Institutionen för husdjursgenetik

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Examensarbete 292
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ABSTRACT

Metabolic traits are of paramount importance in agricultural production, as this group includes most traits of economic interest in livestock improvement. Examples include growth rate, feed efficiency and fat deposition. An improved understanding of the genetic basis of these traits can both improve our understanding of the genes that have been under selection and identify genes and pathways to be included in future breeding programs.

A novel genetic mechanism has been found to regulate growth in chicken lines divergently selected for body weight. A network of four interacting genes explains nearly half of the difference in body weight at 8-weeks of age between the two lines. The central locus in this network is located on chromosome 7 and it has a role in releasing the genetic effects of three other loci in the network located on chromosome 3, 4 and 20. Interestingly, the release of the genetic effects is also reciprocal as the loci on chromosome 3, 4 and 20 jointly release the genetic effect on growth for the QTL on chromosome 7. The original report by Carlborg *et al* report results on body weight and fat deposition, the study does, however not report results on other phenotypes collected on the F_2 individuals. This thesis presents results from analyses to evaluate the effects of the four QTL network on other measured traits in the F_2 population and to see which traits that are useful in further epistatic analyses (CARLBORG *et al.* 2006). Furthermore the study also serves as a replication of the original study by analysing data on a larger number of added genetic markers in the QTL regions.

The four QTL network was shown to have significant effects on body weight at different ages, abdominal fat and body compositions. The effect of body composition is most likely the results of an increase in general body size as the effects were not significant after corrected for body weight in the analyses. The network do, however, appear to have an effect on abdominal fat deposition and breast weight even after correcting for body weight. When corrected for body weight at slaughter (10-weeks of age) there were no significant effects on shank weight. No effects could be shown for the gene pair 7 and 4, and for 7 and 20 for other traits than body weight. The regression analysis indicates that chromosome 3 in a chromosome 7-homozygous low-line (LL) background increases relative abdominal fat and decreases relative breast muscle going from LL to HH (Homozygous high-line). When abdominal fat is not corrected for body weight at slaughter, the increase in fat deposition is proportional with increased body weight. In a chromosome 7-HH background, absolute abdominal fat is increasing with increased body-weight but relative abdominal fat is not when chromosome 3 is going from LL to HH. Relative breast muscle is decreasing, while absolute breast muscle is proportional with an increase in body weight. These results suggest that there is a change in the chicken body composition when selected for higher body weight. Chickens tend to go from lean and muscular to fat and thin.

Understanding the genetic regulation of metabolic traits for which the lines differ is of crucial interest. Therefore we collected information from the literature on the descriptive statistics for these traits. Statistics were used to explore the sample size needed in an experiment to detect genetic effects of various sizes in the high- and low- lines. From these it was concluded that there is a lack of power to detect genetic effects on the network for most tested metabolic traits on the cross and that another experimental strategy is needed to explore this further. Next step in this study will be to introgress the 4-QTLs from the low line chickens into a high line background.

1. INTRODUCTION

Artificial selection of animals started when humans began to domesticate them and can be viewed as a method by which humans influence gene flow of other organisms across generations. In 1957, Prof. Paul B. Siegel at the Virginia Polytechnic Institute and State University (USA) started a selection experiment where he selected chickens for high- and low- body weight. Since the experiment started, one generation has been produced each year. The population has been kept in sufficient numbers to minimize inbreeding (LIU *et al.* 1994).

Selection for high- and low- body weight at eight weeks of age has resulted in a notable selection response and correlated responses in other traits including body composition, appetite, metabolic, reproductive and immune response traits (JACOBSSON 2005). Negative correlated response to selection for body weight includes sexual maturity, egg production and fertility. Positive correlation for body weight are feed consumption, bone length, breast width, weight of fat depots and size of various organs and glands (SIEGEL and DUNNINGTON 1987). Figure 1 shows a nine fold weight difference between a HW chicken to the right, and a LW chicken to the left at 8-weeks of age. Body weight at a specific age is a function of growth of component parts of the body so that selection for high body weight at a particular age generally alters high body weight at other ages (DUNNINGTON and SIEGEL 1996).

Figure 1. The photo shows almost a nine-fold weight difference between the high and low line at 8-weeks of age (Photo: Dr. E.A. Dunnington)

Complex traits like body weight are quantitative, meaning that they has a continuous variation and are determined by the cumulative interaction of multiple genes and their alleles (each gene by itself has a relatively small effect on the trait) in combination with environmental factors. Most biological traits of agricultural and medical importance are quantitative traits for example; growth, fat deposition, asthma and diabetes. A quantitative locus is defined as a chromosomal region harbouring one or several genes that influences a quantitative trait. The procedures to find and locate the QTLs is called QTL mapping. Analyses to identify QTL are based on detecting co-segregation of markers and genes affecting phenotypic trait variation. With QTL mapping it is possible to identify previously unknown genes involved in regulation of a trait. QTL can be detected due to linkage disequilibrium (LD) between a QTL and one or several genetic markers. LD extends over long genomic segments in populations with closely related individuals and in general populations, the range of LD is short (JACOBSSON 2005).

Epistasis means that the effect of a specific genotype on the phenotype depends on the genetic background. This refers to an interaction between two or more loci, in which the phenotypic effect of one locus depends on the other loci. Epistasis describes the situation in which the phenotype of a given genotype cannot be predicted by the sum of its component single locus effect if taking about quantitative traits (CARLBORG *et al.* 2006).

A genetic mechanism has been found to regulate growth in the high- and low- weight selected lines. A network of four interacting genes explains nearly half of the difference in body weight at 8-weeks age between the two lines. The central locus in this network is located on chromosome 7 and it has a role in releasing the genetic effects of three other loci in the network located on chromosome 3, 4 and 20. Interestingly, the release of the genetic effects is also reciprocal as the loci on chromosome 3, 4 and 20 jointly release the genetic effect on growth for the QTL on chromosome 7. The original report by Carlborg *et al* report results on body weight and fat deposition, the study does, however not report results on other phenotypes collected on the F2 individuals. This thesis presents results from analyses to evaluate the effects of the four QTL network on other measured traits in the F_2 population and to see which traits that are useful in further epistatic analyses (CARLBORG *et al.* 2006). Furthermore the study also serves as a replication of the original study by analysing data on a large number of added markers in the QTL regions.

2. LITERATURE REVIEW

2.1 Appetite

There are marked differences in eating behaviour between the selected lines, the high line showing hyperphagia and the low line anorexia. Reduction in food consumption is one correlated response to selection for low body weight. Some chicks in the low-line die within the first week after hatch simply because they don't eat. Some of those pullets in the low-line that consume sufficient amounts of feed to survive don't consume enough feed to achieve sexual maturity (DUNNINGTON and SIEGEL 1996). Appetite differences between the lines have been noticeable after the 5th generation. It has been shown that the feed intake per meal is the same for both lines but the high-line birds have more meals per day (BARBATO *et al.* 1980).

Birds from both lines has been raised and fed together to see if the eating behaviour of the high-line could encourage low-line chickens to eat more. Results showed that the high-line chickens consumed more feed when raised with low-line chickens, whereas the low-line chickens showed no difference in feed intake (JACOBSSON 2005). In one experiment, plasma from feed-deprived high-line chickens was injected in low-line chickens blood. The treatment increased the low-line chickens feed intake. The test was also done on high-line chickens and it didn't show any significant effect on their feed intake. This fact suggests that a factor in the plasma of high-line birds increases their feed intake (BARBATO *et al.* 1980).

A Leptin gene has not been identified in chicken but the leptin receptor is present and apparently has a similar function as in mammals (JACOBSSON 2005). In mammals, it appears that a mutation in either the leptin gene or in its receptor will result in changes in feed intake and lipid stores. It has been shown that a decrease in leptin concentration increases the feed intake in the low-line, but not in the high-line (DUNNINGTON and SIEGEL 1996).

Burkhart, (1983) used electrolytic lesion of the ventro-medial hypothalamus in chickens from the low-line which resulted in hyperphagia. The treatment had no effect on the high-line chickens. These results indicates that a factor in or from the hypothalamus of low-line chickens inhibit feed intake and/or conversion, and that the high-line chickens either lack this feed intake regulator or are insensitive to it´s presence (BURKHART *et al.* 1983).

2.2 Feed efficiency

Under- or over consumption of feed may influence efficiency of feed utilization. The effects of feed efficiency was measured in embryos during early generations of selection and this experiment showed that high-line embryos were more efficient in the utilization of energy and certain amino acids, particularly the sulphur containing amino acids, compared to embryos from the low-line (DUNNINGTON and SIEGEL 1996; LEPORE *et al.* 1963). One experiment showed that the high-line had a higher feed efficiency since they gained more weight on the same amount of feed compared to the low line. Feed efficiency was obtained by dividing body weight gain by feed consumed (BARBATO *et al.* 1983). Feed efficiency in the high-line has been associated with intestinal glucose absorption, oxygen consumption, thermoregulation and rate of feed passage; (DUNNINGTON and SIEGEL 1996). Data over feed efficiency suggests that the genetic relationship between growth and feed efficiency is primarily due to pleiotropi rather than linkage (OWENS *et al.* 1971).

2.3 Body Composition; supply and demand organs

Organs involved in digestion, such as the gastrointestinal tract (GIT), heart, lungs and liver belongs to the supply organs. Organs that make use of body supplies and energy to expand such as skeleton, skin, muscles and feathers are called demand organs. Body composition along with changes in body weight is influenced by selection. Development rate of specific organs varied with age and divergent selection for body weight. Selection for high-body weight at 8-weeks of age resulted in relatively heavier breasts, legs, abdominal fat depots and small intestine. Selection for low-body weight resulted in heavier feathers and gizzards (BURKHART *et al.* 1983; KATANBAF *et al.* 1988).

Increased body fat in the high-line is primarily the result of decreased lipolysis rather than increased lipogenesis. The process of lipogenesis and lipolysis occur at faster rates in low-line chickens than in high-line chickens (CALABOTTA *et al.* 1985; DUNNINGTON and SIEGEL 1996). The lipogenic enzymes, liver acetyl CoA carboxylase and malic enzyme were significantly higher in the low line than in the high line, indicating higher lipogenesis in the low line chickens (CALABOTTA *et al.* 1983). Free fatty acid (FFA) concentration in plasma appear to be an estimate of lipid mobilization from adipose tissue, and the low line males have significantly higher plasma FFA levels than the high line males. Net fat deposition is the result of the complex relationship between lipid- deposition and degradation. The low line birds have a greater capacity for lipid synthesis, but the increased capacity for lipolysis prevents excessive accumulation of fat (CALABOTTA *et al.* 1983).

Since the high line chicken have more muscle tissue they also have more and larger muscle cells (LEPORE *et al.* 1965). Brains are heavier in the low line than in the high line relative to body weight (ANTHONY *et al.* 1991).

2.4 Thermoregulation

Cloacal and surface (foot pad) temperatures were measured 2h after feeding. Low line chickens had generally lower surface temperatures than the high line. Heavier body weight was correlated with higher surface temperatures in the lines. The differences is probably due to greater surface to body mass ratio, reduced feed intake and smaller relative amount of adipose fat in the low line chickens (DUNNINGTON *et al.* 1987). Cloacal temperatures were exceedingly narrow across both populations, probably because of natural selection to maintain an internal temperature (DUNNINGTON *et al.* 1984).

2.5 Diabetes

Plasma concentrations of glucose, lipid and protein from the high and low line were compared at 25 and 61 days of age. High line chickens had higher concentrations of glucose, lipid and protein in plasma at 25 but not at 61 days of age (CHERRY *et al.* 1987). Low line chickens were more able to clear glucose from the blood compared to the high line chickens at all ages. Impaired glucose tolerance in high line chickens was not associated with an insulin insufficiency and it was concluded that excessive fat deposition in the high line was associated with increased concentrations of insulin and glucagons in plasma and perhaps insulin resistance (SINSIGALLI *et al.* 1987);(DUNNINGTON and SIEGEL 1996).

2.6 Growth hormones and Thyroid hormones

Nir *et al.,* (1987) measured plasma concentrations of growth hormone (GH), triiodothyronine (T3) and thyroxine (T4) at 25 and 61 days of age. Differences in hormone levels between the lines were larger at 61 than 25 days of age. Growth hormone decreased and T4 increased with age in the high line chickens. Plasma GH and T4 increased and T3 decreased during days when chickens were not fed (NIR *et al.* 1987). They also showed that GH inhibited lipogenesis and stimulated lipolysis (NIR *et al.* 1987).

Insulin-like growth factor-1 (IGF-1) has been demonstrated to stimulate growth in mammals. Plasma concentrations of IGF-1 was higher in the high line than in low line at young age, but there were no difference between the lines as adults (SCANES *et al.* 1989).

2.7 Digestive enzymes

In feed-restricted chicks, specific activities of pancreatic amylase, trypsin and chymotrypsin were less in the low-line at 25 days of age. It is suggested that high-line chickens need more digestive enzymes since they are heavier, consume more feed, utilize feed more efficiently and has a faster passage rate than low-line chickens. When pancreatic enzyme activities were corrected for body weight, differences in trypsin and chymotrypsin were not significant between the high line and a White Plymouth Rock line, and amylase were significantly higher for the White Plymouth Rock line than for the high line (CHERRY *et al.* 1987). Enzymatic activity in the pancreas increased with age for amylase relative for body weight, total trypsin, total and relative chymotrypsin and total and relative lipase (O'SULLIVAN *et al.* 1992).

Enzymatic activities in the intestinal content differed from those in the pancreas and appeared to be line-dependent. Trypsin was lower in the low line at 3 days of age, but by day 9 the level had increased so that it no longer differed from the high line. Intestinal chymotrypsin increased from day 3 to 9 in all lines with no difference between them. There were no differences between intestinal amylase (NITSAN *et al.* 1991). Levels of digestive enzymes in organs and contents of the GIT were influenced by genetic stock, feed composition and level of feed intake (O'SULLIVAN *et al.* 1992).

Blood glutathione levels were greater for high line chickens during the early post hatch period. One function of glutathione is to maintain enzymes in their active state. It was concluded that in these lines of chickens, higher blood glutathione levels are correlated with heavier body weights and that the difference between the lines was greatest at younger ages (OWENS *et al.* 1970).

2.8 Reproductive traits

Chromosomal analyses of embryos reveal a higher frequency of abnormalities in the high line. The age at which pullets laid their first egg is delayed in both lines, but most in the low line. After generation 38, 8 weeks body weight decreased consistently in the low line, but the proportion of pullets that matured increased. This change suggests that the low line pullets have adjusted their physiological mechanisms to accommodate a lower body weight to achieve sexual maturation (REDDY and SIEGEL 1976).

The low line produced more normal eggs while the high line produced a higher number of total eggs but more eggs were defect. The high line has a greater incidence of multiple-yolked eggs compared to the low line and the high line laid larger eggs with more dry yolk, but less relative dry yolk and shell dry matter than those from the low line (REDDY and SIEGEL 1976; UDALE *et al.* 1972).

There was a significant difference between the lines for age at sexual maturity, pullets from the high-line matured 21 days earlier than the low-line. The difference in age at sexual maturity may be explained by differences in gonadotrophin secretion from the pituitary gland, since age at sexual maturity are influenced by the secretion of gonadotrophins, differences between target organs or both (REDDY and SIEGEL 1976; SIEGEL *et al.* 1968). Pituitaries from the high-line chickens were significantly larger than for the low-line chickens, so more gonadotrophin per pituitary should be secreted in the high-line (SIEGEL *et al.* 1968).

It is well known that there is a positive correlation between semen quality and fertility and between total defective spermatozoa and the fertilizing capacity of cockerels. A study from 1963 reported negative correlations between body weight and semen quality parameters and it has been shown that low line chickens produces semen of higher quality compared to high line cockerels. This was further investigated in 1972, and the most striking observation was that the high-line semen contained approximately twice as many abnormal or non-functional spermatozoa than the low line (EDENS *et al.* 1973).

2.9 Antibody responses to SRBC

The aim to select for immune response is to improve health of the animals and to gain insights into the underlying genetic control of immune responses. Some specific genes (for example MHC) plays a role in immune response and disease resistance, but immune response is generally a polygenic trait (PINARD-VAN DET LAAN *et al.* 1998).

Negative correlations that affect fitness may develop between a selected trait and a correlated trait. The negative correlation between growth and antibody response to SRBC (Sheep red blood cells) has been demonstrated in several experimental lines of chickens (MILLER *et al.* 1992). To measure antibody response, SRBC were injected via the brachial vein into the high and low line chickens to provide a method of ascertaining the chicken's ability to mount a protective response to foreign proteins. The response, five day after injection, for both lines was similar to that of a White leghorn population. 1 to 3 weeks after injection the low line showed the ability to maintain a more persistent antibody level compared to high line chickens (DUNNINGTON and SIEGEL 1996; DUNNINGTON *et al.* 1993; MILLER *et al.* 1992).

3. MATERIAL AND METHODS

3.1 Selection experiment

In 1957, Prof. Paul B. Siegel at the Virginia Polytechnic Institute and State University (USA) started a selection experiment were he selected chickens for high- and low-body weight. The selection stock for this experiment consisted of crosses of seven partly inbred lines of White Plymouth Rocks. Chickens with high body weights at 8-weeks of age were selected as parents for the High Line (HW) and chickens with low body weight at 8-weeks were selected as parents for the Low Line (LW). Selection was thereafter practiced within each closed line for the single trait, body weight (BW) at 8-weeks of age. 8 sires and 48 dams were selected for each line through generation 4, 12 sires and 48 dams from generation 5 to 25 and after generation 25, 14 sires and 56 dams were used. Since the experiment started, one generation has been produced each year. The population has been kept in sufficient numbers to minimize inbreeding (LIU *et al.* 1994).

High- and low- weight chickens are hatched on the first Tuesday in March every year and wing banded to enable identification of their pedigree. If the first hatch don´t give as many animals as needed, a second hatching is produced on the third Tuesday in March. Chicks are reared in the same pens on litter with hot air brooding to 8-weeks of age in all generations. Coccidiostat (an agent that controls coccidiosis in animals) is included in the diet and vaccination for Marek´s disease (commenced after the 17th generation) are the only disease preventions. Marek´s disease is a common virus that causes internal lesions (tumors). The diet includes 20% crude protein and 2,685 kcal metabolizable energy (ME)/kg from 0 to 8 weeks of age, 16% crude protein and 2,761 kcal ME/kg for weeks 8 to 28 weeks of age and 16% crude protein and 2,772 kcal ME/kg for the breeders (>18 weeks) throughout the experiment (LIU *et al.* 1994). Feed intake was restricted from the 18th generation for the HW line because of increased difficulties with reproduction (DUNNINGTON and SIEGEL 1996).

3.2 Mapping Population

An F_2 intercross of 795 individuals was generated between high- and low-line chickens from generation 41 (DUNNINGTON and SIEGEL 1996). All F_2 progeny were from the same hatch and their parents of the same age. Individuals from the F_2 population that survived to 56 days of age (n=795; BW56±SD: 624g±168g) 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 (JACOBSSON *et al.* 2004). F_1 and F_2 progeny had mean body weight values below the arithmetric average for the parental lines, which is consistent with previous findings of negative heterosis in F_1 crosses of these lines (LIU *et al.* 1995). All procedures involving animals in this experiment were carried out in accordance with the Virginia Tech Animal Care Committee animal use protocols.

Table 1. Summary of phenotypic data for the F_2 intercross from generation 41 between the high- and low-selection lines. Data for high- and low-line chickens from the literature is included for comparable traits. Fixed effects and covariates included in the QTL analyses are also given (PARK *et al.* 2006).

Traits	Number of animals	Mean±SD	Fixed effect	Covariate	HW Mean ± SD[a]	LW Mean ±SD[a]
Body Weight Traits (g)						
BW at birth	795	27.8±2.1	Sex		35.8[c]	23.0[c]
BW 2[f]	795	75.2±14.9	Sex		109± 14[d]	55±3[d]
BW 4[f]	795	179.1±56.8	Sex		516.5± 46[b]	61.3±14.6[b]
BW 6[f]	795	365.5±113.1	Sex			
BW 8[f]	795	621.7±186.9	Sex		1433.3± 100.3[b]	167.8± 38.4[b]
BW 10[f]	795	943.3±262.2	Sex			
Body composition at 10-weeks (mg)						
Abdominal Fat	402	54.4±40.7	Sex	BW10[f]		
Sum of Shank	405	424.5±119.4	Sex	BW10[f]		
Sum of Breast[e]	201	910.9±288.1	Sex	BW10[f]		
Sum of Lung	405	65.1±22.4	Sex	BW10[f]		
Spleen	401	13.9±5.1				
Bursa	405	18.5±7.1				
Metabolic parameters						
IGF1 (ng/mL)	614	5.2±1.5				
TG2[g] (ng/mL)		7.6±1.3				
Insulin (microIU/mL)	728	3.7±1.6				
Cholesterol (mg/dL)	785	111.3±19.7	Sex			
Packed Cell Volyme (%)		33.8±4.1				
SRBC		6.7±3.4				
Glucose levels (mg /dL)	782	5.5±0.1				
Glucagon levels (pg/mL)	758	13.1±3.9				
B-Protein[h]		39.3±3.5				

[a] Data from the literature (WILLIAMS *et al.* 2002)
[b] Generation 42
[c] Generation 32
[d] Generation 22, fed *ad lib*
[e] *Pectoralis Major + Pectoralis Minor*
[f] Body weight at 2-,4-,6-,8- and 10-weeks of age
[f] Triacylglycerides
[g] Amount of protein in the blood

3.3 Epistatic QTL analysis

795 F_2 individuals were genotyped and phenotyped for a variation of traits in which the HW and LW birds differ (Table 1). QTL that affected body weight in the F_2 intercross, either alone or by interacting with other loci, were mapped in this F_2 population by Carlborg *et al* (2006) using a method for detection of epistatic interactions (CARLBORG and ANDERSSON 2002; CARLBORG *et al.* 2000; CARLBORG *et al.* 2003). QTL were initially mapped using a simultaneous search for pairs of QTL using a statistical model including the fixed effect of sex and the additive, dominance and all pair wise epistatic effects of QTL pairs. Using a statistical model including epistasis, increases the power to identify loci whose effect is dependent on the genotype at other loci (CARLBORG and HALEY 2004; CARLBORG *et al.* 2003). QTL pairs that reached a 5 % genome-wide significant threshold in a randomization test for the joint effect of the epistatic pair (no QTL vs. two interacting QTL) and a 1% significant threshold in a model-selection randomization test for the joint effect of the epistatic parameters (two non-interacting QTL vs. two interacting QTL) are reported as significant pairs. QTLs were assumed to represent the same locus if mapped within 25 cM of each other. In this initial analysis, a 4 QTL network with loci on chromosome 3, 4, 7 and 20 was identified to have a large effect on body weight. This analysis was repeated using the new genetic map and the results remain significant (CARLBORG *et al.* 2006).

3.4 Network analysis

The network of four interacting loci was in the initial QTL analysis found to influence the trait for which the HW and LW had been selected, body weight at eight-weeks of age, and where they displayed a nine-fold difference. Body weight is truly a complex trait, where an increase or decrease could be due to genetic effects on multiple levels in the body, e.g. fat deposition, muscle growth, levels of growth hormones etc. To gain better insights as to how the four locus network affects body weight, we conducted analyses to explore the effects of the four loci jointly as well as independently on all traits measured in the F_2 population. For these analyses, multiple-regression analysis was performed using R. It was known from the original analysis that the effects of the loci on Gallus Gallus Autosome (GGA) 3, 4 and 20 differed depending on the genotype for the QTL on GGA 7. We therefore used a multiple regression model, where we modelled a common mean and separate effects for the GGA 3, 4 and 20 loci in individuals that were HH, HL and LL for the GGA 7 QTL.

$$y_i = \mu + Z\beta + x_i^{LL} a_{LL} + x_i^{HL} a_{LH} + x_i^{HH} a_{HH} + \varepsilon_{ij}$$

were y_i is the phenotype for F_2 bird *i*, Zβ are the effects of sex (and BW10 when it is used), $a_{LL}/a_{HL}/a_{HH}$ are the additive effects of the tested locus on GGA 3, 4 or 20 in LL/HL/HH genetic backgrounds of the QTL on GGA 7, x_i^{LL}, x_i^{HL}, x_i^{HH} are:

$$x_i^{LL}\begin{cases}\Pr(QQ)-\Pr(qq), Q_{GGA7}\subset\{LL\}\\ 0, Q_{GGA7}\subset\{HL,HH\}\end{cases}$$

$$x_i^{HL}\begin{cases}\Pr(QQ)-\Pr(qq), Q_{GGA7}\subset\{HL\}\\ 0, Q_{GGA7}\subset\{LL,HH\}\end{cases}$$

$$x_i^{HH}\begin{cases}\Pr(QQ)-\Pr(qq), Q_{GGA7}\subset\{HH\}\\ 0, Q_{GGA7}\subset\{LL,HL\}\end{cases}$$

For these analyses, 538 genetically highly informative F_2 individuals were used. The individuals were selected as follows. QTL genotype probabilities were estimated for the four QTL (GGA 3 109 cM, GGA 4 33 cM, GGA 7 63 cM and GGA 20 56 cM) (HALEY *et al.* 2004). Only individuals where the genotype for all four QTL could be assigned with greater than 80% probability were included in the analysis. The set of commands used in R to conduct this analysis is presented in Appendix 2.

In all analyses, the effects of sex were included and for analyses of abdominal fat, breast muscle weight and shank weight, an additional analysis was performed with body weight at 10-weeks of age (weight at slaughter) included as a covariate. These analyses were performed to explore whether the network alters the relative amount of fat, breast muscle and shank in the body rather than the absolute levels.

To evaluate the joint effects of all four loci on the measured traits, the subset of 538 highly genetically informative individuals for the four loci in the genetic network were used to construct a genotype –phenotype map (LE ROUZIC *et al.* In prep.). There are three possible genotypes (LL, HL or HH) at each locus, with twice as many heterozygous individuals as homozygous, due to the F_2 design. Among the $3^4 = 81$ possible genotypes, we obtained information (*i.e.* an estimate of the average phenotype) for 77 genotypes, with between one and 38 phenotyped individuals per genotype. These complete genotype-phenotype maps were plotted by genotype background of the GGA 7 QTL and total number of low line QTL alleles for the GGA 3, 4 and 7 QTL. Regression lines were fitted for the phenotype on the total number of low line QTL alleles for the loci on GGA 3, 4 and 20 in LL and HH genotypic backgrounds for the locus on GGA 7 respectively.

3.5 Descriptive statistics from literature

The high- and low- body weight selected lines show a wide range of correlated responses to selection. By studying the genetic regulation of these traits, one can get more insights to the genetic architecture underlying body weight traits and also the pathways involved in their regulation. To understand which metabolic traits for which the lines display large enough differences to be useful in a gene mapping study, we collected information from the literature on the descriptive statistics for these traits.

The statistics can be used to calculate the sample sizes needed in an experiment to detect individual and combinations of loci that explains a various amount of the high- low- line original difference. From literature data, standard deviations were calculated **($\sigma = \sqrt{\sigma^2}$)** and the standard error of the mean was calculated as **($SE = \sigma / n$),** where σ^2 is the phenotypic variance and n is the number of individuals in the sample.

3.6 QTL introgression line sample sizes

Since the discovery of DNA markers, microsatellites and SNPs (short nucleotide polymorphism), research on detection and mapping of QTL in animal species has been performed. These DNA markers is used to introgress genes from a donor to a recipient animal, a process known as marker-assisted introgression (MAI). Most characteristics of animals are under multigene control and studies on selecting for or introgress more than one QTL has been published (HOSPITAL and CHARCOSSET 1997) (KOUDANDE *et al.* 2000; KOUDANDE *et al.* 2005).

A traditional introgression program consists of the production of an F_1 generation from the founder animals and is followed by a number of backcrossing generations aiming to reduce the proportion of the donor genome. Finally, an intercrossing phase is used to fix the introgression alleles (KOUDANDE *et al.* 2000). The introgression lines planed to comply this experiment will be generated by simultaneously introgressing the four QTL alleles from the low-line to the high-line background.

The genome in this introgression lines will consist mainly of the HW line and to estimate the sample sizes needed to detect genetic effects of various size one cannot use the original HW and LW standard deviations. We thus calculated new SD´s for the various sizes of QTL effects by interpolating the SD´s from HW and LW depending on the size of the genetic effect of the studied network. For example, the estimate we used for the standard deviation when the network effect was 2% of the original line difference, we took 98 % of the high line standard deviation and 2% of the low line standard deviation, **$\sigma_2 = (0{,}98*\sigma_{HW}) + (0{,}02*\sigma_{LW})$.**

Calculating standard deviation for 2% line difference for body weight at 8-weeks;
$\sigma_2 = (0{,}98*100{,}3) + (0{,}02*38{,}4) =$ **99.06**

The population size needed to detect a specific effect size was calculated from a one sided t-distribution;

$$t = \frac{x - y}{\sqrt{\frac{\sigma_x^2}{n_x} + \frac{\sigma_y^2}{\sigma_y}}}$$

$$t = \frac{x - y}{\sqrt{\frac{\sigma_x^2 + \sigma_y^2}{n}}} = \frac{x - y}{\sqrt{\frac{1}{n} \times (\sigma_x^2 + \sigma_y^2)}} = \frac{x - y}{\sqrt{\frac{1}{n}} \times \sqrt{\sigma_x^2 + \sigma_y^2}}$$

$$t\sqrt{\frac{1}{n}} = \frac{x - y}{\sqrt{\sigma_x^2 + \sigma_y^2}}$$

$$\frac{t^2}{n} = \frac{(x - y)^2}{\sigma_x^2 + \sigma_y^2}$$

$$n = \frac{t^2(\sigma_x^2 + \sigma_y^2)}{(x - y)^2} = \frac{2\sigma^2 t^2}{D^2}$$

Population size needed to detect 2% line difference for body weight at 8-weeks of age was calculated as follow:

$n = 2*(1{,}645^2 * 99{,}06^2) / (0{,}02 * (1433{,}3 - 167{,}8))^2 = 82.9$

4. RESULTS AND DISCUSSION

The selection experiment has resulted in a nine-fold weight difference between the high- and low- weight line at 8-weeks of age and correlated responses in other traits including abdominal fat, shank weight and breast muscle weight. The four-QTL network is estimated to predict about 45% of this nine-fold weight difference between the lines. This is the first empirical evidence for how interactions in gene networks cause a response to selection (CARLBORG *et al.* 2006).

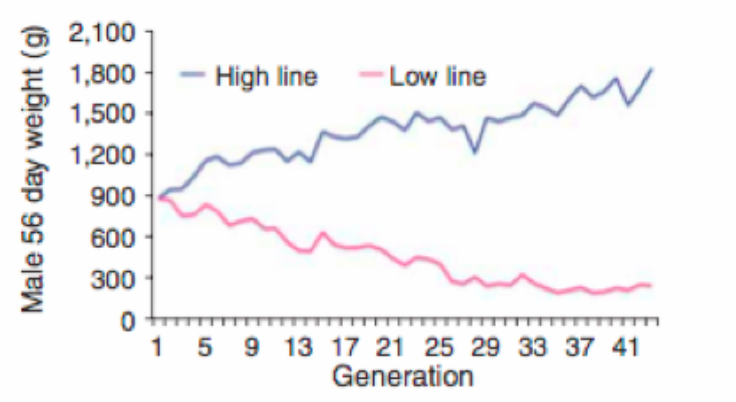

Figure 2. Male body weight at 8-weeks of age in generations 1 to 42 for the high- and low-body weight lines (CARLBORG *et al.* 2006).

The original report by Carlborg *et al* (2006) describes the effects of the network on body weight and fat deposition, the study does not, however report in-depth results on other phenotypes collected on the F_2 individuals. This thesis presents results from analyses to evaluate the effects of the four QTL network on other measured traits in the F_2 population and to see which traits that are useful in further epistatic analyses. The study also serves as a replication of the original study by including data on a large number of additional markers in the QTL regions. The analyses based on the new genetic map with more markers included replicated the large effect of the network on body weight.

It is difficult to evaluate the genetic factors that influence long-term selection of a quantitative trait since big changes occur during the selection. Responses may be influenced by population size, changes in fitness, initial gene frequencies, rate of allelic fixation, mutation, inbreeding, changes in variance, environment, and genetic and physiological limits. A long-term experiment like this can provide insights that can not be obtained from short-term experiment or commercial breeding programs (DUNNINGTON and SIEGEL 1985).

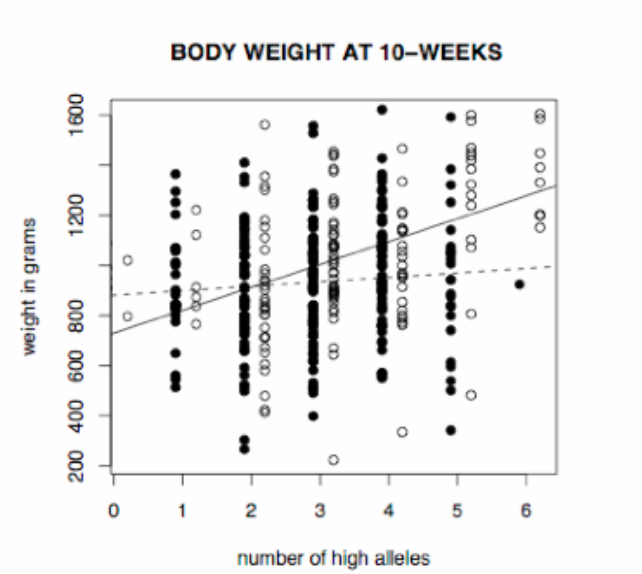

Figure 3. Body Weight at 10-weeks of age. Dotted line refers to the effect of increasing the number of high-line alleles for the loci on chromosome 3, 4 and 20 in a LL-background on chromosome 7 and the blank to their effects in a HH-background. Number of high –line alleles on loci 3, 4 and 20 are shown on the x-axis. Trait levels when homozygous for high-line alleles on chromosome 7 are represented by white dots and the low-line alleles to black dots.

The study of the network effect on all traits measured in the F_2 population show a significant effect on body weight at different ages, lung, spleen, abdominal fat, breast muscle weight and shank weight. Body weight at birth is not significantly influenced, birth weight is highly correlated to the hens body weight. No significant effects were found for the rest of the traits. Large differences between the phenotype values are shown in the graphs, for example, body-weight at 10-weeks of age (figure 3), the measured values vary between 300-1300 grams for the same amount of high line alleles. This is most probably due to dominance between different genotypes rather then environmental effects. The phenotypic values would presumably not differ this much if they only where due to environmental effects.

When corrected for body weight at slaughter (10-weeks of age) there were no significant effects of the network on shank weight. The regression analysis indicates that the only remaining effects where that of chromosome 3, in a chromosome 7 background. In a 7-LL background the relative abdominal fat increases and relative breast muscle decreases when going from LL to HH. When abdominal fat is not corrected for body-weight at slaughter, the increase in fat deposition is proportional with increased body weight while breast muscle is decreasing. In a chromosome 7-HH background, abdominal fat is increasing with body-weight but relative abdominal fat is not when chromosome 3 is going from LL to HH. Relative breast muscle and lung weight is decreasing both in a 7-LL and 7-HH background, lung not corrected for BW10 is proportional with an increase in body-weight.

Table 2. Mean and standard deviation (g) for chickens with different alleles at chromosome 3 and 7.

	HH C7		LL C7	
	AF[b]	BrM[a]	AF[b]	BrM[a]
HH C3	156.1±288.2	988.1±343.6	46.6±40.6	778.6±398.2
LL C3	89.0±199.0	908.1±239.8	29.67±31.63	821.3±204.2

[a] Sum of breast muscle weight
[b] Abdominal fat deposition

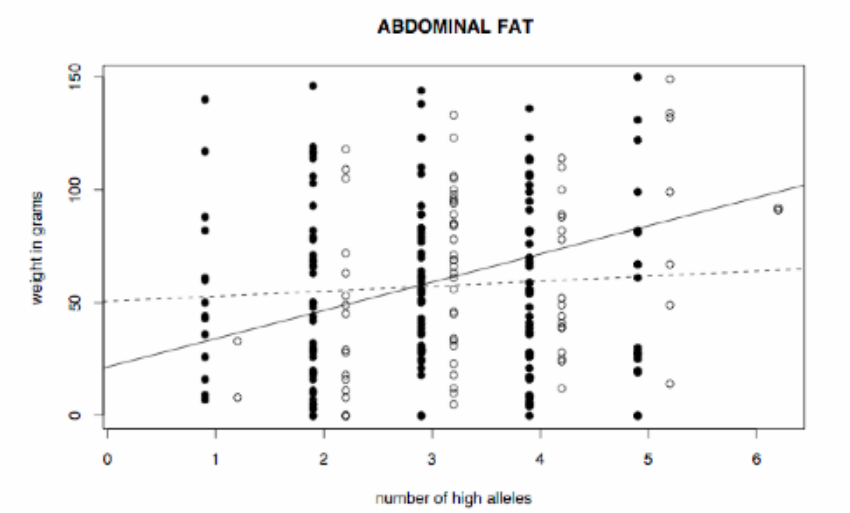

Figure 4. Abdominal Fat not corrected for BW at10-weeks of age. Dotted line refers to LL-background and the blank line to HH-background for loci on chromosome 7. Number of high –line alleles on loci 3, 4 and 20 are shown on the x-axis.

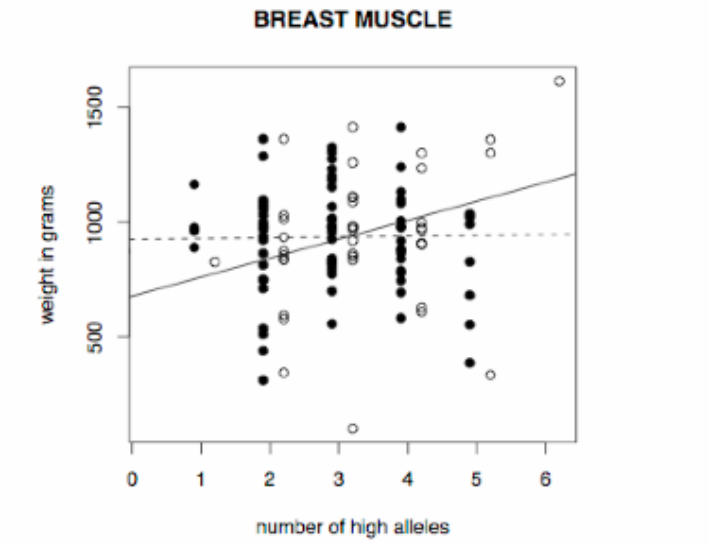

Figure 5. Sum of breast muscle weight, not corrected for BW at 10-weeks age. Dotted line refers to LL-background and the blank line to HH-background for loci on chromosome 7. Number of high –line alleles on loci 3, 4 and 20 are shown on the x-axis.

The QTL on chromosome 3 thus apparently have a complex role in body-weight regulation. The low line alleles increase body weight in a chromosome 7-LL and 7-HL background and HH alleles decreases body weight in a chromosome 7-LL background (Figure 6). The effect is more pronounced at later stages in life (CARLBORG *et al.* 2006).

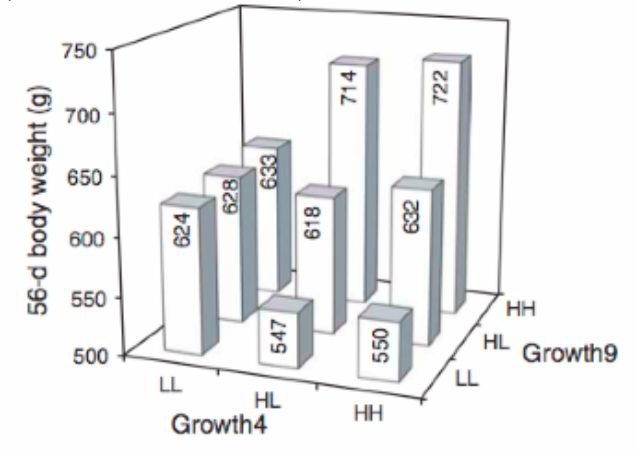

Figure 6. Phenotypic means for Body Weight at 8-weeks of age depending on different genotypes at the loci on chromosome 3 (Growth4) and for different genotype classes in loci on chromosome 7 (Growth 9). Body Weight is decreasing when the genotype for chromosome 3 goes from LL to HH in a LL-background and increasing in a HH-background (CARLBORG *et al.* 2006).

These results suggest that there is a change in the chicken body composition when selected for higher body weight. Chickens tend to go from lean and muscular to fat and thin when selected for higher body-weight. These loci increase relative body weight in the high line, an increase due to increased body fat deposition, but is accompanied by a decrease in the muscle mass in the breast. Selection for lower body weight will decrease body weight by decreasing muscle mass with a continued fat deposition. Body fat is important for the reproduction to start, and a large decrease in fat deposition might therefore prevent the chickens to reproduce. The lines has differences in appetite and feed consumption, which might lead to higher or lower fat deposition in the birds. Some chicks in the low line die within the first week after hatch because they never start to eat. Some of those pullets in the low line that consume sufficient amounts of feed to survive don't consume enough feed to achieve sexual maturity. When force fed, these birds were able to survive and reproduce (DUNNINGTON and SIEGEL 1996).

LL-alleles in the QTL on chromosome 3 might increase body weight in a low line background as a survival factor, if the birds decrease too much in body weight and body fat they would not be able to reproduce or even survive (described in the literature review for reproduction). The age at which pullets laid their first egg is delayed in both lines, but most in the low line. After generation 38, 8-weeks body weight decreased consistently in the low line, but the proportion of pullets that matured increased. This change suggests that the low line pullets have adjusted the

physiological mechanisms to accommodate a lower body weight to achieve sexual maturation (REDDY and SIEGEL 1976).

There are marked differences in eating behaviour between the selected lines, the high line showing hyperphagia and the low line anorexia. Reduction in food consumption is one correlated response to selection for low body weight. It would be interesting to explore if the four QTL network has any affect on appetite and if fat deposition and breast muscle weight is affected by different eating behaviour, not only by the amount. It could also be interesting to explore movement differences between the lines, maybe the high line moves around less than the low line, and thereby loses muscle mass.

Table 3 and Appendix 1, shows the population size needed to detect a genetic effect explaining a given portion of the original line difference using a QTL introgression line. As many of these traits are complicated and costly to measure, we expect that 100 individuals per line is a reasonable starting point, thus we present results for the traits that needs a population size of 200 or less to detect significant differences between the lines. Some of the traits require lower sample sizes than 100. Many of the traits, especially reproductive traits, require larger population sizes to detect significant differences between the lines than what is economically motivated.

Table 3. Number of animal needed to detect a specific effect size of the network (2, 5 and 10%). Data for more traits are found in Appendix 1

Trait	10	5	2
BW[a]			
28d	100	100	100
56 d	100	100	100
168 d	100	100	150
266 d	100	100	
First egg[b]	100	100	150
Shank[c]	100	100	100
GH[d]			
25d	200		
61d	100	100	200
Cumulative Feed Intake[e]			
1d	200		
5d	100	200	
7d	100		
14d	100	100	
21d	100	100	150

[a] Body weight at different days of age
[b] Body weight at first egg
[c] Measured in mm/ 100g BW
[d] Plasma Growth Hormone (ng/ml) 25- and 61- days of age
[e] Cumulative Feed Intake (g) at different days of age

The network mechanism is complex, and a new strategy to study the network is needed. Next step in this study will be to design a breeding scheme to develop a set of QTL introgression lines that can make it possible to explain this network more detailed. Only 1/256 F_2 individuals have the most informative four-QTL genotype (4×HH vs 4×LL) for the gene-network we want to study, and an introgression line with these genotypes will make it possible to study the joint effects of the four-QTL network. The aim is to introgress low line alleles for the four QTL into a high line background. The high line is more fertile compared to the low line and therefore selected as the background since introgression will be performed by marker selected back-crossing to one of the pure lines. The QTL introgression lines would maximize power by only using extreme genotypes in the test and make it easy to perform repeated studies both at one time and across generations. Since the lines are fixed for alternative QTL, genotyping is not required. The background will be more homogenous than for an intercross so the genetic background noise will be reduced. Phenotyping will also be reduced since one can compare only the most divergent genotypes and also perform a series of studies to first explore whether there are any genetic effects at all (using the four-QTL line) before conducting more refined studies based on the 1-, 2- and 3- QTL lines.

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APPENDIX 1

Table 1a. Statistical calculations Body Weight

BW, g[a]	High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
28d	516,5	61,3	133	133	2116	213,16	46,00	14,60	3,99	1,27	(WILLIAMS et al. 2002)
56d	1433,3	167,8	133	133	10060,09	1474,56	100,30	38,40	8,70	3,33	
168d	2494,3	771,8	133	133	35948,16	28056,25	189,60	167,50	16,44	14,52	
266d	3019,7	1152,8	133	133	82311,6	25312,8	286,90	159,00	24,88	13,79	
At first egg, d	2781,3	1122,8	133	133	36825,61	23994,01	191,90	154,90	16,64	13,43	
Feed and water ad lib[b]											(BARBATO et al. 1983)
7d	62	41	5	5	81	16	9,00	4,00	4,02	1,79	
14d	85	52	5	5	144	49	12,00	7,00	5,37	3,13	
21d	111	58	5	5	289	121	17,00	11,00	7,60	4,92	
28d	141	65	5	5	529	196	23,00	14,00	10,29	6,26	
Water restriction[c]											(BARBATO et al. 1983)
7d	58	37	5	5	36	9	6,00	3,00	2,68	1,34	
14d	88	47	5	5	100	25	10,00	5,00	4,47	2,24	
21d	103	61	5	5	289	81	17,00	9,00	7,60	4,02	
28d	128	67	5	5	400	144	20,00	12,00	8,94	5,37	
Feed restriction[d]											(BARBATO et al. 1983)
7d	51	30	5	5	36	9	6,00	3,00	2,68	1,34	
14d	60	39	5	5	49	36	7,00	6,00	3,13	2,68	
21d	75	58	5	5	100	64	10,00	8,00	4,47	3,58	
28d	84	63	5	5	144	100	12,00	10,00	5,37	4,47	
At sexual maturity (g)[e]	1968	796	50	30	192200	38880	438,41	197,18	62,00	36,00	(ZELENKA et al. 1987)
7d											(BARBATO et al. 1984)
Ad lib	61	31	10	10	81	9	9,00	3,00	2,85	0,95	
Overfed	66	46	10	10	49	25	7,00	5,00	2,21	1,58	
14d											
Ad lib	109	55	10	10	196	144	14,00	12,00	4,43	3,79	
Overfed	118	83	10	10	100	81	10,00	9,00	3,16	2,85	
21d											
Ad lib	185	89	10	10	676	529	26,00	23,00	8,22	7,27	
Overfed	199	137	10	10	441	256	21,00	16,00	6,64	5,06	

[a]Body weight at different days of age
[b] Body weight at different age of days when feed and water was restricted
[c] Body weight at different age of days when the amount of water was restricted
[d] Body weight when the amount of feed was restricted
[e] Body weight at sexual maturity when feed *ad lib* or overfed

Table 1b. Estimation of the sample size needed in an experiment to detect individual and combinations of loci that explains a various amount of the low- line original difference (%)

BW[a] g	10	5	2
28d	100	100	100
56 d	100	100	100
168 d	100	100	150
266 d	100	100	
At first egg	100	100	150
Body weight (feed and water ad lib)[b]			
7d	100		
14d	100	200	
21d	100	200	
28d	100	150	
BW at water restriction[c]			
7d	100	150	
14d	100	100	
21d	100		
28d	100	200	
Body weight (feed restriction)[d]			
Days of age 7	100	150	
Days of age 14	100	200	
Days of age 21	150		
Days of age 28	150		
Body Weight at sexual maturity,g[e]	100		
7 d			
Ad lib	100	150	
Overfed	100	200	
14d			
Ad lib	100	100	
Overfed	100	150	
21d			
Ad lib	100	150	
Overfed	100	200	

Table 2a. Statistical calculations on Reproductive traits and spermatozoa

Reproductive traits	High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
Age at first egg[a]	188,2	216,7	133	133	68,89	338,56	8,30	18,40	0,72	1,60	(WILLIAMS et al. 2002)
Hen-day ovulations%	57,2	60,4	133	133	201,64	204,49	14,20	14,30	1,23	1,24	
Hen-day normal eggs%	52,9	60	133	133	174,24	207,36	13,20	14,40	1,14	1,25	
Normal eggs%	94,6	99,5	133	133	26,01	4,84	5,10	2,20	0,44	0,19	
Defective eggs%	5,5	0,5	133	133	27,04	5,29	5,20	2,30	0,45	0,20	
Double yolk%	1,9	0,2	133	133	5,76	1	2,40	1,00	0,21	0,09	
Extra calcified%	1,7	0,1	133	133	7,29	0,4225	2,70	0,65	0,23	0,06	
Broken%	1,2	0,1	133	133	3,24	0,7569	1,80	0,87	0,16	0,08	
Other%	0,6	0,1	133	133	2,7225	0,7569	1,65	0,87	0,14	0,08	
Spermatozoa %											(EDENS et al. 1973)
Trial 1											
Normal	62	78,9	12	13	87,5	47	9,35	6,86	2,70	1,90	
Dead	21,2	12,8	12	13	27	47	5,20	6,86	1,50	1,90	
Total abnormal	16,8	8,3	12	13	63,6	18,7	7,97	4,32	2,30	1,20	
Trial 2											
Normal	58,8	80,5	17	12	258,6	17,3	16,08	4,16	3,90	1,20	
Dead	22,4	7,4	17	12	174,1	12	13,19	3,46	3,20	1,00	
Total abnormal	18,8	12,1	17	12	49,1	12	7,01	3,46	1,70	1,00	

[a] Age at first egg, in days

Table 2b Estimation of the sample size needed in an experiment to detect individual and combinations of loci that explains a various amount of the low- line original difference (%)

Reproductive traits		10	5	2
Spermatozoa % (g36&35)				
Trial 1				
	Normal	200		
	Dead	150		
	Total abnormal	150		
Trial 2				
	Normal			
	Dead	200		
	Total abnormal			

Table 3a. Statistical calculations on Plasma nutrient traits

Plasma glucose mg/100ml	High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
25 days (age)	267	218	5	5	845	405	29,07	20,12	13,00	9,00	(CHERRY et al. 1987)
61 days (age)	298	296	5	5	1620	3380	40,25	58,14	18,00	26,00	
Plasma lipids mg/100ml											
25 days (age)	595	423	5	5	6845	1125	82,73	33,54	37,00	15,00	
61 days (age)	448	387	5	5	6125	2880	78,26	53,67	35,00	24,00	
Plasma protein mg/100ml											
25 days (age)	4,4	3,1	5	5	0,45	0,2	0,67	0,45	0,30	0,20	
61 days (age)	3,4	3,4	5	5	0,45	0,2	0,67	0,45	0,30	0,20	
Liver lipid mg/100ml											
25 days (age)	4,1	1,8	5	5	0,8	0,2	0,89	0,45	0,40	0,20	
61 days (age)	3,4	2,9	5	5	1,25	0,8	1,12	0,89	0,50	0,40	

Table 4a Statistical calculations on Feed intake and feed efficiencies

Cumulative Feed intake[a]	High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
1d	5	2	20	20	4	1	2,00	1,00	0,45	0,22	(BARBATO et al. 1984)
5d	40	21	20	20	49	36	7,00	6,00	1,57	1,34	
6d	73	45	20	20	144	100	12,00	10,00	2,68	2,24	
14d	196	129	20	20	256	144	16,00	12,00	3,58	2,68	
21d	465	274	20	20	484	225	22,00	15,00	4,92	3,35	
Cumulative feed efficiencies[b]											(BARBATO et al. 1984)
7d											
Ad lib	0,44	0,27	10	10	0,0004	0,0016	0,02	0,04	0,01	0,01	
Overfed	0,44	0,42	10	10	0,0025	0,0049	0,05	0,07	0,02	0,02	
14d											
Ad lib	0,41	0,26	10	10	0,0016	0,0004	0,04	0,02	0,01	0,01	
Overfed	0,4	0,34	10	10	0,0049	0,0025	0,07	0,05	0,02	0,02	
21d											
Ad lib	0,33	0,21	10	10	0,0009	0,0001	0,03	0,01	0,01	0,00	
Overfed	0,32	0,32	10	10	0,0036	0,0016	0,06	0,04	0,02	0,01	

[a] Cumulative feed intake in grams at different ages in days
[b] Cumulative feed efficiency in days of age fed *ad lib* or overfed

Table 4b. . Estimation of the sample size needed in an experiment to detect individual and combinations of loci that explains a various amount of the low- line original difference (%)

Cumulative Feed intake,[a]	10	5	2
1d	200		
5d	100	200	
7d	100		
14d	100	100	
21d	100	100	150
Cumulative feed efficiencies[b]			
7d	100	100	150
14d	100	150	
21d	100	100	
Age at first egg, d	100	150	

Table 5a. Statistical calculations on Growth hormones

Plasma growth hormone (GH) (ng/ml)[a]	High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
25d	39,8	67,6	5	5	387	470	19,67	21,68	8,80	9,70	(NIR et al. 1987)
6d1	14,2	87,2	5	5	110	1445	10,49	38,01	4,69	17,00	
Triiodothyronine (T3) (ng/ml)[a]											(NIR et al. 1987)
25d	1,8	1,8	5	5	0,2	0,2	0,45	0,45	0,20	0,20	
61d	1,8	2,6	5	5	0,2	0,2	0,45	0,45	0,20	0,20	
Thyroxine (T4) (ng/ml)[a]											(NIR et al. 1987)
25d	21,8	19,2	5	5	9,8	6	3,13	2,45	1,40	1,10	
61d	24,2	21,2	5	5	0,8	7,2	0,89	2,68	0,40	1,20	
T4:T3[a]											(NIR et al. 1987)
25d	14,8	14,9	5	5	24	36	4,90	6,00	2,19	2,68	
61d	15,6	9	5	5	22	6	4,69	2,45	2,10	1,10	

[a] Amount of growth hormone at 25 and 61 days of ag

Table 5b. Estimation of the sample size needed in an experiment to detect individual and combinations of loci that explains a various amount of the low- line original difference (%)

Plasma growth hormone (GH) (ng/ml)[a]		10	5	2
Age (days)	25	200		
Age (days)	61	100	100	200
Triiodothyronine (T3) (ng/ml)[a]				
Age (days)	61	100		
Thyroxine (T4) (ng/ml)[a]				
Age (days)	61	100	150	
T4:T3[a]				
Age (days)	61	200		

Table 6a. Statistical calculations on Hematocrit and blood Glutathione at different days of age

Hematocrit %	High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
1d	33,5	12,25	12	12	33	6,25	3,5	2,5	1,01	0,72	(OWENS et al. 1970)
4d	30,3	1,96	12	12	30,7	4,42	1,4	2,1	0,40	0,61	
7d	26,8	3,24	12	12	26,9	6,25	1,8	2,5	0,52	0,72	
10d	27,9	7,84	12	12	27,9	7,84	2,8	2,8	0,81	0,81	
13d	27,7	4,42	12	12	26,7	5,29	2,1	2,3	0,61	0,66	
16d	27,7	3,61	12	12	27,7	4,41	1,9	2,1	0,55	0,61	
19d	26,3	6,76	12	12	26,5	4,84	2,6	2,2	0,75	0,64	
28d	27,9	2,56	12	12	26,5	6,76	1,6	2,6	0,46	0,75	
56d	29,4	2,56	12	12	28,1	4,42	1,6	2,1	0,46	0,61	
Blood Glutathione, GSH (mg/100 ml blood)											(OWENS et al. 1970)
1d	54,5	219,04	12	12	43,7	108,16	14,8	10,4	4,27	3,00	
4d	86,6	84,64	12	12	72,3	104,04	9,2	10,2	2,66	2,94	
7d	85	104,04	12	12	79,5	228,01	10,2	15,1	2,94	4,36	
10d	81,1	216,09	12	12	78,3	129,96	14,7	11,4	4,24	3,29	
13d	80,7	56,25	12	12	71,3	90,25	7,5	9,5	2,17	2,74	
16d	79,7	84,64	12	12	77,4	106,09	9,2	10,3	2,66	2,97	
19d	63,6	57,76	12	12	60,1	96,04	7,6	9,8	2,19	2,83	
28d	73,7	50,41	12	12	65,1	158,76	7,1	12,6	2,05	3,64	
56d	70,9	106,09	12	12	59,9	104,04	10,3	10,2	2,97	2,94	

Table 6b. . Estimation of the sample size needed in an experiment to detect individual and combinations of loci that explains a various amount of the low- line original difference (%)

	10	5	2
Hematocrit %			
1d	100	100	
4d	100	100	100
7d	100	100	100
10d	100	100	200
13d	100	100	100
16d	100	100	100
19d	100	100	200
28d	100	100	100
56d	100	100	100
Blood Glutathione, GSH (mg/100 ml blood)			
1d	100	100	100
4d			
7d	150		
10d	100	100	150
13d	100	150	
16d			
19d			
28d	100	150	
56d	100	150	

Table 7a. Statistical calculations on Organs

Organ lenght (mm organ/100g bw)	High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
Shank	6,26	27,58	4	4	1,2544	42,772	1,12	6,54	0,56	3,27	(KATANBAF et al. 1989)
Esophagus	13,24	59,57	4	4	4,75	301,4	2,18	17,36	1,09	8,68	
Small intestine	122,8	463,7	4	4	812,3	28988	28,50	170,26	14,25	85,13	
Tarsus-metatarsus TM lenght (mm)	60	45	50	30	50	30	7,07	5,48	1,00	1,00	(ZELENKA et al. 1987)
Absolute weight (g)[a]											(BARBATO et al. 1984)
Esophagus and crop											
ad lib	1,5	0,7	10	10	0,16	0,09	0,40	0,30	0,13	0,09	
overfed	4,5	3,5	10	10	0,64	0,09	0,80	0,30	0,25	0,09	
Proventriculus											
ad lib	1	0,8	10	10	0,25	0,09	0,50	0,30	0,16	0,09	
overfed	1,6	1,2	10	10	0,01	0,04	0,10	0,20	0,03	0,06	
Gizzard											
ad lib	5,7	4,3	10	10	1,69	0,16	1,30	0,40	0,41	0,13	
overfed	8	6,2	10	10	0,25	1	0,50	1,00	0,16	0,32	
Duodenum											
ad lib	1,4	1	10	10	0,16	0,09	0,40	0,30	0,13	0,09	
overfed	2,4	2,1	10	10	0,16	0,49	0,40	0,70	0,13	0,22	
Intestine											
ad lib	4,9	3,3	10	10	2,89	1	1,70	1,00	0,54	0,32	
overfed	8,9	6	10	10	1,44	1,44	1,20	1,20	0,38	0,38	
Relative weight (g/g bw*100)											(BARBATO et al. 1984)
Esophagus and crop											
ad lib	0,8	0,8	10	10	0,04	0,04	0,20	0,20	0,06	0,06	
overfed	2,3	2,6	10	10	0,09	0,09	0,30	0,30	0,09	0,09	
Proventriculus											
ad lib	0,5	0,9	10	10	0,01	0,04	0,10	0,20	0,03	0,06	
overfed	0,8	0,9	10	10	0,04	0,09	0,20	0,30	0,06	0,09	
Gizzard											
ad lib	3,1	4,8	10	10	0,04	0,16	0,20	0,40	0,06	0,13	
overfed	5	4,5	10	10	0,16	0,09	0,40	0,30	0,13	0,09	
Duodenum											
ad lib	0,8	1,1	10	10	0,01	0,16	0,10	0,40	0,03	0,13	
overfed	1,2	1,5	10	10	0,09	0,04	0,30	0,20	0,09	0,06	
Intestine											
ad lib	2,6	3,5	10	10	0,25	1,96	0,50	1,40	0,16	0,44	
overfed	4,5	4,4	10	10	0,64	1,21	0,80	1,10	0,25	0,35	
Organs											(REDDY and SIEGEL 1976)
Liver											
Absolute weigth (g)	66,7	32,5	40	40	144,4	25,6	12,02	5,06	1,90	0,80	
Adjusted (g./100g bw)	2,1	1,9	40	40	0,4	0,4	0,63	0,63	0,10	0,10	
Thyroid											
Absolute weigth (g)	142,7	66,2	40	40	1 081,6	291,6	32,89	17,08	5,20	2,70	
Adjusted (g./100g bw)	4,46	3,8	40	40	230,4	1,024	15,18	1,01	2,40	0,16	
Pituitary											
Absolute weigth (g)	9,4	6,4	40	40	3,6	3,6	1,90	1,90	0,30	0,30	
Adjusted (g./100g bw)	0,29	0,37	40	40	0,004	0,016	0,06	0,13	0,01	0,02	
Ovary											
Absolute weigth (g)	41,1	21,9	40	40	384,4	67,6	19,61	8,22	3,10	1,30	
Adjusted (g./100g bw)	1,3	1,3	40	40	14,4	0,4	3,79	0,63	0,60	0,10	

[a] Absolute organ weight in gram at 21 days of age, fed *ad lib* overfed

Table 7b. . Estimation of the sample size needed in an experiment to detect individual and combinations of loci that explains a various amount of the low- line original difference (%)

Organ lenght (mm organ/100g bw)	10	5	2
Shank	100	100	100
Esophagus	100	100	100
Small intestine	100	100	100
Tarsus-metatarsus TM lenght (mm)	100		
Absolute weight (g) [a]			
Esophagus and crop			
ad lib	100		
Proventriculus			
ad lib			
overfed	100	100	
Gizzard			
ad lib			
overfed	100	150	
Intestine			
ad lib			
overfed	100		
Relative weight (g/g bw*100)			
Proventriculus			
ad lib	100	100	
overfed			
Gizzard			
ad lib	100	100	150
overfed			
Duodenum			
ad lib	100	200	
overfed			
Intestine			
ad lib	150		
overfed			
Organs, generation 16			
Liver			
Absolute weigth (g)	100	200	
Thyroid			
Absolute weigth (g)	100		
Pituitary			
Absolute weigth (g)	150		

Table 8a. Statistical calculations on Surface and cloacal temperatures

	High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
Cloacal[A]											
Ad lib[a]	41,1	41,7	13	8	0,13	0,08	0,36	0,28	0,10	0,10	(DUNNINGTON et al. 1987)
Surface [A]											
Ad lib[a]	35,6	30,9	13	8	1,17	2	1,08	1,41	0,30	0,50	

[a] Temperature in celcius when fed *ad lib*

Table 8b. Estimation of the sample size needed in an experiment to detect individual and combinations of loci that explains a various amount of the low- line original difference (%)

Cloacal	10	5	2
Ad lib	200		
Surface			
Ad lib	100	100	

Table 9a. Statistical calculations on Oxygen consumption during daytime (6.00 PM-11.00 PM) and evening

		High mv	Low mv	n High	n low	Var high	Var low	SD high	SD low	SE high	SE low	References
Day	4 w	1,43	2,22	14	14	0,144	0,4356	0,38	0,66	0,10	0,18	(OWENS et al. 1971)
Day	8 w	0,57	0,78	14	14	1,21	0,0169	1,10	0,13	0,29	0,03	
Evening	4 w	0,98	1,1	14	14	0,1156	0,0169	0,34	0,13	0,09	0,03	
Evening	8 w	0,54	0,74	14	14	0,0064	0,0169	0,08	0,13	0,02	0,03	

Table 9b. Estimation of the sample size needed in an experiment to detect individual and combinations of loci that explains a various amount of the low- line original difference (%)

Oxygen consumption		10	5	2
Day	4 weeks	100		
Evening	8 weeks	100		

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APPENDIX 2

Commands used in R

```
ny_geno <- read.delim("./Documents/R/san_ALLA_NY.txt", na.strings="*",
header=T)

ny_geno <- merge(raw_nysex, ny_geno, by.x = "anim", all=TRUE)
> ny_geno_pheno <- merge(ny_geno, raw_nyphe, by.x = "anim", all=TRUE)
```

Making factors

```
~~~~~~~~~~~~~
ny_geno_pheno$f_4A <- factor(ny_geno_pheno$fac_4A)
> ny_geno_pheno$f_7A <- factor(ny_geno_pheno$fac_7A)
> ny_geno_pheno$f_22A <- factor(ny_geno_pheno$fac_22A)
```

Linear Regression

```
~~~~~~~~~~~~~~
lm(formula = BW0 ~ SEX + X3A:f_7A + X4A:f_7A + X22A:f_7A, data =
ny_geno_pheno)
```

Linear Regression with BW10 included as a covariate

```
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
lm(formula = S_SHANK ~ SEX + BW10 + X3_131:fac_C7HF + X22_94:fac_C7HF
+ X4_39:fac_C7HF, data = total_data3)
```

Making Graphs

```
~~~~~~~~~~~~~~~~~
read.delim("./Documents/R/ny_genodat_endastA.txt",na.strings="*", header=T)
Genotyperna_sex <- merge(raw_nysex, GENOTYPERNA, by.x = "anim", all=TRUE)
genotyperna_pheno <- merge(Genotyperna_sex, raw_nyphe, by.x = "anim",
all=TRUE)

genotyperna_pheno[genotyperna_pheno[,"CHR7"]==1,] -> data_1
genotyperna_pheno[genotyperna_pheno[,"CHR7"]==0,] -> data_0
genotyperna_pheno[genotyperna_pheno[,"CHR7"]==0,] -> data_m1

data_1[,"CHR4"]+data_1[,"CHR22"]+data_1[,"CHR3"]+3 -> x
data_1[,"BW6"] -> y
plot(x+0.2,y)
reg <- lm(y~x)
abline(coef(reg))

title <- title(main = "BW6")
data_m1[,"CHR4"]+data_m1[,"CHR22"]+data_m1[,"CHR3"]+3 -> x1
data_m1[,"BW6"] -> y1
reg <- lm(y1~x1)
abline(coef(reg), untf = FALSE, lty="dashed")
points(x1-0.1, y1, pch=19)
```