Correlated selection responses in animal domestication:
the behavioural effects of a growth QTL in chickens

Anders Wirén
Cover by Anders Wirén

© Copyright 2011 Anders Wirén, unless otherwise noted

Anders Wirén
Correlated selection responses in animal domestication:
the behavioural effects of a growth QTL in chickens
ISSN: 0345-7524
Linköping Studies in Science and Technology, Dissertation No. 1413
Electronic publication: http://www.ep.liu.se

Printed in Sweden by LiU-Tryck, 2011
Till Framtiden
Abstract

Studying animal domestication offers an opportunity to understand the mechanisms of evolution. Domestication is associated with a change in selection pressures; selection for production traits is introduced, and animals are faced with larger and denser social groups. It is not unexpected then that domestication produces a simultaneous change in a number of traits, both physiological and behavioural. This correlated change in traits, e.g. egg production and social behaviour has been termed the “domestic phenotype”. However, it has been shown that selection for one trait alone among the many associated with the domestic phenotype can lead to simultaneous changes in others. This may be a result of such traits being inherited together because of pleiotropy or close linkage of several genes affecting different traits. A chicken growth QTL has previously been found in an intercross between White Leghorn layers (WL) and their main wild ancestor, the red junglefowl (RJF). This QTL has also been found to influence explorative and social behaviours. This thesis aims to characterize this QTL further with respect to social and emotional behaviours, and tries to clarify whether pleiotropy or linkage is responsible for the many observed effects. This is done using behavioural phenotyping, genetic marker genotyping, QTL- and gene expression analysis of an intercross line between RJF and WL, and to some extent of the parental RJF and WL lines themselves. The results show that domestication in these chickens has led to increased social tolerance to unfamiliar conspecifics and a tendency to a decrease in the propensity of chickens to explore the environment, and that these effects are partly explained by the previously described growth QTL. The results also indicate that close linkage of genes, rather than pleiotropy, may be responsible for the multiple effect of the QTL, as different traits to some extent seem to be influenced by different areas within the larger QTL region. This information, in combination with that of other studies and with existing and upcoming genetic research techniques, may be used in the design of future breeding programs that take animal behaviour and welfare as well as production traits into account. Findings like these may also be of use in directing research in human psychiatric genetics.
Domesticering av djur är ett exempel på en snabb evolutionsprocess. Jämfört med det vilda ställer livet i fångenskap nya krav på djur; avel för produktionsegenskaper (som äggproduktion och tillväxttakt) införs, och djuren måste anpassa sig till att leva i större och tätare grupper än förr. Därför är det inte oväntat att många fysiologiska och beteendemässiga egenskaper förändras samtidigt under processens gång, och det uppkommer vad man brukar kalla en ”domesticerad fenotyp”, d.v.s. en uppsättning egenskaper som är gemensamma för domesticerade djur i jämförelse med deras vilda förfäder. Men avelsförsök har visat att det går att framkalla en förändring i många av de här egenskaperna samtidigt även om man bara avlar för en av egenskaperna. Det tyder på att egenskaperna är genetiskt relaterade till varandra, och det skulle kunna bero på s.k. pleiotropi, d.v.s. att en (eller ett fåtal) gener har flera funktioner och därför påverkar flera av just de här egenskaperna samtidigt. Men det skulle också kunna bero på att flera gener som var och en påverkar enskilda egenskaper är kopplade till varandra, d.v.s. ligger nära varandra i kromosomerna och därför i hög grad ärvs tillsammans från en generation till en annan. Även om man vet att en egenskap i hög grad är ärflig så är det trots stora framsteg inom genforskningen fortfarande svårt att hitta enskilda gener som påverkar olika egenskaper. Men det finns metoder, t.ex. QTL-analys, som åtminstone indikerar vilka större regioner på vilka kromosomer man kan rikta in sitt sökande på. Den här avhandlingen fokuserar på en tidigare identifierat QTL (ett område som påverkar en viss egenskap) för kroppsvikt och tillväxttakt hos värphöns av typen White Leghorn ("WL") och deras vilda förfäder, det röda djungelhönset ("RJF"). WL-DNA i det här området gör höns tyngre än om de har RJF-DNA, men det påverkar också vissa beteendeegenskaper. Den här avhandlingen undersöker i vilken grad QTLen också påverkar sociala och emotionella beteenden, eftersom man skulle kunna förvänta sig att domesticeringen lett till förändringar i dessa, och om de många samtidiga effekterna av QTLen beror på pleiotropi eller koppling mellan gener. Metoderna utgörs av beteendetester och en rad genetiska tekniker (marker assisted selection, QTL- och genuttrycksanalys) på en korsning mellan RJF och WL, och i viss mån på de okorsade raserna själva. Resultaten visar att WL-DNA i QTL-området leder till att höns blir mer socialt toleranta mot andra höns, och att de får en tendens till att utforska sin omgivning mindre än de annars gör. Det här förklarar en del av skillnaden man ser i de egenskaperna mellan okorsade RJF- och WL-höns. I viss mån har
undersökningarna också lyckats visa att QTLens effekter beror på koppling av gener som påverkar olika egenskaper snarare än pleiotropi, och även om det fortfarande inte kan sägas med säkerhet vilka dessa gener är så finns det vissa kandidater som verkar troligare än andra. Tillsammans med resultat från andra studier, och med hjälp av genetiska metoder som redan existerar eller är under utveckling skulle den här informationen kunna hjälpa till att designa bättre avelsprogram i framtiden. Med tanke på att resultaten rör sociala beteenden skulle de också kunna vara till hjälp när man söker efter kandidatgener för psykiska åkommor hos människor.
List of publications

This thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-V).

Paper I

Paper II

Paper III
Wirén A, Wright D, Jensen P (2011) “Effects of a chicken growth QTL on behaviour is due to linkage rather than pleiotropy”. Manuscript

Paper IV
Wirén A, Wright D, Jensen P (2011) “Social preference and support seeking in chickens is related to genotype on a growth-QTL”. Manuscript

*During the course of this project, Anna Wirén has changed her/his sex and name and is now known as Anders Wirén. Thus the first author of papers I and II is listed as “Anna Wirén” whereas the first author of papers III and IV is listed as “Anders Wirén”.

IX
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIL</td>
<td>Advanced Intercross Line</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Double Stranded DNA</td>
</tr>
<tr>
<td>MAS</td>
<td>Marker Assisted Selection</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Quantitative Real-Time Polymerase Chain Reaction</td>
</tr>
<tr>
<td>QTL</td>
<td>Quantitative Trait Locus</td>
</tr>
<tr>
<td>RJF</td>
<td>Red Junglefowl</td>
</tr>
<tr>
<td>SAIL</td>
<td>Selected Advanced Intercross Line</td>
</tr>
<tr>
<td>TI</td>
<td>Tonic Immobility</td>
</tr>
<tr>
<td>WL</td>
<td>White Leghorn</td>
</tr>
</tbody>
</table>
## Contents

1. INTRODUCTION ................................................................................................................................. 1
   1.1 DOMESTICATION ...................................................................................................................... 1
   1.2 BEHAVIOURAL GENETICS AND ITS METHODS ................................................................. 3
   1.3 CHICKENS AS A MODEL OF DOMESTICATION ................................................................. 7
   1.6 THE RJF×WL INTERCROSS .............................................................................................. 8
   1.7 AIMS ......................................................................................................................................... 9

2. METHODS ........................................................................................................................................ 13
   2.1 ANIMALS ............................................................................................................................... 13
   2.2 BEHAVIOUR STUDIES ........................................................................................................ 14
   2.3 QTL ANALYSIS .................................................................................................................. 15
   2.4 GENE EXPRESSION ANALYSIS ..................................................................................... 16

3. SUMMARY OF PAPERS ................................................................................................................ 17
   3.1 PAPER I .................................................................................................................................. 17
   3.2 PAPER II ............................................................................................................................... 17
   3.3 PAPER III ............................................................................................................................. 18
   3.4 PAPER IV ............................................................................................................................. 19

4. DISCUSSION .................................................................................................................................. 21
   4.1 THE EFFECTS OF GROWTH1 ON PHENOTYPE ............................................................. 21
      4.1.1 Effects on social behaviour ......................................................................................... 21
      4.1.2 Effects on emotionality ............................................................................................ 25
      4.1.3 Effects on bodyweight .............................................................................................. 26
   4.2 LINKAGE OR PLEIOTROPY? ............................................................................................... 28
   4.3 GENES IN THE GROWTH1 REGION ................................................................................... 29
   4.3 IMPLICATIONS FOR BREEDING AND MEDICINE ......................................................... 31
      Implications for animal breeding ....................................................................................... 31
      Implications for medicine .................................................................................................. 31
   4.4 PROSPECTS FOR FUTURE RESEARCH ........................................................................... 32

5. CONCLUSION ................................................................................................................................ 33

ACKNOWLEDGEMENTS .................................................................................................................. 35
1. Introduction

1.1 Domestication

Since the beginning of time, or at least since the advent of modern humans, people have been striving to tame the nature around them and adapt it to themselves. The adaptation includes other animals that man has used as a food resource, labour and a means of recreation.

The domestication of animals is believed to have started at least 15,000 years ago (Vigne 2011) and can be described as an example of high speed evolution where animals are subjected to a radical change in selection pressures (Price 1998). Since animals are protected by humans, natural selection due to predation and food shortage is relaxed. At the same time artificial selection for traits preferred by humans (e.g. tameness and production traits such as high milk yield and high growth rate) is introduced. Except for active selection by humans for preferred traits, animals also have to adapt to the new captive environment; those individuals who can better cope with the stress imposed by the physical and social conditions that humans choose to keep them under, will reproduce better and therefore contribute larger numbers of offspring to the next generation. These conditions include the close proximity to humans, and therefore require animals to not be fearful of people. It also often includes the keeping of larger numbers of animals together on smaller spaces than occur in the wild, and this in turn requires the animals to adapt their social behaviour to cope with frequent encounters with other conspecifics.

In small groups animals have the possibility of establishing and maintaining dominance relationships to all other group members based on individual recognition, and thereby avoiding repeated, energy demanding aggressive encounters once the relationship between birds has been established. In larger groups, on the other hand, the number of conspecifics may be too great for an animal’s learning capacity to allow individual recognition of all group members. In that setting an individual could benefit by adopting a social strategy based on tolerance for unfamiliar conspecifics. It has been suggested that inability of
animals to adjust their social behaviour in this way could explain the decrease in production related traits sometimes seen in large as compared to small groups (Keeling et al. 2003).

There are thus many changes in selection pressure associated with the process of domestication, and both the physiology and the behaviour of animals change. There seems to be a complex of traits that are altered simultaneously in the domestication of many different species, forming what Price (1999) has called the “domestic phenotype”. This domestic phenotype is comprised of e.g. earlier sexual maturity (Trut et al. 2009), decreased anti-predator and exploration behaviours (Lindqvist et al. 2002; McPhee 2003), decreased aggression to conspecifics (Künzl & Sachser 1999; Desforges & Wood-Gush 1975; Boice 1972) and less fear of humans (Campler et al. 2009) in domestic animals compared to their wild counterparts. But it is possible that multiple selection pressures is not the only explanation for the simultaneous change in a variety of traits. In the 1960’s, Dimitry Belyaev started a selection experiment on farmed silver foxes using reduced fear towards humans as the only criterion for selection (Trut et al. 2009). Rather than seeing only a change in the proportion of foxes that were less fearful to humans (and a reduction in the extent of fear of the individual animals), Belyaev and his colleagues observed that the foxes also evolved earlier sexual maturation, altered coat colour and later onset of fear response (Trut et al. 2009). Since fearfulness towards humans was the only trait selected for, this correlated response in traits suggested that the development of the involved characteristics were influenced by common a genetic mechanism.

Such a common mechanism could be for example pleiotropy, which is the case when one gene affects several traits (e.g. by the gene product being involved in more than one biochemical pathway). The correlation between traits could also be a result of close linkage of different genes affecting different traits. Linkage occurs when alleles at one genetic locus do not segregate independently of alleles at another locus, due to the physical closeness on the chromosome of the two loci. In other words, if allele 1 at locus A is inherited along with allele 1 at locus B more often than 50% of the time, then the two loci are linked. If allele 1 at locus A gives it’s bearer a greater body mass and allele 1 at locus B increases the deposition of red pigment into the bearer’s fur, then selection for high body
mass can result in a simultaneous increase in the proportion of individuals with red fur.

Understanding the genetic mechanisms responsible for correlated selection responses in unselected traits, such as the domestic phenotype, can be useful for understanding why unwanted side effects of intense selection occur (e.g. poorer immune system and leg problems in broilers, mastitis in dairy cattle (Rauw et al. 1998)) and how to avoid this by designing better breeding programs for domestic animals.

1.2 Behavioural Genetics and its Methods

Biological traits of any organism can be influenced by the environment that the organism develops and lives in, but also by the organism’s genes. Geneticists usually write this in the form of an equation:

\[ P = E + G \]

where \( P \) is phenotype (physical traits or appearance), \( E \) is environment and \( G \) is genes. More formally, the term \( P \) signifies the variance in a particular phenotype (e.g. body weight) among the individuals of a particular population of a species. The term \( E \) correspondingly signifies the fraction of the total phenotypic variance that is caused by variation in the population’s environment (e.g. all members of the population may not have equal access to food). \( G \) represents the fraction of the population’s total phenotypic variance that is caused by genetic variation among the individuals of the population (e.g. all individuals have the same genes, but some have alleles (variants) of those genes that make them grow faster and attain a greater body weight than others). Estimating the relative contributions of environmental and genetic variance to overall phenotypic variance in traits is the aim of the research field of quantitative genetics, and this can be done in all organisms, including humans. More specifically, quantitative genetics deals with explaining variation in quantitative traits (e.g. body weight), i.e. traits that vary on a continuous scale rather than being manifested as a few easily distinguishable phenotypic categories (for example having legs or not rather than having short, intermediate or long legs).
Not only can phenotypic variance be partitioned into environmental and genetic components, but the genetic component (G) can be further divided into additive (A), dominance (D) and epistatic (I) variance. Two alleles at a genetic locus influence the phenotype additively when the heterozygote genotype has a phenotypic value that is intermediate between those of the two homozygotes. In other words, to the extent that genotype affects phenotype for a particular trait, it doesn’t matter how alleles are combined a locus (or among several loci), but the influence of the combination of alleles equals the sum of each allele’s individual contribution. If this is not the case, i.e. if the phenotypic value of the heterozygote genotype is not the average of the two homozygotes, there is dominance (D) at that particular locus. Dominance is an effect of the unique combination of alleles at a locus. Epistasis is also a result of the combination of alleles, but in this case it is the combination of alleles at different loci that affects the phenotype. E.g. if individuals with a $A_1A_1$ genotype at locus A (where the subscript “1” denotes an allele) and $B_1B_1$ at locus B have, on average, a certain phenotypic value but individuals who are $A_1A_1$ and $B_2B_2$ have another average phenotypic value (and this difference cannot be explained by the additive or dominance effects of locus B), then locus B influences the expression of locus A. In other words there is an epistatic interaction between the two loci. Detecting epistatic interactions between loci can be an indication that the gene products of these two loci interact in the same biochemical pathway, and can therefore be a useful analytical tool, but it also requires large sample sizes (Carlborg et al. 2003).

So genetic variation can influence the traits of organisms. Behaviours are also traits, and can be investigated with the same methods used to study other traits. Behaviours are often quantitative rather qualitative (all or none) traits, and has been shown to be influenced (to varying degrees depending on which type of behaviour) by both environmental and genetic variation, also in humans (Anholt & Mackay 2010; Plomin et al. 2002).

But how can genes affect behaviour? How do genes affect traits in general? Genes exert their actions by (in the most typical case) being transcribed into mRNA which is brought from the cell nucleus to the ribosomes in the cytosol to be translated into polypeptides that, alone or together with other peptides and co-factors, form proteins. Proteins are then transported to their designated
location in, on the surface of or outside cells to function as structural proteins, enzymes, transporters, signalling molecules and more. Proteins can for example reside on the surface of one cell and serve as receptors for signalling molecules from another cell (such as a hormone or neuropeptide). When a signalling molecule binds to a receptor it triggers a structural change in the receptor’s intracellular part that in turn causes the activation of an intracellular protein (e.g. a G-protein) which activates other, downstream molecules and this cascade of reactions eventually results in altered function of the receiving cell. The receiving cell can be a neuron, in which case the alteration in function can e.g. affect the way the brain perceives or processes signals from the outside world, or the way the brain sends signals to other organs (muscles, viscera) and thereby mediates a response to stimuli in the outside world (e.g. a behaviour).

If the base pair sequence of a gene is changed by mutation, the mutation can occur either in the coding part of the gene (which specifies the amino acid sequence in the resulting peptide), or in the regulating sequences of the gene (which specify how often/to what extent RNA polymerase can bind to the DNA strand and transcribe the gene, i.e. how much the gene will be expressed). A mutation in the coding sequence of the gene can change the amino acid sequence of the gene’s peptide, and as a result the structure of that peptide. Peptide structure is what determines its function, and therefore a mutation in a gene’s coding sequence can alter, or entirely obliterate, its function. If a mutation occurs in the regulating sequences of the gene, this can alter how much the gene is transcribed, and as a result how much there will be of the gene product. Taking the receptor gene as an example, a mutation in the coding sequence may lead to structural change in the receptor preventing it from binding the signalling molecule and therefore making it unable to transmit the signal from stimuli in the outside world to the brain (or vice versa), preventing the individual to react to those stimuli (e.g. by behaving in a certain way). A mutation in the regulating sequences of the gene can enhance or decrease the transcription of the gene, and therefore the abundance of the gene product. If cells express less of the receptor on their surface, they will not be able to respond and react to the external signal as much or as often as otherwise, leading to a decreased frequency or intensity of the individual’s reaction to the external stimuli (e.g. decreased frequency/intensity of a certain type of behaviour). There
are many other ways than through receptor signalling that genes can influence behaviour, but they shall not all be described here.

Since behaviours often are quantitative rather than all or none traits, it is reasonable to assume that the mutations occurring in a population of animals of the same species, leading to genetic variation among individuals that make them behave differently from each other, will often (but not always) be mutations in the regulating rather than the coding sequences of genes. In addition to exploring to what extent particular behaviours are influenced by genetic variation, and whether the variation is due to additive, dominance or epistatic effects, the aim of behavioural genetics is to pinpoint which genes actually exert that influence.

There are a number of methods used to discover genes, and they shall only be described briefly here. A fuller description of the methods used in this thesis is given in the Methods section. Estimating the influence of genes on a trait requires that the environment that individuals grow up in is standardized, or as standardized as possible, to rule out the effect of environmental variation on the trait. In general, gene-finding methods include detecting correlations between differences in genotype at genetic marker sequences across the genome with differences in phenotypic traits among individuals of a population. Such studies can yield information about in which (often rather large) regions on chromosomes genetic elements are located that influence particular traits. If the genome of the species in question has been sequenced and annotated, there will be information about which genes (known or predicted) reside in that region. Finer marker-phenotype mapping can be carried out using a higher density of markers in that particular region. If genes are indicated by closeness to a marker that is highly correlated to a trait, then their expression (in terms of mRNA and/or protein) can be measured in specific tissues and at specific times in the organisms development and a correlation analysis made between the level of expression and the level of the trait of interest. Such a correlation is not in itself a proof that genotype at a gene is a cause of the variation seen in the trait (it might be an effect rather than a cause), but it does strengthen the hypothesis that the gene is somehow involved in shaping the trait in question. A more direct proof of a genes effect is to experimentally alter its expression, either by destroying the gene all together (knock-out) or by lowering or enhancing the
expression (knock-down, knock-up). This can be done selectively in specific tissues or at specific stages in the organism’s development. If knocked out (or down, or –up) individuals alter the level of the particular trait in the predicted way, and if restoring the gene to its original state (“rescue”) also restores the phenotype, this is a more conclusive evidence that the gene actually causes a change in the trait.

1.3 Chickens as a model of domestication

Chicken domestication is believed to have started in South East Asia approximately 8,000 years ago (West & Zhou 1989). The wild ancestor of all domestic breeds is most likely the Red Junglefowl (*Gallus gallus*), although evidence of genetic contributions from Grey Junglefowl (*Gallus sonneratii*) has also been found (Eriksson *et al.* 2008; Fumihito *et al.* 1994). It is unclear what the initial purpose of domestication was, and whether there was indeed conscious selection going on, but it has been suggested that chickens were first kept for cock fighting (Crawford 1990). It may have been the Romans that first started breeding chickens actively for meat and egg production (Crawford 1990). Today hundreds of different breeds exist, mainly bred for either meat (broilers) or egg production (layers). The Red Junglefowl still exists in the wild, and is also available in zoos and lab populations. This makes possible direct comparisons between domestic chicken breeds and their wild ancestor, although captive populations of Red Junglefowl may differ from each other in terms of genetic drift and adaptation to the captive environment.

Compared to other domestic species, the chicken is small and fairly inexpensive to keep in the large numbers required to provide power to statistical analyses. It also has a relatively short generation time (approximately 5 months) and its genome has been completely sequenced (International Chicken Genome Sequencing Consortium 2004). The diploid chromosome number is 78 and the estimated number of genes 20,000-23,000. Compared to mammals, the recombination frequency is high (2.5–21 cM/MB; human: 1–2 cM/MB; mouse: 0.5–1 cM/MB) (International Chicken Polymorphism Map Consortium 2004), which is useful when generating experimental crosses. The reference sequence is based on the Red Junglefowl, but re-sequencing projects are underway that also include several domestic breeds (Rubin *et al.* 2010).
1.6 The RJF×WL intercross

To study the genetic basis of the many physiological and behavioural changes associated with domestication, members of our research group and others have previously generated an intercross line between the Red Junglefowl (from here on abbreviated “RJF”) and the White Leghorn layer (“WL”, bred for egg production). These two breeds differ in a number of traits (table 1). Most notably, WL have a higher growth rate and body weight, and lay more and heavier eggs. The breeds also differ in a range of emotionality-related behaviours, such as the WL being more active in an open field test, entering tonic immobility easier (at least in females) and showing less fear towards humans. The breeds also differ in their tendency to stay close to (familiar) conspecifics, the direction of the difference depending on context.

A QTL study on the F2 generation of the RJF×WL intercross found several QTLs with a major effect on body weight at different ages and on growth rate between ages (Carlberg et al. 2003; Kerje et al. 2003), where WL alleles consistently gave a higher body weight and growth rate. The QTL with the largest effect (termed “Growth1”) was located on chromosome 1 and explained 35% of the difference in adult body weight between the parental breeds (RJF and WL). It also had an influence on average egg weight, where WL alleles gave heavier eggs, explaining 30% of the difference between the parental breeds in that trait. The QTL was located between the markers MCW0010 and ADL0019, approximately corresponding to 17.2 MB (figure 1). Kerje et al. (2003) concluded that there was very little recombination between these markers, and that the QTL may have been under selection early in chicken domestication since it had not been detected in crosses between domestic breeds. This growth QTL was also found to influence behavioural traits, such as time spent immobile in a tonic immobility test (WL alleles being associated with longer duration (Schütz et al. 2004)), and the propensity of chickens to explore their environment (WL alleles decreasing this propensity (Ahlbeck 2005)). Some of the behavioural influences of the QTL could be more specifically mapped to different areas within the 17.2 MB region, e.g. a tendency to spend more time close to familiar conspecifics in a foraging vs. sociality test (where WL alleles was associated with longer duration) was associated with one area whereas time spent foraging in the same test was associated with another (Väisänen 2005). However, these data should be interpreted with caution, since the confidence
intervals of the latter two genotype-phenotype correlations were not established, and it cannot be excluded that they overlap. Figure 1 summarizes the phenotypic effects of the Growth1 QTL.

1.7 Aims

The many phenotypic effects of the Growth1 QTL is in agreement both with the notion of multiple selection pressures in captivity leading to changes in many traits simultaneously and with the observations that selection for one trait alone (such as egg production) can lead to a correlated response in other traits. However, even though influences of the QTL on some behavioural variables are known, the data are not exhaustive and do not indicate whether there is an effect on social tolerance. It is also not clear if the multiple phenotypic effects are a result of pleiotropy of a few genes or close linkage of many. The aims of this thesis are therefore to

- make a comprehensive characterization of the QTLs influence on behaviour that is likely to have changed as a result of domestication, and in particular emotionality related behaviours and social tolerance.

- if possible, to determine which parts of the QTL region affect which traits and to find candidate genes for the observed effects in the indicated areas of the QTL.
Table 1. Phenotypic differences between Red Junglefowl and White Leghorn layers.

<table>
<thead>
<tr>
<th>Trait</th>
<th>RJF</th>
<th>WL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>Low</td>
<td>High</td>
<td>Kerje et al 2003, Carlborg et al 2003</td>
</tr>
<tr>
<td>Growth rate</td>
<td>Low</td>
<td>High</td>
<td>Kerje et al 2003, Carlborg et al 2003</td>
</tr>
<tr>
<td>No. of eggs</td>
<td>Low</td>
<td>High</td>
<td>Schütz et al 2002, Kerje et al 2003</td>
</tr>
<tr>
<td>Sexual maturity</td>
<td>Late</td>
<td>Early</td>
<td>Schütz et al 2002</td>
</tr>
<tr>
<td>Feeding motivation</td>
<td>Low</td>
<td>High</td>
<td>Väisänen &amp; Jensen 2003</td>
</tr>
<tr>
<td>Information gain during feeding</td>
<td>High</td>
<td>Low</td>
<td>Lindqvist et al 2002</td>
</tr>
<tr>
<td>Open field behaviour (m)</td>
<td>Less active</td>
<td>More active</td>
<td>Schütz et al 2004</td>
</tr>
<tr>
<td>Latency to approach a novel object (m)</td>
<td>Long</td>
<td>Short</td>
<td>Schütz et al 2004</td>
</tr>
<tr>
<td>Tendency to enter tonic immobility (f)</td>
<td>Weaker</td>
<td>Stronger</td>
<td>Schütz et al 2004</td>
</tr>
<tr>
<td>Response to aerial predator</td>
<td>Active</td>
<td>Passive</td>
<td>Schütz et al 2001</td>
</tr>
<tr>
<td>Response to physical restraint</td>
<td>Active</td>
<td>Passive</td>
<td>Schütz et al 2001, Schütz et al 2004</td>
</tr>
<tr>
<td>Fear of humans</td>
<td>More</td>
<td>Less</td>
<td>Campler et al 2009</td>
</tr>
<tr>
<td>Social re-instatement tendency</td>
<td>High</td>
<td>Low</td>
<td>Väisänen &amp; Jensen 2003</td>
</tr>
<tr>
<td>Distance to nearest neighbour</td>
<td>Long</td>
<td>Short</td>
<td>Väisänen &amp; Jensen 2003</td>
</tr>
</tbody>
</table>

RJF = Red Junglefowl, WL = White Leghorn, m = males, f = females
Figure 1. Phenotypic effects of the Growth1 QTL. Blue bar = chromosome. MB = Mega base pairs from the beginning of the chromosome. Confidence intervals for the mapping of traits are indicated as grey bars, followed by a reference to the original paper reporting that phenotype. Abbreviations of phenotypes are shown below the chromosome. “WL +” indicates that a WL allele at the marker/interval associated with a phenotype increases the level of that phenotype, whereas “WL -” indicates that a WL allele decreases the level of the phenotype. Grey bars bordered by a dashed line indicate that 95% confidence intervals have not been established, but the genotype-phenotype correlation has its peak between the markers indicated. Grey bars without border indicate that only one marker was investigated, and therefore no confidence interval could be established. Open ended bars indicate that the confidence interval extends beyond the region investigated in the respective study. Bars with a solid red line represent 95% confidence intervals.
2. Methods

2.1 Animals

The animals used in this study are Red Junglefowl, White leghorn layers, and several generations of an intercross line between them.

The Red Junglefowl were originally wild caught in Thailand (Schütz et al. 2001), but have since then been kept in captivity in zoos and research facilities for at least 10 generations (Schütz et al. 2001; Per Jensen, personal communication), and may be considered inbred.

The White Leghorn line used here originates from the Scandinavian Selection and Crossbreeding Experiment with Laying Hens (Liljedahl & Weyde 1980), and have been bred for high egg production.

Studies of genotype-phenotype correlations require that there is genetic variation in the study population, and therefore we have crossed the two chicken populations we wish to compare. Each generation of crossing introduces more genetic recombination and therefore the genomes of individual birds in successive generations will have a more and more random mixture of RJF and WL DNA. This allows a good substrate for genotype-phenotype correlation mapping, such as QTL analysis.

The birds used in paper I, except for the parental lines (RJF and WL), were derived from the F5 generation of the RJF×WL intercross. F5 individuals were genotyped and selected on the basis of being homozygous for either an RJF allele or a WL allele at the microsatellite marker MCW0106, which is in the centre of the previously reported growth QTL. Homozygous RJF individuals were used as parents to breed a homozygous RJF-MCW line. Homozygous WL individuals were correspondingly used to breed a homozygous WL-MCW line. In papers I and III, these lines are collectively referred to as a Selected Advanced Intercross Line (SAIL), and in paper II as a Locus-controlled Advanced Intercross Line (LAIL). In this thesis they will be designated RJF-
MCW and WL-MCW. These two lines have a controlled genotype at one particular locus, whereas the rest of their genome (except for linked loci close to MCW0106) is a random mixture of RJF and WL DNA. In other words, the phenotypic effects of genotype at this locus can be studied against a random genetic background.

Two different batches of the first generation of the two SAIL lines (referred to as MCW1:1 and MCW1:2) were used in paper I, along with purebred RJF and WL. The offspring of MCW1:2 (referred to as MCW2) were used for the behaviour tests in papers II and III. The MCW2 were also used for the QTL analysis in paper III, whereas the gene expression analysis in that study used purebred RJF and WL. In paper IV parts of the $F_8$ and $F_9$ generations of the RJF×WL intercross were used for QTL analysis rather than the SAIL line. This was done to ensure a greater amount of recombination within the Growth1 QTL region.

2.2 Behaviour studies

The spontaneous, undisturbed behaviour of animals can be observed in their daily lives in the lab, farm or field. This can give valuable information about how they allocate their time between different activities such as foraging, resting, sleeping and interacting with other individuals. However, designing a behavioural test allows the researcher to answer specific hypotheses about how animals will react to stimuli or situations that do not occur often enough or in a sufficiently similar way every time for accurate conclusions to be made about how groups of animals differ systematically from each other. Tests can be performed under standardized conditions to rule out environmental variation as a cause for observed differences in behaviour.

The tests performed in this thesis are described in the published papers and manuscripts, and therefore they will not be described in detail in this section. In general, these tests have been aimed at distinguishing differences in social and emotional behaviours between chickens with different genetic background. This has been done by putting individual animals in a situation where they have a choice between e.g. exploring a new environment or staying in a more familiar one, or between spending time with unfamiliar chickens as opposed to familiar
ones. The outcome, e.g. durations in different parts of the test arena and frequency of behaviours such as perching, preening, flying and jumping has then been observed either directly and/or from video recordings. To rule out further variation, the order in which individual animals have been subjected to a specific test has been (as far as possible) either balanced or randomized with respect to time of day and sex of the animal.

2.3 QTL analysis

When comparing two breeds or varieties of the same species that diverge for a number of traits, an experimental cross can be made between the two. Because of genetic recombination during meiosis, each individual in advanced generations of the intercross will carry a genome that is a more or less (depending on recombination rate) random mixture of alleles from the two breeds (e.g. alleles R and W). By examining the genotype (RR, WW or the heterozygote, RW) at a large number of genetic markers across the genome and correlating the genotype at each marker to the observed level of a (quantitative) trait of interest, a graph showing the strength of the correlation (the LOD score) at each given location along each chromosome can be constructed. The strength of the correlation will depend on the proximity to a QTL and that QTL’s effect size. The peaks of the graph will indicate the locations and effect sizes of QTLs. Furthermore, confidence intervals for the location of QTLs can be estimated. The often low heritabilities of quantitative traits in general (Kearsey 1998), and the fact that any given QTL usually only explains a fraction of this heritability, means that confidence intervals for the location of QTLs are often large. QTL analyses are typically performed on F2 generations from crosses between inbred strains or varieties, backcrosses to one or both parental strains, or recombinant inbred lines. A way of decreasing confidence intervals (i.e. make a more precise estimate of a QTL’s location) is to accumulate more recombinations by breeding the population randomly for more generations, using larger numbers of animals and a higher marker density. Even with these measures, QTL analysis will most often not be able to pinpoint the individual genes responsible for variation in a trait, but it is a good starting point for more fine-tuned analyses.
2.4 Gene Expression Analysis

Gene expression analysis looks at the level of expression of a gene, i.e. mRNA levels, in a specific tissue at a specific stage in an organism’s development. Finding a difference in expression level of a gene between e.g. two breeds can be an indication that the gene is involved in shaping phenotypic differences between them. The gene expression analyses described in this thesis were performed using qRT-PCR on cDNA samples from a selected region of the chicken brain. Because of its PCR step, this method is more sensitive (can detect lower levels of cDNA) than e.g. microarrays, and therefore it is appropriate when the expression levels of only a few gene are being investigated. The specificity of qRT-PCR is based on the fact that primers are designed that are specific for the gene of interest. A fluorophore in the reaction mix emits light when bound to double stranded DNA (dsDNA), which means the fluorescent signal will increase as more PCR product accumulates. Because of this the reaction can be monitored in real time. Since the rate of product formation in the exponential phase of the reaction is more proportional to the initial amount of template than the end point amount of product is, this allows a more accurate estimate of gene expression than end point PCR does. The specificity of the analysis can be further improved by the use of an additional probe (e.g. TaqMan) that binds to an internal part of the amplicon and is cleaved when the DNA polymerase replicates the DNA. If a probe is used, it is the cleaving of the probe that emits a detectable (e.g. fluorescent) signal. The analyses in this thesis were performed using mostly the TaqMan method, but in some cases regular PCR primers were used in combination with a dsDNA binding fluorophore (SYBR Green).
3. Summary of Papers

3.1 Paper I

In paper I, chickens with either an RJF or a WL homozygous genotype at the marker MCW0106 (RJF-MCW and WL-MCW) in the Growth1 QTL were subjected to two tests designed to measure social recognition learning. The marker MCW0106 is located close to the gene AVPR1a, which is similar to the receptor for the peptide hormone vasopressin (vasotocin is the corresponding hormone in birds). This gene has been shown to influence a range of social behaviours in species such as mice, voles, birds and humans (Bielsky et al. 2005; Goodson et al. 2006; Lim et al. 2004; Walum et al. 2008). The behaviours affected by the gene include social recognition learning. When the chickens were 30-32 days old they were repeatedly during one day presented with one familiar and one unfamiliar chicken of the same age, to test their capacity for social recognition learning. There was no indication that such learning took place, but male WL-MCW chickens spent significantly more time close to the unfamiliar conspecific than RJF-MCW males did. This pattern was also seen when comparing parental WL birds to parental RJF birds in the same test. When the chickens became adult they were repeatedly over one day presented with an unfamiliar conspecific of the same sex and genotype, also to test for social recognition learning. There was some indication that birds learned to recognize the unfamiliar individual, and to some extent WL-MCW male birds showed less agonistic behaviours to the unfamiliar individual, compared to RJF-MCW males. The results indicate that the Growth1 QTL influences a characteristic that may be termed preference for social novelty (in young birds) or social tolerance (in adults) in male chickens, but not in females.

3.2 Paper II

In paper II, chickens with alternative homozygous genotypes at the marker MCW0106 (RJF-MCW and WL-MCW) were studied more extensively with regard to emotional and social behaviours, both at a young age and as adults. Young WL-MCW decreased their levels of passive behaviours (standing, sitting, lying, sleeping) and increased perching in response to the presentation of an
aerial predator model, and tended to stay longer close to familiar conspecifics rather than exploring a novel environment. They also interacted more with their own mirror image. As adults, WL-MCW birds showed higher levels of locomotion in a novel environment and did not enter tonic immobility as easily as RJF-MCW. They also had a tendency to engage in agonistic interactions with an unfamiliar individual sooner than RJF-MCW birds did. A Principal Components Analysis (PCA) indicated that decrease in passiveness in response to the aerial predator model and frequent agonistic behaviours towards one’s mirror image were related to each other, as were the tendency to stay close to familiar conspecifics and a short latency to agonistic interaction with an unfamiliar (adult) conspecific. WL-MCW birds tended to have slightly higher scores on both these components. The results suggest that the Growth1 QTL affects many aspects of emotional reactivity and social behaviours in a way that may be expected from domestication theory.

### 3.3 Paper III

In paper III, a refined QTL analysis was performed using the same animal material and a subset of the behavioural data used in paper II, to determine whether the different behavioural and physiological traits influenced by the Growth1 QTL were associated with different locations within the larger QTL region. In addition to this, a gene expression analysis employing qRT-PCR was performed in the lower frontal lobes of the brain from 35 day old Red Junglefowl and White Leghorns. The QTL analysis found a suggestive association between a WL genotype in an unrecombined subset of markers central in the original growth QTL and higher levels of perching behaviour performed by chickens. WL genotypes at this marker set were also associated with the tendency of chickens to stay in a familiar-looking zone of the test arena as opposed to exploring new environments. Furthermore, a significant QTL for weight at 8 days of age (with WL genotype birds being heavier than RJF genotype ones) was centred at the marker 1_36652477. The gene expression analysis showed that some of the genes located in the Growth1 interval were, or had a tendency to be, differentially expressed between RJF and WL. This included $AVPR2$, $AVPR1a$ and $NRCAM$. The results should be interpreted with caution since the confidence intervals for QTLs were large, but they may indicate that weight and emotionality-related behaviours are influenced by
different linked genes in this region rather than the same pleiotropic ones. Although gene expression analysis does not constitute proof of a gene’s involvement in shaping a phenotype, the differentially expressed genes cannot be excluded as candidates for explaining the observed effects on behaviour.

3.4 Paper IV

In paper IV, a test of social preference and social support seeking from conspecifics in response to stress was undertaken using adult birds from the F<sub>8</sub> and F<sub>9</sub> generations of the RJF×WL intercross. A refined QTL analysis using six markers in the Growth1 QTL region was also performed on these animals. The analysis found one suggestive and one significant QTL, adjacent to each other, for a variable reflecting social tolerance/preference for social novelty and for seeking social support from familiar as opposed to unfamiliar conspecifics. These two QTLs affected social support seeking in opposite directions; the significant QTL acted to make birds with a WL genotype seek out familiar birds for social support, the suggestive QTL (which had a smaller effect) to make them seek support from unfamiliars. The net effect when these two QTLs are inherited as a unit would be a slight tendency for WL birds to shift social preference from unfamiliar to familiar birds in response to stress. However, the results indicate that this does not always have to be the case; genetic variation among loci affecting the described traits can be introduced by breeding and possibly used to enhance social tolerance in chickens, if desired.
4. Discussion

4.1 The effects of Growth1 on phenotype

The aims of this thesis were to do a more comprehensive behavioural characterization of the Growth1 QTL with special emphasis on social behaviours and emotionality, and if possible, to map such genotype-behaviour correlations to more specific locations within the ~17 MB QTL region. Figure 2 summarizes data that the thesis has added to previous findings.

4.1.1 Effects on social behaviour

At the outset of this project it was known that birds with a WL genotype in parts of the Growth1 QTL have a propensity to spend more time with other birds than to explore a novel environment (Väisänen 2005), and birds homozygous for WL alleles at the marker MCW0106 exhibited less of some agonistic behaviours (i.e. chasing other chickens) than RJF genotype birds (Ahlbeck 2005). It had been noted that the gene AVPR1a, which encodes a receptor for the hormone and neuropeptide vasotocin, is located close to this marker. AVPR1a plays a role in social affiliation and social recognition learning (Bielsky et al. 2004; Pitkow et al. 2001).

Social recognition learning is a prerequisite for forming stable social relationships in a flock. Paper I of this thesis therefore set out to investigate whether genotype at marker MCW0106 affects social recognition learning. The tests performed on young (MCW1:1) as well as adult (MCW1:2) birds in paper I were based on similar tests in voles and mice, where a test individual is presented with an unfamiliar stimulus individual on repeated occasions. A completely unfamiliar individual should elicit social investigation from the test individual, but this should decrease as the stimulus individual gradually becomes familiar. Neither 30-32 day old nor adult test chickens changed their behaviour towards the stimulus bird over time, but WL-MCW males inspected the stimulus chicken more and
Figure 2. Legend on page 23
Figure 2. Phenotypic effects of the Growth1 QTL. Blue bar = chromosome. MB = Mega base pairs from the beginning of the chromosome. Boxes below the chromosome represent genes, some of which are significantly (red label on blue bar) or suggestively (green label) differentially expressed between RJF and WL in lower frontal lobes of the brain. Confidence intervals for the mapping of traits are indicated as coloured bars, followed by a reference to the original paper reporting that phenotype. Grey bars = data from previous studies. Yellow bars = data added by this thesis. Abbreviations of phenotypes are shown below the chromosome. "WL +" indicates that a WL allele at the marker/interval associated with a phenotype increases the level of that phenotype, whereas "WL -" indicates that a WL allele decreases the level of the phenotype. Bars bordered by a dashed line indicate that 95% confidence intervals have not been established, but the genotype-phenotype correlation has its peak between the markers indicated. Bars without border indicate that only one marker was investigated, and therefore no confidence interval could be established. Bars with a solid border represent 80% confidence intervals. Open ended bars indicate that the confidence interval extends beyond the region investigated in the respective study. Bars with a solid red line represent 95% confidence intervals. Green print indicates suggestive associations (0.05 < p < 0.10).

showed a tendency towards less agonistic behaviours towards unfamiliar conspecifics when adult. This suggests that genotype at MCW0106 does not affect social recognition learning, but does affect something that can be interpreted as a preference for social novelty and/or social tolerance to unfamiliar conspecifics, where WL genotype male birds show more of these two traits. However, the analysis in paper I was based on correlations between behaviour and one single marker genotype. The results give no information about the recombination rate between the marker locus, AVPR1a and other potential candidate gene loci in the region.

The QTL analysis of paper III added information about recombination and linkage relationships in the birds selected for homozygosity at MCW0106; a central haplotype block spanning the region between markers UG0002 and MCW0106 showed no recombination in the second generation of this selected line (MCW2), but this block was flanked by areas of higher recombination. This means that a bird carrying a WL allele at MCW0106 most likely also carries a WL allele of AVPR1a and other genes in the 2.6 MB between the two markers.

Paper IV added support for the finding that genotypes in Growth1 influence social preference and/or social tolerance, and this time in both sexes. If the stranger inspection test of paper I presents a test chicken with a choice between
spending its time with either familiar or unfamiliar conspecifics under undisturbed circumstances, the social preference test of paper IV presents the bird with a similar choice, but compares the bird’s social preference under normal circumstances with that under stressful ones. The result of the QTL analysis in paper IV shows that birds with WL genotypes in an area between markers MCW0106 and 1_37164711 spend more time with unfamiliar chickens than the corresponding RJF genotype birds do under undisturbed circumstances. However, when subjected to a stressful event, birds with a WL genotype in this area shift their preference towards familiar birds. This tendency to seek social support from familiar conspecifics in response to stress has been shown to attenuate the stress response in e.g. voles and humans as reflected by lowered cortisol levels (Kaiser et al. 2003; Kirschbaum et al. 1995). Although no corticosterone (corresponding to mammalian cortisol) measurements were made in this study, it is interesting to observe the behavioural outcome. Particularly interesting was the detection of a second, suggestive, QTL peak affecting the shift in social preference in response to stress, or social support seeking. This peak was centred at the marker UG0022, but unlike the first peak, a WL allele at this locus increased the amount of time birds spent with unfamiliar chickens in response to stress. In other words these birds sought social support preferentially from unknown individuals. This peak did however not influence social preference under undisturbed circumstances.

The sociality vs. exploration test in Paper II, where 21-23 days old WL-MCW birds showed a tendency to stay close to familiar companion birds rather than explore the test arena, adds support to the similar finding by Väisänen (2005). This tendency could however not be more precisely mapped since only a single marker was included in the analysis of paper II.

It seems clear from these findings that, in addition to affecting the tendency for chickens to stay close to other chickens rather than exploring when in a new environment (what might be termed "sociality" or "social motivation") (Väisänen 2005), chickens with a WL genotype in specific areas of the Growth1 chromosomal region, under normal circumstances, spend more time with unfamiliars conspecifics than RJF genotype birds do (papers I and IV). This is in agreement with the difference seen in social preference/tolerance between the two parental RJF and WL lines (paper I). It is also in line with the notion that
domestication should lead to increased social tolerance to enable animals to cope with dense social groups.

In addition, it has to some extent been possible to localize the genetic influence on social tolerance and/or preference for social novelty to two adjacent QTL peaks in the Growth1 region (figure 2).

4.1.2 Effects on emotionality

If social behaviour describes the reactions of individuals to other individuals, their reactions to everything other than that can be described by the term emotionality. This includes e.g. an animal's reaction to perceived predators and new environments. At the start of this project it was known that Growth1 influenced the duration of time spent in tonic immobility, where WL-MCW birds took longer to recover from this state that is believed to reflect the reaction to a predator (Gallup 1977; Schütz et al. 2004). It was also known that the QTL region affected some aspects of explorative behaviour in a novel environment, where WL-MCW birds were less explorative than RJF-MCW (Ahlbeck 2005).

Reaction to predators

Paper II reported that 21-23 day old WL-MCW chickens decreased their level of passive behaviours (standing, sitting, lying, sleeping) and increased perching in response to the presentation of a model of an aerial predator, whereas RJF-WL did not. This seems like a very natural reaction to stress, but it is not obvious why birds with alleles derived from a domestic parent (WL) should show a less passive reaction than those with alleles derived from a wild parent (RJF). On the contrary one might expect domestication to lead to an attenuated response to predators (McPhee 2003). The WL-MCW birds of paper II, as adults, also showed a more active response in the tonic immobility test, in that they required more induction attempts before they entered tonic immobility. This lesser tendency for WL-MCW birds to enter tonic immobility contrasts with Schütz’s (2004) finding that WL alleles in the Growth1 region contribute to longer time till recovery once a bird has entered the state of tonic immobility. Longer duration in tonic immobility can reasonably be interpreted as a more passive way of reacting to a predator. It seems that the Growth1 region certainly does influence the reaction of chickens to predators, but it is not clear if different loci
within the region affect different aspects of this reaction, or how this relates to changing selection pressures during domestication.

Exploration
The WL-MCW chickens of paper II tended to explore a novel environment less than RJF-MCW did in a sociality vs. exploration test, but since the genotype of these birds was not known at other loci than MCW0106, it was not possible to make an assessment of the genomic location of this suggestive genotype effect. The QTL analysis of paper III also detected a tendency for birds with WL alleles to be less explorative in a novel environment, this time in the Complex Environment test, which did not involve a choice between social stimuli and exploring the environment. WL alleles in a suggestive confidence interval upstream of the marker 1_36652477 (to the left in figure 2) was associated with 16-17 day old SAIL birds spending more time in a home-like zone of the test arena (which was also the zone where chickens started the test session), rather than exploring other, more unfamiliar-looking zones. These findings are suggestive, and should be interpreted with a bit of caution. However, a tendency for domestic birds to stay close to social stimuli rather than explore the environment could very well be due to the changing selection pressures that domestication comprises. Birds in captivity do not continuously have to search their environment to find food, since it is usually available in the same place every day, and the selection pressure for exploration may therefore be relaxed.

In summary, this thesis supports the idea that the Growth1 chromosomal region influences the reaction of chickens to predators and probably also to novel environments, but these effects could not be pinpointed to individual loci within the region.

4.1.3 Effects on bodyweight
Unpublished data on body weight at several ages of the MCW1 and MCW2 animals used in papers I, II and III support the hypothesis that genotype at the MCW0106 marker locus influences bodyweight (BW) (figure 3). However, the QTL analysis in paper III, which considered BW at hatch, 8, 46, 112 and 200 days of age, detected only a QTL for BW at 8 days of age. This may seem contradictory considering the fact that previous studies found the Growth1
region to influence weights at all ages throughout the chicken’s development, with the exception of weight at hatch (Carlborg et al. 2003; Kerje et al. 2003). Since the QTL for BW in Kerje and Carlborg were of a considerable magnitude, and that the analyses in this thesis were able to identify smaller QTL for other traits, other BW QTLs should have been detected here, if they were located in the more confined chromosomal area covered by paper III. This lack of BW QTLs may mean that weights at later stages than day 8 are indeed not influenced by the 8 MB region between markers UG0006 and 1_37164711, but by loci outside this interval. However, this would not be in agreement with recent data placing a QTL for adult bodyweight (BW200, weight at 200 days of age) between markers RS14803977 and MCW0106 (Wright et al. 2010, figure 2). The lack of a QTL for adult bodyweight in the present thesis may therefore be
due to reduced statistical power caused by the small sample size of the mapping populations (paper III, N=62 , paper IV, N=68).

From the localization of the BW8 QTL reported in paper III, in combination with the data of Wright et al (2010), it seems that early weight (represented by BW8) and adult weight (represented by BW200) are influenced by different genomic regions. This is in keeping with the view that early and late growth are different physiological processes, where early growth is characterized by development of internal organs whereas later growth involves quantitative deposition of body tissues (Carlborg et al. 2003). Early weight in chickens may also be more related to egg weight than to weight later in life, since the yolk sac provides most of the energy supply for newly hatched chickens.

4.2 Linkage or pleiotropy?

The Growth1 QTL region influences many different traits, but the mechanism for these simultaneous influences has so far not been clear. Simultaneous phenotypic changes, such as the ones emerging as a result of animal domestication, may be due to pleiotropy of one or a few genes, or they may be due to the fact that several different genes, affecting different traits, are closely linked on a chromosome and therefore often inherited together as a unit. This thesis has attempted to address this question by performing refined QTL analyses with higher marker densities in this specific chromosomal region than has been done in previous studies (Kerje et al 2003; Carlborg et al 2003).

It should be remembered that the QTL analyses performed in papers III and IV were performed on a comparatively small number of animals (62 and 68), and therefore do not have the same statistical power that studies on larger populations do. It also should be noted that confidence intervals of some of the indentified QTLs overlap. With this in mind, however, the finding of two different QTL peaks for social behaviour (social preference and social support seeking, paper IV), different QTLs for adult and early body weight (Wright et al 2010, paper III), and the differentiation between a suggestive interval for explorative behaviour and early weight (paper III) within an 8 MB region suggests that linkage plays an important role for the correlation between traits.
This is not to say that pleiotropy does not occur. E.g. the relatively small QTL intervals for social support seeking from familiar individuals (paper IV), social preference for unfamiliars (paper IV) and early bodyweight (BW8, paper III) overlap quite well, and this opens the possibility for the same gene/genes affecting all these traits. Future studies on larger sample populations and denser marker sets, or more candidate gene centred approaches such as knock-outs/downs and transgenics would be required to dissect the more exact genetic relationships within these intervals.

4.3 Genes in the Growth1 region

The main aim of this thesis has not been to pinpoint individual genes responsible for phenotypic differences, but the ultimate goal of genetics is to identify genes and characterize their mechanisms of action and effects on the organism as a whole. Some data emerging from this project may therefore be interesting to discuss in relation to known genes in the Growth1 region.

Reviewing the literature, some genes appear particularly interesting, by virtue of their known effects on behaviour and neural development. Except for AVPR1a, similar to the arginine vasopressin receptors of mammals, which has been studied extensively and is well known for its effects on social behaviour in many different species, including rats, mice, voles, birds and humans (Bielsky et al. 2005; Bielsky et al. 2004; Goodson et al. 2006; Goodson et al. 2004; Lim et al. 2004; Pitkow et al. 2001; Walum et al. 2008), the AVPR2, a homolog of AVPR1a, is also found in the QTL region affecting social support seeking from unfamiliar individuals (paper IV), as well as explorative behaviour (paper III) and adult bodyweight (BW200, Wright et al. 2010). So is NRCAM (Neuronal Cell Adhesion Molecule), which is known for its association with autism in humans, a spectrum of disorders characterized by deficiencies in social cognition and behaviour (Marui et al. 2009).

In paper III, AVPR2 was found to be overexpressed in the lower frontal lobes of 35 day old female RJF chickens, compared to their WL counterparts. This region of the brain contains the medial and lateral septum (Puelles et al. 2007), in which differential expression and receptor density of AVPR1a have been linked to changes in social affiliation in mice, finches and voles (Bielsky et al. 2004; Goodson et al. 2006; Goodson et al. 2004; Lim et al. 2004; Pitkow et al. 2001; Walum et al. 2008).
2005; Goodson *et al.* 2006; Pitkow *et al.* 2001). There was also a tendency for *AVPR1a* and *NRCAM* to be overexpressed in the lower frontal lobes, but this time in WL male chickens compared to RJF male chickens.

In this context it should be said that differential expression of a gene is not necessarily the cause of phenotypic differences, it might be a correlation that is due to the expression of that gene and the phenotype of interest being influenced by the same underlying genetic mechanism, or it may even be an effect of differences in phenotype. It should also be noted regarding the gene expression study in paper III that it was performed on mRNA extracted from quite a large piece of tissue (~0.05 g). This means that no particular smaller brain area or cell population within this region can be pinpointed as the site where differential expression is taking place. It also means that the signal from such a smaller area is "diluted" by large quantities of mRNA from brain tissue where the genes of interest are equally expressed. Because of this dilution it may well be that *AVPR2*, *AVPR1a* and *NRCAM* show a greater expression difference in more restricted areas. It must also be remembered that gene expression analyses are snapshots of expression patterns at the particular developmental stage the animal is in at the time of sampling. Genes influencing behaviour and other phenotypes may act e.g. by shaping individual differences in neural connectivity in regions of the brain early in ontogeny, and these differences in connectivity can be what influences a behavioural phenotype later in life, after differential gene expression has ceased. This makes discovering a causal role of the expression of individual genes even more difficult. All this being said, while differential expression of *AVPR2*, *AVPR1a* and *NRCAM* are not necessarily an indication that they are involved in shaping behavioural phenotypes such as social tolerance and explorative behaviour, it does strengthen them as candidates for further studies.

Other genes in the Growth1 region include *Contactin1* and *AMIGO2* which may be involved in neural development (Chen *et al.* 2011; Stoeckli 2010), *Prolactin-b* (prolactin like protein) whose function in chickens is unknown but is expressed in the chicken brain (Wang *et al.* 2010) and *GRIP1* (Glutamate Receptor Interacting Protein 1) which interacts with and influences the trafficking of AMPA receptors (Bakshi *et al.* 2009; Dong *et al.* 1997). It is particularly interesting to note that *GRIP1* is located in the middle of the QTL
interval affecting early body weight (BW8, paper III), social preference for unfamiliar individuals (paper IV) and social support seeking from familiar conspecifics (paper IV).

4.3 Implications for breeding and medicine

Implications for animal breeding

It is apparent from other studies (Rauw et al. 1998; Turner 2011) that production and behavioural traits sometimes are correlated in a non-favourable way, e.g. leading to disease and harmful behaviours. Detailed knowledge of underlying genetic and/or physiological mechanisms is therefore important if sound breeding programs are to be developed. In the present study, however, it seems that selection for increased production traits (egg production in the White Leghorn), because of close gene linkage, has led to favourable correlated changes in social behaviour (increased social tolerance). The WL line used here is no longer being used commercially, and it isn’t granted that commercial layers carry the same alleles at the loci affecting growth, egg weight and social tolerance. However, the findings presented here could serve as a direction for future research into which loci should be included in more complete genetic surveys of animals used for breeding commercial stocks, so called genome-wide selection (Goddard 2009; Turner 2011). Such genetic selection strategies are based on a large bank of accumulated knowledge of what factors shape animal phenotypes, and this thesis adds some pieces to the puzzle.

Implications for medicine

For breeding purposes it may be sufficient to know the location and genetic markers associated with traits to be selected for or against, but information on genotype-phenotype associations in animals can also be used as to direct research on human disorders (e.g. psychiatric disorders such as autism and anxiety), and in this case more detailed knowledge of causative genes (and preferably their mechanism of action) is required. Since we can breed animals under controlled circumstances and have access to a range of experimental treatments such as gene knock-out, animal studies have greater statistical power for investigating the effects of individual genes on phenotype than human studies do. Some of the genes described in this thesis (NRCAM and the
(such as GRIP1) could also be considered. Progress has been made in developing molecular tools for studying functional genetics in chickens, but even though gene knock-out, knock-down by RNAi and transgenesis are being performed more and more in chicken cell lines, methods knocking out genes or altering expression by RNA interference in live animals are not yet as developed as in e.g. mice (Cogburn et al. 2007). On the other hand, for the sake of directing human psychiatric research, using chickens as a model is not crucial, but well-developed model systems like mice can be employed instead.

4.4 Prospects for future research

Although the results of this research project may have implications for animal breeding and human health, the main objective has been a more basic understanding of evolution in the form of animal domestication. The re-sequencing of more individuals from more chicken lines is providing additional information on genetic polymorphisms. These polymorphisms are being used in denser marker maps in the continuing mapping of genotype-phenotype associations in the RJF×WL and other intercross lines. At the time of writing, the RJF×WL intercross is entering its 11:th generation, more genetically recombined than previous generations, and in combination with denser marker maps it should be possible to narrow down intervals for QTL effects even further, both for the associations presented in this thesis and other ones. Following that, and with future improvements in genetic tools, the roles and function in chickens of indicated candidate genes may be clarified. E.g. it should be possible to quantify differences in brain gene expression, and to localize these differences to specific brain areas with much higher precision than in this thesis, using in-situ hybridization.
5. Conclusion

This thesis shows that the many phenotypic effects of a growth QTL on chromosome 1 explain part of the difference between a domestic chicken line and its wild ancestor, and are probably due to linkage of genes affecting different traits rather than pleiotropy of one or a few. A trait that may be interpreted as social tolerance seems to be influenced by two different QTLs within the larger QTL region. Genes in these QTLs include $AVPR2$, $AVPR1a$, $NRCAM$ and $GRIP1$. This new information on genetic markers associated with favourable behavioural and production traits adds to the accumulated knowledge of what factors shape chicken phenotypes, and may be used in designing future breeding programmes. With the development of genetic tools in chickens, and/or application of other model systems, it may also help direct research on human psychiatric disorders.
There are many people I would like to thank, many who I look up to and am inspired by, and many who have stood by me through thick and thin these past years. I would like to thank:

First of all, my supervisor professor **Per Jensen** - you are an outstanding scientist, deeply committed to research as well as teaching. I admire your communication skills and way of leading our research group, and I keep wondering where you get your boundless energy…

Professor **Leif Andersson**, who has been available with good advice when that was needed.

It is not custom to thank animals in these formal contexts, but I think that **all my chickens**, whose names and numbers are too many to be printed here, and who have given their lives for science, should not be forgotten. It is important to remember that our only justification for using and killing other beings is that we try to give them as good a life as we can while they are still alive.

**My family.** Thank you for everything. No matter how unorthodox my ideas have been, how big the enterprise I embarked on or how dramatic the change that I have gone through, you have always believed in me and supported me. No matter where I go from here or what I do, I will feel safe because of you. You are the best!

**Ganarupan Satha.** It doesn't matter if the world is falling apart, when you are around there is always something to laugh and smile about 😊 Your industriousness and work ethic inspire me. Thank you for a lot of encouragement and support during the process of writing this thesis, and for making my life in Linköping a lot less lonely than it could have been!

**Nadine Reefmann.** Thank you for long discussions about science, life, language and culture. You are one of the top "social enrichments" of my life! I admire
your high ambitions and ability to resolve problems even at the toughest of times.

**Vivian Goerlich.** It has been a pleasure to have you as a colleague and a friend! It doesn’t matter how stressed you seem to be, you are never too tired to share your knowledge, discuss science and come up with new ideas in a very active way. I’m working on becoming more like you. With your fortitude you’ll soon be heading your own research group.

**Anke Suska,** thank you for being my friend through rain and shine, and for putting up with all my little yellow notes teaching you more or less useless (but interesting, if I may say so) Swedish words. I can think of several ways of using “Särinmer”, “guldmyntfot” and “motsols” in the same sentence.

**Cecilia Andrésen - med sol i sinne.** You always meet austerity with a sunny smile – your positive outlook on life – and your great professionalism - are inspiring. Thanks for being my friend 😊

**Patricia Wennerstrand.** Thanks for sharing some of my more interesting discussions on life and language use, and not least for providing me with ideas about what to do in the future. You’ll be the first guest in my talkshow. See you in the studio!

Neither would this thesis have been possible to complete without the help, support and inspiration from (in no particular order) **Ulrika Gunnarsson, Therese Klingstedt, Alva Curtsdotter, Johan Edqvist, Agneta Johansson, Daniel Nätt, Anna-Carin Karlsson, Beatrix Agnvall, Martin Johnsson, Dominic Wright, Lejla Bektić, Gunilla Sjunnesson, Ingevald Abrahamsson, Jennie Westander, Annelie Andersson, Ida Gustafson, Isa Lindgren, Anna Sundin, Jakob Ljungh, Tove Bjerg, Annette Molbaek and Åsa Schippert**

And of course, a big Thank You to the rest of the Biology and Theory and Modelling divisions, a heterogeneous collection of kind, playful, interesting, competitive, critical, hard working people. Going to work has never been boring during my time here.
Many thanks also go to Anna-Lena Larsson, for helping me see things more clearly, Elisabeth Haggård for inspiring me to enter the field of genetics and Alexandra Ahlgren-Berg for being a role model in teaching...

...and to my many students, who I have learned a lot from and some of who have become good friends.

Finally, a very special Thank You to Maya Nair, for trusting me and helping me out when I was in a very difficult situation. Thank you, it changed my life.
References


Chen, Y., Hor, H.H. & Tang, B.L. (2011) AMIGO is expressed in multiple brain cell types and may regulate dendritic growth and neuronal survival. J Cell Physiol -.


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


reveals trade-off in resource allocation between behavior and production traits. *Behav Genet* **32** 423-433.


