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# Many QTLs with minor additive effects are associated with a large difference in growth between two selection lines in chickens

LINA JACOBSSON[1], HEE-BOK PARK[2], PER WAHLBERG[2], ROBERT FREDRIKSSON[3], MIGUEL PEREZ-ENCISO[4,5], PAUL B. SIEGEL[6] AND LEIF ANDERSSON[1,2]*

[1] *Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, BMC, SE-75124 Uppsala, Sweden*
[2] *Department of Medical Biochemistry and Microbiology, Uppsala University, BMC, SE-75124 Uppsala, Sweden*
[3] *Department of Neuroscience, Uppsala University, BMC, SE-75124 Uppsala, Sweden*
[4] *Institut Català de Recerca i Estudis Avançats, 08010 Barcelona, Spain*
[5] *Department de Ciència Animal i del Aliments, Facultat de Veterinária, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*
[6] *Virginia Polytechnic Institute and State University, Department of Animal and Poultry Sciences, Blacksburg, VA 2406-0300, USA*

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**Summary**

Two growth-selected lines in chickens have been developed from a single founder population by divergent selection for body weight at 56 days of age. After more than 40 generations of selection they show a nine-fold difference in body weight at selection age and large differences in growth rate, appetite, fat deposition and metabolic characteristics. We have generated a large intercross between these lines comprising more than 800 $F_2$ birds. QTL mapping revealed 13 loci affecting growth. The most striking observation was that the allele in the high weight line in all cases was associated with enhanced growth, but each locus explained only a small proportion of the phenotypic variance using a standard QTL model (1·3–3·1 %). This result is in sharp contrast to our previous study where we reported that the two-fold difference in adult body size between the red junglefowl and White Leghorn domestic chickens is explained by a small number of QTLs with large additive effects. Furthermore, no QTLs for anorexia or antibody response were detected despite large differences for these traits between the founder lines. The result is an excellent example where a large phenotypic difference between populations occurs in the apparent absence of any single locus with large phenotypic effects. The study underscores the need for powerful experimental designs in genetic studies of multifactorial traits. No QTL at all would have reached genome-wide significance using a less powerful design (e.g. approx. 200 $F_2$ individuals) regardless of the nine-fold phenotypic difference between the founder lines for the selected trait.

## 1. Introduction

A number of selection experiments have revealed that remarkable selection responses can be obtained for almost any multifactorial trait in plants and animals (Falconer & Mackay, 1996; Lynch & Walsh, 1998). An excellent example of this genetic plasticity is two selection lines in chickens that have been established by divergent selection on a single trait: body weight at 56 days of age (Dunnington & Siegel, 1996). This selection experiment was initiated 1957 by crossing seven partially inbred lines of White Plymouth Rock chickens. After more than 40 generations of selection in opposite directions, the high- and low-weight lines show a remarkable nine-fold difference in 56 day body weight (Fig. 1). The strong selection response documents that this trait is highly heritable and the realized heritability through the fourth generation was 0·29 (Siegel, 1962). A number of interesting correlated responses have been observed between the two lines, including large differences in appetite. High-line chickens are hyperphagic whereas low-line chickens have very low appetite and tend to be anorexic (Burkhart *et al.*, 1983). As a consequence,

* Corresponding author. Department of Medical Biochemistry and Microbiology, Uppsala University, BMC, Box 597, SE-75124 Uppsala, Sweden. Tel: +46 18 4714904. Fax: +46 18 4714833. e-mail: Leif.Andersson@imbim.uu.se

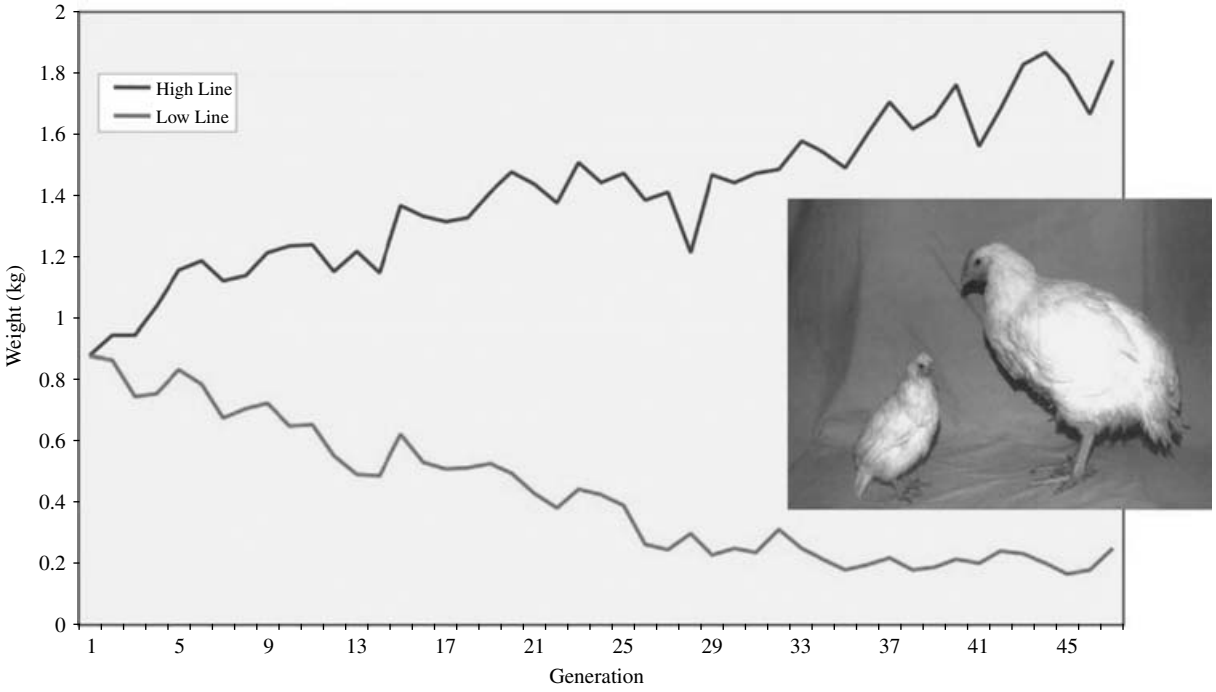


Fig. 1. Body weight at 56 days of age from generation 1 to 47 of males from the chicken lines selected for high and low body weight. The chickens in the photograph are from generation 37 and are 56 days old.

the high-line chickens are feed-restricted after 56 days of age (selection age) to avoid severe metabolic disorders. In contrast, anorexic individuals are observed in the low line. It became evident around generation 25 that a considerable number of females did not enter egg production and this was having an effect on selection intensity (Siegel & Dunnington, 1987). In recent generations 5–20 % of the chicks have failed to survive during the first couple of weeks after hatch because they simply never start to eat and a proportion of the females fails to reach sexual maturity (commence egg production). These individuals can, however, be brought into egg production when force-fed (Zelenka *et al.*, 1988). This anorexic condition is similar to that reported in humans (Frisch, 2002). Furthermore, high-line chickens will become obese as adults unless their feed intake is restricted whereas low-line chickens are extremely lean even when fed *ad libitum*.

A less expected correlated response is that antibody response is greater in the low than high line following immunization with sheep red blood cells (SRBC). Interestingly, two independent studies on divergent selection for SRBC antibody response have revealed a corresponding correlated response so that a higher body weight was obtained in the lines selected for low antibody response (Boa-Amponsem *et al.*, 1998; Paramentier *et al.*, 1996; Pinard van der Laan *et al.*, 1998). Furthermore, a comparison of the immune response of a 2001 commercial broiler line with a 1957 random-bred control line also revealed a negative correlation between growth and antibody production (Cheema *et al.*, 2003). Thus, competition for resources between growth and immunocompetence may cause these correlations.

We have generated a large intercross population between the high and low lines as a resource for genetic dissection of QTLs that have responded to the divergent selection. The size of the experiment (>800 $F_2$ animals in large half-sib families) was chosen to allow the detection of QTLs with small and moderate effects. A genome scan based on 145 genetic markers covering about 80 % of the chicken genome is reported here.

## 2. Materials and methods

### (i) *Animals*

The two selection lines which formed the parental population for the experiment were developed and maintained at the Virginia Polytechnic Institute and State University in Blacksburg, Virginia, USA. The common founder population originated from crosses of seven partially inbred lines of White Plymouth Rock chickens. The two lines have been maintained as closed populations selected for either high or low body weight at 56 days of age (Liu *et al.*, 1994; Dunnington & Siegel, 1996). The only conscious husbandry modification made through time was that vaccination for Marek's disease was commenced in generation 18. From generation 41 of this long-term selection experiment, a reciprocal intercross was

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designed so that 10 high-weight males were mated to 22 low-weight females and 8 low-weight males were mated to 19 high-weight females. From the $F_1$ generation, 8 males and 75 females were reciprocally intercrossed and 874 $F_2$ chickens from a single hatch were used for the QTL study. Matings were at random but matings between sibs were avoided. All phenotype recordings were performed on males and females in the facilities where the selection experiment was conducted using the same dietary formulation of a corn soybean mash ration containing 20% crude protein and 2685 kcal ME/kg of diet. Feed and water were provided *ad libitum*. Rearing was on wood shavings in 16 floor pens of about 50 chickens each in the same windowless house as where the lines underwent selection. Lighting was continuous to day 28 after which the photoperiod was from 0200 to 2200 hours.

Blood samples for DNA preparations were collected at 35 days of age and a second sample was collected at 70 days of age in those cases where the amount of blood obtained at 35 days was too small.

(ii) *Phenotypic traits*

The body weight of each $F_2$ chicken was obtained at hatch, 14, 28, 42, 56 and 70 days of age. Packed cell volume (PCV) was measured at 39 days of age using standard methods with microhaemocrit capillary tubes. Blood protein was measured at 49 days with a Veterinary Refractometer A300CO (Altago, Tokyo, Japan). At 49 days of age chickens received an injection into the brachial vein of 0·1 ml of a 0·5% suspension of SRBC antigen (Siegel & Gross, 1980; Martin *et al*., 1990). Five days later a sample of 0·5 ml of blood was obtained from the brachial vein of each individual and transferred to tubes containing two drops of 5·5% EDTA. Blood samples were stored at 8 °C overnight to allow the blood cells and plasma to separate. Antibody determinations were made following the microtitre haemagglutination procedures of Wegmann & Smithies (1966). Titres are expressed as the $\log_2$ of the reciprocal of the last dilution in which agglutination was microscopically observed.

A number of $F_2$ birds died because of anorexia as we previously observed among the low-line birds (Dunnington & Siegel, 1996). We strongly believe that these birds were anorexic because there was no evidence of infectious agents and of the individuals that were necropsied there was little if any food in the gastrointestinal tract. These $F_2$ individuals died early after hatch because either they did not start to eat after hatch or their feed intake was inadequate for survival. We used two classifications related to the anorexia phenotype: death, defined as 2 if the bird lived throughout the experiment and 1 if it died, and survival, where the birds were assigned the number of weeks they survived.

(iii) *Linkage map*

A genetic map comprising 26 linkage groups was established based on 145 genetic markers (Jacobsson *et al*., 2004). The total map length, summarizing the intervals flanked by markers, was 2521·9 cM. The average distance between adjacent markers assigned to linkage groups was 17·0 cM. However, there were seven gaps greater than 40 cM. The average information content at marker positions was 0·72 when information on flanking markers was taken into account. With few exceptions, the derived linkage map was in excellent agreement with the chicken consensus map (Schmid *et al*., 2000) and with the chicken genome assembly (International Chicken Genome Sequencing Consortium, 2004). We estimated that our linkage map covers about 3180 cM corresponding to approximately 80% of the chicken genome (total map distance is ~4000 cM). This estimate was obtained by adding 20 cM on each side of each linkage group and by counting each single marker, showing no linkage to other markers, as covering 40 cM (20 cM on each side). The estimated map distance exceeding 40 cM for gaps larger than 40 cM was subtracted from the total map length. We assumed that any major QTL located within 20 cM of a single marker should result in at least suggestive evidence for linkage given the large $F_2$ material. This leaves approximately 20% of the chicken genome, including 13 microchromosomes, that was not covered in the present genome scan.

(iv) *Statistical analysis*

An analysis of variance (ANOVA) was performed using the Minitab software (Minitab, 2000) to identify sources affecting phenotypic variation. Effects of sex and/or family were significant and were therefore included as fixed effects in the model for QTL analysis. Residuals derived from the ANOVA were used as dependent variable in the regression analysis for QTL mapping. Fixed effects used in the QTL analysis of each trait are listed in Table 1.

Programs based on the least squares method for outbred populations were employed for QTL analysis of the autosomes (Haley *et al*., 1994). Marker genotypes were used to estimate probabilities of the parental origin of each gamete at 1 cM intervals through the genome. These conditional probabilities given marker genotypes were used to calculate coefficients of additive and dominance components for a putative QTL at each position under the assumption that the QTL was fixed for alternative alleles in the high and low parental line. The phenotypic data were regressed onto these coefficients in intervals of 1 cM. At each position, an *F*-test for QTL segregation was carried out. The Web-based QTL Express program was used

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Table 1. *Summary of the studied phenotypes with fixed effects included in the QTL analyses*

| Trait | *n* | Mean | SD | Fixed effects |
|---|---|---|---|---|
| Body weight (g) | | | | |
| at hatch | 874 | 27·8 | 2·1 | Family |
| at 14 days | 874 | 75·2 | 14·9 | Family, sex |
| at 28 days | 871 | 179·1 | 56·8 | Family, sex |
| at 42 days | 809 | 365·5 | 113·1 | Family, sex |
| at 56 days | 795 | 621·6 | 186·9 | Family, sex |
| at 70 days (g) | 789 | 943·3 | 262·1 | Family, sex |
| Response to SRBC (titre) | 798 | 6·7 | 3·4 | Family |
| Packed cell volume (% cells) | 715 | 33·8 | 4·1 | Family |
| Blood protein (g/100ml) | 800 | 39·3 | 3·5 | Family |
| Growth (g) | | | | |
| 0–14 days | 874 | 47·4 | 14·7 | Family, sex |
| 14–28 days | 871 | 103·8 | 47·3 | Family, sex |
| 28–42 days | 809 | 179·5 | 68·1 | Family, sex |
| 42–56 days | 794 | 251·7 | 88·6 | Family, sex |
| 56–70 days | 788 | 320·7 | 94·9 | Sex |

*n*, number of individuals; SRBC, sheep red blood cells.

for this single QTL analysis (http://qtl.cap.ed.ac.uk; Seaton *et al*., 2002).

The additive and dominance regression indicator variables for the most significant QTLs detected in the initial scan were added as covariates and a new genome scan was done using the updated model. Inclusion of the previously detected QTLs to the model should decrease the residual error variance and thereby increase the statistical power to detect QTLs with smaller effects (Jansen, 1993; Zeng, 1993). Coefficients of additive and dominance components for putative QTLs at each position through the genome computed by QTL Express were transferred to the QTL Fast program (Carlborg & Andersson, 2002; Ljungberg *et al*., 2002) for these analyses. QTL analysis for the Z chromosome was performed using Qxpak based on the dosage compensation model (Pérez-Enciso & Misztal, 2004).

Genome-wide and chromosome-specific empirical significance levels of the test statistic were established by randomization using 1000 permutations of data (Churchill & Doerge, 1994). Genome-wide thresholds for highly significant ($\alpha = 0{\cdot}01$) and significant linkage ($\alpha = 0{\cdot}05$) were employed as proposed by Lander & Kruglyak (1995). Since there is significant length heterogeneity among chicken chromosomes, thresholds for chromosome-wide significance varied considerably among chromosomes depending on the number of markers and the map length. Therefore, the chromosome-wide 5% significance levels for chromosome 4 were used as a suggestive significance threshold for each trait. The value for chromosome 4 was chosen because the map length of this chromosome constitutes approximately 5% of the total chicken genetic map length (i.e. about 4000 cM). Thus, by using this suggestive significance threshold we expected to observe one type I error on average per genome scan and trait. Regression analysis to estimate the residual variance explained by the detected QTLs was conducted using Minitab (Minitab, 2000).

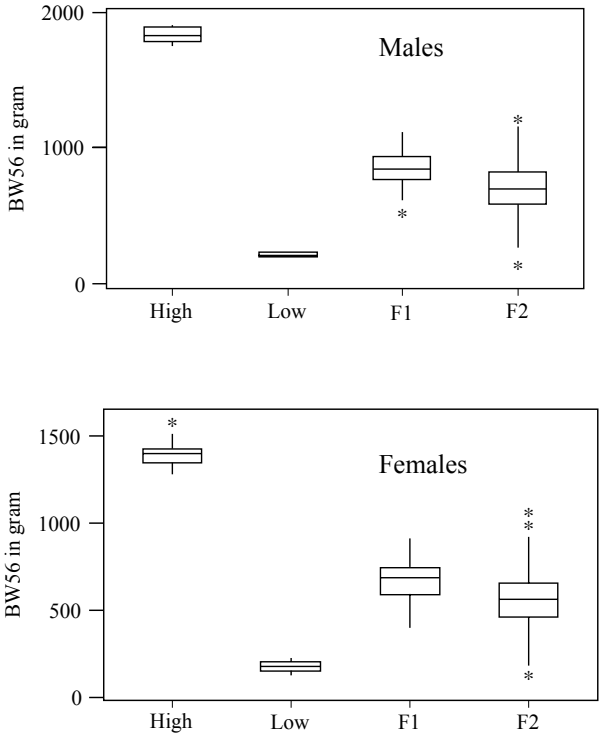


Fig. 2. Box plots for body weight at 56 days of age (BW56) for the high and low body weight lines and their $F_1$ and $F_2$ intercrosses. Outliers are marked by asterisks.

## 3. Results

### (i) *QTL analysis of growth*

Descriptive statistics for the phenotypic traits analysed in this study are compiled in Table 1. Fig. 2 shows box plots for body weight at 56 days, the trait under selection, for the parental lines and their intercrosses. The $F_1$ and $F_2$ progeny had an intermediate body weight but the observed mean values were below the arithmetic average for the parental lines, in particular for the $F_2$ generation. This is consistent with the previous finding of negative heterosis in $F_1$ crosses of these lines from generations 29 through 36 (Liu *et al*., 1993). The variance was, as expected, largest in the $F_2$ generation.

No QTL for weight at hatch was found, which was not unexpected since it has long been known that this trait primarily reflects the phenotype of the dam rather than the genotype of the progeny (Halbersleben & Mussehl, 1922). The results of the

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Table 2. *Quantitative trait loci (QTL) for body weight (BW) and growth (GR) detected in an intercross between two chicken lines divergently selected for growth to 56 days of age*

| QTL | Position Chr : cM | Trait (g) | *F* value[a] | Additive effect ± SE[b] | Dominance effect ± SE[b] | Variance (%)[c] | Marker 1 (cM)/Marker2 (cM)[d] |
|---|---|---|---|---|---|---|---|
| G1 | 1 : 437 | GR56–70 | 11·3** | 19·7 ± 4·2 | −5·2 ± 6·4 | 2·8 | *LEI079* (414·2)/*LEI134* (523·6) |
| G2 | 2 : 115 | BW56 | 6·1† | 26·8 ± 7·9 | 12·8 ± 11·9 | 1·5 | *MCW063* (72·8)/*MCW130* (127·5) |
| G3 | 2 : 253 | BW70 | 6·2† | 37·5 ± 11·2 | −17·2 ± 17·5 | 1·6 | *LEI147* (216·4)/*MCW245* (293·4) |
| G4 | 3 : 123 | BW28 | 5·7† | 10·6 ± 3·2 | −6·5 ± 6·1 | 1·3 | *MCW222* (72·3)/*MCW224* (211·7) |
| G5 | 3 : 243 | BW42 | 6·2† | 21·0 ± 6·0 | −7·5 ± 10·7 | 1·5 | *MCW224* (211·7)/*LEI065* (262·1) |
| | 3 : 252 | BW56 | 5·8† | 35·4 ± 10·4 | 4·3 ± 20·0 | 1·4 | |
| G6 | 4 : 50 | GR42–56 | 6·4† | 23·0 ± 6·6 | −9·5 ± 17·5 | 1·6 | *ADL317* (11·5)/*MCW251* (90·3) |
| | 4 : 51 | BW56 | 7·8† | 54·2 ± 14·0 | −21·0 ± 37·1 | 1·9 | |
| | 4 : 52 | GR56–70 | 9·7** | 30·3 ± 6·9 | 8·5 ± 18·1 | 2·4 | |
| | 4 : 54 | BW70 | 9·0* | 77·8 ± 18·4 | −8·6 ± 47·9 | 2·2 | |
| | 4 : 62 | GR28–42 | 7·2† | 18·2 ± 4·8 | 4·7 ± 11·3 | 1·8 | |
| | 4 : 62 | BW42 | 6·4† | 28·6 ± 8·0 | 4·2 ± 18·7 | 1·6 | |
| G7 | 4 : 148 | GR28–42 | 6·3† | 13·2 ± 3·8 | 4·6 ± 6·9 | 1·5 | *LEI125* (136·4)/*LEI076* (182·5) |
| | 4 : 149 | BW42 | 7·4† | 23·9 ± 6·3 | 9·8 ± 11·7 | 1·8 | |
| | 4 : 151 | BW70 | 8·3* | 56·3 ± 14·0 | 16·6 ± 26·8 | 2·1 | |
| | 4 : 151 | BW56 | 6·8† | 37·4 ± 10·5 | 20·1 ± 20·1 | 1·7 | |
| | 4 : 151 | BW28 | 6·1† | 11·3 ± 3·3 | 4·6 ± 6·3 | 1·4 | |
| G8 | 5 : 107 | BW70 | 7·0† | 42·4 ± 11·8 | 25·3 ± 18·6 | 1·8 | *MCW038* (74·6)/*MCW029* (130·9) |
| G9 | 7 : 42 | GR42–56 | 9·3* | 16·7 ± 3·9 | −4·0 ± 6·1 | 2·3 | *ADL169* (0)/*MCW120* (75·9) |
| | 7 : 43 | BW56 | 12·6** | 41·0 ± 8·4 | −15·0 ± 13·0 | 3·1 | |
| | 7 : 44 | BW42 | 9·3* | 21·7 ± 5·2 | −9·0 ± 8·1 | 2·3 | |
| | 7 : 44 | GR28–42 | 8·5* | 12·3 ± 3·2 | −6·6 ± 4·9 | 2·1 | |
| | 7 : 63 | GR56–70 | 6·5† | 17·8 ± 5·0 | 4·9 ± 9·1 | 1·6 | |
| | 7 : 66 | BW70 | 10·3** | 63·6 ± 14·0 | −13·4 ± 26·6 | 2·6 | |
| G10 | 13 : 0 | BW42 | 5·6† | 16·0 ± 5·5 | 13·8 ± 8·8 | 1·4 | *ROS0325* (0)/*ADL225* (33·2) |
| | 13 : 4 | BW70 | 6·5† | 35·0 ± 11·1 | 27·2 ± 16·4 | 1·6 | |
| | 13 : 4 | GR56–70 | 5·9† | 13·6 ± 4·1 | 5·2 ± 6·1 | 1·5 | |
| G11 | 20 : 9 | BW70 | 6·1† | 17·8 ± 16·2 | −110·5 ± 34·8 | 1·5 | *MCW119* (0)/*ADL125* (31·1) |
| G12 | 20 : 61 | GR0–14 | 13·4** | 4·6 ± 0·9 | −2·1 ± 1·9 | 3·1 | *ADL125* (31·1)/*BMP7* (95·4) |
| | 20 : 62 | BW14 | 12·7** | 4·5 ± 0·9 | −2·1 ± 1·9 | 2·9 | |
| G13 | 28 : 0 | BW14 | 6·0† | 1·3 ± 0·8 | −4·4 ± 1·4 | 1·4 | *MCW227* (0)/*MCW227* (0) |

Body weights were obtained at hatch, and at 14, 28, 42, 56 and 70 days of age. Growth between body weight measurements was also calculated. Test statistics, estimated QTL effects and the percentage of residual variance explained by each QTL are given. The QTLs are numbered *Growth1* (G1) to *Growth13* (G13).

[a] *F* statistic for the QTL and level of significance: ** genome-wide 1% significance; * genome-wide 5% significance; † suggestive 5% significance.

[b] The additive and the dominance effects were defined as the deviation of animals homozygous for the high line allele or heterozygous, respectively, from the mean of the two homozygotes. SE, standard error.

[c] Reduction of residual variance for the $F_2$ population when including a QTL at the given position.

[d] Markers flanking the QTL interval estimated by the one-LOD drop method and their positions in Kosambi cM in our linkage map (Jacobsson *et al.*, 2004).

QTL analysis of growth and body weight traits are summarized in Table 2. Our interpretation of these data is that they reflect 13 different loci, denoted *Growth1* to *Growth13*. The presence of more than one QTL on some chromosomes was investigated by examining the QTL graphs for each chromosome. However, a second QTL was only inferred in those cases where the statistical significance was maintained even when the primary QTL (the one with the strongest statistical support) on the same chromosome was included as a cofactor in the QTL analysis. The allele derived from the high-weight line was associated with enhanced growth for all loci. This suggests that the majority of these loci are true QTLs, although only five reached genome-wide significance. With the exception of *Growth11* and *Growth13*, all loci showed largely additive effects (Table 2). The two suggestive QTLs, *Growth11* and *Growth13*, showed negative overdominance implying a reduced growth in the heterozygotes.

The strong bias for QTL alleles inherited from the high line to be associated with high growth is illustrated in Fig. 3, where the estimated additive (*a*) substitution effect is plotted across the genome. A positive *a* value, implying enhanced growth associated with the allele from the high line, was observed on 22 of 25 autosomes and for 77% of the genome. The data clearly illustrate that many loci across the

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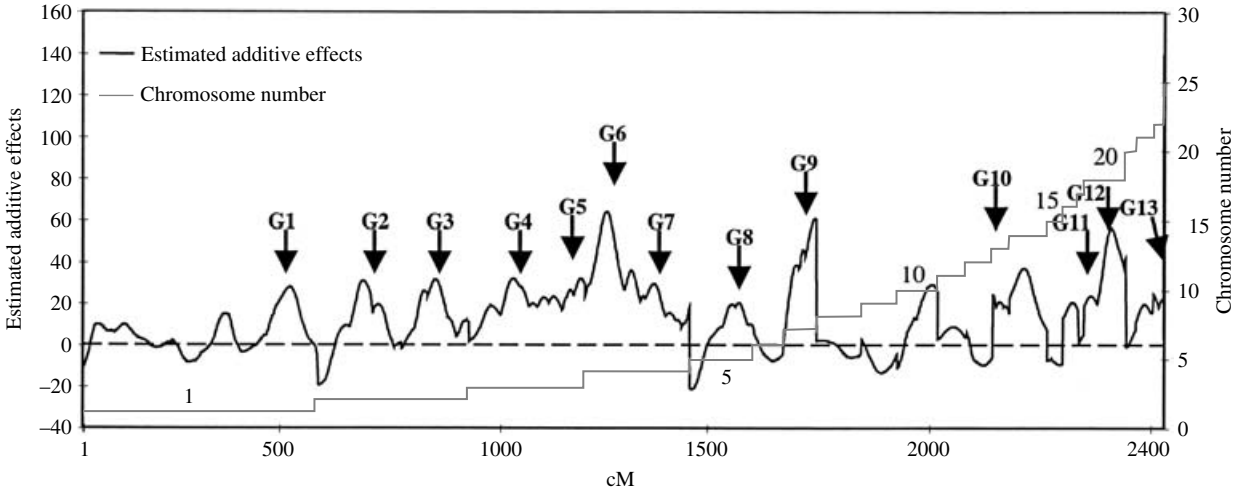


Fig. 3. Plot of the estimated additive (*a*) effects across the chicken genome in a QTL analysis of body weight at 56 days based on an intercross between the high and low growth lines. A positive *a* value indicates that the allele from the high line is associated with high growth. The peak positions of the QTLs detected by segregation analysis are indicated.

genome have responded to the selection. None of the peaks showing a negative *a* value, implying high growth associated with the low-line allele, reached even suggestive significance.

Each QTL explained only a small proportion of the phenotypic variance, 1·3–3·1%, in the $F_2$ generation (Table 2). We included all QTLs (except *Growth11* and *Growth13* which did not show any significant additive effect) in a joint least squares analysis to estimate their individual effect as well as their combined effect on body weight at 56 days (Table 3). Since most QTLs appear to represent true QTLs it was of interest to estimate their effect on body weight at 56 days, the sole criterion for selection when developing the two body weight lines. These 11 loci explain at most 50% of the phenotypic difference between the founder lines and approximately 13% of the residual variance in the $F_2$ generation. This is most likely an overestimation because some QTLs could be false positives and some estimated QTL effects maybe inflated (see Section 4).

### (ii) *QTL analysis of anorexia*

Anorexia occurs regularly in our low line but it has not been observed in our high line or in $F_1$ crosses of the lines. Therefore it was surprising that as many as 18% of the $F_2$ birds died before 56 days of age. We assume that a large proportion of these birds died due to anorexia because there was no evidence of infectious diseases and the veterinary record stated that the chickens were in excellent health. We were able to obtain blood samples for DNA preparation from only 60 of the 176 birds that died. The QTL analysis of this trait did not reveal any significant locus, not even at the suggestive level. We then asked whether any of the 13 growth QTLs had a significant effect on the incidence of anorexia. In this case we could use nominal significance thresholds because we did not conduct a genome-wide search, but no QTL showed a significant effect. However, there was a weak trend that QTL alleles from the high line were associated with higher survival, the estimated additive effect for survival showing a small, but positive value for 11 of 13 growth QTLs.

### (iii) *No evidence for segregation distortion*

If a major susceptibility locus was underlying the high incidence of anorexia in the $F_2$ generation we would expect to observe segregation distortion at that locus because we were unable to sample 118 of the 176 $F_2$ birds that died before 10 weeks of age. We therefore carried out an analysis of segregation distortion in this material using the QTL Express program. We observed in total seven regions that showed a significant deviation at the nominal significance thresholds ($P<0{\cdot}05$) either for the additive component (deviation from 1:1 segregation) or the dominance component (deviation from 50% heterozygotes). This is not more than expected by chance given the large number of tests carried out here; a test was carried out at each centimorgan across our linkage map based on 145 markers. Thus, there was no global evidence for segregation distortion.

We then asked whether there were any signs of segregation distortion at the position for the growth QTLs. No strong deviations were observed; however, there was a trend towards an excess of alleles from the high line at QTLs with 10 of 13 positions being positive (Table 4). One of these deviations was significant (*Growth8* on chromosome 5) and another one approached significance (*Growth2* on chromosome 2). This result is consistent with the QTL

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Table 3. *The body weight at 56 days of age (in grams) in the high (H) and low (L) lines and in the reciprocal $F_1$ progeny as well as the estimated additive (a) effects for 11 quantitative trait loci (QTLs) affecting this trait*

| Parentals | | Mean ± SE |
|---|---|---|
| High line | | 1522 ± 36 |
| Low line | | 181 ± 5·4 |
| H − L difference | | 1341 |
| *Reciprocal $F_1$ crosses* | | |
| H male × L female | | 714 ± 13 |
| L male × H female | | 828 ± 15 |
| *$F_2$ generation* | | |
| QTL[a] | $a \pm$ SE | $2a$[b] |
| *Growth1* | 24·5 ± 12·2 | 49·0 |
| *Growth2* | 22·4 ± 7·5 | 44·8 |
| *Growth3* | 27·9 ± 8·3 | 55·8 |
| *Growth4* | 27·8 ± 9·5 | 55·6 |
| *Growth5* | 37·4 ± 9·8 | 74·8 |
| *Growth6* | 46·0 ± 13·4 | 92·0 |
| *Growth7* | 33·1 ± 10·0 | 66·2 |
| *Growth8* | 21·9 ± 8·6 | 43·8 |
| *Growth9* | 39·3 ± 8·0 | 78·6 |
| *Growth10* | 21·5 ± 7·9 | 43·0 |
| *Growth12* | 15·2 ± 7·8 | 30·4 |
| Sum | | 634·0 |
| % population difference | | 47·3 |
| % residual variance | | 13·3 |

[a] *Growth11* and *Growth13* showed no significant additive effects and were therefore not included in this analysis.
[b] The additive effect represents by definition half the estimated phenotypic difference between the two homozygotes. Therefore we provide the estimates for 2*a* here.

analysis of anorexia showing that although none of the growth QTLs had a major impact on the incidence of anorexia, they may each contribute with a small effect.

### (iv) *QTL analysis of packed cell volume (PCV), blood protein and antibody response to sheep red blood cells (SRBC)*

Metabolic needs for growth, reproduction and immunocompetence vary among the selected lines. For gross measures of physiological demands we measured PCV, which is associated with oxygen carrying capacity, and total blood protein, which is associated with reserves needed for growth and for coping with environmental insults. Comparisons between the parental lines for blood protein have not revealed differences and values are consistent with those seen in the intercross (P. B. Siegel, unpublished data). In contrast, as early as the third generation of selection Washburn & Siegel (1963) noted a difference between the high-line (31%) and low-line (29%) males for PCV at 43 days of age. This difference increases with age, has persisted throughout the selection experiment, and may be associated with the earlier maturation of high- than low-line chickens (Dunnington & Siegel, 1996). Another correlated response resulting in a difference between the selection lines is that the high line shows a poorer antibody response to immunization with SRBC than the low line (Liu *et al.*, 1995); $F_1$ crosses show a higher response than either parental line with a heterosis of 70%. Based on this observed line difference one might expect a negative phenotypic correlation between growth and immune traits in the $F_2$ generation. However, the correlation analysis revealed a weak positive association between 56 day body weight and response to SRBC ($r = 0{\cdot}13$, $P < 0{\cdot}0001$) and PCV ($r = 0{\cdot}09$, $P = 0{\cdot}02$) as well as between the 56 day body weight and blood protein level ($r = 0{\cdot}17$, $P < 0{\cdot}0001$). Furthermore, no significant QTL was detected for these traits and none of the 13 growth QTLs showed a significant effect on SRBC antibody response, not even at the nominal level.

Table 4. *Analysis of segregation distortion at QTL positions in the $F_2$ generation of the high × low intercross*

| QTL | Position Chr : cM | Additive component | | Dominance component | |
|---|---|---|---|---|---|
| | | *a* | *t* | *d* | *t* |
| *Growth1* | 1 : 437 | 0·003 | 0·15 | 0·509 | 0·63 |
| *Growth2* | 2 : 115 | 0·043 | 1·84(*) | 0·484 | −1·07 |
| *Growth3* | 2 : 253 | 0·016 | 0·70 | 0·498 | −0·16 |
| *Growth4* | 3 : 123 | 0·004 | 0·27 | 0·497 | −0·40 |
| *Growth5* | 3 : 252 | −0·004 | −0·21 | 0·495 | −0·64 |
| *Growth6* | 4 : 51 | 0·005 | 0·38 | 0·501 | 0·15 |
| *Growth6* | 4 : 62 | 0·010 | 0·67 | 0·505 | 0·75 |
| *Growth7* | 4 : 151 | 0·011 | 0·96 | 0·508 | 0·51 |
| *Growth8* | 5 : 107 | 0·044 | 2·06* | 0·512 | 0·81 |
| *Growth9* | 7 : 43 | 0·001 | 0·03 | 0·506 | 0·38 |
| *Growth10* | 13 : 4 | −0·005 | −0·56 | 0·493 | 0·51 |
| *Growth11* | 20 : 9 | 0·027 | 0·72 | 0·514 | 1·76 |
| *Growth12* | 20 : 62 | 0·017 | 1·02 | 0·503 | 0·88 |
| *Growth13* | 28 : 0 | −0·020 | −0·89 | 0·502 | 0·11 |

*a*, additive component; a value above 0 indicates an excess of alleles from the high line.
*d*, dominance component; estimated frequency of high/low heterozygotes. The expected frequency is 0·500.
*t*, Student's *t*-test.
* $P < 0{\cdot}05$; (*) $P < 0{\cdot}10$.

## 4. Discussion

This study revealed 13 significant or suggestive QTLs for growth, each explaining only a small proportion of the residual phenotypic variance (1·3–3·1%) in the

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$F_2$ generation. We concluded that the majority of these QTLs are true QTLs, although only five reached genome-wide significance, because the allele from the high line was associated with higher growth at all 13 QTLs (Table 3; Fig. 3). This is an unlikely outcome if many of these loci are false positives. Furthermore, our conclusion is supported by the results of a recent global search for epistatic interaction that revealed five pairs of interactions involving six loci in total (Ö. Carlborg *et al.*, unpublished data). As many as five of these mapped in the near vicinity of QTLs reported in the present study (*Growth2*, *4*, *6*, *9* and *12*). The detected QTLs explained "only" at most 50% of the line difference (Table 3). However, our data on the reciprocal $F_1$ generations indicated that about 100 g of the line difference is due to maternal effects (Table 3). Thus, even if all true QTLs were known they would not explain the entire line difference.

Our observation of many QTLs, each with minor individual effects, is consistent with the steady response to selection, without any major leaps, that has been observed during the course of the selection experiment (Fig. 1). The data show that the dramatic response to selection has not involved any QTL with large individual effects, although we cannot exclude the possibility that a major QTL is hiding in the approximately 20% of the chicken genome that was not covered in this study. The size of the individual QTL effects is difficult to assess after this initial genome scan for several reasons. It is likely that some estimated QTL effects have been inflated since a common problem in QTL studies is that those loci where the effect by chance is overestimated are more likely to reach statistical significance (Mackinnon & Georges, 1992; Goring *et al.*, 2001). It is also possible that individual QTL effects have been overestimated because they represent a haplotype effect of two or more linked QTLs, each with a smaller individual effect. Furthermore, it is possible that some QTL effects have been underestimated if QTLs were not fixed for different alleles in the founder lines. The statistical analysis has been carried out with the assumption that the lines are fixed for different alleles, but if this assumption is not fulfilled the effects are underestimated. Another possible bias when estimating QTL effects may be caused by the fact that a sizable portion of the birds died because of anorexia and this may also diminish the estimated effects. However, the segregation analysis indicated that no or only a very minor segregation distortion occurred at QTLs so this possible bias should not seriously affect the estimates. The importance of the QTLs may have been significantly underestimated due to epistatic interaction since only the marginal effects of the individual loci are revealed in a standard one-dimensional QTL search (Carlborg *et al.*, 2003). In fact, a recent analysis has shown that epistatic interactions among several of the QTLs reported in this study have played a prominent role during the selection response in these lines (Ö. Carlborg *et al.*, unpublished data). Finally, the rather small QTL effects, estimated as the percentage of the residual variance explained by each QTL, are partly due to the large variance observed in the $F_2$ generation. For instance, the additive effect of 41 g of the *Growth9* QTL on chromosome 7 can be compared with a standard deviation of 187 g in the $F_2$ generation but only approximately 120 g for the base population from which the high and low lines were developed. Thus, the phenotypic difference between opposite homozygotes for this QTL corresponds to about 0·67 SD in the base population.

Our finding of many QTLs, each with small individual effects, is in good agreement with the results of some previous QTL studies of intercrosses between mouse lines, divergently selected for growth, and corn lines, divergently selected for oil content in kernels (Cheverud *et al.*, 1996; Morris *et al.*, 1999; Laurie *et al.*, 2004). Similarly, van Kaam *et al.* (1999) detected only a few QTLs each with a small effect using an intercross between broiler lines selected for high growth, despite a powerful experimental design involving progeny testing. However, QTLs with large or moderate effects have been detected in other studies involving divergently selected lines of mice (Moody *et al.*, 1999; Horvat *et al.*, 2000; Allan *et al.*, 2005). The results in the present study are also in sharp contrast to our previous QTL study based on an intercross between red junglefowl and White Leghorn chickens where we documented that a few QTLs with large effects explain a large proportion (approx. 70%) of the difference between the founder lines in adult body weight and a large part ( approx. 30%) of the residual phenotypic variance in the $F_2$ generation (Kerje *et al.*, 2003). The experimental designs, regarding the size of the pedigree and the number of genetic markers, of the two studies are very similar. However, the characteristics of the founder populations are strikingly different. The red junglefowl and White Leghorn chickens have been separated for thousands of years whereas the high and low lines were developed from a common ancestral population during 41 generations of intensive selection for the single trait of 56 day body weight. The former show a two-fold difference in adult body weight whereas the latter show a nine-fold difference in body weight at 56 days of age. The number of QTLs detected in the two studies is similar, but the distribution of effects is very different. There has been no intensive selection for body weight in White Leghorns in recent years. The QTLs with major effects on body weight may have been fixed before advanced forms of animal breeding were implemented. Our results suggest that no QTL with a

large individual effect on growth was segregating in the founder population for the high and low lines. Despite this, a remarkable selection response has been obtained which illustrates the genetic plasticity of most biological traits provided that sufficient genetic diversity exists in the population under selection. In this context it is of interest that a very high nucleotide diversity of about five single nucleotide polymorphisms (SNPs) per kilobase has been documented in comparisons both between and within breeds of domestic chicken (International Chicken Polymorphism Map Consortium, 2004). This is about five-fold higher than the nucleotide diversity occurring in humans across populations (International SNP Map Working Group, 2001). Thus, there must be many variants with minor effects on gene expression or gene function that can contribute to a selection response such as the one observed for our high and low lines. The distribution of observed QTL effects in a QTL mapping experiment will therefore depend on the genetic background of the population(s) investigated and a huge phenotypic difference between two populations does not necessarily imply the existence of QTLs with large effects.

Our high and low body weight lines provide interesting models for metabolic disorders in humans. The low line shows a high incidence of anorexia and is very lean. In contrast the high line shows hyperphagia, obesity and impaired glucose tolerance not associated with insulin deficiency (Dunnington & Siegel, 1996), the last being a classical feature of type II diabetes in humans. Furthermore, electrolytic lesion of the ventro-medial hypothalamus has shown that birds from the high line have a defect in the hypothalamic satiety mechanism (Burkhart *et al*., 1983). The great majority of clinical cases of metabolic disorders in humans have a polygenic background and the present study shows that such disorders may have a strong genetic background even in the absence of mutations with major effects. A very large human dataset would be required to detect loci explaining as little as a few per cent of the phenotypic variance for a disorder. An important question for the usefulness of our chicken intercross as a model for metabolic disorders in humans is whether it is possible to identify the mutations underlying these QTLs despite their minor effects. This should be possible unless the majority of the QTLs are due to the combined effect of several closely linked mutations each with a minute effect. We are maintaining an advanced intercross line (AIL; Darvasi & Soller, 1995) for high-resolution mapping that are now (year 2005) at the $F_8$ generation. High-resolution mapping in the chicken is facilitated by the high recombination rate, which ranges from 2·5 to 21 cM/Mbp depending on chromosome (International Chicken Genome Sequencing Consortium, 2004). Here we have analysed each trait separately, but it is known that multivariate (multitrait) techniques help the resolution of QTLs (Turri *et al*., 2004). The wide collection of correlated traits recorded in this experiment should thus allow us to benefit from multitrait analyses. Positional cloning of QTLs in chicken is now greatly facilitated by the access to a high-quality draft genome sequence (International Chicken Genome Sequencing Consortium, 2004) and a SNP map comprising 2·8 million loci (International Chicken Polymorphism Map Consortium, 2004).

Anorexia has never been observed in the high-line chickens; it was noted prior to generation 25 in the low line (Siegel & Dunnington, 1987) and the incidence in the $F_2$ was considerable. Despite these favourable circumstances no QTL for this condition was detected. We propose that this condition is caused by a threshold effect rather than a few predisposing loci. This means that the combined effect of many QTL alleles reducing appetite at one point makes the feed intake inadequate for survival. The high incidence in the low line combined with the absence of anorexia in the $F_1$ generation (Siegel & Dunnington, 1987; Dunnington & Siegel, 1996) suggested that a few recessive loci with major effects may underlie the incidence of anorexia in this pedigree. However, the incidence in the $F_2$ generation appears to be too high (almost as high as in the low line) to be consistent with a simple recessive model. This is because the allele frequency among the $F_2$ birds of an allele present in the low line, but absent in the high line, should be half the frequency in the low line and the phenotype frequency should thus be one-quarter. Epistatic interaction in the form of unfavourable combinations of alleles/haplotypes selected in the two lines may also contribute to the high incidence of anorexia among the $F_2$ birds. We may also have failed to detect any QTL for anorexia partly because of (i) the weak power of QTL analysis of all-or-none traits, (ii) the fact that we were only able to collect DNA samples from a fraction of the birds that died, and (iii) the fact that some birds died for reasons other than anorexia.

We did not detect any QTL for antibody response to SRBC, packed cell volume or total blood protein. There was a weak but significant correlation between body weight and antibody response. Furthermore, our observation that the QTLs for growth showed no significant effect on antibody response may suggest that there is no direct causal relationship between growth and antibody response. This appears unlikely because there are also two independent experiments where selection for low immune response led to a correlated increase in body weight (Boa-Amponsem *et al*., 1998; Paramentier *et al*., 1996; Pinard van der Laan *et al*., 1998). However, no QTL showing significant effects on both growth and antibody response has yet been revealed (Siwek *et al*., 2004; this study).

This suggests that the association may only be observed when the birds have passed a certain weight threshold where the conflict of resource allocation devoted to growth and the immune system becomes severe. Thus, according to this model, too few birds in the $F_2$ generation showed a sufficiently high growth rate to cause a general correlation between body weight and immune response. This may also explain why we did not detect any significant QTLs for antibody response.

Several previous studies have reported growth QTLs in chickens (https://acedb.asg.wur.nl/). There is some overlap between QTLs found in this study and in those previous studies but the data should be interpreted with caution due to the poor precision in initial QTL mapping experiments. It is therefore not possible to judge whether two overlapping QTLs detected in different studies represent the same locus. However, a QTL at approximately 400 cM on GGA1 and QTLs on GGA4 and 7 detected by Kerje *et al.* (2003) in a red junglefowl/White Leghorn intercross map approximately to the same region as QTLs in our study. Sewalem *et al.* (2002) made a QTL study in an intercross between layer and broiler lines. The location of one of our major QTLs, *Growth9* on GGA7, overlaps with a QTL for 21, 42 and 63 day body weight in that intercross. Also our *Growth1* and *Growth13* overlap with QTLs identified in that intercross. Deeb & Lamont (2003) found a significant effect on 56 day body weight in Fayoumi chickens linked to a marker on chromosome 28, as we did; however, with only one marker on chromosome 28 we cannot judge whether these two QTLs overlap or not.

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## References

Allan, M. F., Eisen, E. J. & Pomp, D. (2005). Genomic mapping of direct and correlated responses to long-term selection for rapid growth rate in mice. *Genetics* **170**, 1863–1877.

Boa-Amponsem, K., Dunnington, E. A. & Siegel, P. B. (1998). Diet and humoral responsiveness of lines of chickens divergently selected for antibody response to sheep red blood cells. *Avian Diseases* **42**, 565–571.

Burkhart, C. A., Cherry, J. A., Van Krey, H. P. & Siegel, P. B. (1983). Genetic selection for growth rate alters hypothalamic satiety mechanisms in chickens. *Behavior Genetics* **13**, 295–300.

Carlborg, O. & Andersson, L. (2002). Use of randomization testing to detect multiple epistatic QTLs. *Genetical Research* **79**, 175–184.

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

Cheema, M. A., Qureshi, M. A. & Havenstein, G. B. (2003). A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. *Poultry Science* **82**, 1519–1529.

Cheverud, J. M., Routman, E. J., Duarte, F. A., van Swinderen, B., Cothran, K. & Perel, C. (1996). Quantitative trait loci for murine growth. *Genetics* **142**, 1305–1319.

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

Darvasi, A. & Soller, M. (1995). Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics* **141**, 1199–1207.

Deeb, N. & Lamont, S. J. (2003). Use of a novel outbred by inbred F1 cross to detect genetic markers for growth. *Animal Genetics* **34**, 205–212.

Dunnington, E. A. & Siegel, P. B. (1996). Long-term divergent selection for eight-week body weight in white Plymouth rock chickens. *Poultry Science* **75**, 1168–1179.

Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*, 4th edn. Harlow, UK: Longman.

Frisch, R. E. (2002). *Female Fertility and the Body Fat Connection*. Chicago, IL: University of Chicago Press.

Göring, H. H., Terwilliger, J. D. & Blangero, J. (2001). Large upward bias in estimation of locus-specific effects from genomewide scans. *American Journal of Human Genetics* **69**, 1357–1369.

Halbersleben, D. L. & Mussehl, F. E. (1922). Relationship of egg weight to chick weight at hatching. *Poultry Science* **1**, 143–144.

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

Horvat, S., Bunger, L., Falconer, V. M., Mackay, P., Law, A., Bulfield, G. & Keightley, P. D. (2000). Mapping of obesity QTLs in a cross between mouse lines divergently selected on fat content. *Mammalian Genome* **12**, 284–290.

International Chicken Genome Sequencing Consortium (2004). Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* **432**, 695–716.

International Chicken Polymorphism Map Consortium (2004). A genetic variation map for chicken with 2·8 million single-nucleotide polymorphisms. *Nature* **432**, 717–722.

International SNP Map Working Group (2001). A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* **409**, 928–933.

Jacobsson, L., Park, H. B., Wahlberg, P., Jiang, S., Siegel, P. B. & Andersson, L. (2004). Assignment of fourteen microsatellite markers to the chicken linkage map. *Poultry Science* **83**, 1825–1831.

Jansen, R. C. (1993). Interval mapping of multiple quantitative trait loci. *Genetics* **135**, 205–211.

Kerje, S., Carlborg, O., Jacobsson, L., Schutz, K., Hartmann, C., Jensen, P. & Andersson, L. (2003). The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs. *Animal Genetics* **34**, 264–274.

Lander, E. & Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics* **11**, 241–247.

Laurie, C. C., Chasalow, S. D., LeDeaux, J. R., McCarroll, R., Bush, D., Hauge, B., Lai, C., Clark, D., Rocheford, T. R. & Dudley, J. W. (2004). The genetic architecture of response to long-term artificial selection for oil concentration in the maize kernel. *Genetics* **168**, 2141–2155.

Liu, G., Dunnington, E. A. & Siegel, P. B. (1993). Maternal effects and heterosis for growth in reciprocal cross populations of chickens. *Journal Animal Breeding and Genetics* **110**, 423–428.

Liu, G., Dunnington, E. A. & Siegel, P. B. (1994). Responses to long-term divergent selection for eight-week body weight in chickens. *Poultry Science* **73**, 1642–1650.

Liu, E., Dunnington, E. A. & Siegel, P. B. (1995). Growth related traits in body weight selected lines and their crosses reared in different regimes. *British Poultry Science* **36**, 209–219.

Ljungberg, K., Holmgren, S. & Carlborg, O. (2002). Efficient algorithms for quantitative trait loci mapping problems. *Journal of Computational Biology* **9**, 793–804.

Lynch, M. & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer Associates.

Mackinnon, M. J. & Georges, M. A. (1992). The effects of selection on linkage analysis for quantitative traits. *Genetics* **132**, 1177–1185.

Martin, A., Dunnington, E. A., Gross, W. B., Briles, W. E., Briles, R. W. & Siegel, P. B. (1990). Production traits and alloantigen systems in lines of chickens selected for high or low antibody responses to sheep erythrocytes. *Poultry Science* **69**, 871–878.

Minitab (2000). *Minitab User's Guide*, release 13. State College, PA: Minitab.

Moody, D. E., Pomp, D., Nielsen, M. K. & Van Vleck, L. D. (1999). Identification of quantitative trait loci influencing traits related to energy balance in selection and inbred lines of mice. *Genetics* **152**, 699–711.

Morris, K. H., Ishikawa, A. & Keightley, P. D. (1999). Quantitative trait loci for growth traits in C57BL/6J × DBA/2J mice. *Mammalian Genome* **10**, 225–228.

Parmentier, H. K., Nieuwland, M. G., Rijke, E., De Vries Reilingh, G. & Schrama, J. W. (1996). Divergent antibody responses to vaccines and divergent body weights of chicken lines selected for high and low humoral responsiveness to sheep red blood cells. *Avian Diseases* **40**, 634–644.

Pérez-Enciso, M. & Misztal, I. (2004). Qxpak: a versatile mixed model application for genetical genomics and QTL analyses. *Bioinformatics* **20**, 2792–2798.

Pinard van der Laan, M., Siegel, P. B. & Lamont, S. J. (1998). Lessons from selection experiments on immune response in the chicken. *Poultry Avian Biology Reviews* **9**, 125–141.

Schmid, M., Nanda, I., Guttenbach, M., Steinlein, C., Hoehn, M., Schartl, M., Haaf, T., Weigend, S., Fries, R., Buerstedde, J. M., Wimmers, K., Burt, D. W., Smith, J., A'Hara, S., Law, A., Griffin, D. K., Bumstead, N., Kaufman, J., Thomson, P. A., Burke, T., Groenen, M. A., Crooijmans, R. P., Vignal, A., Fillon, V., Morisson, M., Pitel, F., Tixier-Boichard, M., Ladjali-Mohammedi, K., Hillel, J., Maki-Tanila, A., Cheng, H. H., Delany, M. E., Burnside, J. & Mizuno, S. (2000). First report on chicken genes and chromosomes 2000. *Cytogenetics and Cell Genetics* **90**, 169–218.

Seaton, G., Haley, C. S., Knott, S. A., Kearsey, M. & Visscher, P. M. (2002). QTL Express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics* **18**, 339–340.

Sewalem, A., Morrice, D. M., Law, A., Windsor, D., Haley, C. S., Ikeobi, C. O., Burt, D. W. & Hocking, P. M. (2002). Mapping of quantitative trait loci for body weight at three, six, and nine weeks of age in a broiler layer cross. *Poultry Science* **81**, 1775–1781.

Siegel, P. B. (1962). Selection for body weight at 8 weeks of age. 1. Short term response and heritabilities. *Poultry Science* **41**, 954–962.

Siegel, P. B. & Dunnington, E. A. (1987). Selection for growth in chickens. *Critical Reviews in Poultry Biology* **1**, 1–24.

Siegel, P. B. & Gross, W. B. (1980). Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poultry Science* **59**, 1–5.

Siwek, M., Cornelissen, S. J., Buitenhuis, A. J., Nieuwland, M. G., Bovenhuis, H., Crooijmans, R. P., Groenen, M. A., Parmentier, H. K. & van der Poel, J. J. (2004). Quantitative trait loci for body weight in layers differ from quantitative trait loci specific for antibody responses to sheep red blood cells. *Poultry Science* **83**, 853–859.

Turri, M. G., DeFries, J. C., Henderson, N. D. & Flint, J. (2004). Multivariate analysis of quantitative trait loci influencing variation in anxiety-related behavior in laboratory mice. *Mammalian Genome* **15**, 69–76.

van Kaam, J. B., Groenen, M. A., Bovenhuis, H., Veenendaal, A., Vereijken, A. L. & van Arendonk, J. A. (1999). Whole genome scan in chickens for quantitative trait loci affecting carcass traits. *Poultry Science* **78**, 1091–1099.

Washburn, K. W. & Siegel, P. B. (1963). Influence of thiouracil on chickens selected for high or low body weights. *Poultry Science* **42**, 161–169.

Wegmann, T. G. & Smithies, O. (1966). A simple hemagglutination system requiring small amounts of red cells and antibodies. *Transfusion* **6**, 67–73.

Zelenka, D. J., Dunnington, E. A., Cherry, J. A. & Siegel, P. B. (1988). Anorexia and sexual maturity in female white rock chickens. I. Increasing the feed intake. *Behavior Genetics* **18**, 383–387.

Zeng, Z. B. (1993). Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Science of the USA* **90**, 10972–10976.

## Web site References:

http://genome.wustl.edu/projects/chicken/; The Genome Sequencing Center at Washington University School of Medicine

http://qtl.cap.ed.ac.uk; Web site for the QTL Express program