratio[1]	Position (cM)	CI[2]	Flanking markers	Additive effect (SE)	Dominance effect (SE)	VP[3] (%)
WFE[4] (with no covariate), g							
2	10.29**	326	246-422	ADL0114 - MCW0056	85 (18.6)	2 (31.2)	1.9
4	36.40**	157	144-184	ADL0241 - MCW0180	381(45.1)	170 (172.3)	7.2
8	10.10**	60	0-74	MCW0100 - ROS0075	110 (25.0)	38 (48.2)	1.9
27	19.00**	0	0	ROS0071	107(17.3)	2 (25.1)	3.7
Z	9.23*	103	63 -127	LEI0111 – LEI0075	96 (31.6)	-	1.0
Z	10.64**	0	0 - 65	ROS0072 – ADLO201	82 (25.0)	-	1.0
WFE (lnABF[5] fitted as covariate), g							
1	11.25**	134	118 – 494	LEI0068 – LEI146	89 (18.2)	12 (27.7)	4.1
2	10.17*	325	246 – 340	ADL0114 – MCW0056	82 (17.9)	52 (31.1)	4.1
4	15.79**	175	147 – 195	ADL0241 – MCW0180	201 (33.8)	-39 (93.1)	6.3
8	7.87†	23	0 – 63	ADL0179 – MCW0095	71 (18.5)	-6 (26.1)	2.4
27	23.30**	0	0	ROS0071	124 (17.0)	-6.8 (26.0)	9.5

Continues on the next page

Table 5.3 cont'd Chromosome, F-ratio, QTL position, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation explained for weight at first egg (WFE) with and without adjustment for covariates in a broiler-layer F_2 chicken population.

Chromosome	F-ratio[1]	Position (cM)	CI[2]	Flanking markers	Additive effect (SE)	Dominance effect (SE)	VP[3] (%)
	WFE[4] (lnAFE94[6] fitted as covariate), g						
1	8.78*	141	90 - 501	LEI0068 – LEI0146	64(16.5)	42(24.3)	1.5
2	16.65**	307	285 -333	ROS0074 – ADL0114	95 (17.2)	-50 (29.7)	3.0
3	7.42†	136	11-250	MCW0127 – LEI0118	43.8 (16.1)	63 (22.4)	1.2
4	45.05**	155	144 -172	ADL00241- MCW0180	401 (41.8)	-94 (164.7)	8.3
8	9.93**	60	12 -78	MCW0100 – ROS0075	100 (22.6)	39 (43.4)	1.7
13	7.43**	54	26 - 68	MCW0340 – ADL0225	101 (27.3)	-85 (57.9)	1.2
27	31.82**	0	0	ROS0071	125 (15.5)	8 (22.6)	5.8
28	6.98†	1	0 -39	ROS0095 – ROS0085	-62 (16.3)	-1 (22.7)	1.1
Z	14.65**	17	0 -113	ROS0072 – ADL0201	116 (30.2)	-	1.6

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

[3] VP% = percentage of phenotypic variation explained by the QTL

[4] WFE = weight at first egg, g

[5] lnABF =ln(abdominal fat, lng)

[6] lnAFE94 = ln(age at first egg-94, d)

Table 5.4 Chromosome, F-ratio, QTL position, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation explained for the natural logarithm transformed age at first egg (lnAFE94[5]) in a broiler-layer F_2 chicken population.

Chromosome	F-ratio[1]	Position (cM)	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP[3] (%)
lnAFE94[4] (with no covariate fitted), ln(d)							
1	5.70[†]	164	0-380	LEI0146 – ADL0319	-0.07 (0.022)	0.06 (0.042)	1.1
2	8.43*	291	115-358	ROS0023 – ADL0236	-0.06 (0.017)	0.04(0.025)	1.7
3	5.50[†]	24	16-185	MCW0083 – HUJ0006	-0.07(0.021)	0.01(0.035)	1.0
13	7.00[†]	0	0-44	MCW0340 – ADL0225	-0.07 (0.018)	0.02 (0.027)	1.4
15	5.47[†]	41	11-49	LEI0083 – MCW0080	-0.08 (0.025)	0.02 (0.052)	1.5

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and ([†]) suggestive

[2] CI = 95% confidence interval

[3] VP% = percentage of phenotypic variation explained by the QTL

[4] lnAFE94 = ln(age at first egg-94d)

Table 5.4 cont'd Chromosome, F-ratio, QTL position, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation explained for the natural logarithm transformed age at first egg (lnAFE94[5]) in a broiler-layer F_2 chicken population.

Chromosome	F-ratio[1]	Position (cM)	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP[3] (%)
lnAFE94[4] (with WFE[5] fitted as a covariate), ln(d)							
1	9.0**	153	30 - 386	LEI0146 – ADL0319	-0.08 (0.019)	0.01 (0.032)	1.7
2	15.4**	291	229 - 298	ROS0023 – ADL0236	-0.08 (0.015)	0.05 (0.023)	3.1
3	8.2*	139	5 - 230	MCW0127 – LEI0118	-0.06 (0.017)	-0.05(0.024)	1.5
3	7.7†	23	9 - 250	MCW0083 – HUJ0006	-0.07 (0.019)	-0.03 (0.031)	1.4
4	8.1*	195	65 - 195	ADL0241 – MCW0180	-0.08 (0.021)	-0.06 (0.037)	1.5
4	6.7†	3	0 - 194	ADL0317 – MCW0295	-0.07 (0.018)	0.01 (0.028)	1.2
13	8.6*	20	0 - 46	MCW0340 – ADL0225	-0.11(0.026)	-0.01 (0.057)	1.6
27	10.1**	0	-	ROS0071	-0.06 (0.016)	-0.04 (0.023)	2.0

[1]Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2]CI = 95% Confidence interval

[3]VP% = Percentage of phenotypic variation explained by the QTL

[4]lnAFE94 = $\log_e$ (age at first egg-94 d)

[5] WFE = weight at first egg

Figure 5.1 Plot of F-ratio versus relative QTL positions on chromosome 1 for WFE (with lnAFE94 as a covariate). The relative position on the linkage map is presented on the x-axis and the F-ratio on the y-axis.

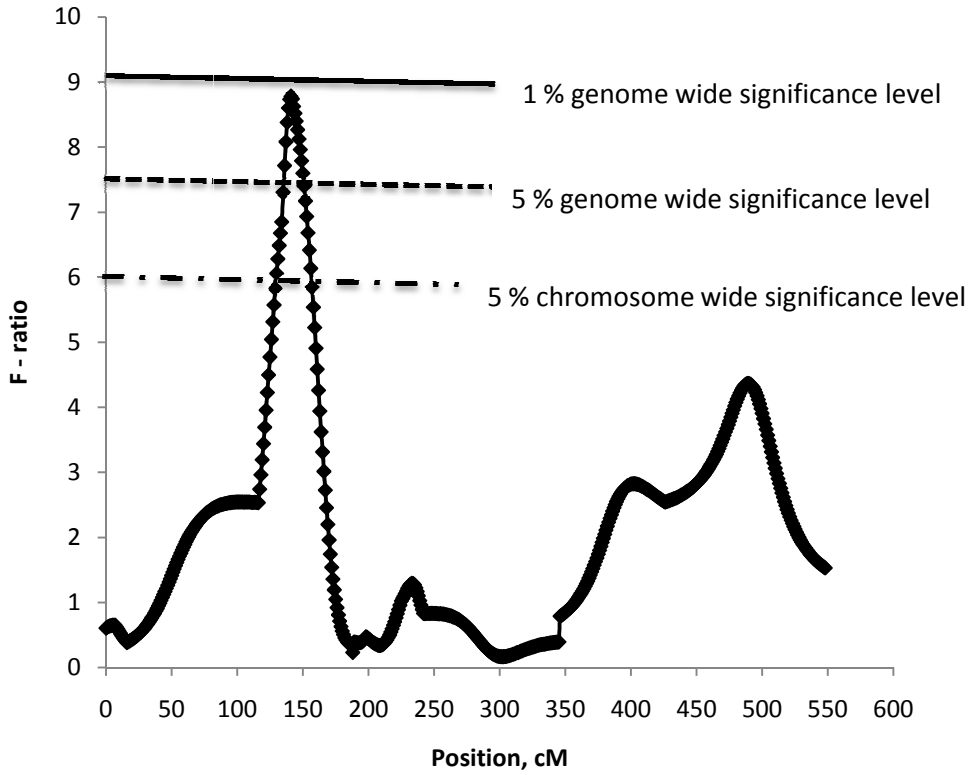
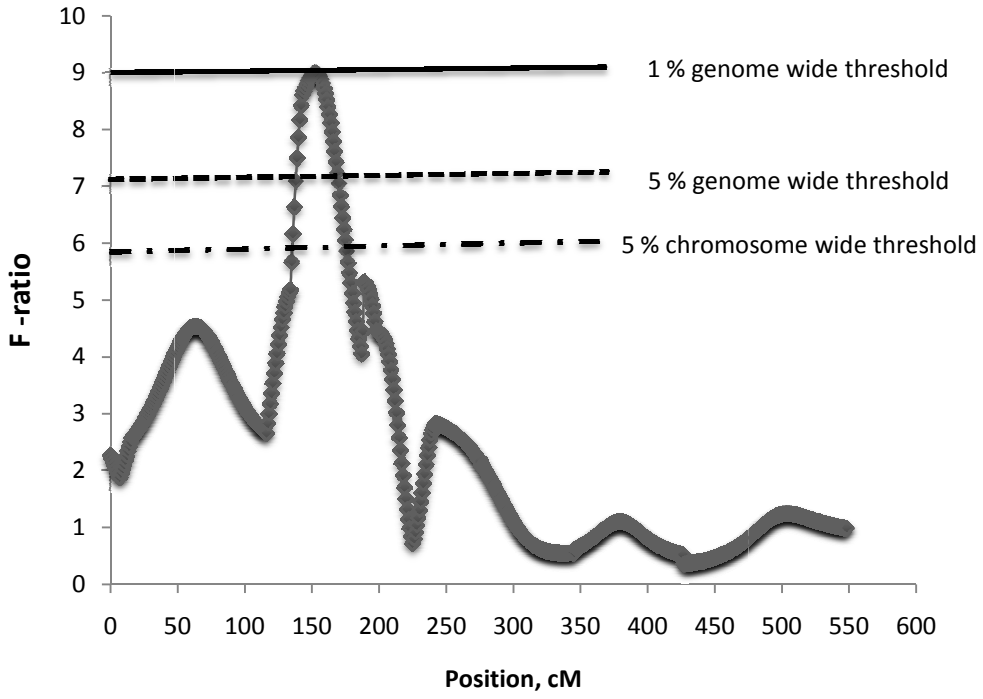


Figure 5.2 Plot of F-ratio versus relative positions on chromosome 1 for lnAFE94 QTL (with WFE as a covariate). The relative position on the linkage map is presented on the x-axis and the F-ratio on the y-axis.



5.3.3 Location of QTL and genetic effects

Without lnAFE94 as a covariate, significant effects were detected on chromosomes 2, 4, 8, 27 and Z (Table 5.3) for WFE. Two peaks of significance were found on the Z chromosome and their confidence intervals (CI) barely overlap. This suggests that two QTL affecting WFE may be located on this chromosome. When including lnABF as a covariate in the analysis of WFE an additional QTL on chromosome 1 became significant but evidence was lost for the Z chromosome QTL. Including lnAFE94 as a covariate for WFE detected a further three QTL on chromosomes 3, 13 and 28, with only one of the original Z chromosome QTL being significant. QTL on chromosomes 4 and 27 accounted for the highest proportion of phenotypic variation (8.3% and 5.8%, respectively) when including lnAFE94 in the analysis. Without WFE as a covariate, only one significant QTL for lnAFE94 was detected, on chromosome 2 with suggestive QTL on chromosomes 1, 3, 13 and 15. With WFE as a covariate the evidence for a QTL became significant in similar regions of chromosomes 1 and 13. In addition, QTL were found on chromosomes 4, 27 and a different region of chromosome 3. Based on location of peaks of significance, and CI, there is also evidence for 2 QTL on both chromosome 3 and chromosome 4 (Table 5.4). The QTL explained relatively small proportions of 1.0 to 3.1% of the phenotypic variation for lnAFE94.

A QTL was detected for lnAFE94 and for WFE in similar regions on chromosome 2, indicating that the same QTL may influence both traits. With no covariate in the

analysis, this was the only QTL found to affect both WFE and AFE. With the alternative trait as covariate in the analysis, however, there was extensive commonality with QTL significant for the two traits on chromosomes 1, 2, 3, 4, 13 and 27. The effects of including a covariate in the analysis will be considered in detail in the Discussion section.

The additive effects for the WFE QTL were all positive, indicating that the increasing allele was from the broiler line (Table 5.3), except for the suggestive QTL on chromosome 28 which had a negative estimate. Dominance effects were not significant indicating that dominance was not an important mode of gene action for this trait. The largest (401 g) and the smallest (64 g) additive effects observed for significant WFE QTL were, respectively, on chromosomes 4 and 1 where lnAFE94 was fitted as a covariate. Inclusion of lnAFE94 as a covariate in the analysis for this trait increased the number of significant linkage groups and slightly increased the magnitude of the effects for each QTL. Fitting lnABF as a covariate had less effect on the results.

The results for the additive and dominance effects for lnAFE94 QTL are presented in Table 5.4. Additive gene action was also important for lnAFE94. The QTL additive effects for lnAFE94 were small and mainly negative. There was no support for epistatic interactions between QTL for either trait.

5.4 Discussion

5.4.1 Effect of including a covariate in the QTL analysis

Including a genetically controlled and phenotypically correlated trait as a covariate in the analysis will affect the evidence for a QTL at a specific location, depending on the direction and magnitude of QTL effects on the two traits (Goddard *et al.* 2001; Neuschl *et al.* 2007; Chiu *et al.* 2010). If the QTL affects the trait, but not the covariate, inclusion of the covariate will increase the evidence for the QTL. This may have happened for the QTL for WFE on chromosome 27, when lnAFE was fitted as covariate. If the QTL affects the covariate but not the trait, inclusion of the covariate will identify a QTL for the covariate rather than a QTL for the trait under investigation. The QTL on chromosomes 4 and 27 originally detected for WFE became significant for lnAFE94 when WFE was included as a covariate, suggesting that these QTL affected WFE rather than lnAFE94. If the QTL affects both the trait and the covariate (i.e. a pleiotropic QTL), the ability to detect it will depend on the QTL and the phenotypic correlations (i.e. the evidence may be lost or enhanced). In this study the traits in the F_2 were positively correlated phenotypically (i.e. individuals with late AFE tended to have high WFE, since these birds had more time to gain weight before first egg). QTL operating in the same direction in both traits, consistent with the phenotypic correlation, will generally lose evidence when one of the traits is fitted as a covariate in the analysis of the other. Apparently this is what happened to the QTL on chromosomes 4, 8 and Z when lnABF was fitted as a covariate to WFE (Table 5.3). The original breed difference, however, implies that birds with later AFE have low WFE and vice versa. This will tend to enhance the

evidence for such a pleiotropic QTL and increase the effect estimates when fitting one of the traits as a covariate in the analysis of the other. The QTL identified for both WFE and lnAFE94 on chromosome 2, could be such a case, as significance for both traits increased when the alternative trait was used as a covariate. The QTL on chromosomes 1 and 13 detected for lnAFE94 became significant when WFE was included as a covariate, and were also identified for WFE when lnAFE94 was included as a covariate. These could be additional examples of pleiotropic QTL, with the QTL affecting both WFE and lnAFE94. Finally, the QTL on chromosome 3 in interval MCW0127 - LEI0118 may be another example as it was detected for both traits but only in the presence of the other as a covariate. In each of these instances, as an alternative to pleiotropy, the co-location of QTL affecting each of the two traits could be due to separate tightly clustered loci that individually influence a single trait (Almasy *et al.* 1997). A better way to detect pleiotropic QTL would be to analyze the traits simultaneously (Knott & Haley 2000).

5.4.2 Importance of the identified QTL

The sum of the additive effects for significant WFE QTL was 0.86 kg accounting for 1.7 kg additive effects difference between the lines. This represents 44 % of the live weight difference (3.9 kg) between the lines at the onset of lay (Table 5.1). For all of these QTL the allele increasing WFE was inherited from the broiler line, which is consistent with the breed difference (Table 5.1). The sum of the additive effects for lnAFE94 (adjusted for WFE) was approximately 12 days. This represents an additive effect difference of 24 days or about half the phenotypic difference between the lines

(Table 5.1). However when the covariate is not fitted the additive effects difference between the lines is about 3.4 days only, suggesting that weight explains much of the observed difference.

Additive effects for lnAFE94 were generally negative (Table 5.4) indicating that the broiler line was earlier maturing than the layer genotype (consistent with the breed differences, Table 5.1) and is consistent with the expectation that fast growing (heavy) birds tend to reach sexual maturity earlier. However, it should be noted that the layer line was unusually late maturing (Table 5.1), possibly due to the lack of photostimulation in this experiment. A constant photoperiod was adopted to avoid confounding differences in maturity with the timing of photostimulation.

5.4.3 QTL affecting early growth rate affect WFE

An earlier study on broiler offspring of the same parents as the present data (Sewalem *et al.* 2002) reported significant QTL for live weight at earlier ages (3 - 9 weeks of age) than in this study on chromosomes 1, 7, 13 and Z (for 3 weeks of age), chromosomes 1, 2, 4, 7, 8, 13 (for 6 weeks of age), and on chromosomes 1, 2, 4, 8, 13 and 27 (for 9 weeks of age). In that study, covariates were not fitted in the analysis. Even though the earlier study used much younger ages (3 - 9 weeks) than in our study (19 weeks of age), the QTL detected for live weight (in the earlier study) and for WFE (without a covariate) in our study were at similar positions across the implicated linkage groups.

Similar results are obtained when comparing our results on WFE with those of other studies on early growth rate. The highly significant QTL for body weight on chromosome 4 detected in this study confirms findings from several studies that have reported large effect body weight QTL on this chromosome (Schreiweis *et al.* 2006). Zhou *et al.*(2006) reported significant QTL for body weight at 8 weeks of age in a broiler-White Leghorn cross on chromosomes 1, 2, 4, 7, 9 and 18. Body weight QTL (at 7 weeks of age) were reported on chromosomes 1, 2 and 13 from a broiler population (Atzmon *et al.* 2006). Ruy *et al.* (2005) reported 4 suggestive QTL on chromosome 3 and three suggestive QTL on chromosome 5 for WFE from a layer-broiler cross. Thus the growth QTL reported in other studies were also in similar chromosome positions to the ones observed in our study and this suggests that sexual maturity QTL are generally not distinct from those for growth.

In contrast to our results, Carlborg *et al.* (2003) observed a pronounced role of epistatic effects on growth prior to 46 days of age in a red jungle fowl - White Leghorn cross and reported significant QTL for body weight in the 1-200 days age-bracket on chromosomes 1, 2, 3, 4, 7, 8, 11, 12, 13, 14, 27 and E27W24. The important contribution of epistasis to early growth (before 6 weeks of age) was also observed in a White Leghorn layer and broiler sire line cross population from the same parents as the present flock (Carlborg *et al.* 2004a).

The WFE QTL on chromosome 27 appears worthy of special attention. When lnABF was used as a covariate, this QTL explained almost 10% of the phenotypic variance in WFE. The QTL is located in a region that contains several growth related

genes (e.g. chicken growth hormone (*cGH*)) (Lei *et al.* 2007). The G+1705A in intron 3 of *cGH* could have a direct effect on chicken growth via an influence on *cGH* gene expression (Nie *et al.* 2005).

There are few specific QTL (on chromosomes 8 and 13) associated with age and weight at sexual maturity that are not found when analysing weight at younger ages. However the magnitude of their additive effects for lnAFE94 is relatively small suggesting that there is limited opportunity to genetically manipulate sexual maturity independently of commercial broiler growth traits.

Schreiweis *et al.* (2006) reported a suggestive AFE QTL on chromosome 3 which is similar to the location of a QTL in the current study. AFE QTL have also been reported on chromosome 4 (Schreiweis *et al.* 2006). However, a QTL for AFE reported on chromosome Z by Tuiskula-Haavisto (2002) and Sasaki (2004) was not detected in this study.

5.4.4 Abdominal fat, body weight and puberty

The high correlation (0.71) between ABF and WFE (Table 5.2) suggests that these traits are controlled by similar factors. Circulating lipids increase at the onset of lay for deposition into the developing yolky follicles (Jaccoby *et al.* 1995). The accumulation of body fat is likely to be important for this reason as it is a source of circulating lipid as well as *de novo* synthesis in the liver. Therefore the use of lnABF to model QTL affecting WFE may identify regions of the genome that are associated

with lean tissue mass at the onset of lay. The fact that the majority of the QTL are not affected by fitting body fat as a covariate suggests that the amount of fat does not explain the onset of sexual maturity in poultry (Soller *et al.* 1984) except in the case of QTL on chromosome 1.

In general the results are consistent with the concept that achieving a minimum body weight is permissive for the attainment of sexual maturity (Brody *et al.* 1984; Eitan & Soller 2001). The power of an F_2 cross lies in the fact that traits that characterise two breeds segregate independently. Furthermore because the estimated WFE QTL effects at these locations are not the result of dietary manipulation this may be one of the clearest demonstrations that genetic determination of growth rate results in correlated effects on puberty. The possibility that the converse is true can be eliminated because the effects of the growth QTL take place before the age of puberty is reached. Clear evidence of genetic correlations between growth rate and puberty are not numerous: studies in female pigs suggests that there is a negative phenotypic correlation between growth and puberty (Hutchens *et al.* 1981) and in humans it was estimated that 57% of the additive genetic effects for the age of menarche and body mass index were common (Kaprio *et al.* 1995). The genetic and physiological determinates of sexual maturity underlying the QTL identified in this study remain to be elucidated through a combination of fine mapping and identification of candidate genes for subsequent validation.

5.5 Conclusions

In conclusion, the QTL for WFE and lnAFE94 detected in this study generally acted additively and the broiler alleles were associated with heavier body weights and earlier ages at the onset of lay. The indication that the loci for growth and puberty are common provides a clear demonstration of the genetic basis for the phenotypic correlation between growth and puberty and that body weight is an important determinant for the attainment of sexual maturity.

CHAPTER SIX

Reproductive Traits QTL

6.1 INTRODUCTION

Understanding the genetic mechanisms influencing the development of the ovary and related traits at sexual maturity is important because the ovary is a vital organ for reproduction in chickens. Identification of quantitative trait loci (QTL) for ovarian traits at puberty and genetic factors influencing reproductive traits at this critical stage of production would provide vital information of biological interest and would also enhance selection efforts targeting reproductive traits. Unravelling the genetic mechanisms controlling traits associated with reproductive function such as ovulation rate is important in the effort to improve poultry productivity and welfare in breeding birds of poultry meat strains (broiler, turkey and duck breeders) (Hocking 2009b).

Identification of QTL in chickens has focussed mainly on broiler traits and a few health related factors, and there are relatively few reported QTL for reproduction traits (Hocking 2005; Abasht *et al.* 2006a). Recent QTL based evidence for epistasis included the detection of an epistatic pair of QTL for body weight at 46-112 days in a red jungle fowl x White Leghorn cross and at 3 and 6 weeks of age in the Roslin broiler - layer cross (Carlborg *et al.* 2003; Carlborg *et al.* 2004b). Cheng *et al.* (2007) reported widespread evidence of epistatic interactions influencing Marek's Disease viraemia levels in chickens. Significant QTL by QTL interactions were reported for both muscle yield and abdominal fatness traits by Ankra-Badu *et al.*, (2010).

Chickens normally recruit a yellow yolky ovarian follicle approximately daily into a hierarchy of increasing follicle size that eventually ovulate 5 to 7 days later as fully formed egg yolks. Broiler chickens exhibit an ovarian dysfunction where the ovary recruits two or more follicles each day producing a double hierarchy, a condition that persists for an extended period of time and results in very low rates of egg production (Hocking *et al.* 2002a; Hocking 2009a). This ovarian dysfunction causing multiple ovulations is a problem in broiler breeders and is controlled by limiting body weight gain at the expense of hunger, creating a welfare dilemma (Griffin & Goddard 1994; Decuypere *et al.* 2006). Genetically manipulating the expression of growth factors might assist in addressing this problem (Onagbesan *et al.* 2009) and identifying the genetic loci involved is crucial to provide poultry breeders with the tools to reduce multiple ovulation by genetic selection.

Only the left ovary and oviduct is functional in female chickens but some birds exhibit persistent right oviducts (Frank 1931) which is transmitted genetically and the prevalence of this condition can be increased by selection (Wakamatsu *et al.* 2000). Some development of the right oviduct is frequently observed in broiler chickens and identification of the mechanism suppressing the right oviduct would be biologically interesting. A search was therefore conducted for QTL for a score of the degree of development of the right oviduct.

The ovary is the site for the production of growth factors that regulate the physiological development of the reproductive process (Onagbesan *et al.* 2009). The relative size of the ovary may be an important component of this process.

Interestingly, the relative comb size reflects the influence of steroid hormones produced by the ovary and is functionally important in achieving mating success (Balthazart & Hendrick 1978; Brodsky 1988). QTL for comb mass have been observed to cluster with QTL for female reproductive and skeletal investment (Wright *et al.* 2008).

Several studies have identified QTL for abdominal fat in juvenile birds using an additive-dominance model ignoring epistasis (Tatsuda & Fujinaka 2001; Ikeobi *et al.* 2002; Tuiskula-Haavisto *et al.* 2002; de Koning *et al.* 2003b; Park *et al.* 2006; Atzmon *et al.* 2008; Liu *et al.* 2008). Abdominal fat is a good indicator of adiposity (Pinchasov & Cahaner 1991). Lipids, particularly triglycerides, may be stored in adipocytes, hepatocytes and growing oocytes in avian species. Besides serving as an energy store for incubation, lipid storage in the oocytes is associated with vitellogenesis and further development of the embryo (Needham 1925; Hermier 1997).

The presence of heterosis for reproductive traits is attributed to non-additive gene action (Williams *et al.* 2002; Melchinger *et al.* 2007). However, there are no reports on the role of epistatic quantitative trait loci (QTL) on reproductive traits in chickens. One of the limitations constraining the conduct of studies on epistasis is the lack of statistical methods with sufficient power to detect epistatic QTL (Falconer & Mackay 1996; Carlborg *et al.* 2004a; Ankra-Badu *et al.* 2010). Detection of epistasis has some inherent problems associated with multiple testing, and is difficult to replicate findings due to variation in gene frequencies in different populations (Hill *et al.*

2008a). Epistasis modelling tools are increasingly being proposed to unravel the genetic architecture of traits of medical, biological and economic importance (Carlborg *et al.* 2003; Carlborg & Haley 2004; Le Rouzic *et al.* 2008; Ankra-Badu *et al.* 2010) and relevant software has recently become available (Seaton *et al.* 2006; Wei *et al.* 2010b).

Accounting for epistasis in whole genome QTL analysis is often ignored although epistasis can be an important component of the genetic architecture of complex traits (Carlborg and Haley, 2004). To investigate the role of epistasis among traits related to reproduction at sexual maturity we used a QTL detection approach which not only applies stringent thresholds through a nested test framework to minimize detection of false positives but is also capable of detecting QTL with weak effects. The software runs on a grid of fast computers (Grid-QTL) for efficient computation (Wei *et al.* 2010a). Specifically, the analysis was conducted to identify QTL for the number of normal yellow follicles, ovary weight, oviduct weight, a score for the degree of development of the right oviduct, comb weight, and abdominal fat weight at first egg in a broiler-layer cross in which the White Leghorn was much later maturing than the male broiler line and there was also a two-fold breed difference in terms of the ovary weight and the number of normal yellow follicles.

6.2 MATERIALS AND METHODS

6.2.1 Production of F_2 Resource Population

The production of parental lines and F_2 resource populations is described in Chapter 3.

6.2.2 Collection of genotype data

This GM9 population was genotyped using a total of 143 microsatellite markers covering 26 autosomal linkage groups including the Z chromosome (Table 6.1). The genotyping, construction of linkage maps and QTL analysis were conducted as described in chapter 3.

6.2.3 Collection of phenotypic data

Data were collected on the age (AFE) and body weight (WFE) of each bird after it laid its first egg. Each bird was removed from the cage within 2 - 3 days after laying its first egg and killed with an overdose of sodium pentabarbitone. The abdominal cavity was opened and the abdominal fat pad and fat surrounding the gizzard and proventiculus were dissected out, weighed, and collectively described as abdominal fat (AFW). The oviduct (ODW) was removed and weighed. Yellow follicles (NYF) with a diameter greater than 8 mm were cut from the ovary, counted and weighed. Atretic yellow follicles were similarly removed and the remaining ovary tissue (OVW) was weighed. The comb (CMW) for each bird was cut off the head and weighed. The right oviduct for each bird in the F_2 was examined and given a score (ROS) ranging from 1 to 4. The scoring was defined as: - absent (0), present (1), magnum tissue present (2), shell gland and magnum tissue present (3), and small oviduct (4).

Table 6.1 The number of microsatellite markers, first and last marker and map length on each linkage chromosome in the QTL analysis of reproductive traits in the broiler-layer cross F_2 population.

Chromosome	Number of markers	First marker	Last marker	Map length (cM)
1	19	ADL0160	LEI0079	581
2	14	LEI0163	MCW0157	401
3	27	ADL0177	MCW0037	278
4	15	ADL0143	LEI0073	244
5	7	LEI0082	ADL0298	142
6	4	ROS0062	ADL0323	59
7	3	LEI0064	ADL0180	94
8	10	ROS0026	ADL0278	136
9	4	ROS0078	MCW0134	124
10	1	ADL0209	ADL0209	-
11	6	LEI0110	ROS00112	67
12	2	ADL0240	ADL0044	53
13	3	MCW0340	ADL0225	72
14	1	MCW0123	MCW0123	-
15	2	LEI0083	MCW0080	50
16	1	LEI0258	LEI0258	-
17	1	ADL0199	ADL0199	-
18	3	MCW0219	ROS0027	25
19	1	MCW0094	MCW0094	-
22	1	ROS0073	ROS0073	-
23	2	ADL0289	MCW0249	50
24	1	ROS0113	ROS0113	-
26	2	ADL0285	LEI0074	-
27	1	ROS0071	ROS0071	-
28	3	ROS0095	ADL0299	42
z	9	ADL0022	LEI0075	155
Total	**143**			**2573**

6.2.4 Data description

A total of 143 microsatellite markers covering 26 linkage groups including the z chromosome were used in this analysis Table 6.1. The original F_2 data of about 500 individuals were edited for genotype and parentage errors. Individuals with missing phenotype records or with the number of normal yellow follicles below 5 were removed to exclude individuals with extreme reproductive problems from affecting the analysis results. The resulting analysis data set had 450 F_2 individuals and 32 full sib families (Table 6.3). The records for WFE, AFW, CMW, OVW and ODW were not normally distributed and were transformed by taking natural logarithms (ln) to approximate normality.

6.2.5 Model selection

Different models with additive, dominance and parent-of-origin genetic effects with family and pen as fixed effects (hatch was confounded with pen) were evaluated in a preliminary analysis. There was no evidence for a parent-of-origin effect (detected as a difference between the alternative heterozygous genotypes that differ in which allele was inherited from each parent) (Knott *et al.* 1998). Therefore, it was not considered in subsequent analyses in this study. The Z chromosome was analysed with an additive genetic effects model for the detection of single QTL with significant marginal effects. The NYF was analyzed with an additive genetic effects model.

Models with and without a covariate were fitted for each trait. LnWFE was fitted as a covariate in the QTL analysis models for lnAFW, lnCMW, lnODW and lnOVW. However, no covariates were fitted in the models for NYF and ROS.

6.2.6 Detection of epistatic QTL

The detection of epistatic QTL for each trait was conducted by fitting models with and without covariate as described above. The epistasis analysis did not include the Z chromosome. The search for epistatic QTL pairs was done using two search approaches as described in detail in Chapter 3. Similarly, as described in Chapter 3, significant additive genetic effects due to interaction between locus 1 (a1) and locus 2 (a2), i.e. a1 x a2 and the dominance genetic effects due to the interaction between locus 1 (d1) and 2 (d2), (i.e. d1 x d2) were detected following Jana (1971).

6.3 RESULTS

6.3.1 Population parameters

The trait means and standard deviations for the F_2 resource population are presented in Table 6.3 and the phenotypic correlations of the analyzed traits are presented in Table 6.4. The arithmetic mean of the parental lines was higher than the mean value for the F_2 population for most traits except ODW and NYF (Tables 6.2 & 6.3).

Table 6.2 Means and standard deviations (SD) for weight, age, abdominal fat, comb weight, ovary weight, oviduct weight, number of normal yellow follicles and right oviduct score at the onset of lay in male line broiler and White Leghorn layer females. Differences between the lines were all significant (P<0.01).

Trait	Male-line broiler (n=10)		White Leghorn layer (n=12)	
	Mean	SD	Mean	SD
Ovary weight (OVW) (g)	10.1	3.1	4.6	1.4
Oviduct weight (ODW) (g)	61.9	5.9	46.2	4.8
Number of normal yellow follicles (NYF)	15.2	4.4	7.8	1.2
Right oviduct score (ROS)	2.44	1.33	0.00	-
Comb weight (CMW) (g)	nr	-	nr	-
Weight at first egg (WFE)(g)	5400	600	1500	100
Age at first egg (AFE) (d)	130	8	177	10
Abdominal Fat weight (AFW) (g)	255	71	44	9

nr = not recorded

Table 6.3 Number of records (N), means, standard deviations (SD) and range for age, weight, abdominal fat, comb weight, ovary weight, oviduct weight, number of normal yellow follicles and right oviduct score at the onset of lay in an F_2 broiler - layer population (n = 450, ln values in parentheses).

Variable	Mean	SD	Min	Max
Ovary weight, OVW (g)	6.2 (1.7)	2.6 (0.4)	5.0 (1.8)	22.3 (3.1)
Oviduct weight, ODW (g)	60.0 (4.1)	11.1 (0.2)	36.0 (3.6)	99.3 (4.6)
Number of Normal yellow follicles, NYF	13.0	3.1	5.0	23.0
Right oviduct score, ROS	0.8	1.0	0	4.0
Comb weight, CMW (g)	5.6 (1.6)	2.5 (0.4)	0.8 (-0.3)	21.4 (3.1)
Abdominal fat weight, AFW (g)	134.0 (4.8)	46.3 (0.4)	17.4 (2.9)	356.6 (5.9)
Weight at first egg, WFE (g)	2975 (8.0)	408 (0.1)	1815 (7.5)	4182 (8.3)
Age at first egg, AFE (d)	146.6 (5.0)	20.6 (0.1)	105.0 (4.7)	218.0 (5.4)

Most of the analysed traits had a low correlation with each other except for the moderately high correlation (0.69) between lnWFE and lnAFW (Table 6.4). All the significant correlations were positive except for the number of normal yellow follicles which had a low negative phenotypic correlation with lnAFE reflecting a mild decline in the number of normal yellow follicles as age at puberty increases.

Table 6.4 Phenotypic correlations for the natural logarithms of age, weight, abdominal fat, comb weight, ovary weight, oviduct weight, number of normal yellow follicles and right oviduct score at the onset of lay in an F_2 broiler - layer population. (P<0.05 unless indicated otherwise).

	lnOVW[1]	lnODW[2]	NYF[3]	lnCMW[4]	ROS[5]	lnAFW[6]	lnWFE[7]	lnAFE[8]
lnOVW	-	0.40	0.38	0.48	0.07	0.20	0.43	0.25
lnODW		-	0.22	0.27	0.14	0.10	0.46	0.13
NYF			-	0.18	-0.06[ns]	0.03[ns]	0.13	-0.27
ROS					-	-0.06	0.03[ns]	0.23
lnCMW						0.23	0.36	0.22
lnAFW						-	0.69	0.21
lnWFE							-	0.26

[1] lnOVW = ln(Ovary weight at first egg), ln(g)

[2] lnODW = ln(Oviduct weight at first egg), ln(g)

[3] NYF = number of normal yellow follicles

[4] lnCMW = ln(Comb weight at first egg), ln(g)

[5] ROS = Right oviduct score at first egg

[6] lnAFW = ln(Abdominal fat weight at first egg), ln(g)

[7] lnWFE = ln(Weight at first egg), ln(g)

[8] lnAFE = ln(Age at first egg), ln(g)

ns = not significant at 0.05 % level of significance

Table 6.5.1 Chromosome, QTL position, F-ratio, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation (VP) explained for the natural logarithm (ln) of ovary weight (lnOVW) at the onset of lay fitted with and without ln (weight at first egg), lnWFE as a covariate in a broiler-layer F_2 population.

Chromosome / Trait	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect (SE)	Dominance effect (SE)	VP (%)
lnOVW, (no covariates) lng							
2	107	7.9†	71 - 300	ADL0176 - ADL0196	-0.18 (0.05)	-0.26 (0.14)	3.2
6	14	5.7†	0 - 57	ROS0062 - ROS003	0.11 (0.03)	-0.03 (0.05)	2.2
8	34	5.4†	1 - 113	MCW0100 - ROS0021	0.09 (0.03)	0.09 (0.05)	2.1
lnOVW, (lnWFE fitted as covariate), lng							
2	79	14.4*	60 - 138	MCW0056 - MCW0157	-0.14 (0.03)	-0.04 (0.04)	6.1
2	298	5.6†	68 - 336	ADL0114 - MCW0056	-0.09 (0.03)	-0.07 (0.05)	2.1
4	243	7.2†	9 - 243	ADL0260 - LEI0073	-0.09 (0.02)	0.04 (0.04)	2.8

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% Confidence interval

Table 6.5.2 Chromosome, QTL position, F-ratio, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation (VP) explained for the natural logarithm (ln) of oviduct weight (lnOVW) fitted with and without ln (weight at first egg), lnWFE as a covariate and the number of normal yellow follicles (NYF) at the onset of lay in a broiler-layer F_2 population.

Chromosome / Trait	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect (SE)	Dominance effect (SE)	VP (%)
lnODW, (no covariates), ln(g)							
4	213	39.52**	204 -223	MCW0180 - ADL0260	0.15 (0.02)	0.02 (0.04)	16.1
7	59	7.3†	0 - 93	LEI0064 - ROS0019	-0.10 (0.03)	0.14 (0.11)	2.6
lnODW, (lnWFE fitted as covariate), ln(g)							
1	271	5.1†	59 - 498	LEI0101 - LEI0088	-0.10 (0.03)	0.14 (0.19)	1.8
2	126	5.7†	2 - 375	ADL0176 - ADL0196	-0.09 (0.03)	-0.07 (0.15)	2.0
3	53	8.1†	0 - 209	MCW0083 - ADL0370	-0.06 (0.01)	0.03 (0.03)	3.0
4	210	18.1**	197 - 233	MCW0180 - ADL0260	0.09 (0.02)	0.00 (0.03)	7.3
7	72	7.6*	34 - 93	LEI0064 - ROS0019	-0.07 (0.02)	0.04 (0.05)	2.8
NYF (no covariates were fitted for NYF)[3]							
9	67	7.3†	0 - 124	ROS0078 - MCW0135	-0.69 (0.26)	-	1.5
27	0	10.2†	-	ROS0071	0.65 (0.22)	-	2.2

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% Confidence interval

[3] QTL detected using an additive model

Table 6.5.3 Chromosome, QTL position, F-ratio, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation (VP) explained for the right oviduct score (ROS) and the natural logarithm of the comb weight (lnCMW) fitted with and without a covariate at the onset of lay in a broiler-layer F_2 population.

Chromosome / Trait	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect (SE)	Dominance effect (SE)	VP (%)
ROS (no covariates were fitted for ROS)							
5	46	7.0†	0 - 120	ADL0292 - ROS0084	-0.14 (0.07)	-0.29 (0.09)	2.8
5	125	6.8†	0 - 136	ROS0013 - ADL0298	-0.18 (0.09)	-0.69 (0.22)	2.7
11	15	6.0†	4 - 67	LEI0072 - ROS0111	0.03 (0.06)	0.35 (0.01)	2.3
lnCMW,(no covariates) ln(g)							
4	10	7.8†	0 - 206	ADL0143 – ADL0317	-0.11 (0.03)	0.07 (0.05)	3.3
9	52	4.8†	0 - 114	ROS0078 – MCW0135	-0.15 (0.05)	-0.01 (0.13)	1.8
lnCMW, (lnWFE fitted as covariate), ln(g)							
4	7	13.7**	0 - 225	ADL0143 - ADL0317	-0.05 (0.03)	0.05 (0.05)	5.9
5	89	6.3†	17 - 127	ROS0084 - ADL0166	-0.13 (0.04)	0.07 (0.08)	2.5
9	39	4.9†	0 - 114	ROS0078 - MCW0135	-0.17 (0.05)	0.09 (0.20)	1.8

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% Confidence interval

Table 6.5.4 Chromosome, QTL position, F-ratio, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation (VP) explained for the natural logarithm (ln) of abdominal fat (lnAFW) fitted with and without ln (weight at first egg), lnWFE as a covariate at the onset of lay in a broiler-layer F_2 population.

Chromosome / Trait	Position (cM)	F-ratio[1]	CI[2]	Flanking markers	Additive effect (SE)	Dominance effect (SE)	VP (%)
lnAFW,(no covariates) ln(g)							
1	140	6.8†	98 - 498	LEI0146 - MCW0007	-0.10 (0.03)	0.0 (0.05)	2.7
4	142	7.5†	67 - 225	ADL0266 - LEI0094	0.07 (0.03)	0.15 (0.05)	3.0
5	10	9.5*	0 -116	LEI0082 - MCW0090	0.12 (0.03)	-0.06 (0.02)	3.9
lnAFW (lnWFE[5] fitted as covariate), ln(g)							
1	123	14.9**	113 - 148	LEI0068 - LEI0146	-0.09 (0.02)	0.01 (0.03)	5.7
2	267	6.1†	41 - 294	ADL0236 - ROS0074	-0.05 (0.020	0.00 (0.02)	2.1
4	215	6.3†	13 - 243	MCW0180 - ADL0260	-0.08 (0.020)	0.06 (0.05)	2.2
9	121	10.8**	90 - 124	ROS0030 - MCW0134	-0.08 (0.02)	0.03 (0.03)	4.0
27	0	19.9**	-	ROS0071	-0.10 (0.02)	-0.00 (0.02)	2.2
28	15	6.8†	41 - 294	ROS0095 - ROS0085	-0.07 (0.02)	0.09 (0.04)	2.4

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% Confidence interval

Table 6.6 Test-thresholds for epistatic QTL and estimated F ratios for marginal-effect QTL and epistatic QTL pairs affecting abdominal fat weight (lnAFW) and ovary weight (lnOVW) in the F_2 broiler-layer cross.

Trait	Genome-wide Significant Marginal Effect QTL				Epistasis Genome-wide Thresholds (corrected for present QTL)							
	Chr[1]	Pos[2]	F ratio		1 D search path				2D search path			
			5% Test Threshold	Estimate	Thresholds		Estimate		Thresholds		Estimate	
					F_{all}[3]	F_{int}	F_{all}	F_{int}	F_{all}	F_{int}	F_{all}	F_{int}
lnOVW	2	80	8.1	12.8**								
	2/8	185/0	-	-	4.2/4.2	5.0/5.0	NE[5]	NE	5.8/5.2	8.9/8.1	5.8**	10.1**
lnAFW	1	125	8.2	13.3**	-	-	-	-	-	-	-	-
	9	120	8.2	9.5*	-	-	-	-	-	-	-	-
	27	0	8.2	16.3**	-	-	-	-	-	-	-	-
	9/12	120/37	-	-	4.7/4.2	5.8/4.8	4.4[†]	5.9*	5.9	9.4	NE	NE

[1] Chromosome and interacting chromosomes pairs e.g. 9/12

[2] Position of QTL on the linkage map, cM

[3] F_{all}: F value for the overall test; the F_{int}: the Fvalue of the interaction test; Significant at experiment-wide 0.05 (*), 0.01 (* *) and ([†]) suggestive levels

[4] Threshold for suggestive epistasis

[5] Not epistatic pair detected through that search path

6.3.2 Main Effects QTL

A summary of the QTL with marginal significant effects detected with an additive-dominance model are listed in Table 6.51 to Table 6.5.4. No covariates were fitted for NYF and ROS no significant QTL were detected for both traits.

QTL for lnODW were detected on chromosome 4 without a covariate and on chromosomes 4 and 7 when lnWFE was modelled in the analysis. The QTL for lnODW (without fitting a covariate) on chromosome 4 explained the highest proportion of the phenotypic variation (16.1%) among all the traits studied. However, that value decreased to 7.3% when the covariate lnWFE was included in the model.

Suggestive QTL for lnCMW were detected on chromosome 4 and on chromosome 9 without fitting a covariate. After fitting lnWFE as a covariate the QTL for lnCMW on chromosome 4 was confirmed and suggestive QTL were detected on chromosomes 5 and 9. The QTL for lnCMW had significant negative additive effects.

A significant QTL for lnAFW was detected on chromosome 5 and fitting lnWFE as a covariate led to the detection of 3 QTL for lnAFW on chromosomes 1, 9, and 27 respectively whereas the QTL on chromosome 5 was not detected when the covariate was fitted (Table 6.5.4). The lnAFW QTL on chromosome 5 had both significant dominance and additive effects.

6.3.3 Test thresholds for epistasis

The 5% genome-wide test threshold F ratio for marginal effect QTL affecting lnOVW was 8.1 compared to the observed F ratio of 12.8 ($p<0.01$) (Table 6.6). For the three marginal effect lnAFW QTL on chromosomes 1, 9 and 27 the 5% genome-wide threshold was 8.2 versus the estimated F ratios of 13.4, 9.5 and 16.3 respectively. The 1D and 2D paths analyses test thresholds corrected for the presence of significant marginal effect QTL used in the detection of epistatic QTL pairs are summarized in Table 6.6.

6.3.4 Epistatic QTL

A pair of significant epistatic QTL pair for lnOVW was detected on chromosomes 2 and 8 respectively at 185 and 0 cM when lnWFE was included as a covariate in the model (Table 6.7). A suggestive QTL x QTL interaction was also detected for lnAFW on chromosomes 9 and 12 where lnWFE was fitted as a covariate. The QTL pair accounted for 7 % of the phenotypic variation in lnAFW where 3 % of the phenotypic variation was accounted for by the interaction component of the QTL pair alone. Among the epistatic QTL pair affecting lnOVW only chromosome 2 had showed the presence of a marginally significant QTL. The locus on chromosome 8 was detected only in the 2D scan suggesting that it had weak effects which were not significant enough to be detected in the 1D scan. The epistatic QTL pair for lnOVW had the highest F-value of 10.1 ($P< 0.01$). The QTL pair accounted for 8 % of the

phenotypic variation of the trait and the interaction also accounted for another 8 % of the phenotypic variation. The additive x additive interaction for lnOVW had the highest and most significant effect (Figure 6.2).

Table 6.7 Epistatic QTL positions, forms of epistasis and phenotypic variance explained for ovary weight and abdominal fat weight at first egg for a layer - broiler cross population with the natural logarithms of weight at first egg (lnWFE) fitted as a covariate

Ch1[1]	Pn1[2] cM	Ch2 cM	Pn2 cM	F_{all}[3]	F_{int}	Var-pair[4] (%)	Var-int[5] (%)	a_1 (SE)	d_1 (SE)	a_2 (SE)	d_2 (SE)	a x a (SE)	a x d (SE)	d x a (SE)	d x d (SE)
lnAFW (lng) (lnWFE, fitted as a covariate)															
9	121	12	35	4.4†	5.9*	6.6	2.9	-0.22 0.08	0.44 0.12	-0.14 0.05	0.50 0.17	-0.03 0.05	0.29 0.17	0.14 0.07	-0.84 0.24
lnOVW (lng) (lnWFE fitted as a covariate)															
2	185	8	0	5.8**	10.1**	8.2	7.9	-0.02 0.03	-0.09 0.05	0.08 0.03	-0.08 0.05	0.21 0.04	-0.05 0.06	-0.10 0.05	0.09 0.08

[1] Chromosome

[2] Position on the linkage map

[3] F_{all}: F value for the overall test; the F_{int}: the Fvalue of the interaction test; Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[4] Proportion of the phenotypic variance explained by the whole epistatic pair

[5] Proportion of the phenotypic variance explained by the interaction component of the pair

Figure 6.1 Mean effects for two QTL one with weak marginal effects. Mean effects for an epistastic QTL pair on chromosomes 2 and 8 interacting to influence lnOVW when lnWFE is fitted as a covariate: the “A” and “B” represents the broiler alleles and the “a” and “b” stands for the layer alleles.

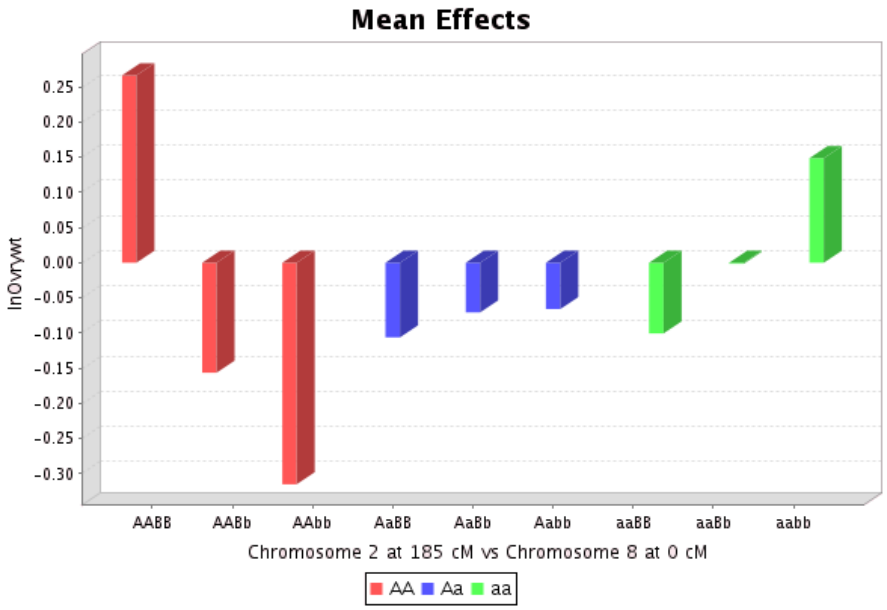


Figure 6.2 Genetic components for the significant epistatic lnOVW QTL where "ai" and "di" represents additive and dominance at respective loci, where i =1 and 2 for locus 1 and 2 respectively.

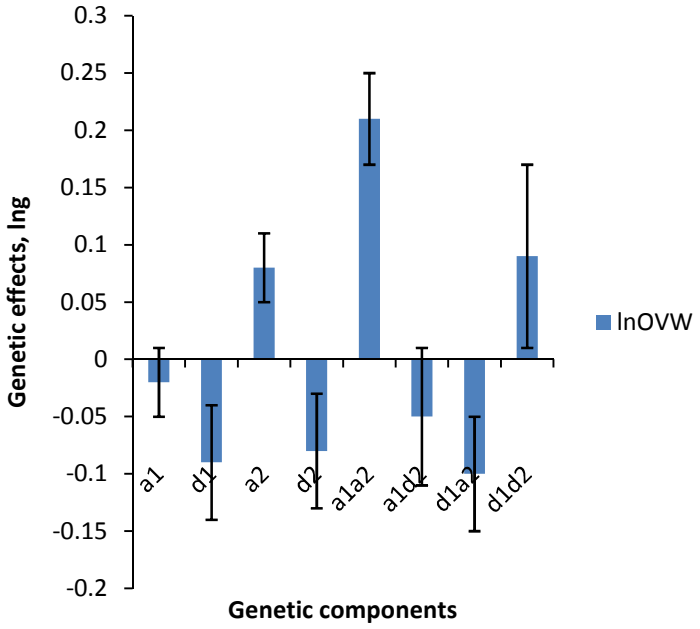


Figure 6.3 Co-adaptive epistasis for ln Ovary weight where "B" represents broiler allele and "L" represents layer allele.

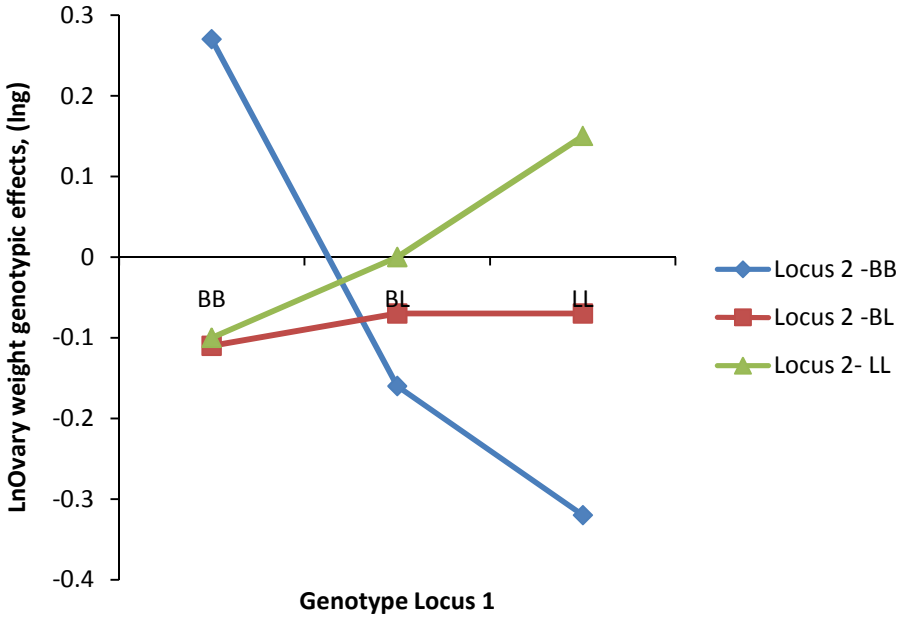


Figure 6.4. Mean effects for two marginal-effects QTL on chromosomes 9 and 12 interacting to influence lnAbdominal fat weight when lnWFE is fitted as a covariate: the "A" and "B" stand for the broiler alleles and the "a" and "b" for the layer alleles.

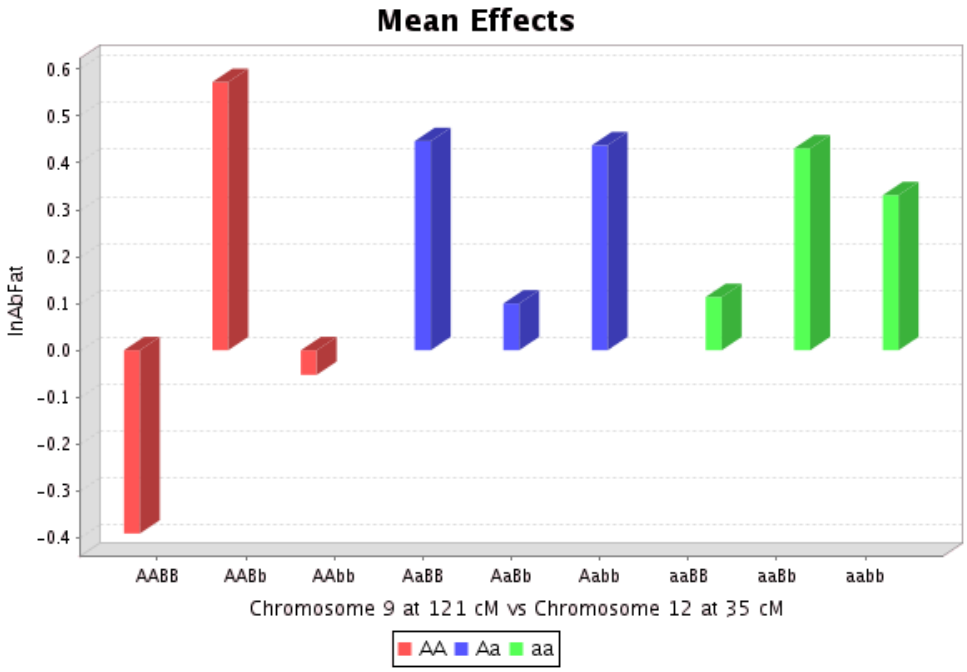


Figure 6.5 Effect estimates for the genetic components of the epistatic lnAFW QTL pair where "a_i" and "d_i" represents additive and dominance at respective loci, where i=1and 2 for locus 1 and 2 respectively

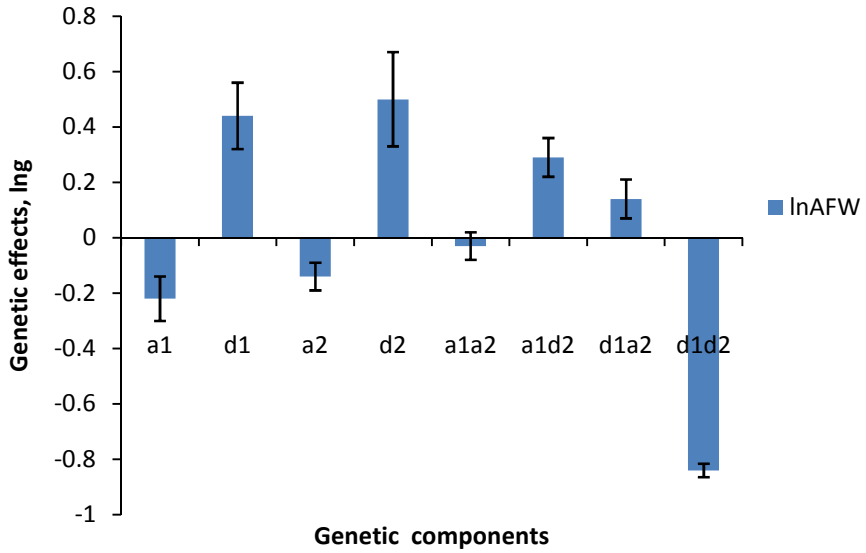
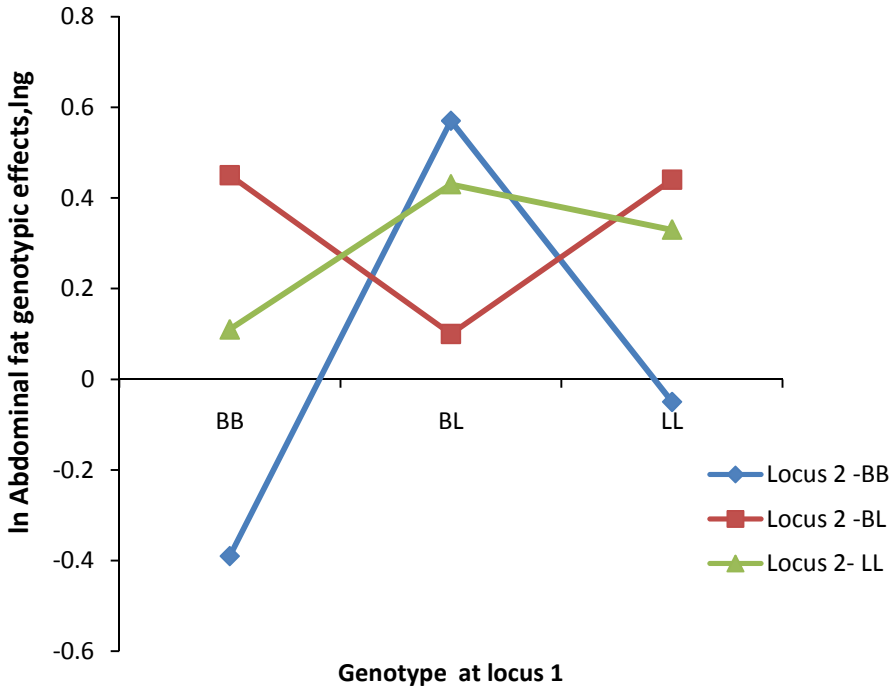


Figure 6.6 Dominant-by-dominant epistasis in the expression of abdominal fat weight where "B" represents broiler alleles and "L" for layer alleles.



A plot of the mean effects for lnOVW (Figure 6.1) for the different genotypic classes shows that birds inheriting at least one layer allele at one locus in the presence of two broiler alleles at the second locus results in a general reduction in ovary weight and the highest reduction occurred when the two loci were completely homozygous for alternate alleles. However, when both loci were homozygous for alleles from any breed it generally increased the ovary weight. The highest increase in ovary weight indicated by the largest positive effects was expressed when both loci were homozygous for broiler alleles but the largest decrease occurred when the interacting loci were each fixed for alternate alleles.

The interaction of alleles for abdominal fat shows that layer alleles generally increased fatness (Figure 6.4). Furthermore, being heterozygous at one locus increased fatness as shown by the positive effects. The highest increase as reflected by the largest positive effect occurred when one locus was heterozygous and the other locus was homozygous for the broiler alleles. However, inheriting both broiler alleles at the two loci lowers the abdominal fat weight as indicated by the largest negative effects for that genotype class.

6.3.5 Pattern of epistatic effects

The *additive x additive* interaction between epistatic loci on chromosomes 2 and 8 was detected for lnOVW (Table 6.7 and Figure 6.1). Both traits had significant *dominance x additive* interaction but the *dominance x dominance* and *additive x dominance* interactions were also only detected between chromosomes 9 and 12 for lnAFW (Table 6.7 and Figure 6.2). A plot of the genotypic values (Figure 6.3 and Figure 6.6) was constructed to identify the patterns of epistasis (Carlborg & Haley 2004; Carlborg *et al.* 2005).

The epistatic pair for lnAFW (Figure 6.4 and Figure 6.6) fit a pattern illustrated by Carlborg and Haley (2004) as indicative of negative dominance x dominance epistasis in which the double heterozygote had a lower phenotype than expected. For lnOVW (Figure 6.1and Figure 6.3), the epistatic pair exhibit co-adaptive epistasis in which there is enhanced performance if an individual has inherited all the four homozygous alleles at the two loci from the same parental line.

6.4 DISCUSSION

QTL for ovarian traits have been reported in cattle (Ponsuksili *et al.* 2006), pigs (Cassady *et al.* 2001; King *et al.* 2003) and mice (Lee *et al.* 2010) but there is paucity of information on QTL compared with gene expression studies on ovary related traits in chickens (Krzysik-Walker *et al.* 2007; Elis *et al.* 2008; Elis *et al.* 2009; Ou *et al.* 2009). This study provides novel QTL information on ovary related traits in chickens to address this gap.

The individual QTL detected explained small to large proportions of the total residual variation (ranging from 2.1 to 16.1 %). The low proportion of the variation explained by the identified QTL for the other traits could be due to the fact that complex traits such as those related to reproduction are influenced by other multiple genetic and environmental factors (Ankra-Badu *et al.* 2010). The reported results are based on 143 markers with a marker density of about 18 cM which is relatively sparse implying that the power to detect QTL with low effects was relatively low. Marker spacing of 10 cM is ideal and power is reduced at a density beyond 50 cM (Darvasi *et al.* 1993; Darvasi & Soller 1997; Mao & Da 2005; Liu *et al.* 2008).

In this study only suggestive QTL were detected on chromosomes 9 and 27 for NYF. An association between three single nucleotide polymorphisms (SNP) within the signal transducers and activators of transcription 5B (STAT5B) gene on chromosome 27 and age at sexual maturity led to the suggestion that this gene may affect sexual maturity by regulating ovary development (Ou *et al.* 2009). While this could be one

of the candidate genes for the NYF QTL, the insufficient number of markers (1) used on this chromosome in our study make it is difficult to draw firm conclusions on the position of the QTL.

A persistent right oviduct in chickens is attributed to mutations in the genes controlling the anti-Mullerian hormone (Wakamatsu *et al.* 2000). From the Ensembl database (www.ensembl.org/) the protein coding transcripts for the anti-Mullerian hormone for chickens were located on chromosome 28. In our study only suggestive QTL for right oviduct score were detected on chromosome 5 and 11 suggesting that some mechanism influenced by loci in these chromosomes might be involved in the outcome related to the right oviduct score.

The detection of QTL for abdominal fat weight at younger ages on chromosome 5 has been reported and confirmed by several studies (Ikeobi *et al.* 2002; Abasht *et al.* 2006b; McElroy *et al.* 2006; Mignon *et al.* 2009). In those studies the position ranged from 62 - 82 cM and was at 10 cM and within the 95% confidence interval (0-116 cM) in our study. The abdominal fat weight QTL positions in the other chromosomes (1, 4, 9 and 27) were comparable to those reported in other studies (Abasht *et al.* 2006b; McElroy *et al.* 2006). The lnAFW QTL on chromosome 28 in this study lies within the confidence interval reported for the same trait by Ikeobi *et al.*, (2002). Ankra-Badu *et al.* (2010) also reported abdominal fat weight QTL on chromosome 2 (at 276 cM compared to the one at 267 cM in this study) in a chicken population that was divergently selected for low and high growth rate. They identified other epistatic

QTL for the same trait on chromosome pairs: (1/1, 1/11, 1/15, 1/18 and 2/18) that were not replicated in this study.

A QTL for lnCMW was detected on chromosome 4 and suggestive QTL were identified on chromosome 5 and chromosome 9. These results differ from the results of an F_2 cross of the White Leghorn and red jungle fowl where female specific QTL were detected (two on chromosome 1 at 87 cM and 193 cM), one on chromosomes 3 and 8 (Wright *et al.* 2008). However, in that study, and in ours, no evidence was found for epistasis among the QTL for comb mass. lnCMW had the highest (0.48) phenotypic correlation with lnOVW which might be a confirmation that comb size could be used as an indicator of fecundity (Wright *et al.* 2008) under an assumption that a large ovary reflects a high steroid output affecting secondary sexual characteristics and puberty.

When both loci were homozygous for alleles from any of the two breeds it generally increased the ovary weight but the highest increase indicated by the largest positive effects was expressed when both loci were homozygous for broiler alleles (Figure 6.1). This suggests that broiler alleles contribute more towards increasing ovary weight in this large framed breed compared to the smaller framed layer. The results on the interaction of alleles for abdominal fat show that inheriting layer alleles generally increased fatness (Figure 6.4) confirms the known biology that layer chickens are relatively fatter than broilers and at sexual puberty layer birds need fat reserves for yolk formation and incubation.

For the significant lnOVW epistatic pair the additive by additive type of interaction had the highest genetic effects (Figure 6.2) and for the dominance by dominance interaction had the highest absolute effects for the suggestive lnAFW epistatic QTL pair (Figure 6.5). The pattern of the interaction for the lnOVW epistatic pair fitted what was described as co-adaptative epistasis (Figure 6.3) while the pattern for lnAFW fitted the negative dominance by dominance epistasis (Figure 6.6) (Carlborg & Haley 2004). The significant additive by dominance epistasis, dominance by additive epistasis and the dominance by dominance epistasis for lnAFW illustrate the importance of the contribution of these forms of epistasis to heterosis and confirms the role of non additive gene action in reproductive traits (Williams *et al.* 2002; Melchinger *et al.* 2007). The observed significant dominance effects for some QTL for lnAFW (Table 6.5.3 and Table 6.5.4) in this study confirms that non-additive gene action plays an important role in some of the reproductive traits associated with sexual maturity.

The epistatic QTL detected in this study explained a relatively significant proportion of the variation of the affected traits but the number of epistatic loci was surprisingly low for reproductive traits in which epistasis is expected to underlie the high heterosis exhibited by such traits (Williams *et al*., 2002). This strengthens the case that epistasis may be a rare occurrence for some reproductive traits, a conclusion that is consistent with observations on loci affecting fecundity in *Drosophila melanogaster* (Leips *et al.* 2006). It is conceivable that more cases of epistatic QTL could be detected from larger F_2 populations especially as new tools are being developed to overcome the challenges for fast computing and the statistical issues

associated with multiple testing. Most of the QTL for the assessed traits acted additively however, there were some in which dominance was important.

The number of epistatic QTL detected in this study was low compared to the epistatic QTL reported for juvenile growth in chickens of the same cross (Carlborg *et al.* 2004a). This could be due to the fact that epistasis is more important at earlier growth stages than at the time of our study. A total of 450 individuals were used in our analysis and this may have reduced the power to detect epistasis because it is generally recommended that at least 500 F_2 individuals should be used (Carlborg *et al.* 2003). However, the approach we used is known to be robust enough to detect epistasis for a minimum of 400 F_2 individuals (Wei *et al.* 2009). The ability to detect epistatic QTL pairs in this study using a small population may be a confirmation of this method's robustness including its ability to identify suggestive evidence for lnAFW QTL on chromosome 12 with weak effects. However, it appears that mainly large effect QTL were detected as reflected by the marginal–effect QTL for lnOVW on chromosomes 2 and 8 which also had significant epistatic effects.

Inclusion of lnWFE as a covariate produced different outcomes across the traits but generally resulted in the detection of more QTL for most of the traits (i.e. lnAFW, lnCMW, lnODW). Adjusting for lnWFE also enabled the detection of epistatic QTL for lnOVW and lnAFW. Fitting lnWFE as covariate enables the comparison of the assessed traits at a constant body weight (Neuschl *et al.* 2007). In some cases the inclusion of the covariate improved the model in explaining the trait phenotypic variation thereby increasing the strength and number of the detected QTL (Zeegers *et*

al. 2004). Body weight and internal organ weights (e.g. abdominal fat weight, ovary weight etc) are correlated (Table 6.4) because body weight includes the contribution of different body organs including internal organs (Neuschl *et al.* 2007). Therefore the detected QTL when a covariate is fitted could also be the result of detecting the QTL for the covariate instead of the QTL for the trait that is being investigated. For example, in this study the QTL for body weight are located on chromosome 4 (Table 4.5.1 to Table 4.5.6, Chapter 4) and evidence for QTL for lnOVW and lnCMW on chromosome 4 tend to appear when lnWFE is fitted as a covariate (Table 6.5.1 and Table 6.5.3).

6.5 CONCLUSIONS

Statistical analysis detected novel significant QTL for lnOVW, lnODW and ROS respectively on chromosomes 4, 2, and 5. Most of the identified QTL acted additively but dominance was also important for some traits. Significant evidence for epistasis was detected for ovary weight and only a suggestive QTL pair was found for abdominal fat weight. Modelling these complex traits by including a covariate resulted in an increase of the estimated effects which consequently increased the chance of finding genes by linkage and identified interacting loci. The analysis confirmed the role of non-additive gene action in influencing some reproductive traits but number of traits affected by epistatic QTL in this study was very low. The size of the data set may have been a limitation in detecting epistasis.

CHAPTER SEVEN

Bone mineral density QTL at sexual maturity and end of lay

7.1 Introduction

Bone mineral density (BMD) is a common measure of susceptibility to osteoporotic fractures in both humans and other species including chickens (Whitehead & Fleming 2000b; Schreiweis *et al.* 2005; Johnson *et al.* 2009). Chicken as a commonly used model organism provides a possible opportunity to understand the biology and genetic mechanism controlling predisposition to osteoporosis in vertebrates. For example, the bone fragility in humans is due to the decline in oestrogen after menopause but in chickens the decline in the structural integrity of the bones is caused by mobilization of cortical bone for egg production which occurs during long periods of production when oestrogen is high (Rubin *et al.* 2007a). Osteoporosis is not only an animal welfare issue but affects productivity and leads to processing losses in the industry (Silversides *et al.* 2006).

Osteoporosis is a common welfare issue among caged layers, which occurs largely through the loss of structural bone caused by the demands of high rates of egg shell formation in modern layers. The long term loss of calcium weakens the bones leading to fractures, particularly of the humerus and keel (Whitehead & Fleming 2000a; Webster 2004). Exercise and good nutrition leads to stronger bones and reduced fractures but the beneficial effects are limited (Fleming *et al.* 2006).

Osteoporosis is evident in laying hens from 35 – 45 weeks of age (Cransberg *et al.* 2001). Osteoporosis in humans is also an age related condition and is influenced not only by genetic factors but also environmental, gene-gene and gene-environmental

interactions (Johnson *et al.* 2009). Age-related changes in avian bone density are a result of changes in oestrogen synthesis and oestrogen receptor populations (Beck & Hansen 2004). As hens approach sexual maturity the oestrogen concentration increases as the reproductive system becomes functional and declines during a moult that induces a period of reproductive rest (Beck & Hansen 2004). The processing of partitioning of key metabolites such as calcium needed for bone deposition, egg production and other homeostatic functions could be controlled by a number of genetic factors or genes, which implies a possible role of epistasis in the biology of a trait such as BMD.

Besides nutritional and environmental interventions, osteoporosis can be combated through selective breeding (Bishop *et al.* 2000; Fleming *et al.* 2006). A selection index (Bone Index) has successfully been used to select against osteoporosis in laying chickens (Bishop *et al.* 2000). A QTL for bone index was reported on chromosome 1 at position 370 cM in an F_2 population produced from two White Leghorn lines divergently selected on the basis of the bone index (Dunn *et al.* 2007). Bone mineral density (BMD) is a traditional measure of bone strength (Hans & Krieg 2008) and identification of QTL for BMD could assist breeding efforts to address osteoporosis. Suggestive QTL for bone mineral density have been detected in an F_2 broiler (Cobb-male) x layer (White Leghorn female) population (Schreiweis *et al.* 2005). Several QTL for BMD were detected and potential gene candidates were proposed based on a QTL study based on two F_2 populations respectively from the crosses of a male broiler line to a White Leghorn line and a male broiler and a Fayoumi chicken line (Zhou *et al.* 2007a).

A number of QTL detection studies have been conducted on poultry to identify loci associated with osteoporosis but none focused specifically at the critical period when birds attain sexual maturity (Schreiweis *et al.* 2005; Dunn *et al.* 2007; Rubin *et al.* 2007b; Zhou *et al.* 2007a). This stage is important for initiating key physiological processes that lead to egg production and which in turn affects bone density. Bone mineral density is important at the onset of lay because birds which attain good bone mineral density have higher reserves of calcium to support subsequent egg production and because deposition of calcium after sexual maturity is prevented by circulating oestrogen.

A QTL analysis was conducted to identify QTL influencing BMD and to investigate the possible role of epistasis in regulating BMD in a F_2 broiler-layer cross population. Osteoporosis as indicated by BMD does occur in broilers but it is mainly a problem of layers at the end of lay (Cransberg *et al.* 2001). The data were from two groups of birds, one that was killed at sexual maturity (GM9) and the other (GM7) at 72 weeks of age at the end of the usually laying period for layers

7.2 Materials and Methods

7.2.1 Measurement of bone mineral density

The population used in the study has been described in detail in Chapter 3. In summary the F_2 populations each had 32 families with a total of 268, 388, 650 individuals respectively for GM7, GM9 and GM7 & GM9 combined (Table 7.1).

The birds were killed with an overdose of sodium pentobarbitone then the ovaries were dissected as described in Chapter 6 and the number of normal yellow follicles greater than 8 mm was counted. The right wing was removed from the carcass (and stored at -20^{0}C) and the humerus was used to determine bone mineral density as described by Hocking *et al*., (2003) in Chapter 3. The BMD was transformed to the natural logarithms of BMD to normalise the residual errors.

Table 7.1 Mean bone mineral density (BMD) and number of yellow follicles (NYF) for the F_2 broiler- layer cross populations

Trait/Population	N	Mean (SE)	Min	Max
GM9 (assessed at first egg)				
Ln(BMD), ln(mm Al[1])	389	0.6 (0.2)	0.18	1.3
NYF	389	12.9 (3.3)	2.0	23
GM7 (assessed at 72 weeks of age)				
BMD, ln(mm Al)	261	2.17 (0.5)	1.3	4.3
Ln(BMD), ln(mm Al)	261	0.76 (0.2)	0.3	1.5
NYF	261	5.8 (2.1)	1	13
Combined GM7 & GM9 populations				
NYF	650	10.0 (4.49)	1.0	23.0
ln(BMD), ln(mm Al)	650	0.67 (0.21)	0.18	1.5
Corrected lnBMD, ln(mm Al)	650	0.67 (0.25)	0.07	1.4

[1] Millimetre of aluminium density equivalent

7.2.2 DNA analysis and map construction

DNA was obtained from blood samples following standard procedures and genotyping was conducted as described in Chapter 3 and by Sewalem *et. al*., (2002). Genetic linkage maps were constructed using the CRIMAP (Green *et al.* 1990) program's prepare, flips and fixed options as described in Chapter 3. The number of markers used in the analysis is presented in Table 3.1 (Chapter 3).

7.2.3 Model fitting

Family and pen were fitted as fixed effects for GM7and GM9. The combined data for GM7 and GM9 was pre-corrected for pen nested within hatch after fitting a model that also included effects for family. The analysis of the combined GM7 & GM9 population included the effect of age at the measurement of BMD and the number of normal yellow follicles (NYF) was fitted as a covariate after excluding birds which had less than 1 NYF from the analysis. The NYF was also fitted as a covariate in the analysis of the GM9 population data but was not fitted for the QTL analysis of GM7 birds which were old and out of lay in which the role of oestrogen in causing osteoporosis was assumed to be negligible..

7.2.4 QTL Analysis

The interval mapping method for QTL analysis in outbred populations (Haley *et al.* 1994) and its adaptation for the detection of epistatic QTL pairs (Wei *et al.* 2010b) was followed as described earlier in Chapter 2.

7.3 Results

The phenotypic correlations between bone mineral density and other traits for the two GM7 and GM9 populations are presented in Table 7.2. The QTL detection results for the individual populations are presented in Table 7.3 and the analysis results of the combined populations are presented in Table 7.4. There was no evidence found for epistatic QTL pairs and therefore the results on epistasis are not presented.

7.3.1 The phenotypic correlations

The highest correlation (0.42) in this analysis was between body weight at 72 weeks (BW72) and BMD in the GM7 population (Table 7.2). There was no correlation between weight at first egg (WFE) and BMD in older birds (GM7) in contrast to when BMD was measured in younger birds (GM9). Ovary weight (OVW) and BMD had the second highest correlation (0.28 – 0.38) for the traits in this analysis followed by the correlation between comb weight (CMW) and BMD. The correlation for NYF and BMD was positive and low (0.22-0.24). The estimated phenotypic correlations were generally low and positive except for the negative correlation between BMD and AFE.

Table 7.2 Phenotypic correlations between bone mineral density and other traits assessed in the F_2 broiler-layer cross GM9 and GM7 populations. (P<0.05 unless indicated otherwise).

BMD[1]	NYF[4]	WFE[5]	AFE[6]	BW72[7]	AFW[8]	OVW[9]	ODW[10]	CMW[11]	ROS[12]
GM7[2]	0.22	0.03ns[13]	-0.20	0.42	0.05ns	0.28	0.19	0.21	0.05ns
GM9[3]	0.24	0.28	-0.18	-	0.11	0.38	0.13	0.37	-0.04ns

[1] Bone mineral density

[2] GM7 population assessed for weight at first egg and for other traits at 72 weeks of age.

[3] GM9 population assessed at first egg

[4] Number of normal yellow follicles (> 8mm)

[5] Weight at first egg

[6] Age at first egg

[7] Body weight at 72 weeks of age

[8] Abdominal fat weight

[9] Ovary weight

[10] Oviduct weight

[11] Comb weight

[12] Right oviduct score

[13] Not significant at p< 0.05

7.3.2 BMD QTL: analyses without NYF as a covariate

Table 7.3 Chromosomes, F ratio, QTL position, confidence interval, flanking markers, additive and dominance effects and proportion of variation explained for BMD in the GM9 (n=388) and GM7 (n=268) broiler-layer cross populations.

Chromosome	F-ratio[1]	Position (cM)	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP[3] (%)
GM9 population							
lnBMD[4] (with no covariate), ln(mm Al)							
1	5.7†	131	29-539	LEI0068 - LEI0146	0.04(0.01)	0.02(0.02)	2.3
1	9.2*	311	103-498	LEI0071-LEI0101	0.06(0.02)	0.02(0.03)	4.1
3	7.3†	57	0-230	HUJ0006 - ROS0001	0.04(0.02)	-0.08(0.03)	3.2
3	5.7†	187	21-213	MCW0252- ADL00306	0.02(0.01)	0.06(0.02)	2.4
4	6.0†	65	21-243	MCW0295 – ADL0241	0.04(0.01)	0.06(0.03)	2.5
8	9.8*	2	0-64	ROS0021 – ROS0026	0.04(0.01)	-0.03(0.02)	4.5

Table 7.3 cont'd Chromosomes, F ratio, QTL position, confidence interval, flanking markers, additive and dominance effects and proportion of variation explained for BMD in the GM9 (n=388) and GM7 (n=268) broiler-layer cross populations.

Chromosome	F-ratio[1]	Position (cM)	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP[3] (%)
lnBM4D all NYF (with NYF fitted as a covariate), ln(mm Al)							
1	10.1**	305	108-498	LEI0071 – LEI0101	0.07 (0.02)	0.06 (0.04)	4.5
1	5.3†	131	46-533	LEI0068 – LEI0146	0.03 (0.01)	0.02 (0.02)	2.1
3	12.6**	57	52-170	HUJ0006 – ROS0001	0.06 (0.02)	-0.10 (0.03)	5.7
3	6.7†	194	25-246	ADL0306 – ADL0237	0.02 (0.01)	0.05 (0.02)	2.8
3	5.3†	105	24-244	ROS001 – LEI0115	-0.04(0.01)	0.0 (0.02)	2.1
5	7.5†	9	0-130	ADL0292 – ROS0084	-0.04(0.01)	0.06(0.02)	3.2
8	8.6*	2	0-81	ROS0021 – ROS0026	0.04(0.01)	-0.02(0.02)	3.7
GM7 Population							
lnBMD with no covariates, ln(mm Al)							
2	6.3†	297	12 -316	ADL0114-MCW0056	0.06(0.02)	0.07 (0.04)	4.9
8	5.3†	27	0-80	ADL0179-MCW0095	0.05(0.03)	0.06 (0.03)	3.9

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

[3] VP% = percentage of phenotypic variation explained by the QTL

[4] lnBMD = natural logarithms of (bone mineral density)

Two significant QTL on chromosomes 1 and 8 respectively and four suggestive QTL on chromosome 1, 4 and two QTL on chromosome 3 were detected in the GM9 population (Table 7.3). A significant detected QTL segregating on chromosome 8 for BMD explained the highest proportion of the phenotypic variation (4.5%) without fitting a covariate in the model. For chickens evaluated at 72 weeks of age (GM7) suggestive QTL were detected on chromosomes 2 and 8. A combined analysis of both populations led to the detection of the same QTL as in the GM9 analysis.

7.3.3 BMD QTL: analyses with NYF fitted as a covariate

Fitting NYF as a covariate in the analysis of GM9 data increased the strength of the signal for detecting QTL and the number of QTL on chromosomes detected on chromosome 3 increased from 2 to 3. A covariate, NYF was not fitted for the GM7 because the birds were out of lay because the influence of oestrogen on BMD from the yellow follicles was assumed to be minimal. For the combined GM7 & GM9 data set two suggestive QTL on chromosomes 1 and one on chromosome 8 persist and a new suggestive QTL was detected on chromosome 9 (Table 7.4).

Table 7.4 Chromosomes, F ratio, QTL position, confidence interval, flanking markers, additive and dominance effects and proportion of variation explained for BMD for and BMD corrected for pen for population GM7&9 (n=650) combined.

Chromosome	F-ratio[1]	Position (cM)	CI[2]	Flanking markers	Additive effect ± SE	Dominance effect ± SE	VP[3] (%)
lnBMD[4] (with no covariate fitted), ln(d)							
1	5.7†	131	38-554	LEI0146 – LEI0068	0.04(0.01)	0.02(0.02)	2.4
1	9.2*	311	72-479	LEI0071 – LEI 0101	0.06(0.02)	0.02(0.03)	4.1
3	7.3†	57	0-250	HUJ0006 – ROS0001	0.04(0.02)	-0.08(0.03)	3.2
3	5.7†	187	25-209	MCW0252 – ADL0306	0.02(0.01)	0.06(0.02)	2.4
4	6.0†	65	21-242	MCW0295 – ADL0241	0.04(0.01)	0.06(0.03)	2.5
8	9.8*	2	0-62	ROS0021 – ROS0026	0.04(0.01)	-0.03(0.02)	4.5
lnBMD (with NYF fitted as a covariate, NYF=0 removed), ln(d)							
1	7.2†	137	109-503	LEI0146 – ADL0319	0.05(0.01)	-0.02(0.02)	1.9
1	5.1†	266	60-606	LEI0071 - LEI0101	0.0(0.02)	-0.02(0.02)	1.3
8	5.9†	15	0-72	MCW0305 – ADL0258	0.03(0.01)	-0.03(0.02)	1.5
9	7.0†	10	0-124	ROS0078 – MCW0135	0.04(0.02)	0.05(0.03)	1.8

[1] Significant at 0.05 (*) and 0.01 (* *) levels experiment-wide, and (†) suggestive

[2] CI = 95% confidence interval

[3] VP% = percentage of phenotypic variation explained by the QTL

[4] lnBMD = natural logarithms of (bone mineral density)

7.3.4 Bone mineral density QTL effects

The QTL effects for the reported QTL are presented in Tables 7.3 and 7.4. The detected QTL explained from 1.3 to 5.7 % of the phenotypic variation in line with the range for most QTL studies (Hocking 2005). The QTL on chromosome 3 explained the highest proportion (5.7%) of the phenotypic variation where NYF was fitted as a covariate. Most of the detected QTL had significant positive additive QTL effects. However, a locus on chromosome 3 at 57 cM had significant dominance action which had a negative effect on BMD.

7.4 Discussion

7.4.1 Significant QTL for BMD

The follicles are a major source of oestrogen with the rest of it coming from the ovary (about 50% each) (Armstrong 1984). To minimize the effect of oestrogen in influencing bone density analysis results the NYF as the source of oestrogen were fitted as a covariate in the QTL analysis of BMD for GM9 and GM7 & GM9. Previously the QTL analysis for NYF in the GM9 population (Chapter 6, Table 6.5.3) detected only suggestive QTL on chromosome 9 and 27 and none of these linkage groups have been detected in the QTL analysis of BMD with NYF fitted as a covariate. Therefore the suspicion that the QTL for the covariate (NYF) were being detected in this analysis instead of the QTL for BMD would not apply. The second BMD QTL detected on chromosome 1 at 311 cM without fitting a covariate in GM9

and GM7 & GM9 populations is similar to the QTL for humeral breaking strength and bone index that were detected on chromosome 1 at 334 cM and 370 cM (Dunn *et al*., 2007). The first QTL on chromosome 1 at 131 cM appears to be similar to a reported significant QTL for femoral BMD at 138 cM (Rubin *et al* 2007) because the QTL confidence intervals overlap and share a common marker flanking marker.

Schreiweis *et al.,* (2005) reported significant BMD QTL on chromosome 3, 4 and 27 compared to our study where chromosome 3 and 4 had suggestive QTL without fitting a covariate. Other QTL detected on chromosomes 2, 3, 5, 8 and 9 in our study are similar to those reported for a Leghorn x red jungle fowl cross (Rubin *et al*, 2007).

For the BMD QTL on chromosome 1 adjusting for the number of yellow follicles did not change the estimated additive effect implying that the difference in BMD between the lines due to the influence of this QTL is independent of the number of yellow follicles.

Most the BMD QTL effects were positive indicating that the broiler allele contributed to the increase in BMD. The highest value of the phenotypic variation explained by the QTL on chromosome 3 is similar to the 6% reported for a whole-body BMD QTL on chromosome 3 (Rubin *et al.* 2007b).

7.4.2 Pleiotropic BMD QTL

The BMD QTL flanked by markers LEI0146 and LEI0068 on chromosome 1 at 131 cM and on chromosome 3 at 57 cM (Table 7.3 & 7.4) overlap with body weight QTL (Table 4.5.1, Chapter 4). This is not unexpected because large framed individuals are expected to have more tissue mass and therefore strong bones to support the weight. Furthermore genes controlling weight and size have pleiotropic effects on skeletal traits (Rubin *et al.* 2007b). It looks like standardizing for weight by fitting it as a covariate which was not done in this analysis would have been interesting.

The positive correlation between BMD and both ovary weight (OVW) and comb weight (CMW) imply that birds with a high BMD tend to have large combs and ovaries. This confirms observations reported by other studies that comb size in females is an indicator of skeletal investment (Wright *et al.* 2008). Furthermore, the relative comb size reflects the influence of steroid hormones produced by the ovary and is functionally important in achieving mating success (Balthazart & Hendrick 1978; Brodsky 1988). This implies that comb size can be used as an easy-to-observe phenotype to select animals with good BMD to minimise the incidence of osteoporosis in chickens. Despite this positive correlation, our results the co-location of BMD QTL and comb weight QTL were not observed.

7.5 Conclusions

Significant BMD QTL detected on chromosomes 1, 3 and 8 confirmed QTL reported by other studies. QTL on chromosomes 1 and 3 overlap with QTL for body weight and suggest that these QTL control both body weight and skeletal phenotypes. The identified QTL is consistent with evidence of genetic basis for the occurrence of osteoporosis that could be used to improve bone strength and reduce bone fractures in commercial layers. Understanding the physiological processes behind the identified QTL would aid effort to solve the problem of osteoporosis in chickens and humans.

CHAPTER 8

Summary and general discussion of conclusions

8.1 Introduction

Chicken are an important farm species contributing about one third of meat produced and consumed globally (Scanes 2007). This role is likely to grow in the future with the anticipated human population growth and the consequent demand for meat, coupled with other production pressures such as climate change, carbon constrained production and animal welfare demands, will require innovative approaches to achieve the competitive production of poultry products (Andersson 2009; Thornton 2010). Impressive advances in increased production of chickens were achieved mainly through traditional practices of selective breeding. In recent years, new genomic tools are increasingly being proposed or adopted to speed up genetic gain or to get a better understanding of traits (Andersson 2001; Meuwissen *et al.* 2001; Andersson 2009; Mackay *et al.* 2009; Wei *et al.* 2010b). The chicken is a popular model species for studying the genetics of different traits as well as understanding diseases in chickens and other organisms including humans (Burt 2002; Stern 2005). Chickens have been divergently selected for growth and egg production creating ideal models to study growth, reproduction, skeletal, disease and welfare traits that are of economic importance to the poultry industry and human health (Andersson 2001; Dodgson 2007; Cheng 2010).

The majority of studies on identification of genes underlying quantitative traits (QTL) in chickens have focussed on early growth and disease traits with limited emphasis on reproductive traits (Hocking 2005; Abasht *et al.* 2006a). Epistasis is known to have a role in the expression of quantitative traits (Cheverud & Routman

1995; Carlborg *et al.* 2003) but most reported QTL analyses have ignored epistasis (Carlborg & Haley 2004). Several studies have estimated growth curve parameters in chickens (Grossman & Bohren 1985; Barbato 1991; Knížetová *et al.* 1991; Pasternak & Shalev 1992; Mignon-Grasteau 1999; Mignon-Grasteau *et al.* 2000; Mignon-Grasteau *et al.* 2001; Aggrey 2002a; Zhu *et al.* 2006; Norris *et al.* 2007). Nonetheless, few studies have reported on QTL affecting growth curve parameters (Le Rouzic *et al.* 2008). The attainment of sexual maturity is a critical physiological phase to commence egg production and other reproductive functions. It is therefore biologically an interesting phase to investigate genetic factors influencing the expression of growth, reproduction and skeletal traits. The occurrence of osteoporosis in layer chickens and humans (although driven by slightly different phenomena), and the similarity of increased fat deposition in early maturing chickens and humans create potential human health benefits from understanding obesity and osteoporosis in chickens.

8.2 Review of objectives

To address the gaps highlighted above, a study was conducted using the Roslin broiler-layer cross resource population to address the objectives listed below.

To detect QTL and investigate the role of epistasis for the following traits:

- Body weight, growth rate and growth curve parameters (Chapter 4).
- Body weight and age at first egg (Chapter 5).
- Reproductive traits (ovarian traits and comb weight) (Chapter 6).
- Bone mineral density at sexual maturity and at slaughter age (Chapter 7).

8.3 Summary of Results

8.3.1 Chapter 4 Growth QTL

Numerous studies have estimated chicken growth curve parameters but information on QTL affecting growth parameter estimates and role of epistasis on these estimates is scarce. To augment the limited information on QTL affecting the growth parameter estimates a study was conducted to detect growth QTL and QTL influencing parameters of the growth curve for chickens aged 3 - 72 weeks of age. Growth was assessed as body weight at 3, 6, 12, 24, 48 and 72 weeks of age. Lastly, detection for QTL affecting growth rates between these age intervals was also conducted. Epistasis models were also run to detect possible epistatic QTL.

Several significant QTL for body weight at specific ages were detected and most of the identified QTL were also detected in the nearest preceding and/or subsequent growth stages due to the high correlation between body weights at nearby growth stages. Most of the detected QTL were reported in other studies and the results confirmed age specific QTL. QTL influencing Gompertz parameters were detected and these QTL also overlapped with loci affecting growth and some carcass traits reported by other studies (Sewalem *et al.* 2002; Navarro *et al.* 2005b). The overlap of body weight, growth rate and QTL Gompertz parameters shows that Gompertz parameters can be used in selection to simultaneously manipulate these traits due to correlated responses. Age specific growth QTL show that there are specific genes and gene actions which orchestrate the developmental process during the different stages of growth. Some loci featured predominantly in early growth to the attainment

of sexual maturity while others were only detected for older ages, post-sexual maturity. Notably, the QTL for body weight and average growth rate from 3-6 weeks of age on chromosome 11 is not detected in other traits including AFE. However, no evidence for epistatic QTL pairs was found. QTL found on chromosome 4 explained much of the observed growth variation across the different ages. Furthermore, most of the QTL for Gompertz parameters were also detected on chromosome 4 confirming the importance of this chromosome in controlling a number of traits of economic importance. Chromosome 4 QTL had relatively little contribution to body weight at very young ages around 3 weeks. The body weight effects were mainly positive as expected indicating that the broiler allele was contributing to the increase in weight as observed in similar studies (Sewalem *et al.* 2002). For the most part, the dominance effects were insignificant. Nevertheless, dominance effects were significant in a few cases and even exceeded the additive effects for body weight QTL from 12 weeks of age and other older ages. Most of the AFE QTL overlap with those for body weight at 12 and 24 weeks of age; indicating the correlation between AFE and weight around sexual maturity.

8.3.2 Chapter 5 Age and weight at first egg QTL

The objective of this study was to identify QTL for age (AFE) and weight (WFE) at first egg in the Roslin broiler-layer cross in which the White Leghorn layer was smaller and later maturing than the heavier broiler. Epistasis among quantitative trait loci (QTL) for early growth have been reported in chickens (Carlborg *et al.* 2003; Carlborg *et al.* 2004a). Therefore, to understand the role played by non-additive gene

action like epistasis in reproductive traits associated with sexual maturity, the possible role of epistasis in AFE and WFE was also investigated. The study provided further light on the hypothesis that the age for the onset of egg laying (AFE) in chickens is dependent on the attainment of a given threshold body weight (WFE).

The QTL for WFE and AFE detected in this study generally acted additively and the broiler alleles were associated with heavier body weights and earlier ages at the onset of lay. Some of the QTL detected co-locate with QTL for the other trait implying that manipulating age independently of weight at sexual maturity will represent a significant challenge. However the indication that the major loci for growth and puberty are common provides a clear demonstration of the genetic basis for the phenotypic correlation between growth and puberty.

8.3.3 Chapter 6 Reproduction traits QTL

The analysis was conducted to identify QTL for abdominal fat weight (AFW), comb weight (CBW), ovary weight (ORW) oviduct weight (ODW), number of normal yellow follicles (NYF) and the right oviduct score (ROS) at first egg in a broiler-layer cross.

Statistical analysis detected novel QTL for lnORW, lnODW and ROS on chromosomes 4, 2, and 5 respectively. The QTL detected for ovarian traits occur on chromosomes bearing candidate genes associated with sexual maturity, fertility or reproductive physiological functions in chickens. For the most part, the identified

QTL for traits related to sexual maturity acted additively but non-additive gene action was also evident in some of the traits. Epistasis played a significant role in the determination of ovary weight. The results also showed suggestive evidence for epistatic gene action in the expression of abdominal fat weight. The number of epistatic QTL pairs detected was lower than expected for these reproductive traits in which epistasis has been proposed as a possible explanation behind the heterosis often expressed by such traits. The size of the data set and the relatively low density of markers may have limited the power to detect epistasis in this analysis.

8.3.4 Chapter 7 Bone density QTL

An investigation was conducted to identify QTL influencing BMD and to investigate the possible role of epistasis in regulating BMD in the F_2 broiler-layer cross population. Osteoporosis as indicated by BMD may occur in broilers. However, it is mainly a problem of layers at the end of lay (Cransberg *et al.* 2001). The data were from two groups of birds, one that was killed at sexual maturity (GM9) and the other at 72 weeks of age at the end of the usual laying period for table egg laying hens.

Significant BMD QTL were detected on chromosomes 1, 3 and 8 and confirm QTL reported in other studies. BMD QTL on chromosomes 1 and 3 overlap with QTL for body weight suggesting that these QTL control both body weight and skeletal phenotypes. The identified QTL are consistent with evidence of a genetic basis for BMD that could be used to improve bone strength and reduce bone fractures in commercial layers. Further resolution of the physiological processes behind the

identified QTL would assist efforts towards solving the problem of osteoporosis in chickens and humans.

8.4 Practical implications of the results

8.4.1 QTL for traits of economic importance identified

This analysis looked at traits of economic importance primarily at the critical physiological growth phase at the attainment of sexual maturity. The results from this thesis do not only have relevance with regard to broadening the knowledge base on the biology underlying complex traits applicable but to livestock breeding, animal welfare and disease, and also have implications to human health. The study primarily established evidence of possible loci which can be targets for further research into indentifying possible genes and other possible uses for the benefit of livestock production and human health.

The study provides novel information on QTL for some ovarian traits as important reproductive traits and confirmed previously reported QTL for growth and bone density. The key findings of the study highlighted the prevalence of the additive mode of gene action in the assessed traits and to a lesser extent the contribution of non-additive gene action which manifested itself in the form of significant dominance effects and even epistasis for a few traits. The number of epistastic QTL pairs detected for these traits was surprisingly low. QTL for growth were detected using three approaches: Using: firstly, specific body weight, secondly growth rate and finally the Gompertz curve parameters. All the three methods identified the same

QTL for mature body weight and growth rate confirming possibilities of using the implicated QTL to manipulate the growth curve in addressing livestock productivity. Body weight, an economically important phenotype was correlated with most traits. The significant portion of variation explained by QTL for body weight at sexual maturity relative to that explained by QTL for age and fatness suggests that it is more critical towards the attainment of puberty. A brief revisit of the afore-mentioned issues and gaps are highlighted in the sections below.

8.4.2 Growth QTL

The QTL for weight at first egg (WFE) detected on chromosomes 1, 2, 3, 4, 8, 13, 27 and Z (Table 5.3, Chapter 5) appear to be the same QTL detected for body weight at different ages (Table 4.5.1 to Table 4.5.6). Notably the WFE QTL on chromosome 4 which explained the highest proportion of the variation for weight has been detected across all the growth traits and almost all the other analyzed traits. The QTL on chromosome 4 were involved throughout the growth stages. In this study, the QTL on chromosome 4 also accounted for the highest phenotypic variation (16.1% for lnODW) (Table 6.5.2). Notably, there was significant evidence for QTL (between marker ADL0241 – MCW0180) segregating on chromosome 4 affecting most of the assessed traits (i.e. body weight at 3 to 72 weeks of age, oviduct weight (lnODW), growth rate at 3-6 and 6-12 weeks of age, age at first egg (LnAFE94), WFE and mature weight (W_A). Suggestive evidence was also observed for the same QTL segregating for other traits (i.e. abdominal fat weight (lnAFW), instantaneous growth rate, L, and growth rate between 24 to 48 weeks of age. The observed co-localization

of QTL on chromosome 4 affecting several traits suggests that QTL on chromosome 4 could be having a pleiotropic effect on these weight related traits.

This study was in accord with previously published chicken QTL studies where the prominent contribution of chromosome 4 on traits of economic important has been reported (Tuiskula-Haavisto *et al.* 2002; Hocking 2005). The involvement of QTL on chromosome 4 throughout the growth stages imply that selection of animals for growth at an early stage not only ensures early growth but also influences adult growth through favourable pleiotropic effects.

QTL that appear important at specific growth phases were detected in this study and this has long been predicted and confirmed in other species (Ma et al. 2002; Leips et al. 2006; Long et al. 2006; Malosetti et al. 2006; Lund et al. 2008). The QTL which are involved early growth before the attainment of sexual maturity are mainly reflecting the importance of physiological processes involved in ensuring readiness for reproduction functions. Studying age dependent QTL could shed light on processes driving the process of senescence because effects on life span have been linked to mutations of the insulin/ insulin like growth factor-1 (IGF-1) pathway (Ricklefs 2008). Growth QTL have also been associated with metabolic factors and hormones, specifically IGF-I and the thyroid hormones triiodothyronine (T3) and thyroxine (T4) (Zhou *et al.* 2007b; Ou *et al.* 2009). From a breeding perspective, some studies have already identified SNPs that are associated with early growth or with sexual maturation in chickens, or both, and they could be used as potential molecular markers for marker assisted selection (Ou *et al.* 2009).

8.4.3 Genetic effects of QTL

Generally, a few QTL of major effect were detected and most the reported QTL in all traits explained only a small proportion of the phenotypic variation in the traits which is consistent with the infinitesimal model (Hill 2010). The majority of QTL detected across the traits predominantly exhibited additive gene action except for ovary weight and growth rate between 24 and 48 weeks. Non-additive gene action in the form of dominance was also observed for some growth QTL such as the body weight QTL across the different ages on chromosome 8 (Table 4.3.3 and Table 4.3.4 Chapter 4). Besides the limitation due to the population size used for the analyses, the few epistatic QTL detected for reproductive traits in this study may be due to the limited sample size used in the analysis or it could be a reflection of the deficiency of the models used which assumed a two-locus case. The models used may be an over simplification of the complex processes observed in biological systems. Nevertheless, the use of much more complex models with higher order effects is computationally intractable. Furthermore, such epistatic effects are likely to be tiny and undetectable if their main effects are already small Hill (2010).

8.4.4 Pleiotropy

The effect of a gene on one or more phenotypes known as pleiotropy (Mackay *et al.* 2009), was not tested in this study. However, the observed overlapping of QTL positions for growth, growth rate and age at the attainment of sexual maturity in this study suggested a possible pleiotropic role of the implicated loci. Establishing the

role of pleiotropy between traits assists in predicting the possible correlated responses to selection (Falconer & Mackay 1996). The high phenotypic correlation and the overlapping QTL for some traits are consistent with the suggestion that pervasive pleiotropy is expected for complex traits (Mackay *et al.,* 2009).

When detected QTL for two or more different traits map to a similar location for correlated traits it does not definitively prove that a single gene is responsible for influencing the assessed traits (Almasy *et al.* 1997; Neuschl *et al.* 2007). It is important to know whether the same QTL could be affecting several traits or whether it is simply two closely linked QTL explaining the variation in the trait (Knott & Haley 2000). Such information is useful in facilitating the accurate cloning of the functional gene and for guiding breeding decisions to avoid unfavourable genetic correlations (Lund *et al.* 2003). Implementing QTL multivariate analysis for correlated traits can improve QTL detection power and better define the QTL pattern and its accurate location in the chromosomal region (Gilbert et al. 2007). In this thesis the traits were analyzed individually and some of the traits had overlapping QTL. Therefore, it would be of interest to conduct a multivariate analysis to establish with certainty whether the implicated QTL are truly pleiotropic (Knott & Haley 2000; Lund *et al.* 2003; Neuschl *et al.* 2007).

8.4.5 Fine mapping of significant QTL

QTL mapping is an initial step to get the general location of QTL after which fine mapping or high resolution mapping is done to limit the region that may contain positional candidate genes (Mackay et al. 2009). The locations of QTL in this study

are imprecise and are characterized by wide confidence intervals, which is characteristic of linkage studies in F_2 experiments in chickens (Abasht *et al.* 2006a) and other farm species (Gautier *et al.* 2006; Hill 2010). Characterization of a QTL involves three key steps: firstly estimating the general QTL position to within ~10 cM, secondly narrowing the confidence interval through fine- mapping and finally identifying the gene and or the causal mutation involved followed by validation (Boichard *et al.* 2006). Association mapping is one of the tools that may be used for fine mapping the location of the causative gene or locus by targeting the specific region using many markers. However this requires a sufficient number of recombination events within the identified interval to have occurred in the study population. The power to fine map QTL regions in an F_2 is therefore limited and additional approaches such as an advanced intercross are needed (Song *et al.* 1999; Abash *et al.* 2006a)

The next step for the QTL detected in this study would be to fine map the QTL especially the QTL for ovarian traits which have not been previously reported. Identification of quantitative trait nucleotides (QTN) affecting the trait has potential to unlock various applications such as marker assisted selective breeding and genetic disease QTN specific therapies. Fine mapping will require the utilisation of several different approaches. Approaches such as whole genome sequencing using over 7000 000 single nucleotide polymorphisms (SNPs) have been used to identify loci under selection during chicken domestication (Rubin *et al*., 2010).

Multidisciplinary approaches are typically used in identifying and verifying genes (Gautier *et al.* 2006) controlling different traits (Mackay *et al.* 2009). Some of these approaches include: positional cloning, candidate gene identification, expression studies, use of knock-down models, comparative studies based on related organisms, all conducted in an integrated frame work with input from different disciplines (Carlborg & Haley 2004).

Some QTL identified in this study were located on chromosomes 1 and 4 which are known to harbour genes which play a role in the expression of growth (insulin-like growth factor 1, IGF1) and obesity (TBC1D1, tre-2/USP6, BUB2, cdc16 domain family member 1), respectively (Rubin *et al*., 2010). Most of the QTL detected in this study had very wide confidence intervals; making the determination of potential candidate genes a speculative endeavour. Nonetheless, the results for chromosome 4 growth QTL are consistent with results from Sewalem *et al.* 2002 and recent whole genome mapping studies (Rubin et al. 2010). A larger population and denser maps would be required to narrow the confidence intervals (Abasht *et al.* 2006a; Liu *et al.* 2008). Using an increased population of 1011 F_2 individuals and 12 microsatellite markers with an average marker density of 2 Mb, QTL on chicken chromosome 1 affecting body weight and abdominal fat percentage were fine mapped from an initial confidence interval spanning 50.8 cM or 24 Mbp to a smaller interval spanning 5.5 and 3.7 Mb, respectively (Liu *et al.,* 2008). SNPs have been successfully used to expedite the fine mapping of body-weight-related QTL on GGA4 that was previously mapped to a 150-cM interval (Sewalem et al. 2002; Ikeobi et al. 2004) by doubling the number of markers resolving the initial QTL into two independent QTL that

affect body weight at 3 and 9 weeks of age (International Chicken Polymorphism Map Consortium 2004). Advances in adopting genome-wide tools such as the 60Kb SNP chips are already in use to study genetic variation in chickens (Marklund and Carlborg, 2010).

8.4.6 The future of mapping quantitative traits and selection approaches

The advent of vast numbers of SNP markers covering most of the genome has led to the exploration of genomic selection in various domesticated species including chickens (Meuwissen et al. 2001; Goddard & Hayes 2009; Meuwissen & Goddard 2010). Genomic selection enables estimation of the genetic merit of an individual by taking into account the influence of all the loci (many SNP) across the genome. Benefits from genomic selection include more rapid genetic gain; lowering generation intervals on traits under selection rendering performance testing in all selection candidates obsolete (e.g. for traits that are difficult or expensive to measure), allowing the evaluation of non-pedigree recorded animals, or sex limited traits (e.g. egg production in males). Genomic selection would also allow for the development of experimental populations to test genotype x environment interactions for global application of the technology. Nevertheless, the rapid genetic progress in application of genomic selection my increase the risk of eroding genetic diversity (Fahrenkrug *et al.* 2010) which is an important attribute that must be maintained (Tixier-Boichard *et al.* 2009). Therefore minimizing genetic erosion must be considered in the application of this technology.

8.5 Application of genomic selection in poultry

The industrial application of genomic selection has been adopted in some livestock sectors such the dairy industry where it is used in the prediction of genetic merit for young bulls (Goddard & Hayes 2009; Hayes *et al.* 2009). However for some species such as chickens the cost of genotyping currently makes the implementation of this technology unprofitable. The cost of genomic selection using the 40 000 SNP marker panel was estimated at about €200 per bird for (Preisinger 2010). There are however indication that as the cost of genotyping declines with technological advancement, genomic selection will become more accessible and cost effective for application in the poultry industry in the foreseeable future (Mackay *et al.* 2009; Fahrenkrug *et al.* 2010).

There are ongoing concerted efforts to implement this approach in poultry breeding (Chen *et al.* 2010; Preisinger 2010). Genome–assisted prediction of economic traits such as feed conversion rate (Gonzalez-Recio et al. 2009) and mortality (Long et al. 2007) have already been demonstrated in chickens.

8.6 Conclusions

This study demonstrated the basis for genetic determination of complex traits associated with growth, reproduction and the skeleton. It provides a basis for the ultimate identification of genes which can be targeted by selection approaches or as drug targets in formulating therapies to deliver optimal welfare and health to chickens and humans. The pervasive additive gene action of QTL with mainly small

to moderately large effects reflects the previous potential for long-term genetic progress by selection over the last 60 years. The epistasis detected on ovary weight confirmed the long-held assumption of the possible role of non-additive action behind the heterosis observed in reproduction traits. The findings from this research are based on current technology and can be enhanced by whole genome based approaches which are increasingly being explored for practical application.

REFERENCES

Abasht B., Dekkers J.C.M. & Lamont S.J. (2006a) Review of quantitative trait loci identified in the chicken. *Poult Sci* **85**, 2079-96.

Abasht B., Pitel F., Lagarrigue S., Le Bihan-Duval E., Le Roy P., Demeure O., Vignoles F., Simon J., Cogburn L., Aggrey S., Vignal A. & Douaire M. (2006b) Fatness QTL on chicken chromosome 5 and interaction with sex. *Genetics Selection Evolution* **38**, 297 - 311.

Aggrey S.E. (2002a) Comparison of three nonlinear and spline regression models for describing chicken growth curves. *Poult Sci* **81**, 1782-8.

Aggrey S.E. (2002b) Comparison of three nonlinear and spline regression models for describing chicken growth curves. *Poultry Science* **81**, 1782-8.

Aksglaede L., Juul A., Olsen L.W. & Sørensen T.I.A. (2009) Age at puberty and the emerging obesity epidemic. *PLoS ONE* **4**, e8450.

Almasy L., Dyer T.D. & Blangero J. (1997) Bivariate quantitative trait linkage analysis: Pleiotropy versus co-incident linkages. *Genetic Epidemiology* **14**, 953-8.

Alvarez-Castro J.M. & Carlborg O. (2007) A nnified model for functional and statistical epistasis and its application in quantitative trait loci analysis. *Genetics* **176**, 1151-67.

Ãlvarez R. & Hocking P.M. (2009) Stochastic modelling of optimum initial body weight, daily weight gain and effect of genetic changes in ovulation rate and age at sexual maturity on total egg production of broiler breeders. *British Poultry Science* **50**, 135 - 43.

Ambo M., Moura A.S.A.M.T., Ledur M.C., Pinto L.F.B., Baron E.E., Ruy D.C., Nones K., Campos R.L.R., Boschiero C., Burt D.W. & Coutinho L.L. (2009)

Quantitative trait loci for performance traits in a broiler × layer cross. *Animal Genetics* **40**, 200-8.

Andersson L. (2001) Genetic dissection of phenotypic diversity in farm animals. *Nat Rev Genet* **2**, 130-8.

Andersson L. (2009) Genome-wide association analysis in domestic animals: a powerful approach for genetic dissection of trait loci. *Genetica* **136**, 341-9.

Andersson L. & Georges M. (2004) Domestic-animal genomics: deciphering the genetics of complex traits. *Nat Rev Genet* **5**, 202 - 12.

Andreescu C., Avendano S., Brown S.R., Hassen A., Lamont S.J. & Dekkers J.C.M. (2007) Linkage disequilibrium in related breeding lines of chickens. *Genetics* **177**, 2161-9.

Ankra-Badu G., Shriner D., Le Bihan-Duval E., Mignon-Grasteau S., Pitel F., Beaumont C., Duclos M., Simon J., Porter T., Vignal A., Cogburn L., Allison D., Yi N. & Aggrey S. (2010) Mapping main, epistatic and sex-specific QTL for body composition in a chicken population divergently selected for low or high growth rate. *BMC Genomics* **11**, 107.

Armstrong D.G. (1984) Ovarian aromatase activity in the domestic fowl (Gallus domesticus). *J Endocrinol* **100**, 81-6.

Atzmon G., Blum S., Feldman M., Cahaner A., Lavi U. & Hillel J. (2008) QTLs detected in a multigenerational resource chicken population. *J Hered* **99**, 528-38.

Atzmon G., Ronin Y.I., Korol A., Yonash N., Cheng H. & Hillel J. (2006) QTLs associated with growth traits and abdominal fat weight and their interactions

with gender and hatch in commercial meat-type chickens. *Animal Genetics* **37**, 352-8.

Balthazart J. & Hendrick J.C. (1978) Steroidal control of plasma luteinizing hormone, comb growth and sexual behaviour in male chicks. *J Endocrinol* **77**, 149-50.

Barbato G.F. (1991) Genetic architecture of growth curve parameters in chickens. *TAG Theoretical and Applied Genetics* **83**, 24-32.

Beck M.M. & Hansen K.K. (2004) Role of estrogen in avian osteoporosis. *Poult Sci* **83**, 200-6.

Bishop S.C., Fleming R.H., McCormack H.A., Flock D.K. & Whitehead C.C. (2000) Inheritance of bone characteristics affecting osteoporosis in laying hens. *British Poultry Science* **41**, 33-40.

Boichard D., Fritz S., Rossignol M.N., Guillaume F., Colleau J.J. & Druet T. (2006) Implemetation of marker-assisted selection: practical lessons from dairy cattle. In: *8th World Congress on Genetics Applied to Livestock Production, August 13-18, 2006, paper no. 22-11*. 8WCGALP, Belo Horizonte, MG, Brazil, Belo Horizonte, MG, Brasil.

Brewer C.J. & Balen A.H. (2010) The adverse effects of obesity on conception and implantation. *Reproduction*, REP-09-0568.

Brodsky L.M. (1988) Ornament size influences mating success in male rock ptarmigan. *Animal Behaviour* **36**, 662-7.

Brody T.B., Siegel P.B. & Cherry J.A. (1984) Age, body weight and body composition requirements for the onset of sexual maturity of dwarf and normal chickens. *British Poultry Science* **25**, 245 - 52.

Broman K.W., Wu H., Sen Ś. & Churchill G.A. (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics* **19**, 889-90.

Bronson F.H. (2001) Puberty in female mice is not associated with increases in either body fat or leptin. *Endocrinology* **142**, 4758-61.

Burt D.W. (2002) Applications of biotechnology in the poultry industry. *World's Poultry Science Journal* **58**, 5-13.

Burt D.W. (2007) Emergence of the chicken as a model organism: implications for agriculture and biology. *Poult Sci* **86**, 1460-71.

Carlborg O., Andersson L. & Kinghorn B. (2000) The use of a genetic algorithm for simultaneous mapping of multiple interacting quantitative trait loci. *Genetics* **155**, 2003-10.

Carlborg Ö., Brockmann G.A. & Haley C.S. (2005) Simultaneous mapping of epistatic QTL in DU6i × DBA/2 mice. *Mammalian Genome* **16**, 481-94.

Carlborg O. & Haley C.S. (2004) Epistasis: too often neglected in complex trait studies? *Nat Rev Genet* **5**, 618-25.

Carlborg O., Hocking P.M., Burt D.W. & Haley C.S. (2004a) Simultaneous mapping of epistatic QTL in chickens reveals clusters of QTL pairs with similar genetic effects on growth. *Genetical Research* **83**, 197-209.

Carlborg O., Kerje S., Schutz K., Jacobsson L., Jensen P. & Andersson L. (2003) A global search reveals epistatic interaction between QTL for early growth in the chicken. *Genome Res.* **13**, 413-21.

Carlborg R., Hocking P.M., Burt D.W. & Haley C.S. (2004b) Simultaneous mapping of epistatic QTL in chickens reveals clusters of QTL pairs with similar genetic effects on growth. *Genetical Research* **83**, 197-209.

Cassady J.P., Johnson R.K., Pomp D., Rohrer G.A., Van Vleck L.D., Spiegel E.K. & Gilson K.M. (2001) Identification of quantitative trait loci affecting reproduction in pigs. *J. Anim Sci.* **79**, 623-33.

Chase K., Adler F.R. & Lark K.G. (1997) Epistat : a computer program for identifying and testing interactions between pairs of quantitative trait loci. *TAG Theoretical and Applied Genetics* **94**, 724-30.

Chen C.Y., Misztal I., Aguilar I., Tsuruta S., Meuwissen T.H.E., Aggrey S.E., Wing T. & Muir W.M. (2010) Genome-wide marker-assisted selection combining all pedigree phenotypic information with genotypic data in one step: an example using broiler chickens. *J. Anim Sci.*, jas.2010-3071.

Chen H., Huang R.L., Zhang H.X., Di K.Q., Pan D. & Hou Y.G. (2007) Effects of photoperiod on ovarian morphology and carcass traits at sexual maturity in pullets. *Poult Sci* **86**, 917-20.

Cheng H.H., Zhang Y. & Muir W.M. (2007) Evidence for widespread epistatic interactions influencing Marek's disease virus viremia levels in chicken. *Cytogenetic and Genome Research* **117**, 313-8.

Cheng H.W. (2010) Breeding of tomorrow's chickens to improve well-being1. *Poult Sci* **89**, 805-13.

Cheverud J.M. & Routman E.J. (1995) Epistasis and its contribution to genetic variance components. *Genetics* **139**, 1455-61.

Chiu Y.-F., Chiou J.-M., Liang K.-Y. & Lee C.-Y. (2010) Incorporation of covariates in simultaneous localization of two linked loci using affected relative pairs. *BMC Genetics* **11**, 67.

Churchill G.A. & Doerge R.W. (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963-71.

Cransberg P.H., Parkinson G.B., Wilson S. & Thorp B.H. (2001) Sequential studies of skeletal calcium reserves and structural bone volume in a commercial layer flock. *British Poultry Science* **42**, 260 - 5.

Darmani Kuhi H., Kebreab E., Lopez S. & France J. (2003) An evaluation of different growth functions for describing the profile of live weight with time (age) in meat and egg strains of chicken. *Poult Sci* **82**, 1536-43.

Darmani Kuhi H., Porter T., LÓPez S., Kebreab E., Strathe A.B., Dumas A., Dijkstra J. & France J. (2010) A review of mathematical functions for the analysis of growth in poultry. *World's Poultry Science Journal* **66**, 227-40.

Darvasi A. & Soller M. (1997) A simple method to calculate resolving power and confidence interval of QTL map location. *Behavior Genetics* **27**, 125-32.

Darvasi A., Weinreb A., Minke V., Weller J.I. & Soller M. (1993) Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. *Genetics* **134**, 943-51.

de Koning D.J., Dekkers J.C.M. & Haley C.S. (2003a) Designs for QTL detection in livestock and theor implications for MAS. In: *Marker assisted selection: A fast track to increase genetic gain in plant and animal breeding?* Food and Agriculture Organisation (FAO) and University of Turin, Turin, Italy.

de Koning D.J., Windsor D., Hocking P.M., Burt D.W., Law A., Haley C.S., Morris A., Vincent J. & Griffin H. (2003b) Quantitative trait locus detection in commercial broiler lines using candidate regions. *J. Anim Sci.* **81**, 1158-65.

Decuypere E., Bruggeman V., Everaert N., Li Y., Boonen R., De Tavernier J., Janssens S. & Buys N. (2010) The broiler breeder paradox: ethical, genetic and physiological perspectives, and suggestions for solutions. *British Poultry Science* **51**, 569 - 79.

Decuypere E., Hocking P.M., Tona K., Onagbesan O., Bruggeman V., Jones E.K.M., Cassy S., Rideau N., Metayer S., Jego Y., Putterflam J., Tesseraud S., Collin A., Duclos M., Trevidy J.J. & Williams J. (2006) Broiler breeder paradox: a project report. *World's Poultry Science Journal* **62**, 443-53.

Dekkers J.C.M. (2004) Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. *J. Anim Sci.* **82**, E313-28.

DiPetrillo K. & Zou F. (2009) QTL mapping in intercross and backcross populations. In: *Cardiovascular Genomics* (pp. 157-73. Humana Press.

Dodgson J.B. (2007) The chicken genome: some good news and some bad News. *Poult Sci* **86**, 1453-9.

Doerge R.W. (2002) Mapping and analysis of quantitative trait loci in experimental populations. *Nat Rev Genet* **3**, 43-52.

Dunn I.C., Fleming R.H., McCormack H.A., Morrice D., Burt D.W., Preisinger R. & Whitehead C.C. (2007) A QTL for osteoporosis detected in an F_2 population derived from White Leghorn chicken lines divergently selected for bone index. *Animal Genetics* **38**, 45-9.

Eitan Y. & Soller M. (2001) Effect of photoperiod and quantitative feed restriction in a broiler strain on onset of lay in females and onset of semen production in males: a genetic hypothesis. *Poult Sci* **80**, 1397-405.

Elis S., Batellier F., Couty I., Balzergue S., Martin-Magniette M.-L., Monget P., Blesbois E. & Govoroun M.S. (2008) Search for the genes involved in oocyte maturation and early embryo development in the hen. *BMC Genomics* **9**, 110.

Elis S., Blesbois E., Couty I., Balzergue S., Martin-Magniette M.-L., Batellier F. & Govoroun M.S. (2009) Identification of germinal disk region derived genes potentially involved in hen fertility. *Molecular Reproduction and Development* **76**, 1043-55.

Fahrenkrug S.C., Blake A., Carlson D.F., Doran T., Van Eenennaam A., Faber D., Galli C., Gao Q., Hackett P.B., Li N., Maga E.A., Muir W.M., Murray J.D., Shi D., Stotish R., Sullivan E., Taylor J.F., Walton M., Wheeler M., Whitelaw B. & Glenn B.P. (2010) Precision genetics for complex objectives in animal agriculture. *J. Anim Sci.* **88**, 2530-9.

Falconer D.S. & Mackay T.F.C. (1996) *Introduction to quantitative genetics*. 4th Edition. Longman Group Ltd, Essex.England.

Feenstra B., Skovgaard I.M. & Broman K.W. (2006) Mapping quantitative trait Loci by an extension of the Haley-Knott regression method using estimating equations. *Genetics* **173**, 2269-82.

Fleming R.H., McCormack H.A., McTeir L. & Whitehead C.C. (2006) Relationships between genetic, environmental and nutritional factors influencing osteoporosis in laying hens. *British Poultry Science* **47**, 742 - 55.

Frank D.M. (1931) A persistent right oviduct in the domestic fowl. *The Anatomical Record* **49**, 51-7.

Frisch R.E. (1994) The right weight: body fat, menarche and fertility. *Proceedings of the Nutrition Society* **53**, 113-29.

Frisch R.E. & Revelle R. (1970) Height and weight at menarche and a hypothesis of critical body weights and adolescent events. *Science* **169**, 397-9.

Gao Y., Hu X.X., Du Z.Q., Deng X.M., Huang Y.H., Fei J., Feng J.D., Liu Z.L., Da Y. & Li N. (2006) A genome scan for quantitative trait loci associated with body weight at different developmental stages in chickens. *Animal Genetics* **37**, 276-8.

Garn S.M., LaVelle M. & Pilkington J.J. (1983) Comparisons of fatness in premenarcheal and postmenarcheal girls of the same age. *The Journal of Pediatrics* **103**, 328-31.

Gautier M., Druet T., Fritz S., Eggen A. & Boichard D. (2006) QTL fine-mapping: experience in french dairy cattle. In: *8th World Congress on Genetics Applied to Livestock Production, August 13-18, 2006, paper no. 22-10, Belo Horizonte, MG, Brasil*. 8WCGALP, Belo Horizonte, MG, Brazil.

Gilbert H.L.N., Le Roy P., Milan D. & Bidanel J.-P. (2007) Linked and pleiotropic QTLs influencing carcass composition traits detected on porcine chromosome 7. *Genetics Research* **89**, 65-72.

Goddard K.A.B., Witte J.S., Suarez B.K., Catalona W.J. & Olson J.M. (2001) Model-free linkage analysis with covariates confirms linkage of prostate cancer to chromosomes 1 and 4. *The American Journal of Human Genetics* **68**, 1197-206.

Goddard M.E. & Hayes B.J. (2009) Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nat Rev Genet* **10**, 381-91.

Gonzalez-Recio O., Gianola D., Rosa G., Weigel K. & Kranis A. (2009) Genome-assisted prediction of a quantitative trait measured in parents and progeny:

application to food conversion rate in chickens. *Genetics Selection Evolution* **41**, 3.

Green P., Falls K. & Crooks S. (1990) Cri-map (Version 2.4). Washington University, School of Medicine, St. Louis, Mo. USA.

Griffin H.D. & Goddard C. (1994) Rapidly growing broiler (meat-type) chickens. Their origin and use for comparative studies of the regulation of growth. *International Journal of Biochemistry* **26**, 19-28.

Grossman M. & Bohren B.B. (1985) Logistic growth curve of chickens: heritability of parameters. *J Hered* **76**, 459-62.

Habier D., Fernando R.L. & Dekkers J.C.M. (2009) Genomic selection using low-density marker panels. *Genetics* **182**, 343-53.

Hadjipavlou G. & Bishop S.C. (2009) Age-dependent quantitative trait loci affecting growth traits in Scottish Blackface sheep. *Animal Genetics* **40**, 165-75.

Haley C.S. & Knott S.A. (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**, 315-24.

Haley C.S., Knott S.A. & Elsen J.M. (1994) Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* **136**, 1195-207.

Hanlon P. & Lorenz A. (2005) A computational method to detect epistatic effects contributing to a quantitative trait. *Journal of Theoretical Biology* **235**, 350-64.

Hans D. & Krieg M.A. (2008) The clinical use of quantitative ultrasound (QUS) in the detection and management of osteoporosis. *Ultrasonics, Ferroelectrics and Frequency Control, IEEE Transactions on* **55**, 1529-38.

Hayes B.J., Bowman P.J., Chamberlain A.J. & Goddard M.E. (2009) Invited review: Genomic selection in dairy cattle: Progress and challenges. *Journal of Dairy Science* **92**, 433-43.

Hermier D. (1997) Lipoprotein metabolism and fattening in poultry. *J. Nutr.* **127**, 805S-.

Hill W.G. (2010) Understanding and using quantitative genetic variation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 73-85.

Hill W.G., Goddard M.E. & Visscher P.M. (2008a) Data and theory point to mainly additive genetic variance for complex traits. *PLoS Genet* **4**, e1000008.

Hill W.G., Goddard M.E. & Visscher P.M. (2008b) Data and Theory Point to Mainly Additive Genetic Variance for Complex Traits. *PLoS Genetics* **4**, e1000008.

Hocking P. (2004) Roles of body weight and feed intake in ovarian follicular dynamics in broiler breeders at the onset of lay and after a forced molt. *Poult Sci* **83**, 2044-50.

Hocking P.M. (2005) Review of QTL mapping results in chickens. *World's Poultry Science Journal* **61**, 215-26.

Hocking P.M. (2009a) Feed restriction. In: *Biology of Breeding Poultry* (ed. by Hocking PM), pp. 307-30. CABI, Abingdon.

Hocking P.M. (2009b) *Feed restriction. Biology of breeding poultry*. CAB International, Wallingford, Oxfordshire, OX10 8DE, UK.

Hocking P.M., Bain M., Channing C.E., Fleming R. & Wilson S. (2003) Genetic variation for egg production, egg quality and bone strength in selected and traditional breeds of laying fowl. *British Poultry Science* **44**, 365-73.

Hocking P.M., Bernard R. & Robertson G.W. (2002a) Effects of low dietary protein and different allocations of food during rearing and restricted feeding after peak rate of lay on egg production, fertility and hatchability in female broiler breeders. *British Poultry Science* **43**, 94-103.

Hocking P.M., Bernard R. & Robertson G.W. (2002b) Effects of low dietary protein and different allocations of food during rearing and restricted feeding after peak rate of lay on egg production, fertility and hatchability in female broiler breeders. *Br Poult Sci* **43**, 94-103.

Hocking P.M., Gilbert A.B., Walker M. & Waddington D. (1987) Ovarian follicular structure of white leghorns fed ad libitum and dwarf and normal broiler breeders fed ad libitum or restricted until point of lay. *British Poultry Science* **28**, 493 - 506.

Hocking P.M. & Robertson G.W. (2000) Ovarian follicular dynamics in selected and control (relaxed selection) male- and female-lines of broiler breeders fed ad libitum or on restricted allocations of food. *British Poultry Science* **41**, 229 - 34.

Hu J., Mungall C., Law A., Papworth R., Nelson J.P., Brown A., Simpson I., Leckie S., Burt D.W., Hillyard A.L. & Archibald A.L. (2001) The ARKdb: genome databases for farmed and other animals. *Nucl. Acids Res.* **29**, 106-10.

Hutchens L.K., Hintz R.L. & Johnson R.K. (1981) Genetic and Phenotypic Relationships between Pubertal and Growth Characteristics of Gilts. *J. Anim Sci.* **53**, 946-51.

Ikeobi C.O., Woolliams J.A., Morrice D.R., Law A., Windsor D., Burt D.W. & Hocking P.M. (2002) Quantitative trait loci affecting fatness in the chicken. *Anim Genet* **33**, 428-35.

Ikeobi C.O.N., Woolliams J.A., Morrice D.R., Law A., Windsor D., Burt D.W. & Hocking P.M. (2004) Quantitative trait loci for meat yield and muscle distribution in a broiler layer cross. *Livestock Production Science* **87**, 143-51.

International Chicken Polymorphism Map Consortium (2004) A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. *Nature* **432**, 717-22.

Jaccoby S., Arnon E., Snapir N. & Robinzon B. (1995) Effects of estradiol and tamoxifen on feeding, fattiness, and some endocrine criteria in hypothalamic obese hens. *Pharmacol Biochem Behav* **50**, 55-63.

Jacobsson L., Park H.-B., Wahlberg P., Fredriksson R., Perez-Encison M., Siegel P.B. & Andersson L. (2005) Many QTLs with minor additive effects are associated with a large difference in growth between two selection lines in chickens. *Genetics Research* **86**, 115-25.

Jana S. (1971) Simulation of quantitative characters from qualitatively acting genes. *TAG Theoretical and Applied Genetics* **41**, 216-26.

Jannink J.-L. & Jansen R. (2001) Mapping epistatic quantitative trait loci with one-dimensional genome searches. *Genetics* **157**, 445-54.

Jansen R.C. (1994) Controlling the type I and type II errors in mapping quantitative trait loci. *Genetics* **138**, 871-81.

Jansen R.C. & Stam P. (1994) High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* **136**, 1447-55.

Johnson M., Lara N. & Kamel M. (2009) How genomics has informed our understanding of the pathogenesis of osteoporosis. *Genome Med* **1**, 84.

Kao C.-H. (2000) On the differences between maximum likelihood and regression interval mapping in the analysis of quantitative trait loci. *Genetics* **156**, 855-65.

Kao C.-H., Zeng Z.-B. & Teasdale R.D. (1999) Multiple interval mapping for quantitative trait loci. *Genetics* **152**, 1203-16.

Kaplowitz P.B. (2008) Link between body fat and the timing of puberty. *Pediatrics* **121**, S208-17.

Kaprio J., Rimpela A., Winter T., Viken R.J., Rimpela M. & Rose R.J. (1995) Common genetic influences on BMI and age at menarche. *Hum Biol* **67**, 739-53.

Kerje S., Carlborg Ö., Jacobsson L., Schütz K., Hartmann C., Jensen P. & Andersson L. (2003) The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs. *Animal Genetics* **34**, 264-74.

Kerje S., Carlborg Ö., Jacobsson L., Schütz K., Hartmann C., Jensen P., & Andersson L. (2003) The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs. *Animal Genetics* **34**, 264-74.

King A.H., Jiang Z., Gibson J.P., Haley C.S. & Archibald A.L. (2003) Mapping quantitative trait loci affecting female reproductive traits on porcine chromosome 8. *Biology of Reproduction* **68**, 2172-9.

Knizetova H., Hyanek J., Knize B. & Roubicek J. (1991) ANALYSIS OF GROWTH-CURVES OF FOWL .1. CHICKENS. *British Poultry Science* **32**, 1027-38.

Knížetová H., Hyánek J., Kníže B. & Roubíček J. (1991) Analysis of growth curves of fowl. I. Chickens. *British Poultry Science* **32**, 1027 - 38.

Knott S.A. & Haley C.S. (2000) Multitrait least squares for quantitative trait loci detection. *Genetics* **156**, 899-911.

Knott S.A., Marklund L., Haley C.S., Andersson K., Davies W., Ellegren H., Fredholm M., Hansson I., Hoyheim B., Lundstrom K., Moller M. & Andersson L. (1998) Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs. *Genetics* **149**, 1069-80.

Koncagul S. & Cadirci S. (2010) Analysis of growth of broilers with restricting and unrestricting initial body weight in Gompertz-Laird model in different environments. *Ital J Anim Sci* **9:e5**, 20-5.

Kruglyak L. & Lander E.S. (1995) A nonparametric approach for mapping quantitative trait loci. *Genetics* **139**, 1421-8.

Krzysik-Walker S.M., Ocón-Grove O.M., Maddineni S.B., Hendricks G.L. & Ramachandran R. (2007) Identification of calcitonin expression in the chicken ovary: Influence of follicular maturation and ovarian steroids. *Biology of Reproduction* **77**, 626-35.

Lacassagne L. & Jacquet J.P. (1965) Maturite sexuelle et apparition doeufs a double jaune et sans coquille . Role du regime lumineux et du niveau alimentaire (Sexual maturity and appearance of double-yolked and shell-less eggs. Role of light treatment and nutritional level). *Annales de Zootechnie* **14**, 169-79.

Lande R. & Thompson R. (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics* **124**, 743-56.

Lander E. & Kruglyak L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* **11**, 241-7.

Lander E.S. & Botstein D. (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185-99.

Law A.S. & Archibald A.L. (2000) Farm animal genome databases. *Brief Bioinform* **1**, 151-60.

Le Rouzic A., Alvarez-Castro J.M. & Carlborg O. (2008) Dissection of the genetic architecture of body weight in chicken reveals the impact of epistasis on domestication traits. *Genetics* **179**, 1591-9.

Lee S.-h., Ichii O., Otsuka S., Hashimoto Y. & Kon Y. (2010) Quantitative trait locus analysis of ovarian cysts derived from rete ovarii in MRL/MpJ mice. *Mammalian Genome* **21**, 162-71.

Lei M., Luo C., Peng X., Fang M., Nie Q., Zhang D., Yang G. & Zhang X. (2007) Polymorphism of growth-correlated genes associated with fatness and muscle fiber traits in chickens. *Poult Sci* **86**, 835-42.

Leips J., Gilligan P. & Mackay T.F.C. (2006) Quantitative trait loci with age-specific effects on fecundity in *Drosophila melanogaster*. *Genetics* **172**, 1595-605.

Liu P., Zhu J., Lou X. & Lu Y. (2003) A method for marker-assisted selection based on QTLs with epistatic effects. *Genetica* **119**, 75-86.

Liu X., Zhang H., Li H., Li N., Zhang Y., Zhang Q., Wang S., Wang Q. & Wang H. (2008) Fine-mapping quantitative trait loci for body weight and abdominal fat traits: Effects of marker density and sample size. *Poult Sci* **87**, 1314-9.

Ljungberg K., Holmgren S. & Carlborg O. (2004) Simultaneous search for multiple QTL using the global optimization algorithm DIRECT. *Bioinformatics* **20**, 1887-95.

Long F.E.I., Qing Chen Y., Cheverud J.M. & Wu R. (2006) Genetic mapping of allometric scaling laws. *Genetics Research* **87**, 207-16.

Long N., Gianola D., Rosa G.J.M., Weigel K.A. & Avendaño S. (2007) Machine learning classification procedure for selecting SNPs in genomic selection: application to early mortality in broilers. *Journal of Animal Breeding and Genetics* **124**, 377-89.

Lopez S., France J., Gerrits W.J., Dhanoa M.S., Humphries D.J. & Dijkstra J. (2000) A generalized Michaelis-Menten equation for the analysis of growth. *J. Anim Sci.* **78**, 1816-28.

Lund M.S., Sorensen P., Guldbrandtsen B. & Sorensen D.A. (2003) Multitrait fine mapping of quantitative trait loci using combined linkage disequilibria and linkage analysis. *Genetics* **163**, 405-10.

Lund M.S., Sorensen P., Madsen P. & Jaffrézic F. (2008) Detection and modelling of time-dependent QTL in animal populations. *Genet. Sel. Evol.* **40**, 177-94.

Ma C.-X., Casella G. & Wu R. (2002) Functional mapping of quantitative trait loci underlying the character process: A theoretical framework. *Genetics* **161**, 1751-62.

Mackay T.F.C., Stone E.A. & Ayroles J.F. (2009) The genetics of quantitative traits: challenges and prospects. *Nat Rev Genet* **10**, 565-77.

Malmberg R.L. & Mauricio R. (2005) QTL-based evidence for the role of epistasis in evolution. *Genetics Research* **86**, 89-95.

Malosetti M., Visser R., Celis-Gamboa C. & van Eeuwijk F. (2006) QTL methodology for response curves on the basis of non-linear mixed models, with an illustration to senescence in potato. *TAG Theoretical and Applied Genetics* **113**, 288-300.

Mao Y. & Da Y. (2005) Statistical power for detecting epistasis QTL effects under the F-2 design. *Genetics Selection Evolution* **37**, 129 - 50.

Marklund S. & Carlborg O. (2010) SNP detection and prediction of variability between chicken lines using genome resequencing of DNA pools. BMC Genomics 2010, 11:665. doi:10.1186/1471-2164-11-665

Martínez O. & Curnow R.N. (1992) Estimating the locations and the sizes of the effects of quantitative trait loci using flanking markers. *TAG Theoretical and Applied Genetics* **85**, 480-8.

McElroy J.P., Kim J.J., Harry D.E., Brown S.R., Dekkers J.C. & Lamont S.J. (2006) Identification of trait loci affecting white meat percentage and other growth and carcass traits in commercial broiler chickens. *Poult Sci* **85**, 593-605.

McKay J.C. (2009) The genetics of modern commercial poultry. In: *Biology of Breeding Poultry* (ed. by Hocking PM). CAB International, Wallingford.

Melchinger A.E., Utz H.F., Piepho H.-P., Zeng Z.-B. & Schon C.C. (2007) The role of epistasis in the manifestation of heterosis: A systems-oriented approach. *Genetics* **177**, 1815-25.

Meuwissen T. & Goddard M. (2010) Accurate prediction of genetic values for complex traits by whole-genome resequencing. *Genetics* **185**, 623-31.

Meuwissen T.H.E. & Goddard M.E. (1996) The use of marker haplotypes in animal breeding schemes. *Genet. Sel. Evol.* **28**, 161-76.

Meuwissen T.H.E., Hayes B.J. & Goddard M.E. (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* **157**, 1819-29.

Mignon-Grasteau S. (1999) Genetic parameters of growth curve parameters in male and female chickens. *British Poultry Science* **40**, 44 - 51.

Mignon-Grasteau S., Beaumont C. & Ricard F.H. (2001) Genetic analysis of a selection experiment on the growth curve of chickens. *Poult Sci* **80**, 849-54.

Mignon-Grasteau S., Piles M., Varona L., de Rochambeau H., Poivey J.P., Blasco A. & Beaumont C. (2000) Genetic analysis of growth curve parameters for male and female chickens resulting from selection on shape of growth curve. *J. Anim Sci.* **78**, 2515-24.

Mignon G.L., Pitel F., Gilbert H., Bihan-Duval E.L., Vignoles F., Demeure O., Lagarrigue S., Simon J., Cogburn L.A., Aggrey S.E., Douaire M. & Roy P.L. (2009) A comprehensive analysis of QTL for abdominal fat and breast muscle weights on chicken chromosome 5 using a multivariate approach. *Animal Genetics* **40**, 157-64.

Morgan P.H., Mercer L.P. & Flodin N.W. (1975) General model for nutritional responses of higher organisms. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 4327-31.

Muir W.M. & Aggrey S.E. (2003) *Poultry genetics, breeding and biotechnology*. CABI Publishing, Oxon, UK.

N'Dri A.L., Mignon-Grasteau S., Sellier N., Tixier-Boichard M. & Beaumont C. (2006) Genetic relationships between feed conversion ratio, growth curve and body composition in slow-growing chickens. *British Poultry Science* **47**, 273 - 80.

Narushin V.G. & Takma C. (2003) Sigmoid model for the evaluation of growth and production curves in laying hens. *Biosystems Engineering* **84**, 343-8.

Navarro P., Visscher P.M., Knott S.A., Burt D.W., Hocking P.M. & Haley C.S. (2005a) Mapping of quantitative trait loci affecting organ weights and blood variables in a broiler layer cross. *Br Poult Sci* **46**, 430-42.

Navarro P., Visscher P.M., Knott S.A., Burt D.W., Hocking P.M. & Haley C.S. (2005b) Mapping of quantitative trait loci affecting organ weights and blood variables in a broiler layer cross. *Br Poult Sci* **46**, 430-42.

Needham J. (1925) The metabolism of the developing egg. *Physiol. Rev.* **5**, 1-62.

Neuschl C., Brockmann G.A. & Knott S.A. (2007) Multiple-trait QTL mapping for body and organ weights in a cross between NMRI8 and DBA/2 mice. *Genetics Research* **89**, 47-59.

Nie Q., Sun B., Zhang D., Luo C., Ishag N.A., Lei M., Yang G. & Zhang X. (2005) High diversity of the chicken growth hormone gene and effects on growth and carcass traits. *J Hered* **96**, 698-703.

Norris D., Ngambi J.W., Benyi K., Makgahlela M.L., Shimelis H.A. & Nesamvuni E.A. (2007) Analysis of growth curves of indigenous male Venda and Naked Neck chickens. *South African Journal of Animal Science* **37**, 21-6.

Onagbesan O., Bruggeman V. & Decuypere E. (2009) Intra-ovarian growth factors regulating ovarian function in avian species: A review. *Animal Reproduction Science* **111**, 121-40.

Ou J.T., Tang S.Q., Sun D.X. & Zhang Y. (2009) Polymorphisms of three neuroendocrine-correlated genes associated with growth and reproductive traits in the chicken. *Poult Sci* **88**, 722-7.

Park H.-B., Jacobsson L., Wahlberg P., Siegel P.B. & Andersson L. (2006) QTL analysis of body composition and metabolic traits in an intercross between chicken lines divergently selected for growth. *Physiol. Genomics* **25**, 216-23.

Pasternak H. & Shalev B.A. (1992) An algorithm to fit the Gompertz function to growth curves. *Comput. Appl. Biosci.* **8**, 239-41.

Payne R.W., Murray, D.A., Harding, S.A., Baird, D.B. & Soutar, D.M. (2007) In: *GenStat for Windows (12th Edition) Introduction*. VSN International, Hemel Hempstead.

Phillips P.C. (2008) Epistasis - the essential role of gene interactions in the structure and evolution of genetic systems. *Nat Rev Genet* **9**, 855-67.

Pinchasov Y. & Cahaner A. (1991) The possible prediction of fatness in broiler chickens by biochemical measurements on adipose tissue. *British Poultry Science* **32**, 783 - 7.

Ponsuksili S., Brunner R.M., Goldammer T., Kühn C., Walz C., Chomdej S., Tesfaye D., Schellander K., Wimmers K. & Schwerin M. (2006) Bovine NALP5, NALP8, and NALP9 genes: Assignment to a QTL region and the expression in adult tissues, oocytes, and preimplantation embryos. *Biology of Reproduction* **74**, 577-84.

Preisinger R. (2010) Genome-wide selection in poultry. In: *Lohmann Information*, pp. 18-21. Lohmann Tierzucht GmbH, Germany, Cuxhaven.

Reddish J.M., Nestor K.E. & Lilburn M.S. (2003) Effect of selection for growth on onset of sexual maturity in randombred and growth-selected lines of Japanese quail. *Poult Sci* **82**, 187-91.

Ricklefs R.E. (2008) The evolution of senescence from a comparative perspective. *Functional Ecology* **22**, 379-92.

Ricklefs R.E. (2010) Embryo growth rates in birds and mammals. *Functional Ecology* **24**, 588-96.

Robinson F.E., Renema R.A., Oosterhoff H.H., Zuidhof M.J. & Wilson J.L. (2001) Carcass traits, ovarian morphology and egg laying characteristics in early versus late maturing strains of commercial egg-type hens. *Poult Sci* **80**, 37-46.

Rodriguez-Zas S.L., Southey B.R., Heyen D.W. & Lewin H.A. (2002) Detection of quantitative trait Lloci influencing dairy traits using a model for longitudinal data. *J. Dairy Sci.* **85**, 2681-91.

Rothschild M.F. & Plastow G.S. (2008) Impact of genomics on animal agriculture and opportunities for animal health. *Trends in Biotechnology* **26**, 21-5.

Roush W.B., Dozier W.A. & Branton S.L. (2006) Comparison of Gompertz and neural network models of broiler growth. *Poultry Science* **85**, 794-7.

Rubin C.-J., Lindberg J., Fitzsimmons C., Savolainen P., Jensen P., Lundeberg J., Andersson L. & Kindmark A. (2007a) Differential gene expression in femoral bone from red junglefowl and domestic chicken, differing for bone phenotypic traits. *BMC Genomics* **8**, 208.

Rubin C.J., Brandstrom H., Wright D., Kerje S., Gunnarsson U., Schutz K., Fredriksson R., Jensen P., Andersson L., Ohlsson C., Mallmin H., Larsson S. & Kindmark A. (2007b) Quantitative trait loci for BMD and bone strength in an intercross between domestic and wildtype chickens. *J Bone Miner Res* **22**, 375 - 84.

Rubin C.J., Zody M.C., Eriksson J., Meadows J. R. S., Sherwood E., Webster M.T., Jiang L., Ingman M., Sharpe T., Ka S., Hallböök F., Besnier F., Carlborg O., Bed'hom B., Tixier-Boichard M., Jensen P., Siegel P., Lindblad-Toh K & Andersson L. (2010) Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature* **464**, 587-591. doi:10.1038/nature08832.

Ruy D.C., Nones K., Baron E.E., Ledur M.C., Melo C.M.R.d., Ambo M., Campos R.d.L.R. & Coutinho L.L. (2005) Strategic marker selection to detect quantitative trait loci in chicken. *Scientia Agricola* **62**, 111-6.

Sasaki O., Odawara S., Takahashi H., Nirasawa K., Oyamada Y., Yamamoto R., Ishii K., Nagamine Y., Takeda H., Kobayashi E., Furukawa T. (2004) Genetic mapping of quantitative trait loci affecting body weight, egg character and egg production in F2 intercross chickens. *Animal Genetics* **35**, 188-94.

Scanes C.G. (2007) The global importance of poultry. *Poult Sci* **86**, 1057-8.

Schreiweis M.A., Hester P.Y. & Moody D.E. (2005) Identification of quantitative trait loci associated with bone traits and body weight in an F2 resource population of chickens. *Genet. Sel. Evol.* **37**, 677-98.

Schreiweis M.A., Hester P.Y., Settar P. & Moody D.E. (2006) Identification of quantitative trait loci associated with egg quality, egg production, and body weight in an F2 resource population of chickens. *Anim Genet* **37**, 106-12.

Seaton G.H.J., Grunchec J.A., White I., Allen J., De Koning D.J., Wei W., Berry D., Haley C. & Knott S. (2006) GridQTL: A grid portal for QTL mapping of compute intensive datasets. In: *Proceedings of the 8th World Congress on Genetics Applied to Livestock Production*, Belo Horizonte, Brazil.

Sen S. & Churchill G.A. (2001) A statistical framework for quantitative trait mapping. *Genetics* **159**, 371-87.

Sewalem A., Morrice D.M., Law A., Windsor D., Haley C.S., Ikeobi C.O., Burt D.W. & Hocking P.M. (2002) Mapping of quantitative trait loci for body weight at three, six, and nine weeks of age in a broiler layer cross. *Poult Sci* **81**, 1775-81.

Sharman P.W., Morrice D.R., Law A.S., Burt D.W. & Hocking P.M. (2007) Quantitative trait loci for bone traits segregating independently of those for growth in an F2 broiler x layer cross. *Cytogenet Genome Res* **117**, 296-304.

Silversides F.G., Korver D.R. & Budgell K.L. (2006) Effect of strain of layer and age at photostimulation on egg production, egg quality, and bone strength. *Poult Sci* **85**, 1136-44.

Soller M., Brody T., Eitan Y., Agursky T. & Wexler C. (1984) Minimum weight for the onset of sexual maturity in ffemale chickens: heritability and phenotypic correlations with early growth rate. *Poult Sci* **63**, 2103 -13.

Song J.Z., Soller M. & Geneizi A. (1999) The full-sib intercross line (FSIL): a QTL mapping design for outcrossing species. *Genetics Research* **73**, 61-73.

Stern C.D. (2005) The chick: A great model system becomes even greater. *Developmental Cell* **8**, 9-17.

Tatsuda K. & Fujinaka K. (2001) Genetic mapping of the QTL affecting body weight in chickens using a F2 family. *British Poultry Science* **42**, 333-7.

Terčič D., Holcman A., Dovč P., Morrice D.R., Burt D.W., Hocking P.M. & Horvat S. (2009) Identification of chromosomal regions associated with growth and carcass traits in an F3 full sib intercross line originating from a cross of

chicken lines divergently selected on body weight. *Animal Genetics* **40**, 743-8.

Thornton P.K. (2010) Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 2853-67.

Tixier-Boichard M., Bordas A. & Rognon X. (2009) Characterisation and monitoring of poultry genetic resources. *World's Poultry Science Journal* **65**, 272-85.

Tuiskula-Haavisto M., Honkatukia M., Vilkki J., de Koning D.J., Schulman N.F. & Maki-Tanila A. (2002) Mapping of quantitative trait loci affecting quality and production traits in egg layers. *Poult Sci* **81**, 919-27.

Uemoto Y., Sato S., Odawara S., Nokata H., Oyamada Y., Taguchi Y., Yanai S., Sasaki O., Takahashi H., Nirasawa K. & Kobayashi E. (2009) Genetic mapping of quantitative trait loci affecting growth and carcass traits in F2 intercross chickens. *Poult Sci* **88**, 477-82.

Varona L., Gómez-Raya L., Rauw W.M., Ovilo C., Clop A. & Noguera J.L. (2005) The value of prior information for detection of QTL affecting longitudinal traits: an example using Von Bertalanffy growth function. *Journal of Animal Breeding and Genetics* **122**, 37-48.

Visscher P.M., Thompson R. & Haley C.S. (1996) Confidence intervals in QTL mapping by bootstrapping. *Genetics* **143**, 1013-20.

Wade M.J. (2001) Epistasis, complex traits, and mapping. *Genetica* **112-113**, 59-69.

Wakamatsu M., Yamagata T., Namikawa T. & Mizutani M. (2000) Hereditary persistent right oviduct in the chicken PNP/DO line. *Poult Sci* **79**, 1075-81.

Wang Z. & Zuidhof M.J. (2004) Estimation of growth parameters using a nonlinear mixed Gompertz model. *Poult Sci* **83**, 847-52.

Webster A.B. (2004) Welfare implications of avian osteoporosis. *Poult Sci* **83**, 184-92.

Wei W.H., Duan Y., Haley C.S., Ren J., Koning D.J.d. & Huang L.S. (2010a) High throughput analyses of epistasis for swine body dimensions and organ weights. *Animal Genetics. Online advance publication: (DOI): 10.1111/j.1365-2052.2010.02082.x.*

Wei W.H., Knott S., Haley C.S. & de Koning D.J. (2009) Controlling false positives in the mapping of epistatic QTL. *Heredity*.

Wei W.H., Knott S., Haley C.S. & de Koning D.J. (2010b) Controlling false positives in the mapping of epistatic QTL. *Heredity* **104**, 401-9.

Whitehead C.C. & Fleming R.H. (2000a) Osteoporosis in cage layers. *Poult Sci* **79**, 1033-41.

Whitehead C.C. & Fleming R.H. (2000b) Osteoporosis in cage layers. *Poult Sci* **79**, 1033 - 41.

Williams S.M., Price S.E. & Siegel P.B. (2002) Heterosis of growth and reproductive traits in fowl. *Poult Sci* **81**, 1109-12.

Winsor C.P. (1932) The Gompertz curve as a growth curve. *Proceedings of the National Academy of Sciences* **18**, 1-8.

Wright D., Kerje S., Brändström H., Schütz K., Kindmark A., Andersson L., Jensen P. & Pizzari T. (2008) The genetic architecture of a female sexual ornament. *Evolution* **62**, 86-98.

Wu R., Ma C.-X., Lin M. & Casella G. (2004) A general framework for analyzing the genetic architecture of developmental characteristics. *Genetics* **166**, 1541-51.

Wu W., Zhou Y., Li W., Mao D. & Chen Q. (2002) Mapping of quantitative trait loci based on growth models. *Theoretical and Applied Genetics* **105**, 1043-9.

Xu S. (2003) Theoretical basis of the Beavis effect. *Genetics* **165**, 2259-68.

Yandell B.S., Mehta T., Banerjee S., Shriner S., Venkataraman R., Young Moon J.Y., Neely W.W., Wu H., von Smith R. & Yi N. (2007) R/qtl bim: QTL with Bayesian Interval Mapping in experimental crosses. *Bioinformatics* **23**, 641 - 3.

Yang J., Zhu J. & Williams R.W. (2007) Mapping the genetic architecture of complex traits in experimental populations. *Bioinformatics* **23**, 1527-36.

Yang R., Tian Q. & Xu S. (2006) Mapping quantitative trait loci for longitudinal traits in line crosses. *Genetics* **173**, 2339-56.

Yannakopoulos A.L., Christaki E. & Florou-Paneri P. (1995) Effect of age and carcase composition on the onset of sexual maturity in quail under normal feeding regimens. *British Poultry Science* **36**, 771 - 7.

Yi N. & Shriner D. (2007) Advances in Bayesian multiple quantitative trait loci mapping in experimental crosses. *Heredity* **100**, 240-52.

Yi N., Yandell B.S., Churchill G.A., Allison D.B., Eisen E.J. & Pomp D. (2005) Bayesian model selection for genome-wide epistatic quantitative trait loci analysis. *Genetics* **170**, 1333 - 44.

Yi N., Zinniel D., Kim K., Eisen E., Bartolucci A., Allison D.B. & Pomp D. (2006) Bayesian analyses of multiple epistatic QTL models for body weight and body composition in mice. *Genet Res* **87**, 45 - 60.

Zeegers M., Rijsdijk F. & Sham P. (2004) Adjusting for Covariates in Variance Components QTL Linkage Analysis. *Behavior Genetics* **34**, 127-33.

Zeng Z.-B., Wang T. & Zou W. (2005) Modeling quantitative trait loci and interpretation of models. *Genetics*, genetics.104.035857.

Zeng Z.B. (1994) Precision mapping of quantitative trait loci. *Genetics* **136**, 1457-68.

Zhang L., Li H., Li Z. & Wang J. (2008) Interactions between markers can be caused by the dominance effect of quantitative trait loci. *Genetics* **180**, 1177-90.

Zhou H., Deeb N., Evock-Clover C.M., Ashwell C.M. & Lamont S.J. (2006) Genome-wide linkage analysis to identify chromosomal regions affecting phenotypic traits in the chicken. I. Growth and average daily gain. *Poult Sci* **85**, 1700-11.

Zhou H., Deeb N., Evock-Clover C.M., Mitchell A.D., Ashwell C.M. & Lamont S.J. (2007a) Genome-Wide Linkage Analysis to Identify Chromosomal Regions Affecting Phenotypic Traits in the Chicken. III. Skeletal Integrity. *Poult Sci* **86**, 255-66.

Zhou H., Evock-Clover C.M., McMurtry J.P., Ashwell C.M. & Lamont S.J. (2007b) Genome-wide linkage analysis to identify chromosomal regions affecting phenotypic traits in the chicken. IV. metabolic traits. *Poult Sci* **86**, 267-76.

Zhu Z., Qiangba Y., Zhu M., Ji S., Tang X., Mo D. & Liu B. (2006) Comparative study on analysis and fitting of Tibetan chicken growth curve. *Scientia Agricultura Sinica* **39**, 2159-62.

APPENDIX

Appendix I

Published manuscript on: Overlap of quantitative trait loci for growth rate and for body weight and age at the onset of sexual maturity in chickens.

Overlap of quantitative trait loci for early growth rate, and for body weight and age at onset of sexual maturity in chickens

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Abstract

Critical age, weight and body composition have been suggested as necessary correlates of sexual maturity. A genome scan to identify quantitative trait loci (QTL) for age and body weight at first egg (AFE and WFE) was conducted on 912 birds from an F_2 broiler–layer cross using 106 microsatellite markers. Without a covariate, QTL for body WFE were detected on chromosomes 2, 4, 8, 27 and Z and a single QTL for AFE was detected on chromosome 2. With AFE as a covariate, additional QTL for body WFE were found on chromosomes 1 and 13, with abdominal fat pad as covariate a QTL for body WFE was found on chromosome 1. With body WFE as covariate, additional QTL for AFE were found on chromosomes 1, 3, 4, 13 and 27. The QTL generally acted additively and there was no evidence for epistasis. Consistent with the original line differences, broiler alleles had positive effects on body WFE and negative effects on AFE, whereas the phenotypic correlation between the two traits was positive. The mapped QTL for body WFE cumulatively accounted for almost half the body weight difference between the chicken lines at puberty. Overlapping QTL for body WFE and body weight to 9 weeks of age indicate that most QTL affecting growth rate also affect body WFE. The co-localisation of QTL for body weight, growth and sexual maturity suggests that body weight and growth rate are closely related to the attainment of sexual maturity and that the genetic determination of growth rate has correlated effects on puberty.

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Introduction

Understanding the genetic mechanism between growth rate and the onset of puberty is of significant biological and agricultural interest. Identification of quantitative trait loci (QTL) and their related genetic relationships is important in understanding the genetic factors controlling reproductive traits associated with sexual maturity, such as age and weight at puberty. Several studies across species have shown that the attainment of puberty (sexual maturity) is dependent on a number of factors such as age, minimum weight and body composition (Frisch 1994, Yannakopoulos *et al.* 1995, Eitan & Soller 2001).

In humans, the attainment of a critical body weight of 48 kg and a fat percentage of 22% for the onset of puberty in girls was proposed in the early 1970s (Frisch & Revelle 1970). Recent interest in this issue has been reignited by the observed early puberty in girls associated with increasing levels of obesity (Kaplowitz 2008, Aksglaede *et al.* 2009). Similarly, agricultural species that have been intensely selected for early growth, such as broiler chickens, have also become heavier and higher in body fat content, with negative effects on their reproductive performance as adults (Hocking *et al.* 2002, Brewer & Balen 2010). Early maturity has also been associated with reproductive problems such as abnormal ovarian hierarchies in chickens (Lacassag & Jacquet 1965, Hocking *et al.* 1987, Hocking 2004), and understanding the genetic mechanisms influencing these conditions could shed light on reproductive dysfunction in other species (Onagbesan *et al.* 2009).

The existence of a threshold level of weight or fatness that is critical for menarche has been disputed (Garn *et al.* 1983). The hypothesis that puberty depends on a critical amount of body fat has been rejected repeatedly by experimentalists (Bronson 2001). The linkage between body fat and the reproductive axis in girls is thought to be the result of an evolutionary mechanism in mammals for ensuring that pregnancy will not occur unless there are adequate fat stores to sustain both the mother and the growing foetus (Kaplowitz 2008). According to Kaplowitz (2008), published evidence suggests that obesity may be causally related to earlier puberty in girls. Rodent and human studies suggest that leptin is the critical link between body fat and early puberty but the question of whether earlier puberty is

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the cause or the result of increased body fat has not been resolved (Kaplowitz 2008).

An alternative view based on chicken studies is that the fat deposition is a result of processes associated with steroidogenesis driven by the development of the ovary (Hocking & Robertson 2000). Some authors have suggested that there is a minimum fat requirement and that body weight is not a limiting factor for achievement of sexual maturity (Robinson *et al.* 2001) whereas Soller *et al.* (1984) showed that a critical body fat content was not required for the attainment of puberty. While some data point to a possible minimum fat requirement to attain sexual maturity, a cautious approach is needed to identify the actual mechanisms involved (Chen *et al.* 2007) and that there is possible distortion due to effects of selection (Reddish *et al.* 2003).

The chicken (*Gallus gallus domesticus*) is a model organism used in genetic studies with implications for agriculture and biology (Griffin & Goddard 1994, Burt 2007). The relative ease of using chickens to generate DNA-based genetic data and the similarities in the sexual maturity phenomena across species makes the chicken a relevant model to gain more understanding of the genetic relationship between growth rate and the onset of sexual maturity across species. Interactions of several loci have been reported to influence early growth in chickens, for example (Carlborg *et al.* 2003), but genetic mechanisms involved in the interplay of other factors impacted by early growth remain to be elucidated.

This study adopted a QTL approach to address four issues. First, to investigate the relationship between growth rate and sexual maturity by identifying the QTL for age at first egg (AFE) and weight at first egg (WFE) in the Roslin broiler–layer cross in which the White Leghorn layer was much later maturing than the male broiler line. Secondly, to identify the relative importance of age and body weight for the attainment of puberty in chickens. Thirdly, reproductive traits are known to exhibit high heterosis (Williams *et al.* 2002), which may be explained partly by epistasis, the non-additive interaction of genes with one another (Williams *et al.* 2002, Carlborg *et al.* 2004, Melchinger *et al.* 2007). For this reason, the effects of epistasis on AFE and WFE were investigated using recently developed software (Wei *et al.* 2009). Finally, we investigated the effect of adiposity on puberty by using abdominal fat weight (ABF) as a covariate due to its high correlation with WFE and total body fat.

Results

Broiler and layer phenotypes

The mean AFE and WFE of the broiler male line and the White Leghorn layer line are presented in Table 1. At first egg, the broiler line was heavier (5.4 vs 1.5 kg) and earlier maturing than the layer (130 vs 177 days).

Table 1 Means and s.d.s for age, abdominal fat and weight at the onset of lay in male line broiler and White Leghorn layer chicken females.

	Male line broiler ($n=10$)		**White Leghorn layer** ($n=12$)	
Trait	Mean	s.d.	Mean	s.d.
Weight at first egg, WFE (kg)	5.4	0.6	1.5	0.1
Age at first egg, AFE (days)	130	8	177	10
Abdominal fat, ABF (g)	255	71	44	9

F_2 phenotypes

Trait means, s.d.s, ranges and phenotypic correlations between the phenotypic traits in the F_2 population are given in Table 2. AFE for the F_2 population ranged from 99 to 226 days. The mean AFE for the broiler male line stock and F_2 were similar (130 and 134 days respectively) and lower than that of the White Leghorn layer line (177 days). The Pearson correlation between WFE and the natural logarithm of AFE minus 94 days (lnAFE94) in the F_2 population was low (0.31; Table 2). The transformation of AFE was done to normalise residual errors.

Location of QTL and genetic effects

Without lnAFE94 as a covariate, significant effects were detected on chromosomes 2, 4, 8, 27 and Z (Table 3) for WFE. Two peaks of significance were found on the Z chromosome and their confidence intervals (CI) barely overlap. This suggests that two QTL affecting WFE may be located in this chromosome. When including lnABF as a covariate in the analysis of WFE, an additional QTL on chromosome 1 became significant but evidence was lost for the Z chromosome QTL. Including lnAFE94 as a covariate for WFE detected a further three QTL on chromosomes 3, 13 and 28, with only one of the original Z chromosome QTL being significant. The mapped QTL varied widely in their effects explaining from 1.0 to 9.5% of trait variance in WFE, with half of the QTL

Table 2 Number of records (*N*), means, s.d.s, range and phenotypic correlations for age, weight, natural logarithm of age-94 days and abdominal fat at the onset of lay in an F_2 broiler–layer chicken population.

						Correlation	
Variable	***N***	**Mean**	s.d.	**Min**	**Max**	AFE	WFE
Weight at first egg, WFE (kg)	912	2.9	0.4	1.4	4.2	0.32	–
Age at first egg, AFE (days)	912	134.2	21.1	99	226.0	–	0.32
ln(AFE94), ln(days)	912	3.56	0.54	1.61	4.88	–	0.31
Abdominal fat, ABF (g)	455	134.5	48.0	17.4	409.2	0.27	0.71
lnABF, ln(g)	455	4.84	0.37	2.86	6.01	0.18	0.69

Table 3 Chromosome, *F* ratio, quantitative trait loci (QTL) position, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation explained for weight at first egg (WFE) with and without adjustment for covariates in a broiler–layer F_2 chicken population.

Chrom	*F* ratio[a]	Position (cM)	CI	Flanking markers	Additive effect (S.E.M.)	Dominance effect (S.E.M.)	VP (%)
WFE (with no covariate) (g)							
2	10.29†	326	246–422	ADL0114–MCW0056	85 (18.6)	2 (31.2)	1.9
4	36.40†	157	144–184	ADL0241–MCW0180	381 (45.1)	170 (172.3)	7.2
8	10.10†	60	0–74	MCW0100–ROS0075	110 (25.0)	38 (48.2)	1.9
27	19.00†	0	0	ROS0071	107 (17.3)	2 (25.1)	3.7
Z	9.23*	103	63–127	LEI0111–LEI0075	96 (31.6)	–	1.0
Z	10.64†	0	0–65	ROS0072–ADL0201	82 (25.0)	–	1.0
Total					861	212	16.7
WFE (lnABF fitted as covariate) (g)							
1	11.25†	134	118–494	LEI0068–LEI146	89 (18.2)	12 (27.7)	4.1
2	10.17*	325	246–340	ADL0114–MCW0056	82 (17.9)	52 (31.1)	4.1
4	15.79†	175	147–195	ADL0241–MCW0180	201 (33.8)	−39 (93.1)	6.3
8	7.87‡	23	0–63	ADL0179–MCW0095	71 (18.5)	−6 (26.1)	2.4
27	23.30†	0	0	ROS0071	124 (17.0)	−6.8 (26.0)	9.5
Total					567	21.2	26.4
WFE (lnAFE94 fitted as covariate) (g)							
1	8.78*	141	90–501	LEI0068–LEI0146	64 (16.5)	42 (24.3)	1.5
2	16.65†	307	285–333	ROS0074–ADL0114	95 (17.2)	−50 (29.7)	3.0
3	7.42‡	136	11–250	MCW0127–LEI0118	43.8 (16.1)	63 (22.4)	1.2
4	45.05†	155	144–172	ADL00241–MCW0180	401 (41.8)	−94 (164.7)	8.3
8	9.93†	60	12–78	MCW0100–ROS0075	100 (22.6)	39 (43.4)	1.7
13	7.43†	54	26–68	MCW0340–ADL0225	101 (27.3)	−85 (57.9)	1.2
27	31.82†	0	0	ROS0071	125 (15.5)	8 (22.6)	5.8
28	6.98‡	1	0–39	ROS0095–ROS0085	−62 (16.3)	−1 (22.7)	1.1
Z	14.65†	17	0–113	ROS0072–ADL0201	116 (30.2)	–	1.6
Total					983.8	−78	25.4

Chrom, chromosome; CI, 95% confidence interval; VP%, percentage of phenotypic variation explained by the QTL; lnABF, $\log_e$ (abdominal fat weight); lnAFE94, $\log_e$ (age at first egg minus 94 days).
[a]Significant at 0.05 (*) and 0.01 (†) levels experiment-wide, and (‡) suggestive.

explaining 2.4% or more of the trait variance. QTL on chromosomes 4 and 27 accounted for the highest proportion of phenotypic variation (8.3 and 5.8% respectively) when including lnAFE94 in the analysis. Without WFE as a covariate, only one significant QTL for lnAFE94 was detected on chromosome 2 with suggestive QTL on chromosomes 1, 3, 13 and 15. With WFE as a covariate, the evidence for a QTL became significant in similar regions of chromosomes 1 and 13. In addition, QTL were found on chromosomes 4, 27 and a different region of chromosome 3. There was also evidence for two QTL on both chromosomes 3 and 4 (Table 4). The QTL explained relatively small proportions of 1.0–3.1% of the phenotypic variation for lnAFE94; only two of the QTL had effects explaining 2.0% or more of trait variation.

A QTL was detected for lnAFE94 and for WFE in similar regions on chromosome 2, indicating that the same QTL may influence both traits. With no covariate in the analysis, this was the only QTL found to affect both WFE and AFE. With the alternative trait as covariate in the analysis, however, there was extensive commonality with QTL significant for the two traits on chromosomes 1, 2, 3, 4, 13 and 27. The effects of including a covariate in the analysis will be considered in detail in the 'Discussion' section.

The additive effects for the WFE QTL were all positive, indicating that the increasing allele was from the broiler line (Table 3), except for the suggestive QTL on chromosome 28, which had a negative estimate. Dominance effects were not significant indicating that dominance was not an important mode of gene action for this trait. The largest (401 g) and the smallest (64 g) additive effects observed for significant WFE QTL were respectively on chromosomes 4 and 1 when lnAFE94 was fitted as a covariate. The mapped QTL accounted for almost half of the difference in trait value between the two parental lines, and 25% of F_2 trait variance. Inclusion of lnAFE94 as a covariate in the analysis for this trait increased the number of significant QTL and slightly increased the magnitude of the effects for each QTL. Fitting lnABF as a covariate had less effect on the results.

The results for the additive and dominance effects for lnAFE94 QTL are presented in Table 4. Here, too, the dominance effects were not significant. Similar to WFE, the additive gene action was also important for lnAFE94. The QTL additive effects for lnAFE94 were small and mainly negative. The mapped QTL accounted for 14% of F_2 trait variance, but only a small proportion of the difference between the two parental lines. There was no support for epistatic interactions between QTL for either trait.

Table 4 Chromosome, *F* ratio, quantitative trait loci (QTL) position, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation explained for the natural logarithm transformed age at first egg (lnAFE94) in a broiler–layer F_2 chicken population.

Chr	*F* ratio[a]	Position (cM)	CI	Flanking markers	Additive effect ± S.E.M.	Dominance effect ± S.E.M.	VP (%)
lnAFE94 (with no covariate fitted), ln(days)							
1	5.70‡	164	0–380	LEI0146–ADL0319	−0.07 (0.022)	0.06 (0.042)	1.1
2	8.43*	291	115–358	ROS0023–ADL0236	−0.06 (0.017)	0.04 (0.025)	1.7
3	5.50‡	24	16–185	MCW0083–HUJ0006	−0.07 (0.021)	0.01 (0.035)	1.0
13	7.00‡	0	0–44	MCW0340–ADL0225	−0.07 (0.018)	0.02 (0.027)	1.4
15	5.47‡	41	11–49	LEI0083–MCW0080	−0.08 (0.025)	0.02 (0.052)	1.5
Total					−0.35	0.15	6.7
lnAFE94 (with WFE fitted as a covariate), ln(days)							
1	9.0†	153	30–386	LEI0146–ADL0319	−0.08 (0.019)	0.01 (0.032)	1.7
2	15.4†	291	229–298	ROS0023–ADL0236	−0.08 (0.015)	0.05 (0.023)	3.1
3	8.2*	139	5–230	MCW0127–LEI0118	−0.06 (0.017)	−0.05 (0.024)	1.5
3	7.7‡	23	9–250	MCW0083–HUJ0006	−0.07 (0.019)	−0.03 (0.031)	1.4
4	8.1*	195	65–195	ADL0241–MCW0180	−0.08 (0.021)	−0.06 (0.037)	1.5
4	6.7‡	3	0–194	ADL0317–MCW0295	−0.07 (0.018)	0.01 (0.028)	1.2
13	8.6*	20	0–46	MCW0340–ADL0225	−0.11 (0.026)	−0.01 (0.057)	1.6
27	10.1†	0	–	ROS0071	−0.06 (0.016)	−0.04 (0.023)	2.0
Total					−0.61	−0.12	14

Chr, chromosome; CI, 95% confidence interval; VP%, percentage of phenotypic variation explained by the QTL; lnAFE94, $\log_e$ (age at first egg minus 94 days); WFE, weight at first egg.
[a]Significant at 0.05 (*) and 0.01 (†) levels experiment-wide, and (‡) suggestive.

Discussion

Effect of including a covariate in the QTL analysis

Including a genetically controlled and phenotypically correlated trait as a covariate in the analysis will affect the evidence for a QTL at a specific location, depending on the direction and magnitude of QTL effects on the two traits (Goddard *et al.* 2001, Neuschl *et al.* 2007, Chiu *et al.* 2010). If the QTL affects the trait, but not the covariate, inclusion of the covariate will increase the evidence for the QTL. This may have happened for the QTL for WFE on chromosome 27, when lnAFE was fitted as covariate. If the QTL affects the covariate but not the trait, inclusion of the covariate will identify a QTL for the covariate rather than a QTL for the trait under investigation. The QTL on chromosomes 4 and 27 originally detected for WFE became significant for lnAFE94 when WFE was included as a covariate, suggesting that these QTL affected WFE rather than lnAFE94. If the QTL affects both the trait and the covariate (i.e. a pleiotropic QTL), the ability to detect it will depend on the QTL and the phenotypic correlations (i.e. the evidence may be lost or enhanced). In this study, the traits in the F_2 were positively correlated phenotypically (i.e. individuals with late AFE tended to have high WFE, since these birds had more time to gain weight before first egg). QTL operating in the same direction in both traits, consistent with the phenotypic correlation, will generally lose evidence when one of the traits is fitted as a covariate in the analysis of the other. Apparently, this is what happened to the QTL on chromosomes 4, 8 and Z when lnABF was fitted as a covariate to WFE (Table 3). The original breed difference, however, implies that birds with later AFE have low WFE and vice versa. This will tend to enhance the evidence for such a pleiotropic QTL and increase the effect estimates when fitting one of the traits as a covariate in the analysis of the other. The QTL identified for both WFE and lnAFE94 on chromosome 2 could be such a case, as significance for both traits increased when the alternative trait was used as a covariate. The QTL on chromosomes 1 and 13 detected for lnAFE94 became significant when WFE was included as a covariate, and were also identified for WFE when lnAFE94 was included as a covariate. These could be additional examples of pleiotropic QTL, with the QTL affecting both WFE and lnAFE94. Finally, the QTL on chromosome 3 in interval MCW0127–LEI0118 may be another example as it was detected for both traits but only in the presence of the other as a covariate. In each of these instances, as an alternative to pleiotropy, the co-location of QTL affecting each of the two traits could be due to separate tightly clustered loci that individually influence a single trait (Almasy *et al.* 1997). A better way to detect pleiotropic QTL would be to analyse the traits simultaneously (Knott & Haley 2000).

Importance of the identified QTL

The sum of the additive effects for significant WFE QTL was 0.86 kg accounting for 1.7 kg additive effects difference between the lines. This represents 44% of the live weight difference (3.9 kg) between the lines at the onset of lay (Table 1). For all of these QTL, the allele increasing WFE was inherited from the broiler line, which is consistent with the breed difference (Table 1).

The sum of the additive effects for lnAFE94 (adjusted for WFE) was ~12 days. This represents an additive effect difference of 24 days or about half the phenotypic difference between the lines (Table 1). In comparison to this analysis, when the covariate was not fitted the sum of the additive effects was only 3.4 days, explaining <15% of the line difference. This is consistent with the conclusion that QTL for weight rather that age explain far more of the observed difference in age at onset of puberty between the lines.

Additive effects for lnAFE94 were generally negative (Table 4) indicating that the broiler line was earlier maturing than the layer genotype (consistent with the breed differences, Table 1) and is consistent with the expectation that fast growing (heavy) birds tend to reach sexual maturity earlier. However, it should be noted that the layer line was unusually late maturing (Table 1), possibly due to the lack of photostimulation in this experiment. A constant photoperiod was adopted to avoid confounding differences in maturity with the timing of photostimulation.

QTL affecting early growth rate affect WFE

An earlier study on broiler offspring of the same parents as the present data (Sewalem *et al.* 2002) reported significant QTL for live weight at earlier ages (3–9 weeks of age) than in this study on chromosomes 1, 7, 13 and Z (for 3 weeks of age), chromosomes 1, 2, 4, 7, 8, 13 (for 6 weeks of age) and on chromosomes 1, 2, 4, 8, 13 and 27 (for 9 weeks of age). In that study, covariates were not fitted in the analysis. Even though the earlier study used much younger ages (3–9 weeks) than in our study (19 weeks of age), the QTL detected for live weight (in the earlier study) and for WFE (without a covariate) in our study were at similar positions across the implicated linkage groups.

Similar results were obtained when comparing our results on WFE with those of other studies on early growth rate. The highly significant QTL for body weight on chromosome 4 detected in this study confirm findings from several studies that have reported large effect body weight QTL on this chromosome (Schreiweis *et al.* 2006). Zhou *et al.* (2006) reported significant QTL for body weight at 8 weeks of age in a broiler–White Leghorn cross on chromosomes 1, 2, 4, 7, 9 and 18. Body weight QTL (at 7 weeks of age) were reported on chromosomes 1, 2 and 13 from a broiler population (Atzmon *et al.* 2006). Ruy *et al.* (2005) reported four suggestive QTL on chromosome 3 and three suggestive QTL on chromosome 5 for WFE from a layer–broiler cross. Thus, the growth QTL reported in other studies were also in similar chromosome positions to the ones observed in our study and this suggests that sexual maturity QTL are generally not distinct from those for growth.

In contrast to our results, Carlborg *et al.* (2003) observed a pronounced role of epistatic effects on growth prior to 46 days of age in a red jungle fowl–White Leghorn cross and reported significant QTL for body weight in the 1–200 days age bracket on chromosomes 1, 2, 3, 4, 7, 8, 11, 12, 13, 14, 27 and E27W24. The important contribution of epistasis to early growth (before 6 weeks of age) was also observed in a White Leghorn layer and broiler sire line cross population from the same parents as the present flock (Carlborg *et al.* 2004).

The WFE QTL on chromosome 27 appears worthy of special attention. When lnABF was used as a covariate, this QTL explained almost 10% of the phenotypic variance in WFE. The QTL is located in a region that contains several growth-related genes (e.g. chicken growth hormone (*GH*); Lei *et al.* 2007). The G+1705A in Intron 3 of *GH* could have a direct effect on chicken growth via an influence on *GH* gene expression (Nie *et al.* 2005).

There are specific QTL (on chromosomes 8 and 13) associated with age and weight at sexual maturity, which are not found when analysing weight at younger ages. However, the magnitude of their additive effects for lnAFE94 is relatively small, suggesting that there is limited opportunity to genetically manipulate sexual maturity independently of commercial broiler growth traits.

Schreiweis *et al.* (2006) reported a suggestive AFE QTL on chromosome 3, which is similar to the location of a QTL in this study. AFE QTL have also been reported on chromosome 4 (Schreiweis *et al.* 2006). However, a QTL for AFE reported on chromosome Z by Tuiskula-Haavisto *et al.* (2002) and Sasaki *et al.* (2004) was not detected in this study.

Abdominal fat, body weight and puberty

The high correlation (0.71) between ABF and WFE (Table 2) suggests that these traits are controlled by similar factors. Circulating lipids increase at the onset of lay for deposition into the developing yolky follicles (Jaccoby *et al.* 1995). The accumulation of body fat is likely to be important for this reason, as it is a source of circulating lipid as well as *de novo* synthesis in the liver. Therefore, the use of lnABF to model QTL affecting WFE may identify regions of the genome, which are associated with lean tissue mass, at the onset of lay. The fact that the majority of the QTL are not affected by fitting body fat as a covariate suggests that the amount of fat does not explain the onset of sexual maturity in poultry (Soller *et al.* 1984) except in the case of QTL on chromosome 1.

In general, the results are consistent with the concept that achieving a minimum body weight is permissive for the attainment of sexual maturity (Brody *et al.* 1984, Eitan & Soller 2001). This is interesting because the estimated WFE QTL effects at these locations are genetically determined and are not the

result of dietary manipulation. Therefore, this may be one of the clearest demonstrations that genetic determination of growth rate results in correlated effects on puberty. The possibility that the converse is true can be eliminated because the effects of the growth QTL take place before the age of puberty is reached. Clear evidence of genetic correlations between growth rate and puberty are not numerous: studies in female pigs suggest that there is a negative phenotypic correlation between growth and puberty (Hutchens *et al.* 1981) and in humans it was estimated that 57% of the additive genetic effects for the age of menarche and body mass index are common (Kaprio *et al.* 1995). The genetic and physiological determinates of sexual maturity underlying the QTL identified in this study remain to be elucidated through a combination of fine mapping and identification of candidate genes for subsequent validation.

In conclusion, the QTL for WFE and lnAFE94 detected in this study generally acted additively and the broiler alleles were associated with heavier body weights and earlier ages at the onset of lay. The indication that the loci for growth and puberty are common provides a clear demonstration of the genetic basis for the phenotypic correlation between growth and puberty and that body weight is an important determinant for the attainment of sexual maturity.

Materials and Methods

Parent lines

One-day-old female chicks were obtained from the Ross 308 male line broiler (Aviagen, Newbridge, UK) and a White Leghorn egg laying line maintained at the Roslin Institute. The chicks were brooded and reared under conventional husbandry practices in floor pens. At 12 weeks of age, 12 birds from each line were randomly allocated to individual cages to record phenotypic data on the age and weight at the onset of lay. However, for the broilers, only ten birds that survived were used.

Animals and genotyping

The F_2 population was created by crossing two males and two females from the broiler male line with two females and two males from the White Leghorn line to produce an F_1 generation (Sewalem *et al.* 2002). Eight males and 32 females of the F_1 generation were selected and mated in a balanced mating scheme to produce over 2000 F_2 birds (one F_1 female died and was replaced making a total of 33 full sib families). The female birds were reared in floor pens and moved to individual cages that measured 40 cm wide×45 cm deep×80 cm high at 12 weeks of age. The birds were fed *ad libitum* on a conventional poultry diet. The birds were exposed to a constant photoperiod of 14 h per day from hatch to the end of the experiment. WFE and AFE were recorded at the onset of lay, which was defined as the day of first recorded oviposition. In a subset of the birds (about half of the hatches), the birds were killed and the weight of abdominal fat was recorded. AFE was transformed to natural logarithms of (AFE − 94 days) to normalise residual errors. The 94 days was chosen as the lowest AFE in the unedited data. The QTL effects on the original scale were calculated from the back-transformed means of the three genotype classes. The additive effects were obtained from the average of the back-transformed means of the two homozygotes.

Blood samples were obtained after caging for extracting DNA by standard methods (Sewalem *et al.* 2002). Genotyping was conducted using 106 microsatellite markers covering 25 autosomal linkage groups and the Z sex chromosome (Table 5) on the 8 F_0 grandparents, 41 F_1 and 912 F_2 offspring with data on age and WFE. All pedigree, marker genotypes and recorded traits were stored in the resSpecies database (Law & Archibald 2000). The 2005 consensus genetic linkage map (ArkDB, 2007) was used to add new markers to a linkage map based on the same population used in the analysis (Navarro *et al.* 2005). The total map length was 2479 cM (Table 5).

QTL analysis

The interval mapping method (Haley *et al.* 1994) for QTL detection was conducted using a newly developed module for analysis of epistasis in GridQTL (Seaton *et al.* 2006, Wei *et al.* 2009). The programme initially conducts the standard processes of QTL searching, testing, permutation and bootstrapping for a single QTL F_2 analysis. Genome scans were conducted iteratively using forward selection of significant QTL for each trait (Carlborg *et al.* 2004). Analyses were performed at 1 cM

Table 5 The number of microsatellite markers, first and last marker and map length on each linkage chromosome in the quantitative trait loci analysis of the chicken broiler–layer cross.

Chromosome	Number of markers	First marker	Last marker	Map length (cM)
1	17	ROS0008	MCW0107	548
2	13	LEI0163	MCW0157	473
3	15	MCW0169	MCW0037	286
4	4	ADL0317	MCW0180	195
5	5	ROS0013	ADL0298	119
6	4	ADL0323	ADL0142	113
7	3	LEI0064	ADL0180	109
8	9	ROS0021	ROS0075	92
9	4	ROS0078	MCW0134	132
10	1	ADL0209.2	ADL0209	–
11	5	LEI0110	ROS00112	71
12	2	ADL0240	ADL0044	34
13	2	MCW0340	ADL0225	68
14	1	MCW0123	MCW0123	–
15	2	LEI0083	MCW0080	49
16	1	LEI0258	LEI0258	–
17	1	ADL0199	ADL0199	–
18	2	ROS0022	ROS0027	24
19	1	MCW0094	MCW0094	–
22	1	ROS0073	ROS0073	–
23	1	MCW0249	MCW0249	–
26	2	ADL0285	LEI0074	–
27	1	ROS0071	ROS0071	–
28	3	ROS0095	ADL0299	39
z	6	ROS0072	LEI0075	127
Total	106			2479

intervals. Exhaustive QTL searches with an updated model were implemented by fitting the significant QTL as co-factors (Jansen 1994, Zeng 1994) until no additional significant QTL were detected.

Epistatic QTL were mapped simultaneously by extending the method for a single QTL search using a linear model with marginal effects for a pair of QTL and their four possible pairwise interactions in 1 cM genome scans (Wei *et al.* 2009). A significant outcome in the overall test of the marginal effects and all two-way interactions between the additive and dominance effects at two loci and of this test compared to the two-locus model with no inter-locus interactions is indicative of epistasis. The method detects epistatic pairs of QTL by using two complementary search approaches. In the first stage, the algorithm automates a genome scan to identify interactions between pre-identified single QTL with all the other genomic positions. In the second approach, a two-dimensional genome scan involving all combinations of two positions in the genome is conducted to search for epistatic interactions regardless of the locations of the pre-identified marginal effect QTL.

Determination of significance thresholds

In single QTL detection, significance thresholds were determined by conducting 5000 permutations (Churchill & Doerge 1994), and 1000 bootstraps were used to generate 95% CI (Lander & Botstein 1989, Visscher *et al.* 1996). A QTL was considered as being significant if it had an F value greater than the $P \leq 0.05$ experiment-wide threshold value and highly significant if the F value exceeded the $P \leq 0.01$ threshold (Kruglyak & Lander 1995). Alternatively, the QTL was considered to be suggestive if it had an F value exceeding the $P \leq 0.05$ chromosome-wide threshold. All significant and suggestive QTL were used in the first approach for detection of epistatic QTL pairs.

Significance testing for epistatic pairs in both approaches used F ratio tests for model comparisons in a nested test framework. Permutations based on 1000 replications were used to determine genome-wide thresholds. In the first stage, exhaustive genome scans were performed on permuted data to derive thresholds for each pre-identified marginal effect QTL. The DIRECT algorithm (Ljungberg *et al.* 2004) was used to perform fast two-dimensional genome scans in permutations to derive genome-wide thresholds for the second stage (Wei *et al.* 2009).

Models

In a preliminary analysis, different models with additive, dominance and parent-of-origin genetic effects with family and pen as fixed effects were analysed (hatch was confounded with pen). There was no evidence for parent-of-origin effects (detected as a difference in effect between the alternative heterozygous genotypes that differ in which allele has been inherited from each parent), and a simple additive–dominance model was adopted for subsequent analyses. Additive genetic effects were defined as half the difference between the broiler and layer homozygotes and dominance effects as the difference between the heterozygote and the mean of the two homozygotes (Falconer & Mackay 1996). A positive additive effect indicates that the QTL allele originating from the broiler line increased the trait value relative to that from the layer line. The Z chromosome was analysed with an additive genetic effects model.

For each trait, models with and without a covariate were fitted. Covariates were included in the model of analysis to detect differences in the assessed trait at a fixed level of the covariate trait (Kerje *et al.* 2003, Park *et al.* 2006). A regression analysis on lnAFE94 and WFE showed that lnAFE94 explained 29% of the variation in WFE where pen and family were included as fixed effects. Conversely, fitting the same effects, WFE explained a moderately high proportion of the variation (64%) in lnAFE94. In the QTL analysis for WFE, lnAFE94 was added in the model as a covariate. Additionally, for the subset of the individuals with ABF recorded, another model was run for WFE with lnABF as a covariate. Similarly, WFE was fitted as a covariate in the model for lnAFE94.

The percentage of the F_2 phenotypic variance explained by the QTL was calculated as percentage variance = ((RRMS − FRMS)/RRMS) × 100 (Zhou *et al.* 2006), where RRMS is the residual mean square from the reduced model in which all the effects including background QTL effects are fitted but the QTL is omitted. The FRMS is the residual mean square from the model in which all the effects and QTL are fitted.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Aksglaede L, Juul A, Olsen LW & Sørensen TIA 2009 Age at puberty and the emerging obesity epidemic. *PLoS ONE* **4** e8450. (doi:10.1371/journal.pone.0008450)

Almasy L, Dyer TD & Blangero J 1997 Bivariate quantitative trait linkage analysis: pleiotropy versus co-incident linkages. *Genetic Epidemiology* **14** 953–958. (doi:10.1002/(SICI)1098-2272(1997)14:6<953::AID-GEPI65>3.0.CO;2-K)

Atzmon G, Ronin YI, Korol A, Yonash N, Cheng H & Hillel J 2006 QTLs associated with growth traits and abdominal fat weight and their interactions with gender and hatch in commercial meat-type chickens. *Animal Genetics* **37** 352–358. (doi:10.1111/j.1365-2052.2006.01487.x)

Brewer CJ & Balen AH 2010 The adverse effects of obesity on conception and implantation. *Reproduction* **140** 347–364. (doi:10.1530/REP-09-0568)

Brody TB, Siegel PB & Cherry JA 1984 Age, body weight and body composition requirements for the onset of sexual maturity of dwarf and normal chickens. *British Poultry Science* **25** 245–252. (doi:10.1080/00071668408454863)

Bronson FH 2001 Puberty in female mice is not associated with increases in either body fat or leptin. *Endocrinology* **142** 4758–4761. (doi:10.1210/en.142.11.4758)

Burt DW 2007 Emergence of the chicken as a model organism: implications for agriculture and biology. *Poultry Science* **86** 1460–1471.

Carlborg O, Kerje S, Schutz K, Jacobsson L, Jensen P & Andersson L 2003 A global search reveals epistatic interaction between QTL for early growth in the chicken. *Genome Research* **13** 413–421. (doi:10.1101/gr.528003)

Carlborg O, Hocking PM, Burt DW & Haley CS 2004 Simultaneous mapping of epistatic QTL in chickens reveals clusters of QTL pairs with similar genetic effects on growth. *Genetical Research* **83** 197–209. (doi:10.1017/S0016672304006779)

Chen H, Huang RL, Zhang HX, Di KQ, Pan D & Hou YG 2007 Effects of photoperiod on ovarian morphology and carcass traits at sexual maturity in pullets. *Poultry Science* **86** 917–920.

Chiu Y-F, Chiou J-M, Liang K-Y & Lee C-Y 2010 Incorporation of covariates in simultaneous localization of two linked loci using affected relative pairs. *BMC Genetics* **11** 67. (doi:10.1186/1471-2156-11-67)

Churchill GA & Doerge RW 1994 Empirical threshold values for quantitative trait mapping. *Genetics* **138** 963–971.

Eitan Y & Soller M 2001 Effect of photoperiod and quantitative feed restriction in a broiler strain on onset of lay in females and onset of semen production in males: a genetic hypothesis. *Poultry Science* **80** 1397–1405.

Falconer DS & Mackay TFC 1996 *Introduction to Quantitative Genetics*, 4th edn. Harlow: Longman Group Ltd.

Frisch RE 1994 The right weight: body fat, menarche and fertility. *Proceedings of the Nutrition Society* **53** 113–129. (doi:10.1079/PNS19940015)

Frisch RE & Revelle R 1970 Height and weight at menarche and a hypothesis of critical body weights and adolescent events. *Science* **169** 397–399. (doi:10.1126/science.169.3943.397)

Garn SM, LaVelle M & Pilkington JJ 1983 Comparisons of fatness in premenarcheal and postmenarcheal girls of the same age. *Journal of Pediatrics* **103** 328–331. (doi:10.1016/S0022-3476(83)80379-5)

Goddard KAB, Witte JS, Suarez BK, Catalona WJ & Olson JM 2001 Model-free linkage analysis with covariates confirms linkage of prostate cancer to chromosomes 1 and 4. *American Journal of Human Genetics* **68** 1197–1206. (doi:10.1086/320103)

Griffin HD & Goddard C 1994 Rapidly growing broiler (meat-type) chickens. Their origin and use for comparative studies of the regulation of growth. *International Journal of Biochemistry* **26** 19–28. (doi:10.1016/0020-711X(94)90190-2)

Haley CS, Knott SA & Elsen JM 1994 Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* **136** 1195–1207.

Hocking P 2004 Roles of body weight and feed intake in ovarian follicular dynamics in broiler breeders at the onset of lay and after a forced molt. *Poultry Science* **83** 2044–2050.

Hocking PM & Robertson GW 2000 Ovarian follicular dynamics in selected and control (relaxed selection) male- and female-lines of broiler breeders fed *ad libitum* or on restricted allocations of food. *British Poultry Science* **41** 229–234. (doi:10.1080/713654904)

Hocking PM, Gilbert AB, Walker M & Waddington D 1987 Ovarian follicular structure of white leghorns fed *ad libitum* and dwarf and normal broiler breeders fed *ad libitum* or restricted until point of lay. *British Poultry Science* **28** 493–506. (doi:10.1080/00071668708416983)

Hocking PM, Bernard R & Robertson GW 2002 Effects of low dietary protein and different allocations of food during rearing and restricted feeding after peak rate of lay on egg production, fertility and hatchability in female broiler breeders. *British Poultry Science* **43** 94–103. (doi:10.1080/00071660120109908)

Hutchens LK, Hintz RL & Johnson RK 1981 Genetic and phenotypic relationships between pubertal and growth characteristics of gilts. *Journal of Animal Science* **53** 946–951.

Jaccoby S, Arnon E, Snapir N & Robinzon B 1995 Effects of estradiol and tamoxifen on feeding, fattiness, and some endocrine criteria in hypothalamic obese hens. *Pharmacology, Biochemistry, and Behavior* **50** 55–63. (doi:10.1016/0091-3057(94)00251-D)

Jansen RC 1994 Controlling the type I and type II errors in mapping quantitative trait loci. *Genetics* **138** 871–881.

Kaplowitz PB 2008 Link between body fat and the timing of puberty. *Pediatrics* **121** S208–S217. (doi:10.1542/peds.2007-1813F)

Kaprio J, Rimpela A, Winter T, Viken RJ, Rimpela M & Rose RJ 1995 Common genetic influences on BMI and age at menarche. *Human Biology* **67** 739–753.

Kerje S, Carlborg Ö, Jacobsson L, Schütz K, Hartmann C, Jensen P & Andersson L 2003 The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs. *Animal Genetics* **34** 264–274. (doi:10.1046/j.1365-2052.2003.01000.x)

Knott SA & Haley CS 2000 Multitrait least squares quantitative trait loci detection. *Genetics* **156** 899–911.

Kruglyak L & Lander ES 1995 A nonparametric approach for mapping quantitative trait loci. *Genetics* **139** 1421–1428.

Lacassag L & Jacquet JP 1965 Maturite sexuelle et apparition doeufs a double jaune et sans coquille. Role du regime lumineux et du niveau alimentaire (Sexual maturity and appearance of double-yolked and shell-less eggs. Role of light treatment and nutritional level). *Annales de Zootechnie* **14** 169–179. (doi:10.1051/animres:19650204)

Lander ES & Botstein D 1989 Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121** 185–199.

Law AS & Archibald AL 2000 Farm animal genome databases. *Briefings in Bioinformatics* **1** 151–160. (doi:10.1093/bib/1.2.151)

Lei M, Luo C, Peng X, Fang M, Nie Q, Zhang D, Yang G & Zhang X 2007 Polymorphism of growth-correlated genes associated with fatness and muscle fiber traits in chickens. *Poultry Science* **86** 835–842.

Ljungberg K, Holmgren S & Carlborg O 2004 Simultaneous search for multiple QTL using the global optimization algorithm DIRECT. *Bioinformatics* **20** 1887–1895. (doi:10.1093/bioinformatics/bth175)

Melchinger AE, Utz HF, Piepho H-P, Zeng Z-B & Schon CC 2007 The role of epistasis in the manifestation of heterosis: a systems-oriented approach. *Genetics* **177** 1815–1825. (doi:10.1534/genetics.107.077537)

Navarro P, Visscher PM, Knott SA, Burt DW, Hocking PM & Haley CS 2005 Mapping of quantitative trait loci affecting organ weights and blood variables in a broiler layer cross. *British Poultry Science* **46** 430–442. (doi:10.1080/00071660500158055)

Neuschl C, Brockmann GA & Knott SA 2007 Multiple-trait QTL mapping for body and organ weights in a cross between NMRI8 and DBA/2 mice. *Genetical Research* **89** 47–59. (doi:10.1017/S001667230700852X)

Nie Q, Sun B, Zhang D, Luo C, Ishag NA, Lei M, Yang G & Zhang X 2005 High diversity of the chicken growth hormone gene and effects on growth and carcass traits. *Journal of Heredity* **96** 698–703. (doi:10.1093/jhered/esi114)

Onagbesan O, Bruggeman V & Decuypere E 2009 Intra-ovarian growth factors regulating ovarian function in avian species: a review. *Animal Reproduction Science* **111** 121–140. (doi:10.1016/j.anireprosci.2008.09.017)

Park H-B, Jacobsson L, Wahlberg P, Siegel PB & Andersson L 2006 QTL analysis of body composition and metabolic traits in an intercross between chicken lines divergently selected for growth. *Physiological Genomics* **25** 216–223. (doi:10.1152/physiolgenomics.00113.2005)

Reddish JM, Nestor KE & Lilburn MS 2003 Effect of selection for growth on onset of sexual maturity in randombred and growth-selected lines of Japanese quail. *Poultry Science* **82** 187–191.

Robinson FE, Renema RA, Oosterhoff HH, Zuidhof MJ & Wilson JL 2001 Carcass traits, ovarian morphology and egg laying characteristics in early versus late maturing strains of commercial egg-type hens. *Poultry Science* **80** 37–46.

Ruy DC, Nones K, Baron EE, Ledur MC, Rodrigues de Melo CM, Ambo M, Campos RDLR & Coutinho LL 2005 Strategic marker selection to detect quantitative trait loci in chicken. *Scientia Agricola* **62** 111–116. (doi:10.1590/S0103-90162005000200003)

Sasaki O, Odawara S, Takahashi H, Nirasawa K, Oyamada Y, Yamamoto R, Ishii K, Nagamine Y, Takeda H, Kobayashi E *et al.* 2004 Genetic mapping of quantitative trait loci affecting body weight, egg character and egg production in F_2 intercross chickens. *Animal Genetics* **35** 188–194. (doi:10.1111/j.1365-2052.2004.01133.x)

Schreiweis MA, Hester PY, Settar P & Moody DE 2006 Identification of quantitative trait loci associated with egg quality, egg production, and body weight in an F_2 resource population of chickens. *Animal Genetics* **37** 106–112. (doi:10.1111/j.1365-2052.2005.01394.x)

Seaton GHJ, Grunchec JA, White I, Allen J, De Koning DJ, Wei W, Berry D, Haley C & Knott S 2006 GridQTL: a grid portal for QTL mapping of compute intensive datasets. In *Proceedings of the 8th World Congress on Genetics Applied to Livestock Production*, Belo Horizonte, MG, Brazil.

Sewalem A, Morrice DM, Law A, Windsor D, Haley CS, Ikeobi CO, Burt DW & Hocking PM 2002 Mapping of quantitative trait loci for body weight at three, six, and nine weeks of age in a broiler layer cross. *Poultry Science* **81** 1775–1781.

Soller M, Brody T, Eitan Y, Agursky T & Wexler C 1984 Minimum weight for the onset of sexual maturity in female chickens: heritability and phenotypic correlations with early growth rate. *Poultry Science* **63** 2103–2113.

Tuiskula-Haavisto M, Honkatukia M, Vilkki J, de Koning DJ, Schulman NF & Maki-Tanila A 2002 Mapping of quantitative trait loci affecting quality and production traits in egg layers. *Poultry Science* **81** 919–927.

Visscher PM, Thompson R & Haley CS 1996 Confidence intervals in QTL mapping by bootstrapping. *Genetics* **143** 1013–1020.

Wei WH, Knott S, Haley CS & de Koning DJ 2009 Controlling false positives in the mapping of epistatic QTL. *Heredity* **104** 401–409. (doi:10.1038/hdy.2009.129)

Williams SM, Price SE & Siegel PB 2002 Heterosis of growth and reproductive traits in fowl. *Poultry Science* **81** 1109–1112.

Yannakopoulos AL, Christaki E & Florou-Paneri P 1995 Effect of age and carcase composition on the onset of sexual maturity in quail under normal feeding regimens. *British Poultry Science* **36** 771–777. (doi:10.1080/00071669508417821)

Zeng ZB 1994 Precision mapping of quantitative trait loci. *Genetics* **136** 1457–1468.

Zhou H, Deeb N, Evock-Clover CM, Ashwell CM & Lamont SJ 2006 Genome-wide linkage analysis to identify chromosomal regions affecting phenotypic traits in the chicken. I. Growth and average daily gain. *Poultry Science* **85** 1700–1711.

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