Articles in PresS. Physiol Genomics (March 23, 2010). doi:10.1152/physiolgenomics.00149.2009

# Genetic analysis of metabolic traits in an intercross between body weight selected chicken lines

**Weronica Ek[1], Lina Strömstedt[1], Per Wahlberg[3,4], Paul Siegel[2], Leif Andersson[1,3] and Örjan Carlborg[1]**

[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, SE-75007 Uppsala, Sweden
[2] Virginia Polytechnic Institute and State University, Department of Animal and Poultry Sciences, Blacksburg, VA 24061-0306, USA
[3]Department of Medical Biochemistry and Microbiology, Uppsala University, BMC, SE-75124 Uppsala, Sweden
[4]Present address: INRA, UMR 1313 de Génétique Animal et Biologie. Intégrative, Domaine de Vilvert, 78350 Jouy-en-Josas, France

Running head: Genetic analysis of metabolic traits in chickens

[*]Corresponding author
Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, SE-75007 Uppsala, Sweden. Tel: +46 18 672056. e-mail: Weronica.ek@hgen.slu.se

**ABSTRACT**

A network of four interacting loci has previously been reported to influence growth in two lines of chickens divergently selected for body weight at 56 days of age. Located on chromosomes 3 (*Growth4)*, 4 *(Growth6*), 7 (*Growth9*) and 20 (*Growth12*), they explained nearly half of the difference in body weight at selection age between the two lines. The original study reported effects on body weight and fat deposition, but no attempts were made to explore the effects on the network on other phenotypes measured in the $F_2$ population. In this study we conduct further analyses to evaluate the specific effects of the four locus network on other metabolic traits as well as refining results from the original study by including a larger number of genetic markers in the QTL regions.

We confirm the previously described effect of the epistatic network on body weight and show that the network increases the total amount of muscle and fat as well as the weight of the internal organs. The network as a whole did not change the relative content of any studied organs or tissues in the body. There was, however, a significant interaction between the loci on chromosome 3 and 7 that changed the relative proportion of abdominal fat and breast muscle in the chicken by increasing abdominal fat weight without a corresponding increase in muscle mass.

QTL; gene interaction; breast muscle; abdominal fat

## INTRODUCTION

IT IS DIFFICULT TO EVALUATE how genetic factors influence long-term response to selection for a quantitative trait. This is because responses are influenced by numerous factors including population size, intensity of selection, initial gene frequencies, mutation, inbreeding, changes in variance, environment as well as genetic and physiological limits. Long-term selection experiments can, however, provide insights that cannot be obtained from short-term experiments or commercial breeding programs. Such an experiment, conducted with White Plymouth Rock chickens, has resulted in a nine-fold body weight difference between the high (HWS) and low-weight selected (LWS) chickens at age of selection (56 days) (2). Moreover, there have been correlated responses in other traits including body composition, appetite, metabolism, reproduction and immunocompetence (3).

A network of four interacting loci explains nearly half of the difference in body weight between the lines at selection age (1). The central locus in this network is located on chromosome 7 and acts by releasing the genetic effects of the other three loci, which are located on chromosomes 3, 4 and 20. The release of the genetic effects is reciprocal as the loci on chromosomes 3, 4 and 20 also jointly release genetic effects on growth for the QTL on chromosome 7. The original study (1) reported effects on body weight and fat deposition in an $F_2$ intercross between the two divergently selected lines. Here we explore the genetic effects of the network on other growth traits in more detail.

## MATERIAL AND METHODS

### Mapping population

The selection lines are the result of a long-term experiment where White Plymouth Rock chickens have been divergently selected for high or low body weight at 56 days of age from a common founder population for more than 40 generations (2). The lines have been kept in sufficient numbers and subjected to mating restrictions to reduce the rate of inbreeding (5). Body weight at a specific age is a function of the growth of component parts of the body. Selection for high or low body weights at a particular age will therefore also lead to heavier or lighter weights at other ages (2).

A reciprocal $F_2$ intercross consisting of 795 individuals was generated from generation 40 of the HWS and LWS lines. DNA was extracted from blood by AGOWA (Berlin, Germany) from all individuals included in the intercross pedigree (P, $F_1$ and $F_2$ chickens). All $F_2$ progeny were from the same hatch and their parents were of the same age. Those that survived to 56 days of age (n=795; BW56±SD: 624 g±168 g) were genotyped for 145 markers covering 2427 cM on 25 linkage groups and subsequently for an additional 350 markers to generate a total map covering ~3100 cM (10). Traits measured included body weight at hatch, 14, 28, 42, 56 and 70 days of age and weight of abdominal fat, breast muscle, lungs, shanks (metatarsus including toes), bursa and spleen at 70 days of age (Table 1). The abdominal fat weight did not include mesenteric fat and gizzard fat. Weights of pectoralis major and pectoralis minor muscles were obtained as a measurement of breast muscle weight. Lung and shank weights were recorded separately for left and right side (6). $F_1$ and $F_2$

progeny had mean body weights below the arithmetic average for the parental lines, which confirmed earlier findings of negative heterosis in crosses of these lines (4). All procedures involving animals in this experiment were carried out in accordance with the Virginia Tech Animal Care Committee animal use protocols.

**Network analysis**

To obtain a better insight into how the four locus network influenced body weight, we estimated the individual effect of each locus, the interaction effect for every possible pair of loci as well as the joint effect of all four loci for each trait measured in the $F_2$ population (Table 1). At each locus, there are three possible genotypes: LL (LWS homozygote), HL (heterozygote) or HH (HWS homozygote). Due to the $F_2$ design, there are twice as many heterozygotes as each of the homozygotes. The original analysis (1) showed that the effects of the loci on GGA (Gallus Gallus Chromosome) 3, 4 and 20 differed depending on the genotype for the QTL on GGA7. Therefore, multiple regression was used to model a common mean and separate effects of *Growth4* (GGA3), *Growth6* (GGA4) and *Growth12* (GGA20) in individuals with different genotypes (HH, HL or LL) for *Growth9* (GGA7). For these analyses, the model (7) was

$$Y_i = \mu + Z\beta + \beta_1 X_{Growth4} + \beta_2 X_{Growth6} + \beta_3 X_{Growth12} + \varepsilon_{ij}$$

where $Y_i$ is the phenotype for $F_2$ bird *i* with *Growth9* HH, HL or LL, $Z\beta$ is the fixed effects of sex (and body weight at 70 days of age, when used), $\beta_1$, $\beta_2$ and $\beta_3$ are the additive effects, $X_{Growth4}$ /$X_{Growth6}$ /$X_{Growth12}$ are indicator variables for the genotype of the tested loci *Growth4, Growth6* and *Growth12* within each of the three

different backgrounds on *Growth9* and $\varepsilon_{ij}$ is the error term. QTL genotype probabilities were estimated for the four QTL: *Growth4* (GGA3 109 cM), *Growth6* (GGA4 33 cM), *Growth9* (GGA7 63 cM) and *Growth12* (GGA20 56 cM). Only individuals where the genotype for all four QTL could be assigned with more than 80% probability were included in the analysis (n=538). Individuals were classified according to the most probable genotype (i.e. HH, HL, LL) and the effect of sex was included in all analyses. For abdominal fat, breast muscle and shank weights an additional analysis was performed with body weight at 70 days of age (i.e. weight at slaughter) included as a covariate. These analyses were performed to explore whether the network altered relative amounts of abdominal fat, breast muscle and shanks in the body rather than the absolute levels.

## RESULTS

The four locus network jointly affected body weight at different ages as well as absolute weights of lungs, spleen, abdominal fat, breast muscle and shanks ($P < 0.01$). However, when corrected for body weight at slaughter (70 days of age) the joint effect of the four locus network was not significant for any of the component traits. A significant two locus interaction was detected between *Growth9* and *Growth4*. In a *Growth9* LL background, relative abdominal fat weight increased and relative breast muscle weight decreased when the *Growth4* genotype changed from LL to HH (Figure 1). When abdominal fat was not corrected for body weight at slaughter, the increase in fat deposition was proportional to the increase in body weight whereas breast muscle weight decreased. In a *Growth9* HH background, relative and absolute abdominal fat increased with body weight when *Growth4* changed from LL to HH.

Relative breast muscle weight and lung weight decreased in a *Growth9* LL background; lung weight not corrected for body weight at slaughter, however, was proportional with the increase in body weight. *Growth4* LL alleles appear to have a recessive effect by increasing breast muscle weight independently of *Growth9*. At a homozygous state for both loci, LL/LL and HH/HH and for *Growth4* LL and *Growth9* HH, relative breast muscle weight increased significantly compared with other genotypes (Figure 1).

Although the chromosomal regions for the observed QTL were too large to reveal specific candidate genes, some genes known to affect growth are located in these regions. Examples include the gene encoding latent transforming growth factor beta

binding protein 1 (*LTBP1*) located in the region on chromosome 3 and insulin-like growth factor binding protein 2 and 5 (*IGFBP2* and *IGFBP5*) located in the region on chromosome 7 (8; 9).

## DISCUSSION

The study was designed to explore how a previously reported QTL network in chicken (1) affected different component traits underlying body weight rather than finding unreported loci associated with these traits. Carlborg *et al.* 2006 (1) showed that this network had strong effects on body weight. Because body weight is a function of component parts of the body, we wanted to see how these four QTL influenced traits that might increase or decrease body weight. With this focus, we can rely on nominal significance testing thresholds in our analyses instead of having to use the very stringent genome-wide thresholds needed in a scan for novel interacting loci affecting the component traits.

The results from this study, with more added markers, on body weight was consistent with those reported previously (1) as we again found that these four loci jointly determine almost 50% of the weight difference between the lines. The epistatic loci also cause an increase in the total amount of muscle and fat in the body of the chickens as well as an increase in the weight of the internal organs. The four loci did, however, not jointly change the relative amount of any measured trait in the body. A significant two-way interaction between *Growth4* and *Growth9* was, however, evidenced and it was shown that these loci caused heavier chickens to have relatively more body fat and less breast muscle than smaller ones, except when both loci were

199 homozygous for HH alleles, in which case the breast muscle weight also increased.
200 This means that during selection for high body weight, alleles at these two loci
201 increased body fat. Homozygosity for HH alleles at both loci did, however, have a
202 slightly different effect causing a relative increase in both body fat and breast muscle.
203 During selection for low body weight, the LL alleles at *Growth4* and *Growth9*
204 decreased body weight by reducing absolute amounts of breast muscle and fat.
205 Relative muscle mass did, however, increase and relative abdominal fat weight
206 decrease. The lines differ in appetite and feed consumption (2), which might lead to
207 higher or lower fat deposition. Body fat is important for the onset of egg production,
208 and a decrease in fat deposition can therefore prevent the chickens from reproducing.
209 For example, some LWS chicks die within the first week after hatch because they
210 never start to eat, while others consume sufficient amounts of feed to survive but not
211 enough to achieve sexual maturity. When force-fed, these chickens will reproduce (2).
212
213 Carlborg *et al.* (1) found that the LL alleles at *Growth4* actually have a switched
214 effect in a *Growth9* LL background, where they actually increased body weight.
215 *Growth4* might increase body weight in a low-line background as a survival factor; if
216 the chickens have too low body weight and too little body fat, they are not able to
217 reproduce or even survive.
218
219 In this study we replicate the finding of a four locus network affecting growth in an
220 intercross between two divergently selected chicken lines. Our explorations of the
221 effects of the network on component traits indicate that the major contribution of the
222 network is to increase the body-size through a symmetric growth of all organs rather
223 than by changing the relative amount of different body components. One of the QTL

pairs in the network does, in addition to contributing to the general increase in body weight, also alter the relative amount of breast muscle and fat in the carcass. Further studies are needed to explore the physiological mechanisms by which the genes underlying the network mediate their effects.

## GRANTS

This work was funded by the Swedish Foundation for Strategic Research, FORMAS and a EURYI-Award to Örjan Carlborg.

## REFERENCES


1. **Carlborg O, Jacobsson L, Ahgren P, Siegel P, Andersson L.** Epistasis and the release of genetic variation during long-term selection. *Nat Genet* 38: 418-420, 2006.
2. **Dunnington E.A, Siegel P.B.** Long-term divergent selection for eight-week body weight in White Plymouth Rock chickens. *Poult Sci* 75: 1168-1179, 1996.
3. **Dunnington E.A, Siegel P.B.** Long term selection for 8-week body weight in chickens –direct and correlated responses. *TAG* 71, 1985.
4. **Liu G, Dunnington E.A, Siegel P.B.** Growth related traits in body weight selected lines and their crosses reared under different nutritional regimens. *Br Poult Sci* 37: 209-219, 1995.
5. **Liu G, Dunnington E.A, Siegel P.B.** Responses to long-term divergent selection for eight-week body weight in chickens. *Poult Sci* 73: 1642-1650, 1994.
6. **Park H, Jacobsson L, Wahlberg P, Siegel P, Andersson L.** QTL analysis of body composition and metabolic traits in an intercross between chicken lines divergently selected for growth. *Physiol. Genomics* 25: 216-233, 2006.
7. **R Development Core Team**. R : a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org
8. **Wolf E, Lahm H, Wu M, Wanke R, Hoeflich A.** Effects of IGFBP-2 overexpression in vitro and in vivo. *Pediatric Nephrology* 14: 572-578, 2000.
9. **Öklu R, Hesketh T.R, Metcaife J.C, Kemp P.R.** Expression of alternatively spliced human latent transforming growth factor β binding protein-1. *FEBS Letters* 435:143-148, 1998.
10. **Wahlberg P, Carlborg Ö, Foglio M, Tordoir X, Syvänen A.C, Gut I.G, Siegel P.B, Andersson L.** Genetic analysis of an F2 intercross between two chicken lines divergently selected for body-weight. *BMC Genomics* 10: 248, 2009

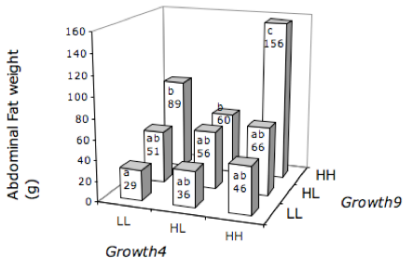
## FIGURE LEGEND

Figure 1. Two locus genotype-phenotype maps for A) abdominal fat weight (corrected for body weight at slaughter) and B) breast muscle weight (corrected for body weight at slaughter). Different letters indicate that the genotype combinations are significantly different from each other ($p < 0.05$).

## TABLE LEGEND

Table 1. *Summary of phenotypic data for the $F_2$ intercross from generation 40 between the high- and low-selection lines. Fixed effects and covariates included in the QTL analyses are also given (6).*

**A**




**B**

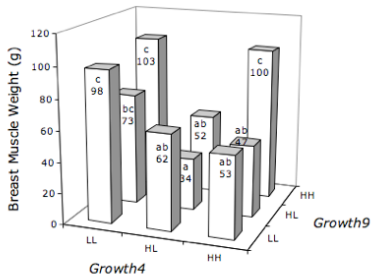



Figure 1. Two locus genotype-phenotype maps for A) abdominal fat weight (corrected for body weight at slaughter) and B) breast muscle weight (corrected for body weight at slaughter). Different letters indicate that the genotype combinations are significantly different from each other ($p < 0.05$).

Table 1. *Summary of phenotypic data for the $F_2$ intercross from generation 40 between the high- and low-selection lines. Fixed effects and covariates included in the QTL analyses are also given (6).*

| Traits | Number of chickens | Mean±SD | Fixed effect | Covariate |
|---|---|---|---|---|
| Body Weight (BW) (g) | | | | |
| at hatch | 795 | 27.8±2.1 | Sex | |
| 14[a] | 795 | 75.2±14.9 | Sex | |
| 28[a] | 795 | 179.1±56.8 | Sex | |
| 42[a] | 795 | 365.5±113.1 | Sex | |
| 56[a] | 795 | 621.7±186.9 | Sex | |
| 70[a] | 795 | 943.3±262.2 | Sex | |
| Body traits at 70 days of age (g) | | | | |
| Abdominal Fat | 402 | 5.4±4.1 | Sex | BW70[a] |
| Shanks | 405 | 42.5±11.9 | Sex | BW70[a] |
| Breast[b] | 201 | 91.1±28.8 | Sex | BW70[a] |
| Lungs | 405 | 6.5±2.2 | Sex | BW70[a] |
| Spleen | 401 | 1.4±0.5 | | |
| Bursa | 405 | 1.9±0.7 | | |

[a] Body weight in days of age
[b] Pectoralis major + Pectoralis minor