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Quantitative trait loci for egg quality and production in laying hens

Doctoral Dissertation

Maria Tuiskula-Haavisto

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Abstract

A genome scan for quantitative trait loci (QTL) for egg quality and production traits was carried out in laying hens. The resource population was an F_2 cross and parental lines were chosen to exhibit the maximum divergence for egg quality and molecular genetic markers. Both the Rhode Island Red (RIR) and synthetic White Leghorn (WL) lines from MTT Agrifood Research Finland were examined. This study represents the first genome scan for a cross between two modern egg layer lines. The crossing between lines was performed reciprocally to allow an analysis of sex chromosome Z and parent-of-origin effects of QTL. Eight animals (four males and four females) from each line were selected to produce the reciprocal F_1 generation, while the subsequent F_2 generation consisted of 305 females. Egg quality and production traits were recorded during the entire production period from 18 to 60 weeks of age. Animals in all three generations were genotyped with 99 polymorphic microsatellite markers. A genetic linkage map was constructed for 13 autosomes and sex chromosome Z for a whole genome scan that covered 2311 cM (centi Morgan) (Haldane) in total. QTL analysis was performed by regression using a statistical model that included dominance, parent-of-origin, background genetic effects and a two QTL model. Several QTLs for different egg quality and production traits were identified. The most interesting QTLs located on chromosome 4 influenced body weight and egg weight, and an area on chromosome Z affected the number and weight of eggs. The QTL affecting body weight explained about 25 % of the phenotypic variation in the F_2 population. Several QTLs with an effect on egg white quality and egg weight were found on chromosome 2. Fine mapping and the use of a back cross (BC) generation, performed by crossing the F_2 generation with males from RIR and WL lines allowed the QTL regions on chromosome 2 to be narrowed down. This approach provided evidence that two QTLs on chromosome 2 affected the same trait. The results of parent-of-origin effects of QTLs indicated that differences can be expected when the QTL allele is inherited from the sire compared with the dam. Three such QTLs affecting body weight, age at the first egg laying and feed intake were clustered on chromosome 1. This is one of the first reports of production QTLs with parent-of-origin effects in avian species.

Key words: laying hens, QTL, egg quality, mapping, parent-of-origin effect

Kananmunan laatuun ja tuotantoon vaikuttavien kromosomialueiden kartoittaminen munijakanalla

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Tiivistelmä

Munijakanan koko genomin kartoitus tehtiin kananmunan laadun ja tuotannon vaihteluun vaikuttavien kromosomialueiden (QTL) selvittämiseksi. Kartoitus tehtiin F_2-risteytyspopulaatiosta, jonka takana olevissa vanhempaislinjoissa munan laatu ja molekyyligeneettiset markkeerit olivat hyvin erilaisia. Linjat olivat MTT:ssä ylläpidetyt Rhode Island Red (RIR) ja valkoinen Leghorn (WL). Tutkimus on ensimmäinen munijalinjojen risteytyksestä tehty koko perimän kartoitus. Risteytys tehtiin vastavuoroisesti, jolloin pystyttiin analysoimaan sukupuolikromosomi Z ja yksilön vanhemman sukupuolesta johtuva vaikutus geenin ilmenemiseen (parent-of-origin-vaikutus). Molemmista linjoista valittiin neljä kukkoa ja neljä kanaa, jotka tuottivat F_1-sukupolven, seuraava F_2-sukupolvi koostui 305 kanasta. Munan laatu ja tuotanto mitattiin koko tuotantokauden ajalta (18-60 viikon iässä). Kaikkien kolmen sukupolven yksilöt genotyypitettiin 99 mikrosatelliitin suhteen. Geneettinen kytkentäkartta muodostettiin 13 autosomille ja Z-kromosomille, jotka kattoivat yhteensä 2311 cM (centi Morgan) (Handane). QTL-testit tehtiin regressioanalyysillä käyttämällä tilastollista mallia joka sisälsi myös dominanssin, parent-of-origin-vaikutuksen, geneettisen taustan ja kahden QTL:n tapauksen. Munanlaatu- ja tuotanto-ominaisuuksiin löydettiin useita QTL:iä. Mielenkiintoisimmat QTL-alueet sijaitsivat kromosomissa 4 vaikuttaen kanan ja munanpainoon ja kromosomissa Z vaikuttaen munanmäärään ja munan painoon. Kanan painoon vaikuttava QTL selitti noin 25 % F_2-sukupolven vaihtelusta. Kromosomissa 2 löydettiin alue, joka vaikuttaa valkuaisen laatuun ja munan painoon. Hienokartoituksella ja takaisinristeytyspolven analysoinnilla saatiin tämä QTL-alue tarkennetuksi. Lisäanalyysit osoittivat, että tässä kromosomissa laatuun vaikuttavia QTL:iä oli kaksi. Takaisinristeytyspolvi muodostettiin risteyttämällä F_2-sukupolven kanoja RIR- ja WL-kukkojen kanssa. Tulokset parent-of-origin-vaikutuksesta osoittivat, että sillä on merkitystä kummalta vanhemmalta QTL-alleeli on peritty. Kolme tällaista QTL-aluetta jotka vaikuttivat kananpainoon, sukukypsyysikään ja rehun syöntiin olivat ryppäänä kromosomissa 1. Tämä on ensimmäinen raportti lintulajien QTL:stä joissa on parent-of-origin-vaikutus tuotanto-ominaisuuksiin

Avainsanat: Munijakana, QTL, munanlaatu, kartoitus, parent-of-origin vaikutus.

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October 2004

Maria Tuiskula-Haavisto

List of original articles

The thesis is a summary and discussion of the following articles, which are referred to by their Roman numerals:

I Vanhala, T., M. Tuiskula-Haavisto, K. Elo, J. Vilkki, and A. Mäki-Tanila, **1998**. Evaluation of Genetic Variability and Genetic Distances Between Eight Chicken Lines Using Microsatellite Markers. Poultry Sci. 77: 783-790.

II Tuiskula-Haavisto, M., M. Honkatukia, J.Vilkki, D-J. de Koning, N.F. Schulman, and A. Mäki-Tanila, **2002**. Mapping of Quantitative Trait Loci Affecting Quality and Production Traits in Egg Layers. Poultry Sci. 81: 919-927.

III Tuiskula-Haavisto, M., D-J de Koning, M. Honkatukia, N. F. Schulman, A. Mäki-Tanila, and J. Vilkki **2004**. Quantitative trait loci with parent-of-origin effects in chicken. Genet. Res.,Camb. 84: 57-66

IV Honkatukia, M., Tuiskula-Haavisto, M., de Koning D-J., Virta A., Mäki-Tanila, A., and Vilkki, J. Fine-Mapping of a Region on Chicken Chromosome Two Affecting Egg White Thinning. Submitted to Poultry Science

Author's contribution

I Sample collection and participation in the interpretation of data analysis and in writing the manuscript.

II Experimental planning, collecting and handling of experimental data, genotyping, statistical analysis, interpretation of results and writing the manuscript.

III Data analysis and writing the manuscript.

IV Organisation of the back cross (BC) population and assisting in the interpretation of experimental data and preparation of the manuscript.

Symbols and abbreviations

AFE	age at first egg
BAC	Bacterial Artificial Chromosome
BC	back cross
BW40	body weight at 40 weeks
CIM	composite interval mapping
cM	centi Morgan
ENa	number of eggs between 18 and 40 weeks of age
ENb	number of eggs between 41 and 60 weeks of age
ESa	egg shell strength at 40 weeks of age
ESb	egg shell strength at 60 weeks of age
EWa	egg weight between 18 and 40 weeks of age
EWb	egg weight between 41 and 60 weeks of age
FE40	kg feed / kg eggs at 40 weeks of age
FI40	mean daily feed intake at 40 weeks
HU	Haugh-unit
HU40	Haugh-unit at 40 weeks of age
HU60	Haugh-unit at 60 weeks of age
IC	information content
IGF-I	insulin-like growth factor I
IGF2	insulin-like growth factor II
J	Jokioinen
MTT	Agrifood Research Finland
QTL	quantitative trait loci
RIR	Rhode Island Red
RLFP	restriction fragment length polymorphisms
SD	standard deviation
SG40	specific gravity at 40 weeks of age
SG60	specific gravity at 60 weeks of age
SNP	single nucleotide polumorphism
VLDLR	very low density lipoprotein receptor
WL	White Leghorn

Contents

1 Introduction

Rapid developments in poultry breeding and production have taken place during the last 60 years. Advances in population genetics, statistical methology and computing capacity have made this progress possible. The intensification of the whole egg production system has been possible for several reasons. Incubation and brooding can now be mechanised, while short generation intervals enables the rapid multiplication of selected populations. Initially, pure line breeding was used, which was superseded by crossbreeding, while modern day production animals for laying hens are hybrids, i.e. line crosses. Over the some time national breeding programmes have been replaced by large commercial companies, which operate worldwide. Currently, as little as three breeding companies provide more than 90 % of the world supply of layer breeding stock.

Recent developments in chicken genomics including the use of polymorphic markers, automatic genotyping and availability (March 2004) of genome sequences has made it possible to understand the physiological basis of gene expression, and its influence on economically important quantitative and qualitative traits in poultry.

1.1 Domestication of chicken

The earliest signs of domestication in the chicken have been traced back to around 8000 years ago from remains at 16 neolithic sites along the Chinese Yellow River. This environment in China has been a semi-arid steppe at least for the last 10 000 years, which is not the natural environment for jungle fowl. West and Zhou, (1988) proposed that domestication occurred in southeast Asia, within the natural range of red jungle fowl. Restriction fragment length polymorphisms (RFLP) in mitochondrial DNA of various gallinaceous birds indicates that all domestic fowl originate from the red jungle fowl (*Gallus gallus gallus)* (Fumihito et al., 1994; Fumihito et al., 1996). Domestication probably arose in Thailand and its immediate surroundings over 8000 years ago. Domestic birds were initially used for cultural purposes having an important role in religion, divination, black and white magic, but also featured in decorative arts and for entertainment purposes, e.g. cockfighting. Only much later were poultry meat and eggs valued as a food (Crawford, 1995). The main period of chicken dispersion throughout Europe occurred around 1050-800 BC. Several routes across Asia and Europe have been postulated, one from Iran, across the Aegean Sea to Greece and Italy, and the other from Iran to the Black Sea, via Scythia and southern Russia to central Europe (Crawford, 1995).

The Romans were responsible for dispersing domestic chickens both within and outside the empire. The Romans reared chickens both for food and reli-

gious activities. Furthermore, the Romans are known to have practised caponising and force feeding, but also recognised the value of hybrid vigor and used a variety of breeds and types of chicken (Crawford, 1995).

Nearly all modern-day breeds and varieties were developed in the late 19th century. The main emphasis was in breeding for plumage and conformation to improve success at exhibitions. Little attention was paid to production parameters. Most of these breeds and varieties continue to exist in the hands of specialised breeders.

The food industry rejected the decorative characteristics and focused on the quantity and quality of food. Pure breeding was used initially, while crossbreeding to exploit heterosis became common between 1930 and 1950. Currently crosses of strains and synthetic lines are used. White Leghorns dominate white egg production. The Rhode Island Red and some others lines are used for the production of brown eggs, while the White Cornish and White Plymouth Rock are used extensively for meat production (Crawford, 1995).

1.2 Modern Poultry Breeding

In 1971, egg production of a commercial layer was around 245 eggs within in a calendar year, associated with a feed conversion ratio of 2.8:1**.** (Flock, 1999). The current management guidelines for both brown and white layers of a number of breeding companies stipulate that commercial layers are expected to produce about 300 eggs with a total egg mass of 19 kg by 72 weeks of age at a feed conversion ratio of 2.1:1. Between 1961 and 2001, annual world egg production increased by more than 3.5-fold. Production from all fowl has reached almost 57 million tonnes. In the next 30 years, egg production is expected to approach 90 million tonnes. This large increase will arise from rapid expansion of egg production in the USA and developing countries, primarily China, India, Brazil and Mexico (Gillen, 2002).

At present more than 90 % of the world market for layer breeding stock is supplied by only three companies (Albers and Van Sambeek, 2002). The parental and commercial layers have to produce eggs economically under very different conditions and management systems that vary from high density cages (particularly in North America), open houses (Asia and South America), and alternative housing systems adopted in Western Europe (Besbes, 2002). The main methods used for poultry breeding have utilised pure line selection and evaluation of crosses to exploit heterosis and the large amount of data from reciprocal effects. Developments of quantitative genetics, statistical approaches and advances in computing have been the most important factors behind the genetic progress during the last 50 years. The progress would not have been possible without simultaneous improvements in nutrition, disease control and housing.

Selection has typically been performed utilising phenotypic records and identifying genetically elite birds through their own performance and physical characteristics and those of their relatives. However, breeding companies regime new methods to identify the best pedigree animals. Utilising results from genomics will have a significant impact on layer breeding in the future (Albers and Van Sambeek, 2002).

1.3 Use of the chicken in gene mapping research

1.3.1 Model Animal

The chicken is an ideal animal model for research, including genetic mapping of complex traits, due to a high breeding capacity and short life cycle. Artificial insemination makes it possible to create sophisticated mapping populations with large full or half sib families. A controlled environment during hatching, rearing and the production period secures reliable data for genetic studies. Several economically important production and health traits can be measured with relatively small inputs compared with pigs or cattle. In the chicken, nucleated red blood cells can be used as a rich source of DNA, allowing large amounts to be extracted from a relatively small amount of blood. Feathers can also be used, allowing large numbers of animals to be screened for specific DNA markers.

1.3.2 Single genes

The first classical gene linkage map of the chicken with 18 genes and 5 linkage groups was published in 1936 by Hutt (Bitgood, 1993). Thereafter, several maps have been published. The latest classical map includes 119 loci in 6 linkage groups. Most are single genes affecting exterior traits (Bitgood and Somes, 1993). Wide collections of chicken genes are documented in Bitgood and Somes (1990) and Lamont and Dietert (1990) including those for plumage colour, blood grouping, immunoglobulins and morphological traits. Further development of mapping techniques has made it possible to locate chromosomal positions for loci affecting important quantitative production traits.

1.4 Recent developments of avian genomic research

1.4.1 Chicken genome analysis

1.4.1.1 Physical map

The avian genome contains 38 pairs of autosomes and the sex chromosomes Z and W. The female is the heterogametic sex (ZW). Autosomes can be classified by size to eight large 'macrochromosomes' and 30 pairs of cytologically indistinguishable, 'microchromosomes' (Ladjali-Mohammedi et al., 1999; Smith and Burt, 1998). The length of the total avian haploid genome is 1200 x 10^6 bp and about 4000 cM (Schmid et al., 2000). Cytogenetic characterization of each chicken chromosome (karyotype) has been established (Masabanda et al., 2004). Physical maps of several chromosomes have been assembled by overlapping Bacterial Artificial Chromosome (BAC) clones by collaboration between several research groups in Europe and the USA (Burt and Pourquie, 2003; Crooijmans et al., 2000). A BAC contig physical map based on over 133 000 BAC fingerprints is comprised of about 260 contings, nearly 89 % of which have been anchored to the genetic linkage/chromosome map. This map has provided an essential companion to the sequence assembly process (http://www.animalsciences.nl/ChickFPC/). Analysis of sequence samples of cloned genomic DNA and the distribution of genes mapped by linkage and physical mapping suggest that microchromosomes are more gene-dense than macrochromosomes (Smith et al., 2000). The density of genes may approach one per 22 kb on macrochromosomes compared with 17 kb on microchromosomes. Macrochromosomes and microchromosomes in the chicken have been estimated to contain 38 000 and 21 000 genes, respectively (Smith et al., 2000). Earlier estimates suggested values of 42 000 and 13 000, respectively (McQueen et al., 1998). Analysis of the published complete chicken genome sequence (see 1.4.1.3) will in the forthcoming years shed more light on the differences between chromosome types.

1.4.1.2 Chicken reference maps

The most commonly used genetic markers for the chicken are anonymous molecular DNA markers such as highly repetitive microsatellites. The core of the microsatellite marker is a tandem- repeated sequence, usually two nucleotides long. Although the same tandem repeats might be represented many thousands of times in the whole genome, each repetitive sequence is flanked by a unique DNA sequence. PCR is used with DNA primers that recognise the flanking sequences and initiate the amplification of a unique allele marker at each locus. Microsatellite markers are highly polymorphic, making it possible to trace the inheritance of alternative alleles in a pedigree.

The development of DNA markers has enabled effective and reliable construction of genetic linkage maps in production livestock species. Linkage maps are primarily used for the identification of genes that control the expression of economically important traits. Three international reference populations have been created for the construction of linkage maps in the chicken: the Compton population at the Institute for Animal Health, UK (Bumstead and Palyga, 1992), the East Lancing population at Michigan State University, USA (Crittenden et al., 1993) and the Wageningen population, The Netherlands (Groenen et al., 1998). Linkage information from these three populations have been integrated into one consensus map (Groenen et al., 2000).

A variety of mapping databases for farm animals and other species of animals can be found on the web, including Arkdb (http://www.thearkdb.org). This website provides the latest maps and mapping data, lists of published microsatellites, descriptions of microsatellite kits, cytogenetic maps and access to other diverse genetic information for the chicken. The chicken database includes 2412 loci of which 665 are designated as genes and 1277 as microsatellite markers.

1.4.1.3 Chicken genome sequencing

The final step in the understanding of the genomic structure was achieved through sequencing of the whole chicken genome. The chicken is the first domestic animal to have its genome sequenced. The National Human Genome Research Institute announced on 1 March 2004 that the first draft of the chicken genome sequence was available via free public databases for research use around the world. The website http://www.ensembl.org /Gallus_gallus/documents the first database for the chicken genome. This work has been conducted at the Washington University School of Medicine Genome Sequencing Center. The high-quality-draft genome sequence is based on 6.6 x Whole-Genome-Shot-Gun sequence of genomic DNA from a single female red jungle fowl. This sequence will be complemented by a sequencing project at the Beijing Genomics Institute using genomic DNA from three breeds of domestic chickens (Andersson and Georges, 2004).

1.4.2 Advances in quantitative trait loci (QTL) mapping methods

The most important implication of the creation of genomic information for animal breeding is the ability to identify genes that control the expression and variation of production traits. Most of these traits are quantitative and are controlled by a large number of QTL and enviromental components. If a QTL is very tightly linked to a marker, then different marker alleles will be associated with different effects of the QTL on the trait. This association can

be quantified using a range of statistical methods such as regression and maximum likelihood analysis.

The principle underlying identification of QTLs by linkage is conceptually simple; individuals are scored for genotype at the marker locus and phenotype for the quantitative trait. The large undefined segment of a chromosome affecting a trait is defined as the quantitative trait locus (Falconer and Mackay, 1996). If there is a difference in mean phenotype among marker genotype classes, then it can be inferred that a QTL is linked to the marker. Marker loci can be considered either singularly or simultaneously (Falconer and Mackay, 1996). The ability to examine the effect of gene action underlying quantitative variation has been greatly enhanced by the rapid development of genetic maps based on DNA markers in combination with statistical methods, which allow some of the loci responsible for variation of a quantitative variation trait to be mapped (Haley et al., 1994). Several statistical approaches (regression or least squares likelihood and Bayesian approaches) have been proposed to locate individual QTL and estimate their effects. Although these methods require different degrees of statistical sophistication, they all result in similar levels of reliability (Knott et al., 1996). For example, Bayesian methods identified the same QTL as that determined by regression of body weight at 48 d and growth traits in a cross between two broiler dam lines (Van Kaam et al., 2002).

1.4.2.1 Single markers and associated genes

Single marker analysis is based on a comparison between marker genotype and phenotypic means and application of t-tests, variance analysis, likelihood ratio tests or regression. One marker at a time is analysed. If the difference between marker genotypes is significant, a QTL is indicated. Single marker analysis offers the benefits of simple analysis of data and no requirement for a marker map. However, there are also several weaknesses with this approach, due to an inaccurate positioning and biased estimates of the QTL (Ben Hui Liu, 1998).

Many single marker studies have been completed. For example, a recent study reported the presence of single markers (23 markers on 5 largest chromosomes) on loci affecting egg production and egg quality traits in a F_2 mapping population (519 females) from a cross between a brown egg layer line and native land race line (Wardecka et al., 2002). Several markers on chromosomes 1, 3 and 4 were significantly associated with egg production and egg quality traits.

Another approach has been to concentrate on association analysis of genes which are expected to cause variation in the studied trait (candidate gene approach). The transforming growth factor-β genes have been found to be associated with growth and development (Li et al., 2003). Different growth

hormone alleles co-selected with disease resistance have been demonstrated to alter the onset of ovulation and egg production (Kuhnlein et al., 1997). The Insulin-like growth factor I (IGF-I) locus has been reported to be associated with changes in mean egg and egg shell weight (Nagaraja et al., 2000). Association studies have also shown that there are candidate genes or markers affecting the variation in salmonella counts in several organs (Kramer et al., 2003) and in immune response (Yonash et al., 2001).

1.4.2.2 Interval mapping

The interval mapping approach is based on joint frequencies of a pair of adjacent markers and the putative existence of a QTL flanked by both markers. Parameters are calculated based on maximum likelihood or regression (Lander and Bostein 1989; Haley and Knott 1992).

In the regression method, both additive and dominance effects, as well as the location of the putative QTL can be estimated. The phenotypic values are regressed against transmission probabilities calculated for putative QTL alleles at fixed positions. For each putative position (1 cM intervals) simple linear least squares can be used to regress the trait value for each animal against the respective probabilities for additive and dominance effects. The best estimate of a QTL is obtained when the residual sum of squares is minimized. For the QTL position the ratio of the regression mean square to the residual mean square provides the usual variance (F) ratio test statistic (Haley et al., 1994; Haley and Knott, 1992). Least-square models also provide estimates of additive and dominance effects, the contribution of QTL to total phenotypic variance and an indication of the putative QTL position (Knott et al., 1998). To establish the significance threshold for a given QTL the permutation test of Churchill and Doerge, (1994) is widely used. To obtain the genome-wide level of significance, the chromosome-wide threshold is adjusted by Bonferroni correction that accounts for the length of the analysed genome. Level of significance criteria usually follow Lander and Kruglyak, (1995) who proposed the use of two levels of significance: suggestive and significant. For suggestive linkage, one false positive is expected in the genome scan. For determining significant linkage, a 5% genome-wide significance level is usually applied. Confidence intervals for QTL positions can be obtained by bootstrapping in which complete combinations of phenotype and genotype are resampled with a replacement to test the sensitivity of parameters. Sorted F-ratios can used to determine the test statistic value corresponding to a desired 90 % confidence interval (de Koning et al., 2000). Alternatively the best QTL position of all replicates can be stored to determine an empirical confidence interval (Knott et al., 1998).

1.4.2.3 Extended models

The simple regression model does not account for complicated genetic models, such as two QTL on one chromosome, epistatic effects, parent-of-origin effects or pleiotropic effects of one QTL. To test for the existence of two QTLs the regression method can be extended to perform grid searching for two QTL on one chromosome (Spelman et al., 1996). With a single QTL model, two QTLs cannot be detected if the QTL reside between markers which are less than 20 cM apart (Haley and Knott, 1992). To ensure that the QTL have some distance between them, only those positions where they are separated by an empty marker bracket are evaluated. For the two QTL model, two tests are calculated: One test statistic compares the fit of one or two QTL model versus no QTL at all. The second test statistic determines if the two QTL model explains significantly more of the variance than the single QTL model (Spelman et al., 1996).

A method to test for epistatic interactions between QTL effects has been implemented (Carlborg et al., 2003) in the chicken QTL mapping study. These tests indicated that the epistatic influence had a strong influence on growth before 48 days of age, whilst additive effects explained most of the genetic variance during later life. Epistasis has been shown to affect the susceptibility to Marek's disease in a F_2 population created from two inbred lines differing in disease susceptibility (Vallejo et al., 1998).

A QTL that has an effect on one trait can also have pleiotropic effects on other traits. To test for pleiotropic effects of QTL affecting correlated traits, a multitrait QTL analysis is required. Multitrait analysis increases the power to distinguish pleiotropic effects of a QTL from more than one linked QTL and improve the precision of location estimates (Knott and Haley, 2000). In dairy cows, there is evidence of a pleiotropic QTL for fat yield and protein yield in BTA6 (Freyer et al., 2003). There are no reports of pleiotropic QTL effects in the chicken.

To increase the power of interval mapping on each chromosome, QTL on other chromosomes (fitting background genetic effects) can be used as cofactors (Jansen, 1993; Zeng, 1993). This analysis termed composite interval mapping (CIM) increases the power to detect a QTL and the precision of estimates of marker positioning. The CIM approach is essentially a combination of simple interval mapping and multiple linear regression. Interval mapping based on CIM integrates information from multiple markers outside the known interval and on other chromosomes (Rodriguez-Zas et al., 2002).

Mendelian principles provide no explanation for reciprocal differences in crossed populations known to occur in poultry (Fairfull et al., 1983; Wearden et al., 1965). An advantage of crossing outbred lines is that the parental origin of alleles can be traced back from the F_2 population to F_1 parents, which is a

prerequisite for testing parent-of-origin effects such as genomic imprinting (Knott et al., 1998). Genomic imprinting is one of the epigenetic phenomena that are inconsistent with Mendelian laws. The consequences of imprinting relate to the silencing of effects from one of the two alleles depending on the sex of the parent from which it was inherited. The hypothesis predicts a conflict between maternal and paternal genes in relation to the transfer of nutrients from the dam to the offspring. The most imprinted paternal allele promotes embryonic growth, while the maternal allele has an opposite effect. This model suggests that imprinting should not occur in birds, since the amount of yolk is determined at fertilisation (Moore and Haig, 1991). However it is unclear if parent-of-origin effects exist in the chicken. There are studies on allelic expression of the insulin-like growth factor II (IGF2) gene in chicken embryos (Koski et al., 2000; Nolan et al., 2001; Yokomine et al., 2001). Koski et al. (2000) reported monoallelic expression in chicken embryos, but these findings have not been confirmed (Nolan et al., 2001; O'Neill et al., 2000). Even though parent-of-origin effects in the chicken remain uncertain it is worthwhile to test the possibility with statistical tools. De Koning et al. (2000) developed a model for the detection of parent-of-origin effects including a paternal and maternal component in the model and compared this with a Mendelian model that included an allowance for dominance. On this basis, de Koning et al. (2000) suggest that the genome should be scanned using a reduced imprinting model with either paternal or maternal expression. This approach has been used in pigs and the results show that imprinting has an important role in determining carcass composition. Parent-of-origin effects have also been found to affect different pork quality traits (de Koning et al., 2001). Buitenhuis et al. (2003) identified a putative maternally expressed QTL on chromosome 5 that alters corticosterone response in a F_2 population of chickens using the model of de Koning et al. (2000).

1.4.2.4 Fine mapping

Fine mapping can be performed for significant QTL to improve the precision of estimates of the QTL location. A common method is to increase marker density around the putative region. In fine mapping the marker interval is generally 1-3 cM. To reduce confidence intervals for a QTL and define its location, the number of events of recombination becomes the limiting factor rather than the number of markers (Van Raden and Weller, 1994). Backcross (BC) generations can also be used to increase mapping resolution by introducing new recombinants (Weller, 2001). Fine mapping is most useful when a QTL accounts for a large proportion of total variance, but this method only results in small reductions in mean error for QTLs that account for a limited amount of total variance (Atwood and Heard-Costa, 2003). An advanced intercrossed line is another approach for reducing the CI of a QTL map location. Such a line is produced by randomly and sequentially intercrossing a population that initially originated from two inbred lines or variants thereof.

However, this method requires that the size of breeding population should not fall below 100 animals per generation (Darvasi, 1995).

1.4.3 Chicken QTL analyses

The chicken has been a target for different mapping studies that have demonstrated the possibility of identifying genes affecting quantitative and qualitative traits. Several QTL results from different line crosses have been published within the last five years. The majority of the QTLs reported affect production traits (Ikeobi et al., 2002; Jennen et al., 2004; Kerje et al., 2003; Sewalem et al., 2002; Tatsuda and Fujinaka, 2001; van Kaam et al., 1998; van Kaam et al., 1999). QTL have also been detected for the incidence of feather pecking and other behavioural traits (Buitenhuis et al., 2003) and the susceptibility to Marek's disease (Yonash et al., 1999) and coccidiosis (Zhou et al., 2003). The genetic basis of these traits is of interest both for animal breeding and human health.

The experimental design in these experiments has been based on an intercross between two different lines: including two broiler dam lines from the White Plymounth Rock breed (van Kaam et al., 1998), a commercial broiler line and an egg layer line (Ikeobi et al., 2002; Sewalem et al., 2002), the White Plymounth Rock broiler and the native Japanese Satsumadori breed (Tatsuda and Fujinaka, 2001), or between the White Leghorn egg layer line and red jungle fowl (Schutz et al., 2002).

The population size in these experiments has varied between 227 (Tatsuda and Fujinaka, 2001) and 851 (Kerje et al., 2003) individuals. van Kaam et al. (1998) used a F_2 population of 470 animals with marker genotypes in which phenotypes were measured in the F_3 generation consisting of over 2000 broilers. The power to detect QTL explaining more than 5 % of the total phenotypic variance with these designs and population size is over 95%, even if the QTL are acting in a dominance fashion (see the review by Lynch and Walsh, 1998). These designs implicitly assume that the lines are fixed for alternative alleles at the QTL loci. If this assumption does not hold true, such designs then have less power to simultaneously detect the QTLs segregating within lines, in which case recent and more generalised methods should be applied (Perez-Enciso et al., 2001).

Most of the identified QTLs in these studies have been associated with growth, feed intake, fatness and body weight, using the regression interval mapping method. Phenotypic variation explained by the QTL affecting body weight at different ages varied between 3.0 and 41.7 % (Tatsuda and Fujinaka, 2001). The effects for body weight were mainly additive (Kerje et al., 2003; Sewalem et al., 2002), whilst dominance effects for abdominal fat weight, fatness and fat distribution have been reported (Ikeobi et al., 2002).

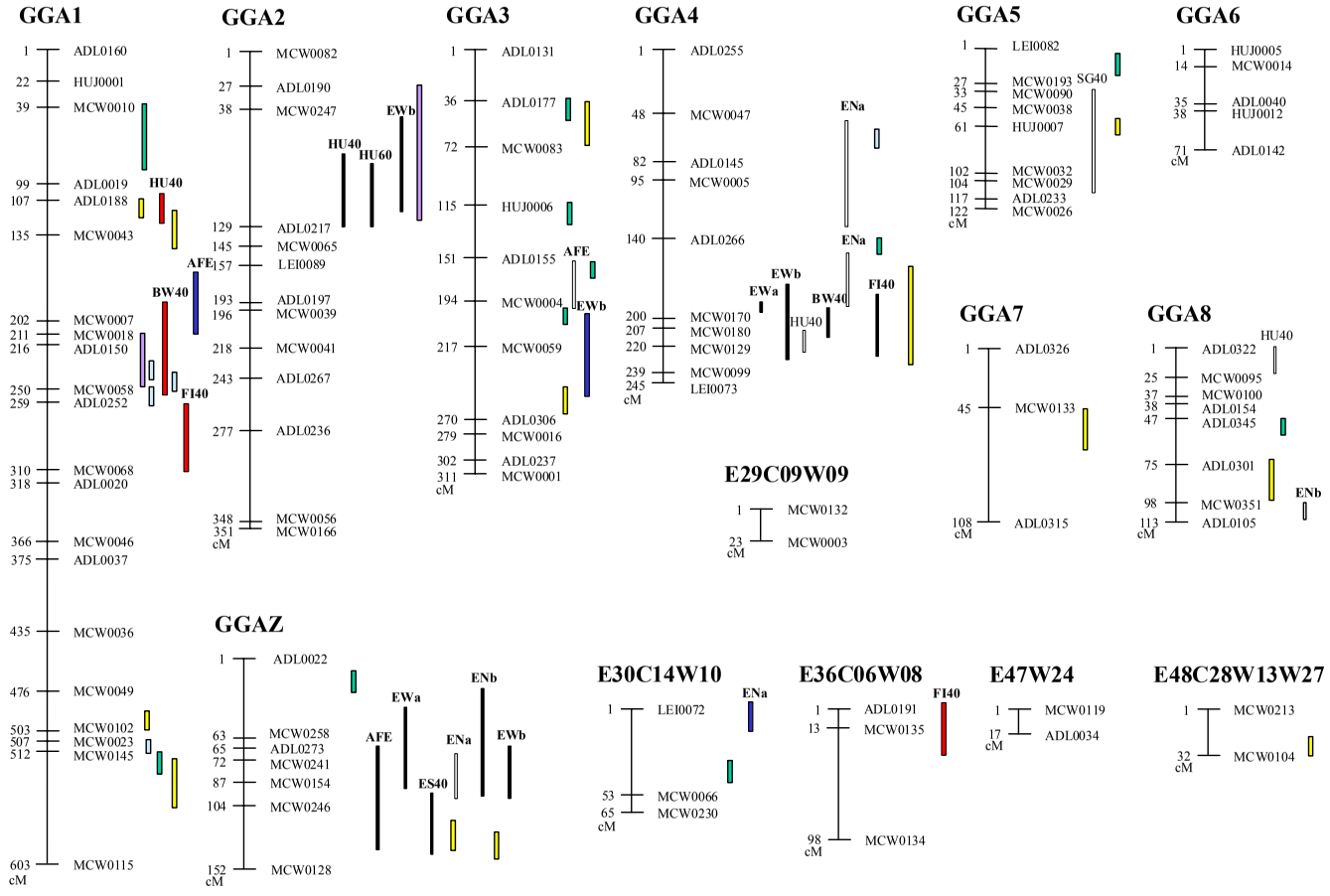

GGA1
1 ADL0160
22 HUJ0001
39 MCW0010
99 ADL0019
107 ADL0188
135 MCW0043
202 MCW0007
211 MCW0018
216 ADL0150
250 MCW0058
259 ADL0252
310 MCW0068
318 ADL0020
366 MCW0046
375 ADL0037
435 MCW0036
476 MCW0049
503 MCW0102
507 MCW0023
512 MCW0145
603 MCW0115
cM
HU40
AFE
BW40
FI40
GGA2
1 MCW0082
27 ADL0190
38 MCW0247
129 ADL0217
145 MCW0065
157 LEI0089
193 ADL0197
196 MCW0039
218 MCW0041
243 ADL0267
277 ADL0236
348 MCW0056
351 MCW0166
cM
HU40
HU60
EWb
GGA3
1 ADL0131
36 ADL0177
72 MCW0083
115 HUJ0006
151 ADL0155
194 MCW0004
217 MCW0059
270 ADL0306
279 MCW0016
302 ADL0237
311 MCW0001
cM
AFE
EWb
GGA4
1 ADL0255
48 MCW0047
82 ADL0145
95 MCW0005
140 ADL0266
200 MCW0170
207 MCW0180
220 MCW0129
239 MCW0099
245 LEI0073
cM
ENa
EWb
EWa
HU40
BW40
FI40
GGA5
1 LEI0082
27 MCW0193
33 MCW0090
45 MCW0038
61 HUJ0007
102 MCW0032
104 MCW0029
117 ADL0233
122 MCW0026
cM
SG40
GGA6
1 HUJ0005
14 MCW0014
35 ADL0040
38 HUJ0012
71 ADL0142
cM
GGA7
1 ADL0326
45 MCW0133
108 ADL0315
cM
GGA8
HU40
1 ADL0322
25 MCW0095
37 MCW0100
38 ADL0154
47 ADL0345
75 ADL0301
98 MCW0351
113 ADL0105
cM
ENb
E29C09W09
1 MCW0132
23 MCW0003
cM
GGAZ
1 ADL0022
63 MCW0258
65 ADL0273
72 MCW0241
87 MCW0154
104 MCW0246
152 MCW0128
cM
AFE
EWa
ES40
ENa
ENb
EWb
E30C14W10
1 LEI0072
53 MCW0066
65 MCW0230
cM
ENa
E36C06W08
1 ADL0191
13 MCW0135
98 MCW0134
cM
FI40
E47W24
1 MCW0119
17 ADL0034
cM
E48C28W13W27
1 MCW0213
32 MCW0104
cM

Figure 1. QTLs in the chicken with confidence intervals for different traits (refer to the text for details) (Jennen et al., 2004; van Kaam et al., 1998; van Kaam et al., 1999) light blue bars, (Ikeobi et al., 2002; Sewalem et al., 2002) yellow bars, (Kerje et al., 2003) green bars, (Tatsuda and Fujinaka, 2001) violet bars. Dotted black bars indicate regions with genome wide Mendelian QTL and the white bars refer to chromosome wide Mendelian QTL (Table 6). Parent-of-origin QTL paternally expressed (blue bars) or maternally (red bars) expressed QTL (Table 2, III). ADL = Avian Disease and Oncology Laboratory, Michigan State University, East Lancing, MI; HUJ = Hebrew University of Jerusalem, Israel; LEI = University of Leicester, Leicester, UK; MCW = Microsatellite chicken Wageningen, The Netherlands. HU40 = Haugh unit at 40 wk of age, HU60= Haugh-unit at 60 wk of age, AFE = age at first egg, BW40 = bodyweight at 40 wk of age, EWa = average egg weight between 18 and 40 wk of age, EWb = average egg weight between 41 and 60 wk of age, ENa = total number of eggs between 18 and 40 wk of age, ENb = total number of eggs between 41 and 60 wk of age, SG40 = specific gravity at 40 wk of age, ES40 = egg shell strength at 40 wk of age, FI40 = daily feed intake between 37 and 40 wk of age.

The QTL results from the four crossbred populations for the chromosomes included in this study are shown in Figure 1. The coloured bars indicate confidence intervals for significant QTLs of different traits. The locations were deduced from the closest flanking markers and comparisons with the consensus map (Groenen et al., 2000). They provide a visual overview of how different QTL results are distributed over the chicken genome. Information of all detected QTL has recently been published on the internet https://acedb.asg.wur.nl/.

There is a clustering of QTLs within chromosomes 1, 2, 4 and Z, while others are more widely distributed throughout the whole chromosome, such as on chromosome 3. Even though the traits measured and the crosses are different, it is interesting to note that the relatively large confidence intervals are to some extent overlapping. In such areas on chromosome 1, 4 and Z, there are many candidate genes affecting different metabolic pathways for growth (Schmid et al., 2000). The QTL results affecting growth on chromosome 4 have been confirmed (de Koning et al., 2003) based on data from populations of commercial broilers over three generations. Information of QTL on chromosomes 1 and 4 represents a solid foundation for the fine mapping of genes affecting growth.

Furthermore, there are many single areas affecting different traits on other chromosomes (5, 7, 8, 11, 13). In conclusion, several QTL areas have been identified in spite of relatively small experimental populations and the limited marker maps used. The results from these studies are consistent, even though the measured traits were not identical. Whilst microchromosomes have been estimated to be more gene-dense, QTL results from the macrochromosomes and chromosome Z appear to be the most promising. Further studies are required to localize the QTL more precisely and to distinguish between effects due to multiple QTL within a region, and those arising from a single pleiotropic effect of QTL. Studies of QTL reported in the literature have been based on the analysis of crossbred generations derived from different line types (broiler and layer, broiler and broiler, broiler and native line, white leghorn and wild jungle flow). Thus far, no QTL analysis has been conducted in chicken populations where crosses have been made between two laying lines. Therefore, this study was conducted to examine QTLs for traits important in the breeding of laying hens, such as egg quality and egg production

2 The aim of this thesis

The aim of the studies described in this thesis was to identify loci affecting economically important traits in egg layers using F_2 generation mapping population. More specifically this research was conducted to:

- Select genetically divergent egg layer lines representing extremes of the two main egg quality traits (egg white thinning and egg shell strength). [I].

- Generate reciprocal crosses of grandparent lines, appropriate design of F_1 matings to produce the F_2 generation and collection of phenotypic records. [II].

- Selection of informative microsatellite markers to construct a linkage map for a whole genome scan. [II].

- Genotyping of individuals across all three generations. [II].

- Analysis of QTL data using regression including the sex chromosome and reciprocal effects. [II and III].

- Fine mapping of interesting QTL regions using an additional backcross generation. [IV].

3 Materials and methods

3.1 Mating plan

3.1.1 F_2 population

The mapping population was based on a reciprocal intercross in order to exploit all genomic information including sex chromosome and parent-of-origin effects across both genders from both lines, White Leghorn (WL) and Rhode Island Red (RIR) [II, III]. The parent generation consisted of 4 animals (2 males and 2 females) per line. Production of the F_2 population was achieved by mating each F_1 male with two females from the same cross and with two females from the opposite cross, excluding matings between siblings. Figure S1 illustrates the mating plan for the F_2 population [III].

Approval to conduct all animal experimentations was granted by the local ethics committee of MTT Agrifood Research Finland in accordance with the Animals (Scientific Procedures) Act of 1996.

3.1.2 Backcross population

The phenotypic value of Haugh-unit (HU) was used as the selection criterion for parents of the BC generation. In order to narrow down the QTL region by reducing the proportion of other parental chromosomes, extreme F_2 individuals (18 hens) of high and low HU were selected as the maternal sources for the first BC generation. Females with a high HU were mated to RIR, while females of low HU were mated to WL.

3.2 Phenotypic data

Because layer parental lines were used, different egg quality and egg production traits were measured for F_1, F_2 and BC generation. Egg shell quality was recorded as egg shell strength at 36-40 and 56-60 weeks of age (ES40 and ES60) and specific gravity at 36-40 and 57-60 weeks of age (SG40 and SG60). Internal egg white quality was also measured in Haugh-units at 36-40 and 57-60 weeks of age (HU40 and HU60). Estimates of the Haugh unit are based on measurements of the egg weight and the height of thick albumen, and calculated as the $\log_{10}$ of weight corrected.

Egg production was measured as the total number of eggs produced during 18 to 40 weeks of age (ENa) and between 41 and 60 weeks of age (ENb) and mean egg weight between 18-40 (EWa) and 41-60 weeks (EWb). Because mapping is expensive and extremely time consuming it is worthwhile to re-

cord as many traits as possible. Therefore traits not directly related to the main objective of the study were also recorded. Age at first egg (AFE), body weight at 40 weeks of age (BW40), daily feed intake (FI40) and corresponding feed conversion ratio (kg feed /kg eggs) (FE40) at 36-40 weeks of age were used to assess production potential. A detailed description of measurements of egg quality and egg production traits is provided in [II]. The effects of hatch were tested and phenotypic values were corrected using the GLM procedure of SAS (SAS Institute Inc.Cary, NC).

3.3 Genome analysis (chromosomes, markers and genotyping)

Genomic DNA was extracted from chicken blood according to [I]. Microsatellite loci were amplified by PCR using standard procedures. Products of PCR were multi loaded and analysed with ALF or ALF Express, while the analysis of fragments was performed using the Fragment Manager (version 1.2) software. Methods used for genotyping are documented in [II]. To avoid genotyping errors, genotyping was determined by two independent assessors. In total 99 microsatellite markers spanning the nine largest linkage groups (1, 2, 3, 4, 5, 6, 7, 8 and sex chromosome Z) and five smaller linkage groups were analysed [II]. Four of the five small linkage groups have been identified as chromosomes. E29C09W09 corresponds to chromosome 10, E30C14W10 to chromosome 11, E36C06W08 to chromosome 9, and E48C28W13W27 to chromosome 13 (http://www.thearkdb.org/browser?species=chicken). Genetic map construction involves both the ordering of loci and the measurement of distances between them. Mapping of loci on chromosomes relies on the frequency of chiasmata and cross overs between loci. Mapping functions attempt to predict the number of cross overs from recombination frequencies. The Haldane mapping function was applied for the construction of the linkage map used in these studies. Linkage analysis was performed using the CRI-MAP program package (Green et al., 1990).

The 99 microsatellite markers used in this study are listed in Appendix V, including information on the map position allele length and distribution of alleles in the parent generations. For other details refer to http://www.ri.bbsrc.ac.uk/chickmap, and http://www.zod.wau.nl/vf/ chickensite/chicken.html. For fine mapping on chromosome 2, a shorter marker interval and BC generation were used. In the confidence interval of QTL for HU40 and HU60 there were several possible candidate genes affecting egg white quality. *Vimentin* was selected, since this is a member of a family of intermediate filaments, which are important in maintaining the mechanical integrity of the cell [IV]. A single nucleotide polymorphism (SNP) was used as a marker for the *vitmentin* gene, that was identified following the sequencing of some extreme animals within the mapping population [IV].

3.4 Statistical methods

The regression approach was used to locate QTLs and estimate their effects. The F_2 data was thereby analysed following line cross concepts. Marker alleles in F_2 animals were traced back to Rhode Island Red or White Leghorn origins, among individuals of the P generation. In the line cross model it is assumed that the two founder lines are fixed for alternative alleles at the QTL affecting the traits of interest even though these may segregate at marker loci. For every F_2 individual an inferred probability of inheritance from two RIR alleles, two WL alleles or one from each line at 1-cM intervals across the genome are determined. The resultant inheritance probabilities are used as estimators for fitting a single biallelic QTL on a chromosome, assuming an additive and dominance effect across all F_2 animals and additive effects on chromosome Z. In the line cross additive (a) and dominance (d) effects are estimated by regression of phenotype against probabilities of inheriting two alleles from the same line (homozygous for one of the two QTL allele) $p_a = p_{11}-p_{22}$ or one allele from both lines (heterozygous for the two QTL alleles) $p_d=p_{12}+p_{21}$. Calculation of inheritance probabilities and QTL effects are detailed elsewhere (Haley et al., 1994).

Multiple QTL analysis was performed using interesting regions (including Z chromosome) as cofactors. For all interesting areas the possibility of two QTL being present was tested by performing a grid search of all possible combinations of positions on a studied chromosome (including chromosome Z). Results for the two QTL model were calculated using *QTLexpress* (Seaton et al., 2002). Robustness of the results generated was evaluated by reanalyses of experimental data following the removal of offspring from individual sires and/or dams with the same parents.

An outbred line cross design provides the possibility of tracing the parental origin of alleles in F_2 individuals back to F_1 parents. This enables the determination of parent-of-origin effects. The model for imprinting (Knott et al., 1998) was reparameterised to carry out a direct test for the contribution of inherited paternal and maternal effects according to de Koning et al. (2000). To test for the influence of maternal effects and sex-linked genes, the parent-of-origin QTLs were re-analysed using Mendelian QTL from chromosome Z as co-factors. For all traits, grand maternal effects were tested and reanalysed. To confirm the results of parent-of-origin effects, the QTL were reanalysed using freely available commercial software. For the best QTL position from *QTLexpress* (Seaton et al., 2002) probabilities were extracted and re-analysed using the raw phenotypes by GENSTAT.

Significance thresholds for individual chromosomes were determined empirically by permutation (Churchill and Doerge, 1994). The chromosome-wide *P*-value for suggestive linkage of a specific chromosome is deduced from the contribution to total genome length, that was calculated by dividing the

length of the chromosome by that of the genome (2311 cM for Mendelian QTL and 2159 cM for QTL with parent-of-origin effect). To derive genome-wide levels of significance from the chromosome-wide significance, the Bonferroni correction was applied.

Confidence intervals for QTL positions were obtained by bootstrapping in which complete combinations of phenotypes and genotypes were resampled with replacement to test the sensitivity of the parameters. Sorted F-ratios were used to determine the test statistic value corresponding to a desired 90 % confidence interval (de Koning et al., 2000). For the two QTL analysis, bootstrapping with *QTLexpress* generates the confidence intervals for the best positions (Seaton et al., 2002).

4 Results and discussion

4.1 Genetic diversity of chicken lines

The probability of finding QTL was maximized by the use of highly divergent lines. Genetic variability and the divergence of chicken lines was assessed using nine microsatellite markers. Chicken lines included three WL lines, three Finnish Landrace lines, RIR line and one broiler line. All microsatellite loci were found to be polymorphic, with the number of alleles varying from 4 to 13 per locus and 1 to 10 per line, respectively. The smallest genetic distance was found between J (Synthetic line of Jokioinen) and LSL (Lohmann Selected Leghorn) (0.117) and the largest between RIR and LSL (0.476). A phylogenetic consensus tree was constructed using the neighbour-joining method. The lines were grouped into three clusters. The first group was formed from the three WL lines, the second cluster consisted of the two Landrace lines, while the third was comprised of RIR, the broiler and one Landrace line. Although the phylogenetic tree was based on only nine microsatellite markers, the reliability estimates gained through bootstrapping were relatively high. Takezaki and Nei, (1996) suggested that for attaining a reliable phylogeny at least 30 markers should be used. However, in the simulation study of Takezaki and Nei, (1996) the expected level of genetic divergence between populations (or species) was lower than in this study. The relatively high level of divergence of D_s (0.117 to 1.117) accounts almost entirely for the high degree of reliability of the tree topology [I].

Two extreme lines were crossed to create the mapping population (F_2 generation). The lines used were RIR obtained from a Finnish breeder and a synthetic WL line J that has been maintained at MTT (Jokioinen) since 1987. These two lines showed the largest pairwise genetic distance among the eight lines that were examined for genetic diversity [I]. The selected parental animals differed in several traits, the RIR individuals were approximately 1 kg heavier (standard deviation (SD) 250g, cf. Table 1, [I]) eggs from these animals were on average 5 g heavier (5g), with a 40 (10) units lower Haugh value and 0.02 (0.005) higher specific gravity.

4.2 Mapping population, phenotypic data and correlations between traits

The F_2 population included 305 females from three hatches (hatch 1: 114, hatch 2: 79, and hatch 3: 112 females) produced by F_1 reciprocal crosses (Figure S1 [III]). Of the 32 full sister groups, one had to be eliminated due to an erroneous mating. Three animals were eliminated because their age at first

egg exceeded 250 days. The numbers of full and half sib families are presented in Table 1. During the production period 22 females died, 16 of them before 40 weeks of age. Cause of death was in most cases due to fatty liver syndrome and peritonitis. All abnormal trait records were omitted. In total, all 14 traits were recorded in 250 hens.

The reliability of phenotypic data is central to genetic research projects such as QTL analysis. Recorded traits are summarised in Table 2 including information on the variation in the F_2 generation. For all egg quality traits, the standard deviation for measurements at 60 weeks of age was higher than at 40 weeks. This is a common observation in older hens. Age at first egg varied between 104 and 162 days. Birds entered the adult lighting programme at the same time but at different ages, which may have underlined the variation of age at first egg. Variation in BW40 is also related to hatching time, such that the difference between the heaviest and lightest birds was 1549 gr. Mean daily feed intake (109 g/d) was in the normal range reported for egg layers (105-115 g/d) (Summers and Robinson, 1995). Animals with a daily feed intake below 90 g/d produced a small number of eggs.

Chicks were hatched in three batches. Hatching time had a significant effect on AFE, ENa, EWa, BW40, ENb, FI40, FE40 and egg shell strength at 40 weeks (ES40). The distribution of the phenotypic data before and after correction for AFE, ENa, EWa, BW40, ENb, FI40, FE40 and ES40 (different bar for different hatch) and raw data for HU40, HU60, ES60, EW60, SG40, SG60 and EW60 are shown in Figure 2. For ES40 and ES60, phenotypic distributions had two peaks that may indicate one major QTL segregating in the population. For hatch three the late production period was only 54 weeks long, that explains the significance of hatch effect on ENb. Distributions for ENa and ENb were skewed due to low producing hens. Feed intake was recorded during a four week period. Feed intake was normally distributed, and varied between 61.1 and 147.4 g /day. Feed conversion efficiency range

Table 1. Number of full and half sib families in the analysed F2-population.

F1 male	cross	Number of females in the four full sib families				Number of half sibs	Total
482	RIR X WL	9	9	5	---	23	
529	RIR X WL	9	16	13	11	49	
2386	RIR X WL	4	5	15	9	35	
2449	RIR X WL	8	9	12	10	39	146
2507	WL X RIR	15	7	8	14	44	
2587	WL X RIR	9	7	15	15	46	
570	WL X RIR	7	14	12	6	39	
629	WL X RIR	2	5	17	6	30	159
							305

Table 2. Description of the traits analysed in the F2 population.

Trait	Abbreviation	Min[1]	Max[2]	Mean[3]	SD[4]	SD after correction for hatch	n[5]
Haugh-unit at 40 wk	HU40	55.1	95.7	78.3	7.1	7.1	284
Haugh-unit at 60 wk	HU60	11.8	89.2	68.6	12.1	12.1	251
Eggshell strength at 40 wk (kilopond)	ES40	1.9	5.6	3.7	0.6	**0.6**	284
Eggshell strength at 60 wk (kilopond)	ES60	0.6	5.6	3.4	0.7	0.7	251
Specific gravity at 40 wk	SG40	1.063	1.097	1.083	0.005	0.005	284
Specific gravity at 60 wk	SG60	1.033	1.148	1.077	0.013	0.013	250
Age at first egg (d)	AFE	104	162	132	10.2	**9.7**	304
Body weight at 40 wk (g)	BW40	1233.0	2782.0	1853.9	252.8	**244.0**	289
Egg weight between 18 and 40 wk (g)	EWa	41.7	72.9	58.5	4.9	**4.9**	298
Egg weight between 41 and 60 wk (g)	EWb	52.7	83.8	66.2	5.3	5.3	284
Number of eggs between 18 and 40 wk	ENa	39	148	122.3	18.1	**17.2**	290
Number of eggs between 41and 60 wk	ENb	31	152	88.7	23.6	**18.9**	282
Mean daily feed intake at 40 wk (g/d)	FI40	60.1	147.4	109.9	14.9	**14.7**	291
kg feed/ kg eggs (kg) at 40 wk	FE40	1.57	6.9	2.35	0.7	**0.7**	286

[1] Minimum

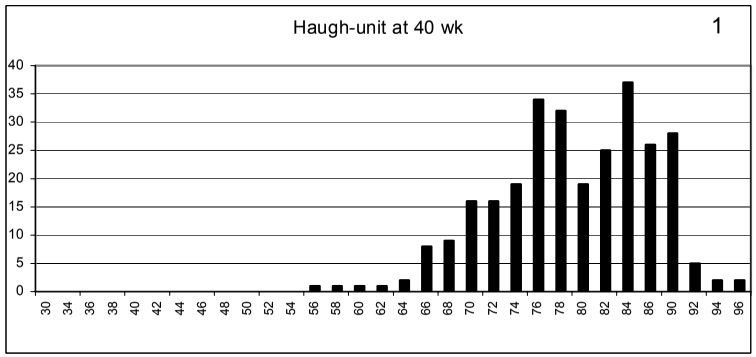
[2] Maximum

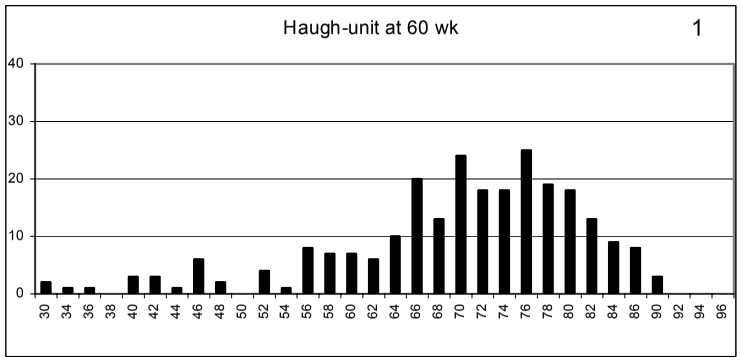
[3] Mean

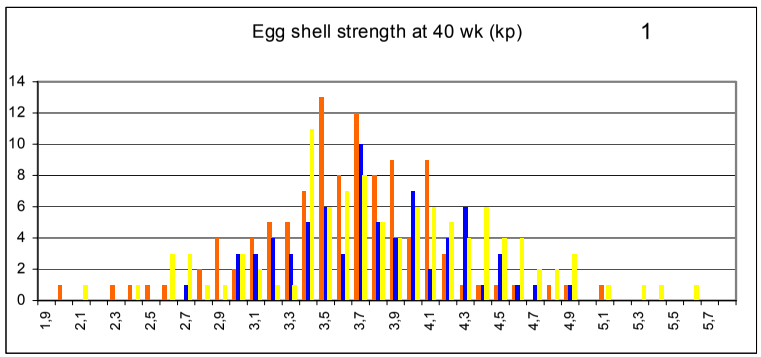
[4] Standard deviation

[5] Number of individuals

Hatch effect corrected values indicated in bold







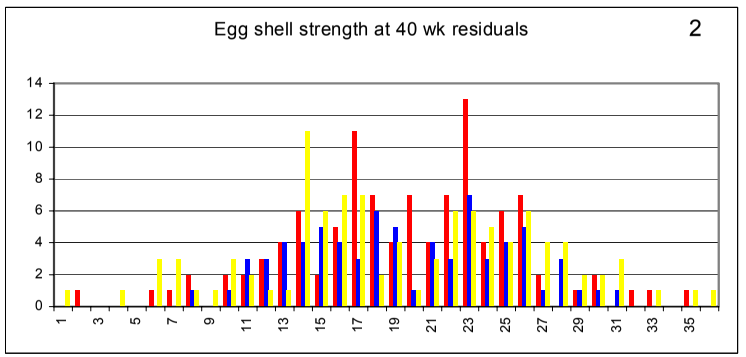
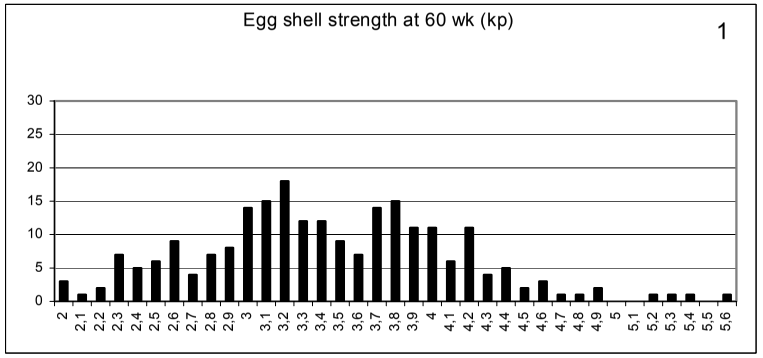
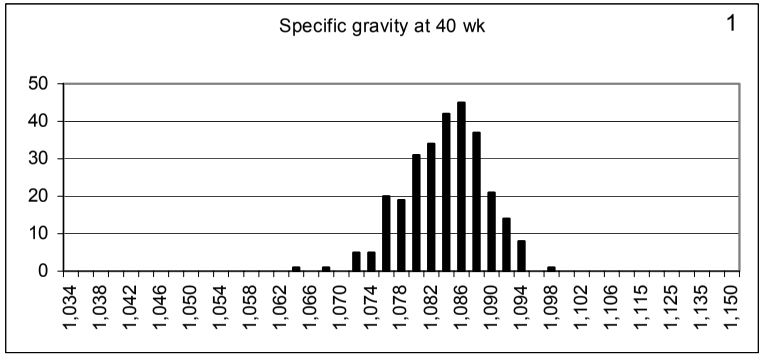
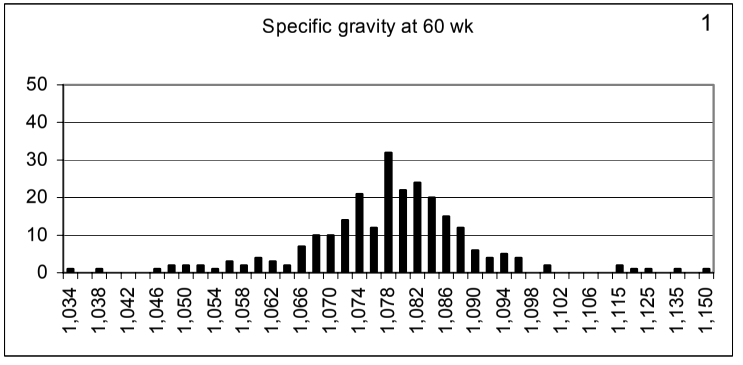


Figure 2. Phenotypic distribution for traits recorded for the F_2 generation. Two distributions of traits for which the hatch effect was significant are presented. Individuals from the first, second and third hatch are indicated by red, blue and yellow bars respectively.

Figure 2. continued







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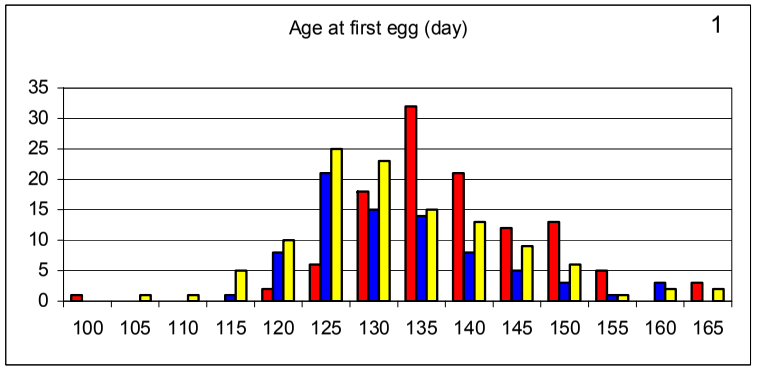

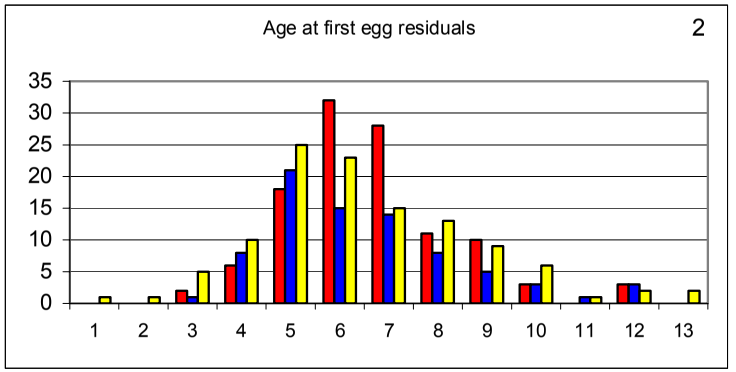

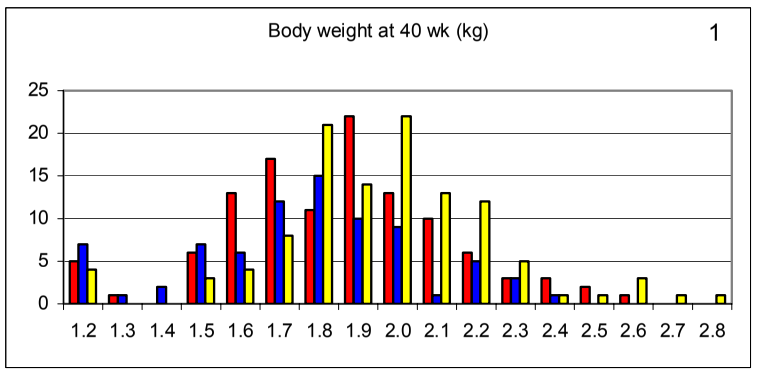

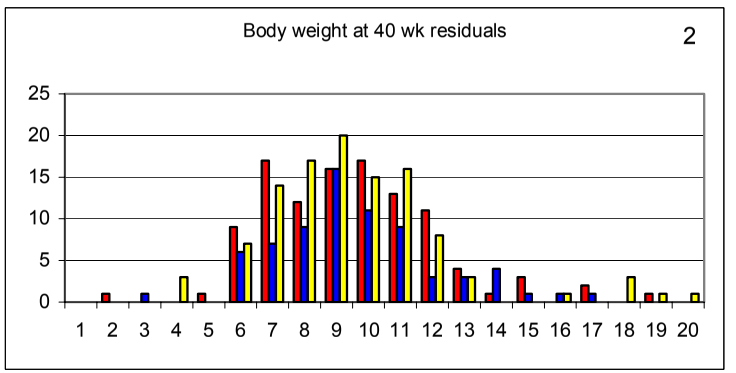

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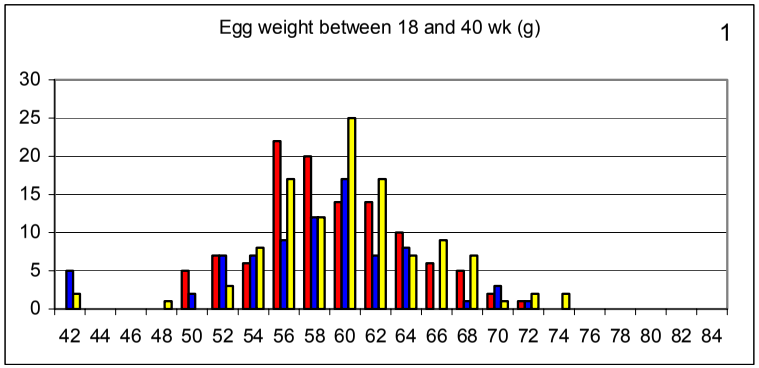

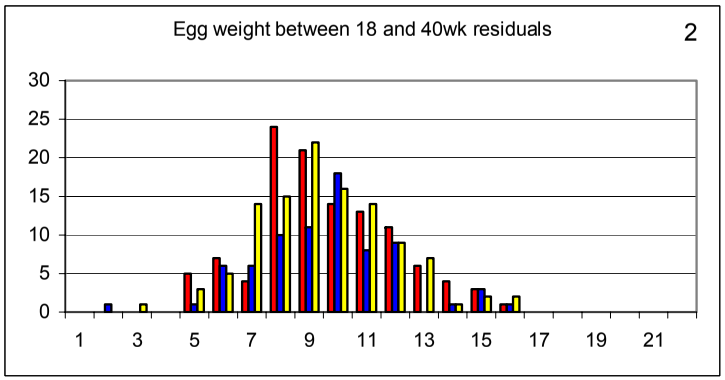

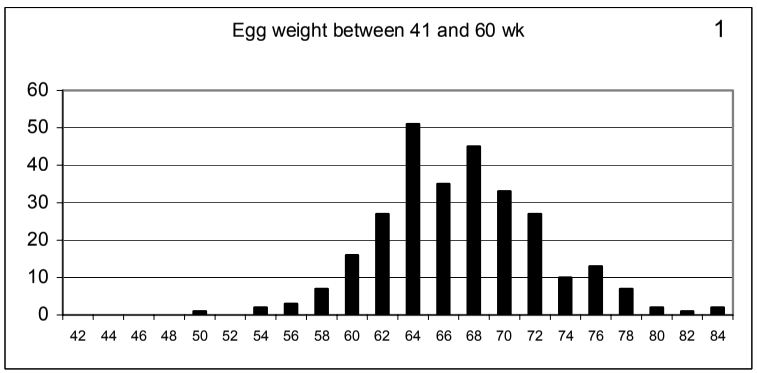

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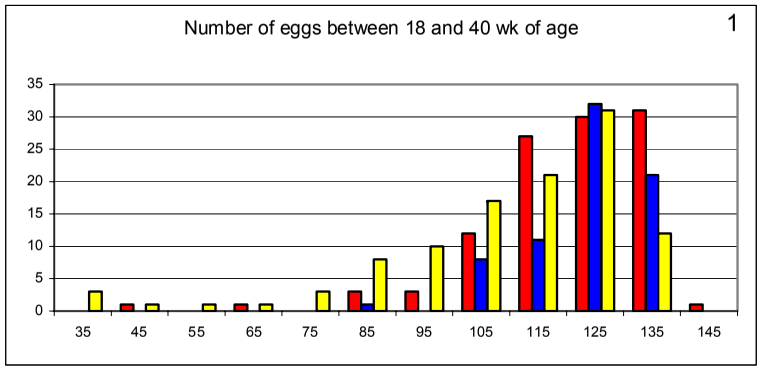

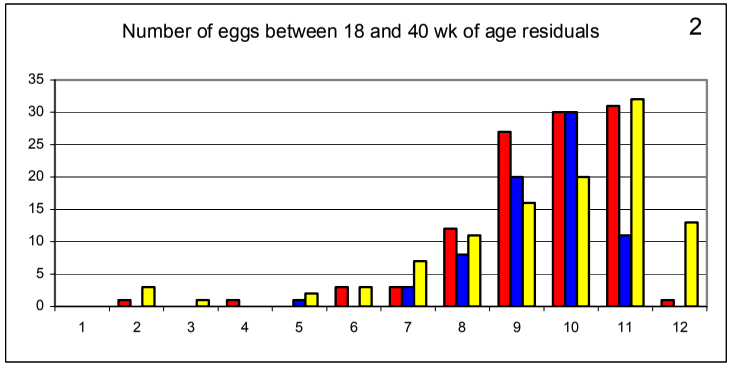

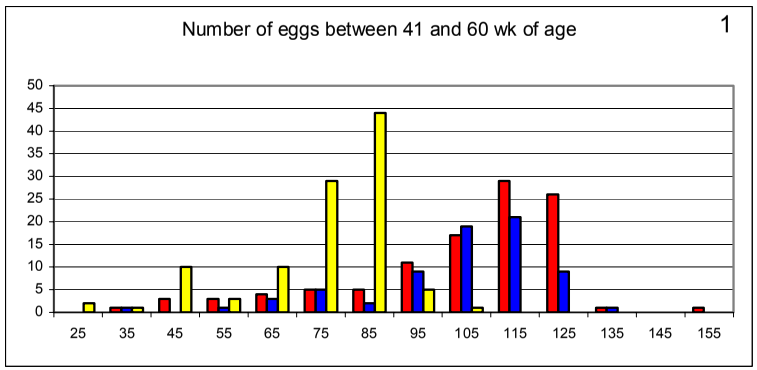

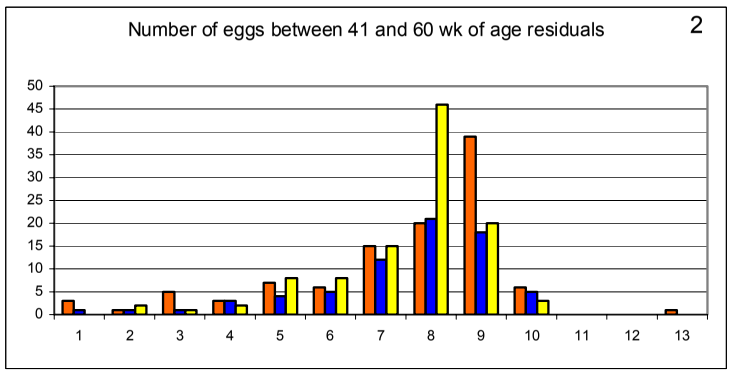

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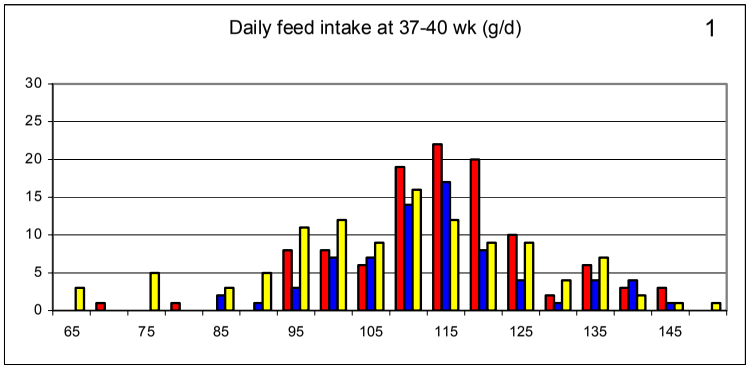
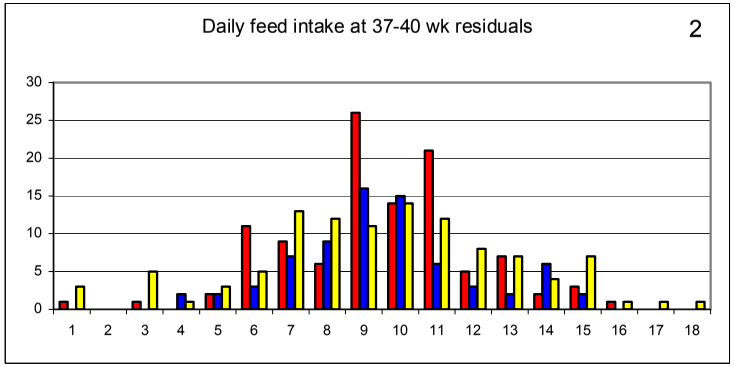
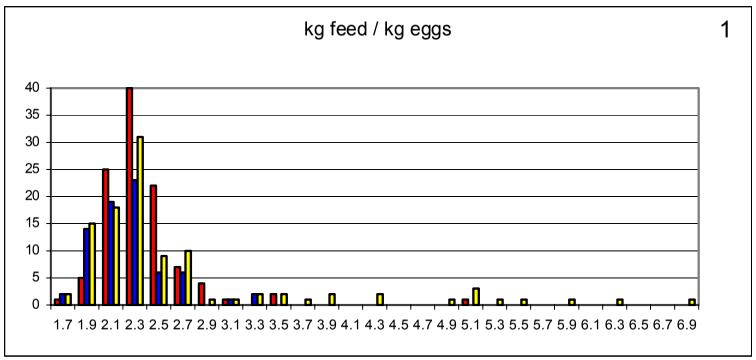






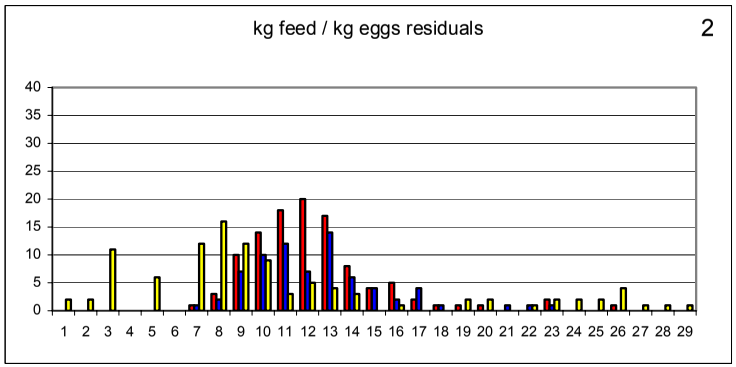


Table 3. Phenotypic correlations between egg quality and egg production traits for the F_2 generation.

		HU40	HU60	ES40	ES60	SG40	SG60	AFE	BW40	EWa	EWb	ENa	ENb	FI40	FE40
Haugh 40 wk (HU)	**HU40**	1													
Haugh 60 wk (HU)	**HU60**	0,48	1												
Egg shell strength at 40 wk (kp)	**ES40**	NS	NS	1											
Egg shell strength at 60 wk (kp)	**ES60**	NS	NS	0.54	1										
Specific gravity at 40 wk	**SG40**	NS	NS	0.67	0.47	1									
Specific gravity at 60 wk	**SG60**	0.3	0.18	0.25	0.32	0.3	1								
Age at first egg (d)	**AFE**	NS	NS	NS	NS	NS	NS	1							
Body weight at40 wk (g)	**BW40**	NS	NS	NS	NS	NS	NS	0.24	1						
Egg weight between 18-40 wk (g)	**EWa**	0.14	NS	NS	NS	-0.22	NS	0.29	0.31	1					
Egg weight between 41-60 wk (g)	**EWb**	NS	NS	NS	NS	-0.19	NS	0.22	0.35	0.89	1				
Number of eggs between 18-40 wk	**ENa**	NS	NS	-0.13	NS	NS	NS	-0.52	-0.22	-0.31	-0.31	1			
Number of eggs between 41-60 wk	**ENb**	0.18	NS	NS	NS	NS	NS	NS	NS	NS	-0.13	0.31	1		
Daily feed intake between 37-40 wk (g/d)	**FI40**	NS	NS	NS	NS	NS	-0.16	NS	0.32	0.32	0.21	0.14	NS	1	
kg feed / kg eggs	**FE40**	NS	NS	0.19	0.22	NS	0.15	0.13	0.13	NS	NS	-0.56	-0.2	NS	1

between 1.6 and 6.9 kg food / kg eggs. Only 25 animals had a FE40 above 3.0. These animals caused the distribution to be very skewed, since FI40 was normal and egg production very low.

Phenotypic correlations were computed to assess the level of dependence between traits (Table 3.). Among egg quality traits, the highest correlations were seen between the early and late period measurement of Haugh-unit and for both egg shell quality traits. The correlation between ES40 and SG40 was 0.67. Early and late periods of egg production were highly correlated (0.89). Unfortunately, the number of observations was too small to calculate genetic correlations or heritabilities. However, calculations of these values would not be representative since the genetic variation is maximised in F_2 generations.

4.3 Marker map

4.3.1 Markers

A total of 117 microsatellite markers were tested for informativity in the parental lines. Several markers were discarded due to the reasons outlined in II. The average allele number across all 99 markers was 3.9. There were 19 line specific (RIR/WL) markers. A maximum number of alleles (9) was found at loci ADL0131 and MCW0029. The minimum was 2 which was observed for 15 different markers. The average distance between markers was 23.3 cM. On the chromosome Z 5 of the 7 markers were line specific. Marker ADL0020 on chromosome 1 (problems in PCR reaction for one male) and ADL0105 in chromosome 8 (zero allele for two females) could not be genotyped for all 8 grandparents. Detailed information of markers and genotypes of the parental individuals in WL and RIR lines is given in Appendix V. On average, 90.9 % of F_2 animals could be genotyped for each marker. The lowest success rate was on linkage group E30C14W10 for marker MCW0230 that could be genotyped for only 58 per cent of individuals of the F_2 population. A total of 16 markers were genotyped with 98 % success, and 80 markers at over 90 % success. In most cases, the reasons for missing genotypes were related to problems in PCR reactions and electrophoresis.

4.3.2 Information content

Information content (IC) was calculated as the proportion of the maximum variance for all linkage groups at each cM (Spelman et al., 1996). Marker heterozygosity resulting from the number and frequency of alleles at the marker loci is the main determinant of IC. IC for additive effects of all 14 studied linkage groups is presented in Figure 3. Mean IC across all linkage groups was 0.49, varying both between- and within-different linkage groups.

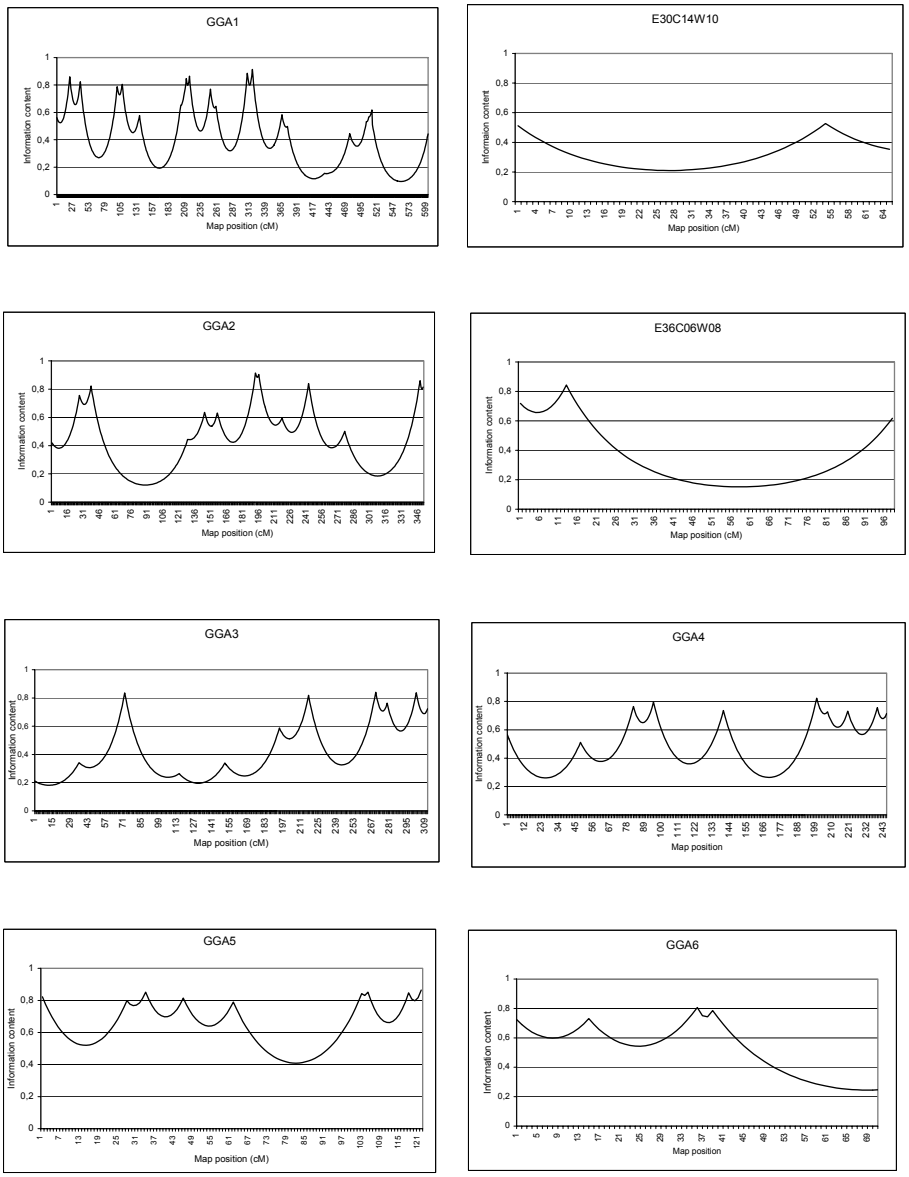
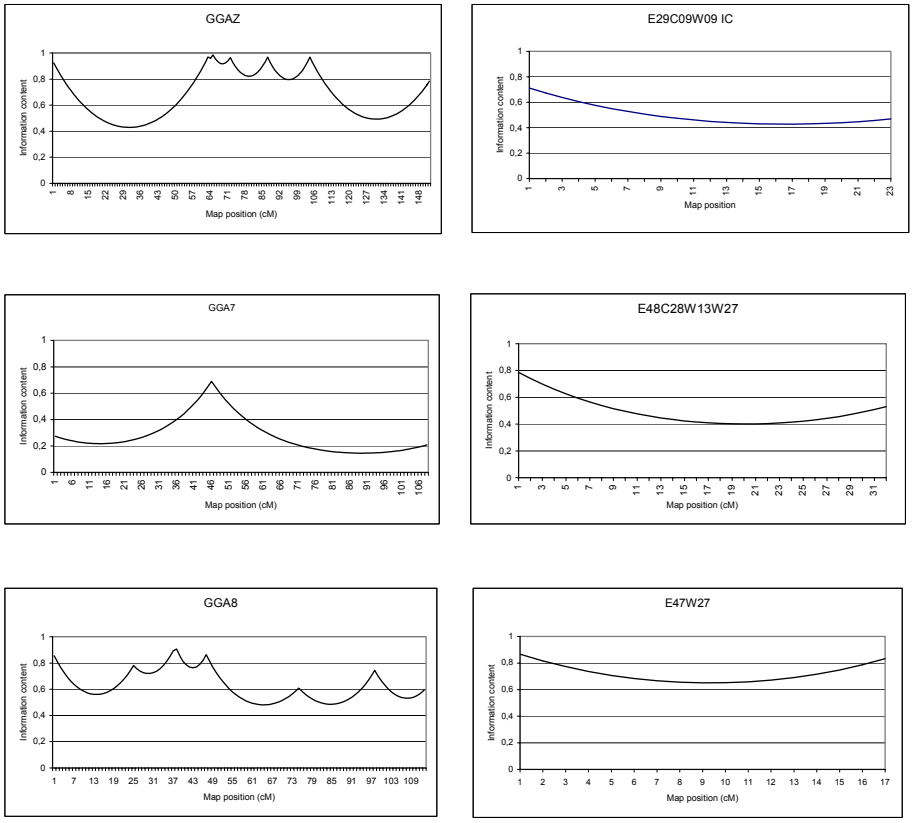


Figure 3. Information content for all studied chromosomes and linkage groups. Map positions are indicated using the Haldane mapping function.

Figure 3. continued



The minimum average IC was 0.28 on chromosome 7 and reached a maximum of 0.72 in linkage group E47W24. On chromosome areas where the distance between markers is over 30 cM and the flanking markers are not fully informative, IC is below 0.2 (refer to chromosomes 1, 2 and 7). These factors can affect the power to estimate the positioning and effect of a QTL. At the time of genotyping, polymorphic markers were not freely available to fill in all the gaps. IC is slightly lower for computing dominance effects (data not shown), which is also reflected as larger standard errors of their estimates compared to additive effects (Table 5).

4.3.3 Genetic linkage map

The 14 linkage groups covered 2311 cM (Haldane) with an average spacing of 23.3 cM between markers. Linkage (Figure 1) maps generated from this study are in general agreement with the order of markers reported in the chicken consensus linkage map (Groenen et al., 2000). The only exception is marker HUJ0012, which maps to chromosome 6 with a two-point LOD of

93.3 and an estimated recombination fraction of 0.05 between HUJ0012 and ADL0345. Marker MCW0170 has only been mapped in the East Lancing population while marker MCW0129 is only reported in the Compton map, both of which are on chromosome 4 (http://www.thearkdb. org/browser?species=chicken).

4.4 QTL analysis

The marker and phenotypic records of F_2 animals were analysed by the least squares method to reveal the association between these two sources of variation, and thereby the size and location of QTL.

4.4.1 Mendelian QTL

The Mendelian QTL results of 14 traits and 14 chromosomes are shown in Table 4. In total 12 genome-wide and 7 chromosome-wide QTL areas were found on chromosomes 2, 3, 4, 5, 8 and Z. When analysing data for publication II, phenotypic data from individual hens was only removed if the age at first egg exceeded 250 d. For some individuals single abnormal trait values were removed. It was known that the mortality before 40 weeks of age was for some sire families higher (20 %) than the average of 0-5 %. Hens died after laying only 2-60 eggs, which is considered as a low ENa record. Peritonitis and fatty liver were the main causes of death. The underlying cause may be genetic, but it was not investigated further in this study. After establishing the reasons for low values the whole data was carefully checked and erroneous records of ENa, and also for ENb (2 animals) and EWb (7 animals) were removed. The hatch effect was estimated and all QTL analysis were repeated. The revised results for significant Mendelian QTL are given in Table 5. Mendelian QTL were checked to ensure that these were not caused by a chance segregation in single families. Background genetic effects due to other QTLs on other chromosomes including chromosome Z, had no effect, indicating that there were no random associations between the analysed chromosomes.

The structure and size of the crossing experiment and the model used in the analysis allowed Mendelian QTL to be detected. These accounted for 5- 8 % of the phenotypic variation in the F_2 population. The QTL for egg weight and body weight explained 16 % and 25 % of the phenotypic variance, respectively. Data of Kerje et al. (2003) and Sewalem et al. (2002) indicate a similar percentage of variance being explained by a growth related QTL. On chromosome 4, the QTL for ENa has two peaks, at 90 and 171 cM. A two QTL model explained significantly more of the variation than a single QTL model. The two QTLs were segregating in different families, while the additive effect of both QTLs was negative, the dominance effect of one of the QTLs was highly positive (13.9 ± 4.7).

Table 4. Highest F ratios and location of QTL for all traits and chromosomes. Chromosome location and genomewide significant F ratios are indicated in bold.

	Trait													
Chromosome	HU40	HU60	ES40	ES60	SG40	SG60	AFE	BW40	EWa	EWb	ENa	ENb	FI40	FE40
1	3.09 504	3.67 272	4.16 101	2.55 105	4.18 101	3.8 603	5.99 1	5.19 298	2.65 187	3.5 229	4.26 71	3.53 281	4.56 22	3.65 119
2	**10.66** **99**	**8.16** **100**	2.1 1	1.1 164	2.87 351	1.83 348	2.02 243	3.7 32	5.44 103	**9.41** **86**	4.7 248	2.6 167	2.86 145	3.31 38
3	2.9 302	4.05 289	5.77 181	4.38 194	5.8 142	3.33 311	**7.58** **182**	1.86 7	1.78 232	2.69 239	3.37 108	1.56 153	3.46 78	4.13 206
4	**6.57** **215**	3.06 209	3.7 119	3.1 188	4.4 63	2.95 164	4.1 172	**49.7** **208**	**28.11** **214**	**28.5** **212**	**7.5, 7.4** **90, 171**	3.76 178	**8.65** **217**	4.86 184
5	1.74 89	1.9 61	5.03 32	1.27 78	**6.2** **81**	1.61 36	2.82 5	4.57 9	3.2 5	2.97 6	4.04 77	3.6 77	1.23 104	1.4 89
6	2.29 57	1.76 1	0,9 71	3,8 2	1,4 71	3,15 67	0,7 44	3,67 36	3,8 45	3,8 41	2,87 40	3,07 44	0,78 57	1,4 36
7	0,6 1	2,5 72	0,36 63	3,4 102	0,34 72	0,42 63	2,07 27	2,45 1	2,5 15	3,85 10	0,45 108	0,94 61	1,9 63	1,61 10
8	**5,9** **11**	3,14 96	3,03 96	2,96 11	3,59 42	2,82 12	3,55 61	4,09 113	4,09 9	3,15 8	2,57 54	**7,37** **113**	1,67 98	1,41 110

Table 4. continued

	Trait													
Chromosome	HU40	HU60	ES40	ES60	SG40	SG60	AFE	BW40	EWa	EWb	ENa	ENb	FI40	FE40
	1,08	1,88	1,18	2,98	0,51	0,54	0,49	3,94	1,85	0,74	0,21	3,69	1,39	0,42
E29C09W09	12	22	1	18	12	8	23	23	21	19	1	23	23	23
	0,83	2,1	0,92	1,83	0,39	1,36	1,03	2,54	1,24	0,96	2,72	2,88	2,29	1,37
E30C14W10	47	15	1	1	47	12	65	1	37	14	65	27	6	54
	2,5	0,97	1,48	2,72	2,08	1,44	4,2	2,17	2,68	1,93	3,61	5,54	5,15	0,76
E36C06W08	98	36	3	1	5	11	54	13	53	2	98	1	1	13
	2,34	1,18	0,55	3,39	0,59	1,54	0,22	0,8	2,14	2,29	1,69	1,98	2,07	4,99
E47W24	17	17	11	6	1	15	1	1	13	6	17	1	1	17
	1,88	4,03	2,13	0,69	3,26	0,67	2,09	1,53	1,8	1,21	2,93	0,22	1,37	0,49
E48C28W13W27	32	32	14	1	23	7	1	32	1	1	1	1	32	1
	5,53	1,09	**14,33**	3,5	6,67	3,01	**22,04**	2,96	**21,14**	**30,69**	**10,85**	**13,77**	1,21	4,6
Z	104	104	**118**	65	114	34	**92**	63	**78**	**80**	**84**	**83**	152	85

HU40 = Haugh unit at 40 wk of age, HU60= Haugh-unit at 60 wk of age, ES40= eggshell strength at 40 wk of age, ES60= eggshell strength at 60 wk of age, SG40= specific gravity at 40 wk of age, SG60= specific gravity at 60 wk of age, AFE = age at first egg, BW40 = bodyweight at 40 wk of age, EWa = average egg weight between 18 and 40 wk of age, EWb = average egg weight between 41 and 60 wk of age, ENa = total number of eggs between 18 and 40 wk of age, ENb = total number of eggs between 41 and 60 wk of age, FI40 = daily feed intake between 37 and 40 wk of age, FE40 = kg feed / kg eggs at 40 wk.

Table 5. Revised summary of genome-wide and chromosome wide significant QTL for egg quality and production traits on different chromosomes.

Genome wide QTL								
Trait		Linkage group	F-ratio [1]	*p*-value [2]	Confidence [3] interval 90 %	Additive [4] effect ± SE	Dominance [5] effect ± SE	R^2 %
Haugh-unit at 40 wk (HU)	HU40	2	10.66	0.004	75-133	-5.3±1.3	-3.8±3.3	7.0
Haugh-unit at 60 wk (HU)	HU60	2	8.16	0.036	85-122	-8.6±2.1	-4.0±5.9	6.0
Eggshell strength at 40 wk (kp)	ES40	Z	14.33	0.014	97-142	-0.16±0.04		5.0
Age at first egg (d)	AFE	Z	22.04	0.0003	65-137	2.76±0.59		6.8
Egg weight between 18 and 40 wk (g)	EWa	4	28.11	<0.0001	186-197	3.1±0.4	-0.8±0.7	16
		Z	21.14	< 0.0001	39-94	1.4±0.3		6.6
Egg weight between 41and 60 wk (g)	EWb	4	28.5	<0.0001	173-230	3.4±0.5	-1.0±0.7	16.9
		Z	30.69	<0.0001	65-92	1.8±0.3		8.2
		2	9.41	0.013	44-120	-3.8±0.9	-1.4±2.7	6.0
Number of eggs between 41and 60 wk	ENb	Z	13.77	0.025	15-95	-4.4±1.1		4.7
Body weight at 40 wk	BW40	4	49.70	<0.0001	194-216	189±19.0	-15.8±29.0	25.8
Daily feed intake at 40 wk of age (g/d)	FI40	4	8.65	0.028	181-227	5.22±1.34	3.8±2.02	5.0

Table 5. continued

Chromosome wide QTL Trait		Linkage group	F-ratio [1]	*p*-value [2]	Confidence [3] interval 90 %	Additive [4] effect ± SE	Dominance [5] effect ± SE	R^2 %
Haugh-unit at 40 wk (HU)	HU40	4	6.57	0.02	209-223	1.5±0.7	-2.8±1.0	4.5
		8	5.94	0.026	1-21	-0.3±0.7	3.9±1.1	4.0
Age at first egg (d)	AFE	3	7.58	0.009	153-201	-2.6±1.1	6.4±2.1	5.0
Number of eggs between 18 and 40 wk	ENa	4	7.50	0.012	30-	-6.4±1.7	-0.7±2.4	
		4	7.40	0.014	178	-3.4±2.3	13.9±4.7	
		Z	10.90	0.007	68-101	-3.5±1.1		3.6
Number of eggs between 41and 60 wk	ENb	8	7.37	0.006	99-113	8.14±2.0	4.2±2.8	5.0
Sppecific gravity of egg at 40 wk	SG40	5	6.18	00196	32-112	0.0016±0.0005	-0.0006±0.0007	4.0

[1] F ratio of regression analysis.
[2] Chromosome wide *P* value.
[3] 90 % confidence interval for the QTL position.
[4] Additive effect reported for the Rhode Island Red QTL allele is half of the average phenotypic difference between animals carrying two Rhode Island Red alleles and those carrying two White Leghorn alleles; estimates are given with standard errors.
[5] Dominance effect is the deviation of the phenotypes of heterozygous individuals from the mean of the two groups of homozygous individuals: Estimates are given with standard errors (SE).
R^2 refers to the proportion (%) of phenotypic variance explained by the QTL.

4.4.2 QTL with parent-of-origin effects

To understand the reasons for differences in reciprocal crosses parent-of-origin effects were examined. Such effects were found on chromosomes 1, 3, E30C14W10 and E36C06W08, whilst no Mendelian QTL was identified in these areas. On chromosome 1 there were three QTLs with maternal expression and one with paternal expression. Confidence intervals of QTLs were concentrated in the middle of chromosome 1. All QTL with parent-of-origin effects explained 3-5% of total phenotypic variance, which was less than that explained by Mendelian QTLs (5-25%). To validate the occurrence of parent-of-origin effects a number of additional tests were performed. The effect of chromosome Z as a possible source of error was eliminated through its use as a co-factor. A grandmaternal effect would be expected to be confounded with mitochondrial or W-chromosomal effects, whereas a maternal effect can reflect these as well as any differences in autosomal gene expression or deposition of proteins in the egg. Grandmaternal effects were significant for AFE, EWb and HU40, but did not influence parent-of-origin effects. To check that the parent-of-origin effects were not segregated in only a few animals, offspring from each of the four grandparents were selectively removed in turn; by (1) omitting offspring of the first F_1 sire; (2) omitting offspring of the second F_1 sire; (3) omitting offspring of F_1 dams; and (4) omitting all offspring from one parent. These procedures were repeated for all the four grand parents.

The results were robust in most cases against such a subdivision. Additional analysis using *QTLexpress* and GENSTAT were used to demonstrate that the same estimates could be attained independently. More details concerning the testing of parent-of-origin effects are reported in III.

This analysis revealed parent-of-origin QTLs for production traits (FI40, BW40, AFE, ENa) and egg white quality (HU40). The genes with parent-of-origin effects found in other livestock species, notably in pigs (Nezer et al. 1999, de Koning et al.2000) and sheep (Charlier et al, 2001) are related to growth. In chicken, Ikeobi et al (2002) and Sawalem et al. (2002) found no evidence for parent-of-origin effects in fatness or growth traits using the model of Knott et al. (1998). In chicken, the genes exhibiting parent-of-origin regulation of gene expression may be different. The current study demonstrates with the use of appropriate statistical tools that the observed reciprocal differences are not caused by sex-linked or mitochondrial genes. Further studies examining gene expression are required to understand the mechanisms underlying reciprocal differences. If the parent-of-origin effects or related mechanisms are involved, this represents an important biological finding.

4.4.3 Egg quality traits

4.4.3.1 F_2 QTL

The main objective of this study was to localise genome areas affecting egg quality traits. One genome-wide QTL for HU40 and HU60 (GGA2), two chromosome-wide QTL for HU40 (GGA4, GGA8), one genome-wide QTL affecting ES40 (chromosome Z) and one chromosome-wide QTL affecting SG40 (GGA5) was identified. It was expected that areas affecting Haugh-unit would be identified, because the grand parental lines differed in these traits [II]. There is one previous report on the relationship between marker allele MCW0051 and Haugh-unit at 53 weeks of age on chromosome 4 (Wardecka et al., 2002). A genome-wide significant QTL affecting HU40 and HU60 was found on chromosome 2 between 75 and 133 cM [II]. The confidence intervals of QTLs for these related traits were only marginally different, and the highest F- value was at 99 cM and 100 for HU40 and HU60, respectively, while the effect of the RIR allele was negative (–5.3) for HU40 and (–8.6) for HU60. These effects correspond to a phenotypic SD of 0.7 for both traits [II]. These QTLs explained 7 % and 6 % of the phenotypic variance in F_2. The other chromosome-wide significant QTLs for HU40 were located on chromosomes 4 and 8. However the chromosome-wide QTL on chromosome 8 had no significant additive effect and only the dominance effect was significant (3.9$\pm$1.1) (Table 6). On chromosome 4 the dominance effect was higher, and of an opposite sign to the additive effect. These results show that dominance variation is an important factor affecting egg white quality. One of the maternally expressed QTL affecting HU40 was located on chromosome 1 at 121 cM, which is close to the marker ADL0188, but the effect of this QTL was relatively small (+1.8$\pm$0.5 HU).

4.4.3.2 Fine mapping

The QTL on chromosome 2 affecting HU40 and HU60 was examined more closely. The distance between markers (MCW247 and ADL217) flanking the interesting QTL area on chromosome 2 was very high (90 cM). To refine these observations, fine mapping using shorter marker intervals and a BC generation were used. The confidence interval for HU40 and HU60 indicated several candidate genes. Of these *vimentin* was selected for sequencing [IV]. A single nucleotide polymorphism (SNP) was found between exons 4 and 5 in the *vimentin* gene after sequencing a few animals from the mapping population. These SNP VIMint4 were used as a marker in linkage analysis [IV]. The use of seven additional microsatellite markers and the SNP on chromosome 2 improved the resolution (IC>0.6). Significant evidence for the existence of two QTLs affecting HU40 and HU60 located on both sides of the former marker gap was obtained. Ambivalence in the exact number and position for the EWb QTL complicates the interpretation of experimental data.

The F statistic curves indicate three separated QTL peaks within a 180 cM area. Both first and second QTL do not overlap with the HU QTL, while the third peak at 141 cM overlaps with the QTL for HU40 and HU60 raising the possibility of pleiotropic effects. Fine mapping based on the BC did allow the QTL position to be refined, but more information of the role of this area on egg white quality and egg weight is required. However the efficiency of this approach was weakened by the lack of informative markers and the low number of individuals analysed.

4.4.4 Egg production traits

There were several QTL areas found for all measured production traits (except FE40). Most of the QTL are located on chromosomes 4 and Z. Most of the parent-of-origin QTLs are located on chromosome 1. For production traits a number of QTLs were distributed over chromosomes, such as the QTLs for AFE on chromosomes 3 and Z, for EWa on chromosomes 4 and Z and for EWb on chromosomes 2, 4 and Z.

The additive effects of different QTLs were rather similar, e.g. EWa QTL had an effect of +3.1 g on chromosome 4 and +1.4 g on chromosome Z. There were also cases of these effects acting in opposite directions. The QTL for AFE on chromosome 3 had an effect of –2.6 d and on chromosome Z an effect +2.76 d. The dominance effects were clearly significant for the three production traits with the dominance effect for AFE on chromosome 3 and for ENa QTL at 171 cM on chromosome 4 being higher than the additive effect and for ENb on chromosome 8.

The CI for the respective QTLs on chromosomes 4 and Z overlapped. Additive effects of the QTLs increased egg weight and decreased the number of eggs during both production periods. These findings indicate that the same QTLs affect egg production for the whole production period. Multi-trait models will yield more information of possible pleiotropic effects.

After adding more markers on chromosome 2, the EWb QTL divided into two QTLs with a larger negative additive effect [IV]. Kerje et al. (2003) found a QTL for egg weight at 29 weeks of age on chromosomes 1, 3 and 4 in a cross between Red Jungle fowl and White Leghorn, but these QTLs are not consistent with those identified in this study.

Age at first egg and egg number are important traits in laying hens. AFE affects egg number during the early production period. In this study, Mendelian and parent-of-origin differentiated QTLs were shown to affect AFE, ENa and ENb (Figure 1). CI on chromosome Z were overlapping for AFE, ENa and ENb. It appears as if the same area on chromosome Z is affecting egg production during the whole production period. On the autosomes there is only

one QTL affecting only one trait, namely the QTL for AFE on chromosome 3 and ENb on chromosome 8. CI on chromosome 1 for the paternally expressed QTL affecting AFE and maternally expressed QTL for BW40 partly overlap. Additive effects are in the same direction and are both negative [III].

4.4.5 Growth related QTLs

In this study the growth traits were BW40, FI40 and FE40, all of which were measured in mature animals. There are no previous QTL results for growth traits of crosses exclusively between laying lines. Therefore comparisons are made with the results obtained from crosses where one or both parental lines included broilers or exotic breeds.

Mendelian QTLs were found for BW40 and FI40 at the end of chromosome 4. The QTL for BW40 explained 25.8 % of the variance with an additive effect of 189 g. CI for BW40, FI40, EWa and EWb were overlapping. Sewalem et al. (2002) identified a QTL on the same area that affected body weight (at 6 and 9 weeks of age) in a broiler x layer cross, with an additive effect of 249 g. A QTL for body weight and egg weight in the vicinity of the same region has also been reported (Kerje et al., 2003). de Koning et al. (2003) observed similar results on chromosome 4 for a population of three generations produced using commercial broiler lines. Overall, the results tend to suggest that there are genes in this area that affect juvenile growth in broilers and mature body weight in layers.

No significant Mendelian QTL on chromosome 1 (Table 4) were identified. After analysing chromosome 1 including an allowance for parent-of-origin effects three growth related genome-wide QTL affecting AFE, BW40 and FI40 were found [III]. These QTLs were located between markers ADL0188 and MCW0046 in the middle of the chromosome. CI for AFE and BW40 overlapped. The region where the parent-of-origin QTL for BW40 was located is much the same area where Mendelian QTL have been identified for different growth traits: body weight at 13 and 16 weeks in a F_2 population originating from a male native Japanese meat chicken male and female White Plymouth Rock heavy broiler female (Tatsuda and Fujinaka, 2001), a QTL for abdominal fat percentage of 10 weeks (Jennen et al., 2004) and also a QTL for feed intake between 23 and 48 d (van Kaam et al., 1999). This area on the longest chicken chromosome (chromosome 1) shows conversation areas with part of the human map on chromosomes 22, 12 and 17. There are many mapped genes in this area known to affect metabolic pathways (Schmid et al., 2000). At the beginning of chromosome 1 (at 68 cM) Kerje et al. (2003) have identified a QTL affecting growth and body weight in a cross between red jungle fowl and WL. However these findings were not verified in this study.

4.4.6 Chromosome Z

Genome-wide significant QTLs for ENb, EWa, EWb, ES40 and AFE and a chromosome-wide QTL for ENa were found on chromosome Z. The respective F ratio profiles and CI are presented in Figure 4. The area between MCW0258 and MCW0246 was the most interesting. This area affected egg number and egg weight during both production periods. One potential gene in this region Z is the VLDLR (Very Low Density Lipoprotein Receptor) gene, which is located 3 cM from MCW0154 on the consensus chicken map (https://acedb.asg.wur.nl/chickdb/generic/pic?name=GGAZ;class=Map). The genetic and biochemical functions of the VLDLR have been documented. The receptor is known to interact with the key step in the deposition of yolk components of chicken oocytes (Nimpf and Schneider, 2000). The absence of VLDLR function leads to almost a complete lack of egg production (Elkin and Zhong, 2002). This gene is a suitable candidate for controlling variation in egg weight and egg number.

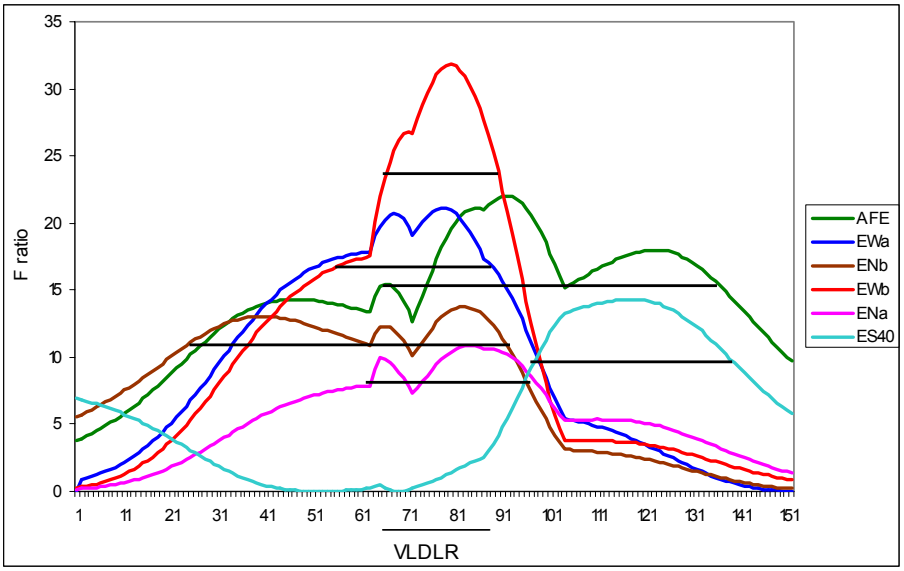


Figure 4. Multiple marker analysis using regression for QTL on chromosome Z in a F_2 population. All marker map positions were calculated using the Haldane mapping function. The F ratio profiles are for genome-wide significant QTL affecting average egg weight between 18 and 40 wks of age (EWa), average egg weight between 41 and 60 wks of age (EWb), total number of eggs between 18 and 40 wks of age (ENa), total number of eggs between 41 and 60 wk of age (ENb), age at first egg (AFE) and average egg shell strength at 40 wk of age (ES40). Black lines represent 90 % confidence intervals for the QTL position. The localisation of the VLDLR gene (Schmid et al., 2000) is indicated by a line under the horizontal axis.

CI for ES40 and AFE were overlapping in the region between MCW0246 and MCW0128. At the end of the chromosome there is an interesting candidate gene i.e. the lipoprotein lipase gene (LPL). Lipoprotein lipase is involved in the transport and transformation of yolk lipids and in the metabolism of adipose tissue (Speake et al., 1998). The gene is mapped at the end of chicken chromosome Z (Crooijmans et al., 1995). A significant QTL affecting live weight at 3 wk of age and a putative QTL affecting abdominal fat weight at 9 wk of age has been identified on chromosome Z in a QTL study where an egg layer and broiler line were crossed (Ikeobi et al., 2002; Sewalem et al., 2002). The QTL confidence intervals for live weight at 3 wk, abdominal fat weight and abdominal fatness covered the same area as for AFE and ES40 in this study. There was no information on abdominal fat content in the current experimental animals but a minimum level of abdominal fatness is known to be required for the onset of sexual maturity (Chambers, 1990). In a three-generation half-sib analysis stemming from broiler dam lines no QTL affecting growth traits were found on chromosome Z (Hamoen et al., 2001; Emara and Kim, 2003). One possible explanation for overlapping CI for AFE and ES40 QTLs is the association between increasing fatness and the body weight with decreases in egg production and increased incidence of abnormal egg shell quality demonstrated in a broiler breeder experiment (Richards et al., 2003). AFE is dependent on live weight and body composition. The overlapping region for AFE and ES40 is particularly long (40 cM) in this study. This area contains some 400 genes (Emara and Kim, 2003). The LPL gene includes two microsatellite markers (MCW0070 and ROS0100). For confirmation the effects of LPL on AFE and ES40 specific genotyping for this gene in the current experimental animals would be required.

5 Conclusions

This study is the first to map QTLs for egg quality and production traits in a cross between two contemporary egg layer lines. Most of the published QTL studies thus far have dealt with fat and growth traits in broilers. The results from this study indicate that there are overlapping areas for egg production and growth traits on chromosomes 1, 4 and Z.

The power of the experimental design allowed for the detection of a QTL explaining 5-8 % of phenotypic variance. QTL were found for all recorded traits except egg shell quality traits during the late production period and feed efficiency. The most significant findings relate to the identification of QTLs that affected body weight and egg weight on chromosome 4. These QTLs explained 25.8 % of phenotypic variation for body weight at 40 weeks of age and 16.9 % for egg weight at 40 weeks of age.

Egg white quality (HU) and egg weight QTLs were found on chromosome 2. These affected HU both in the early and late production period and egg weight during the late production period. More detailed analysis utilising more markers in combination with the BC generation provided evidence that the QTL region does in fact contain two QTLs for Haugh-unit (both in the early and late production period) and at least two QTL for egg weight. The egg weight at 41-60 weeks of age (EWb) and Haugh-unit (HU40 and HU60) QTL are located in different areas, with the exception of the third QTL for EWb and second QTL for HU which may possibly reflect pleiotropic effects.

One of the important features of the current study was the reciprocality of the cross. The reciprocal nature of the experimental design allows for the detection of QTLs on the sex chromosome and also to identify potential parent-of-origin effects of QTL. It has been postulated that chromosome Z is one important reason underlying reciprocal differences between egg layer lines. Analysis of chromosome Z demonstrated the importance of this chromosome to egg production and growth traits. The parent-of-origin effects of QTLs showed that there are differences when the allele is inherited maternally or paternally. These findings are based on statistical methods developed specifically for this purpose. This phenomenon should not occur in birds because the genes that are imprinted affect foetal growth in eutherian mammals. In this study there were QTLs found with paternal or maternal expression affecting growth and production traits in addition to egg white quality. Similar to some of the Mendelian QTL, several QTLs with parent-of-origin effects were cryptic so that the direction of the effect was in complete contrast to the grand parental lines. Overlapping confidence intervals on chromosome 1 for several previously identified Mendelian QTL for body weight and fat traits and the parent-of-origin QTL for body weight at 40 weeks of age raise hopes of detecting candidate genes that affect growth in this region.

The poultry breeding industry is keen to adopt markers or candidate genes as selection tools. Based on this study and other experiments it is clear that there are several interesting chromosomal areas harbouring potential genetic variation for production traits (Ikeobi et al., 2002; Jennen et al., 2004; Kerje et al., 2003; Sewalem et al., 2002; Tatsuda and Fujinaka, 2001; van Kaam et al., 1998; van Kaam et al., 1999). Before the industry can implement new selection tools more detailed research is required. Current QTLs are family based and mainly responsible for population differences. The industry is more concerned in within population variation and w-ould therefore require markers that are valid over families and more direct evidence of genes that affect production or health traits. The chicken genome sequence and comparative mapping should provide new tools to improve the understanding of the physiological basis of growth and egg production, as well as generating information for gene expression studies. More sophisticated models (multiple QTL and multi-trait models) should also be used to establish the existence of epistatic and pleiotropic effects.

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