Department of Physics, Chemistry and Biology

Master Thesis

Correlations between fearfulness and social behaviours in an F7 intercross of red junglefowl and White Leghorn layers

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Content

1 Abstract
The aim of this thesis was to study chickens of an F7 intercross between red jungle fowl and White leghorn layers in five behavioural tests to see if there are any correlations between traits in the intercross. 80 animals were used (40 males, 40 females); they were tested in a TI test, an open field, a fear of human test, an aggression test and lastly a sociality test. The results indicate a couple of correlations between the different variables; chickens with a long TI duration seemed to be less aggressive, and chickens with a high fear of humans seemed to be more social towards other chickens, which could suggest a correlation between fear and social behaviour/aggression. We also found support for previous studies showing that one QTL control chickens behaviour in the TI test based on the strong correlations we found between the variables in the TI test. We could also see differences between the genders in which variables that correlated with each other; this could lead to a speculative suggestion that those behaviours are controlled by genes on the X-chromosome. There was also a significant relationship between the weight of the male chickens' and their behaviour in the open field test and in the fear of human test, where the heavier males were less fearful than the lighter ones.

Keywords:
Aggression, correlations, fearfulness, intercross, leghorn layers, red junglefowl, sociality, weight.

2 List of abbreviations

WL – White Leghorn layers
JF – Red junglefowl
PCA – Principal component analysis
OF – Open Field
QTL – Quantitative trait loci
Agg - Aggression
TI – Tonic Immobility
ST – Social test
FOH – Fear of Human

3 Introduction
There are three different processes that affect animals during domestication (Schütz et al., 2001); 1) relaxed natural selection, 2) intentional selection for desired traits and 3) correlated selection caused by unintentional selection for traits correlated with traits that are selected for. It is through these processes domestication affects different traits, physiological as well as behavioural, e.g. social behaviours and fearfulness. Selection in modern hens for egg production has been on feed conversion efficiency, i.e. the hens have been selected based on their egg production. One area of research is to look into how this selection has affected other traits and which the genetic mechanisms behind it are. This thesis work is part of a larger research project concerning exactly that; the mechanisms behind domestication and its effect upon other traits.

One of the more famous experiments done on domestication was on silver foxes (Trut, 1999). The foxes were selected based only on their tameness, and the results were groundbreaking. The selected foxes differed markedly both in physiology and behaviour from their wild ancestors; e.g. the development of the domesticated foxes fear response was delayed, their coat colour was different (piebald), their ears were floppy and their tails became rolled. In later generations (15-20 generations) shorter tails and legs in the selected foxes started to appear. All of these characteristics are traits seen in domesticated animals all over the world. The process of domestication is one example of correlated selection; when you select for one thing and end up with different changes not selected for. Two genetic mechanisms can be used to explain genetic correlations, either one individual gene is affecting many traits (pleiotropy), or traits can be affected by different sets of gene, which through non-random association of alleles at two or more loci (linkage disequilibrium) causes correlations (Oers, et al. 2004). For this to occur combinations of alleles at a particular locus must be generated and preserved. Albert et al. 2008 has done further studies on correlated selection, on rats. They selected the rats based on tameness or aggression, and found behavioural differences as well as physiological differences between the two lines, such as smaller adrenal glands and larger spleens and lower serum corticosterone levels in the tame rats. The physiological changes indicate a difference in the rats' stress response between the lines.

The chicken is used as a model animal of domesticated animals for a number of reasons (Jensen and Andersson, 2005); *i)* the wild ancestor of domesticated hens (the red junglefowl) is available through populations kept in zoos throughout the world *ii)* chickens exhibit a large breed variability compared to many other domesticated animals *iii)* the environment of the offspring can be controlled from the point of egg laying, which means that the genetic variation will account for a large proportion of the phenotypic variation in the behaviour, and lastly *iiii)* selection for production traits in poultry have been very intense, either selection for egg production or selection for rapid growth. In addition to this the chicken genome has been sequenced, as the first bird genome ever to be sequenced, which gives it even more advantages in genetic studies.

Four major aspects of behaviour have been shown to differ when comparing the wild ancestor of the domesticated hen, the red junglefowl, with domesticated hens, e.g. white leghorn layers (Jensen, 2006). The layers were less active than the junglefowl, with reduced foraging behaviour and reduced exploratory behaviour; they had a less intense social behaviour with lower frequency of social interactions; they had a modified and less intense antipredatory behaviour; and they had a modified foraging strategy. Junglefowl has also been shown to be able to use information from their explorations to cope better with changes in their environment, than layers (Väisänen et al., 2005).

In social behaviours the red junglefowl show more aggression towards unfamiliar birds, and form a dominance hierarchy within the group which is maintained by individual recognition and remembered assessment of status. This is true for laying hens as well, when kept in small groups (Keeling and D'Eath, 2003). Laying hens kept in larger groups (>100 individuals) have been shown to have a low stable level of aggression (Hughes et al., 1997, and Nicol et al., 1999). The type of social behaviour expressed in the two lines of birds is otherwise the same (Jensen, 2006). Schütz et al., 2001, suggests that there is a correlation between aggression and breeding on production traits, at least in males. In their study leghorn layer males were more likely to be aggressive toward humans than red junglefowl males were.

Selection on production traits cause side-effects on other traits, e.g. sociality and foraging, which has been shown by Väisänen et al. (2005). They used animals from an F3 generation of an intercross between red junglefowl and white leghorn layers, and examined production traits such as feed consumption and egg production, as well as social behaviour. They found that F3 birds with higher levels of production related traits behaved more like leghorns than junglefowl in their sociality and explorative behaviour. They suggest that their results could indicate a genetic linkage between these traits.

Quantitative traits, such as weight and length, show a continuous distribution of phenotypic values rather than the distinct values observed for a qualitative trait (traits expressed in descriptive terms) (Andersson, 2001). Quantitative traits are usually controlled by multiple genes and by environmental factors. A quantitative trait loci (QTL) is defined as a locus with significant effect on a quantitative trait (Jensen and Andersson, 2005), The presence of a QTL is detected by gene mapping studies that show significant differences in phenotypic traits between individuals that have inherited different QTL alleles from their parents. (Andersson, 2001) To locate a QTL for a behaviour is one step towards finding the gene/s that affect a phenotypic trait. Kerje et al. 2003 showed in a QTL study that four major growth QTLs explained a large portion of the difference in adult body weight for the birds used in their study. One of these QTLs for growth, located on chromosome 1, also appeared to have a pleiotropic effect on feed consumption, egg production and behaviour. They also saw strong positive correlations between adult body weight and average egg weight, which partly explain the increase in body size for laying hens compared to red junglefowl.

In this thesis birds of an F7 intercross between Red Junglefowl and White leghorn layers will be put through a series of behavioural tests to see whether there are any correlations between different behaviours in the intercross, and if there are any differences in the responses of males and females. When crossing different strains of birds, for example White leghorn layers with its wild ancestor, genetic recombinations on the chromosomes will appear. These recombinations affect and change the birds, both morphological and behavioural wise. The first generation of birds will be heterogametic, having one chromosome from each of its parents, but then recombinations will occur which will affect the birds phenotype. If correlations between traits can be found in a generation so far away as the F7, it means that the traits in question have not been affected by the recombinations during the crossing. Either theses traits are controlled by one gene, or by two genes situated so closely on a locus that the recombinations have not affected them.

Fear is generally defined as a reaction to the perception of actual danger (Forkman et al. 2007), it is a complex response, and as such many methods to assess an animals fear response has been designed. In this study an open field test (novel arena test), a tonic immobility test and a fear of human test were used to study fear behaviour. The open field is believed to measure not only general fear, but also the effect of social isolation/dependence (Forkman 2007). According to Suarez and Gallup (1983) some variables in the open field are more influenced by fear than social motivation, e.g. the duration of freezing, and ambulation. One QTL that is closely related to the chickens response in the open field has been identified (Buitenhuis et al., 2004 cited by Forkman), although this seems to be different for adult animals compared to young ones. In the tonic immobility test the experimenter stimulates a predator attack which in turn elicits a predator response from the animal, "death feigning". The death feigning, tonic immobility, TI, is an unlearned response to physical restraint, characterized by motor inhibition, reduced responsiveness to external stimulation and temporary suppression of the righting response (Jones, 1986). The idea with this response is to be able to escape when the predator relaxes its concentration (Forkman, 2007). There are studies that has found a QTL specific for chickens' response in the TI test (Schütz, et al., 2004), which indicates stability in the behavioural response. The fear of human test was done to measure the birds fear reactions towards humans. Even though reduced fear towards humans is considered to be one of the most important parts of domestication, even domesticated animals show predator avoidance behaviour towards humans (Price, 1984).

Two tests were used to measure the birds' social behaviours; one measured sociality and explorative behaviour, and one measured aggressiveness towards an unfamiliar bird. The social arena used in this study has been used in previous studies in order to measure sociality vs. exploration tendencies by exposing a test bird to a free choice situation between familiar social stimuli and the opportunity to explore a novel space at a distance from social partners at the opposite end of the test arena (Väisänen et al., 2005). To measure aggressive behaviour an experimental setup with a mirror was used. Since chickens are not known to recognize their own reflection and do not show any of the behaviours that could indicate self-recognition in animals (Prior et al., 2008), the mirror in this test exposed the chicken to another chicken which responded in exactly the same way as the test animal when encountering a stranger. An aggressive chicken met an equally aggressive chicken in the mirror, whereas a nonaggressive chicken met a chicken with the same type of behaviour.

The object of this thesis is to see if there are any correlations between fearfulness and social behaviours in the F7 birds, if so there could be genetic mechanisms behind the traits which would be of interest for further studies. Of interest is also to see if there are any differences between the genders, and if weight can be connected to any of the behaviours. The hypothesis of the study is that we will see correlations between fear and social behaviours in the chickens, and that there will be clear gender difference in some variables.

4 Materials and methods

4.1 Animals

80 chickens, 40 males and 40 females, of a F7 intercross between White Leghorn layers and red junglefowl were used in the tests. One male red junglefowl and three White Leghorn females were used as F0 animals. The red junglefowl were derived from a Swedish zoo population which had been kept at the research facility since 1998. The white leghorn originated from a selection line, SLU 13, bred at the Swedish University of Agricultural Sciences, and had been selected for egg mass since about 1970. The chickens came from three different batches of F7 birds, the first were hatched 2008-01-07, the second 2008-02-25 and the third 2008-03-17. All the chickens followed the same standard routines with vaccination and weighing. The birds were kept in adjacent, identical pens in the same room (3.1m×2.5m×3.0 m; *W*×*L*×*H*), with full visual and auditory contact between the pens. The pens contained food ad libitum, water, perches, nest boxes (from 10 weeks of age) and wood shavings on the floor. The birds were kept at a 12:12 h light: dark cycle and the room temperature were maintained at 19 ◦C. The 80 birds that took part in the tests were randomly chosen at the beginning of the first test from the two home pens. The order of the 80 test birds in each of the five behavioural tests was likewise randomized.

4.2 Behavioural tests

The chickens were tested in five behavioural tests assessed to measure the birds fear response, its aggressiveness and its sociality. During the test period the animals were 24-42 weeks old. The tests were carried out consecutively during 3 months.

4.2.1 Tonic immobility

At the age of 24-33 weeks the birds were tested in a Tonic immobility test (TI test). The test was carried out outside the birds' home pen. The birds were caught in total darkness and carried to the test table. A spotlight directed away from the bird was switched on before the test began. The bird was placed on its back in a cradle and TI was induced by the test person who gently put a pressure on the birds' chest for 10 seconds, and then carefully released the bird. If the bird moved during the first 5 seconds the procedure was repeated. Time was measured to the first head movement of the bird, and the time until righting (Jones and Faure, 1981). If the bird had not moved for 600 seconds the test was stopped. Maximum number of

induction attempts was 5, and each bird got a score based on the number of induction attempts (1-5). If the bird was not able to induce into TI it got the score 7. Animals with longer TI duration and fewer induction attempts are normally considered to be more fearful than animals with shorter TI duration or more induction attempts. (Schütz et.al, 2004)
How difficult each bird was to catch was measured when catching the bird in its home pen before the TI test. The test person made a descriptive analyse of the birds using a scale of 1-5, 1 being easy to catch and 5 being difficult.

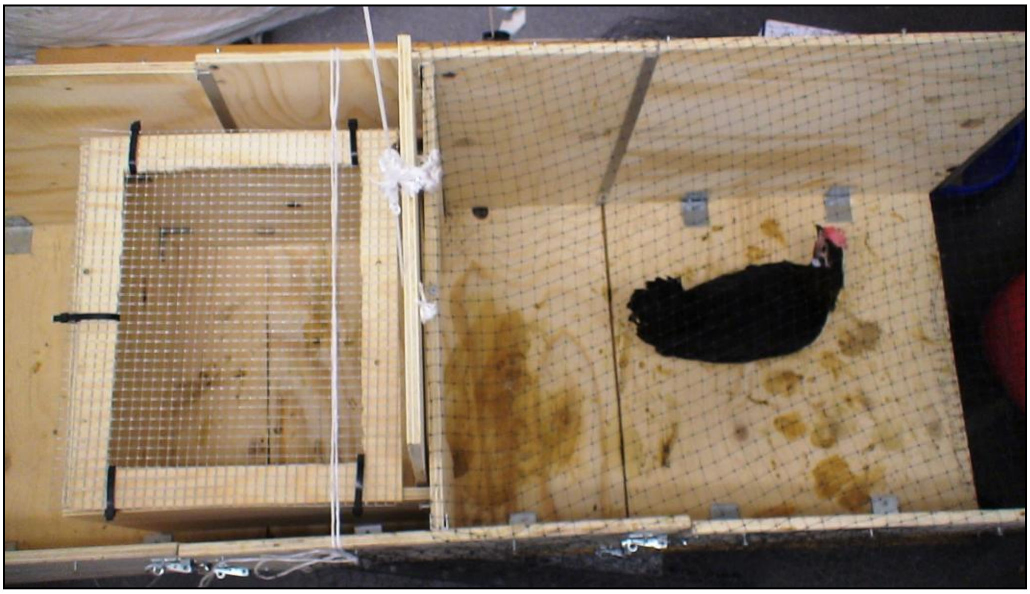
4.2.2 Fear of humans

During the fear of humans test the birds were between 26-35 weeks old. The bird was placed, in darkness, in a secluded compartment (*L*×*W*×*H*: 40cm×40cm×50cm) with a solid sliding door in one direction. Outside the sliding door was an area measuring 35cm×40cm×50cm (*L*×*W*×*H*), where the bird could enter freely once the sliding door had been opened (fig. 1). The short end of this area, opposite to the start box, had open sight out through a wire mesh. Outside the mesh, a human was sitting quietly, facing the arena, with an open hand placed just inside the mesh wall. The hand was filled with standard chicken food. The time of the test was 10 minutes, starting with 120 seconds of habituation. After the test the lights were turned of and the bird put back in its home pen. Each bird was filmed from above with a video camera. No. of pecks in the hand and no. of bouts of pecks were measured continuously by the observer, who also made a descriptive analyse of each birds plumage colour. Each bird's vocalizations and escape attempts were counted through analyse of the film, as well as the latency of the birds to leave the cage (with 50% of its body), the latency to approach the human (measured through the bird crossing a line 0.25m from the human with more than 50% of its body) and the latency to start eating. All birds were weighed directly after the test.

Fig.1. Arena used in the fear of human test.

4.2.3 Aggression test

The aggression test was carried out when the birds were 28-37 weeks old. The birds were caught in their home pen and placed in a test arena in total darkness. The arena measured



70x140cm and one short side was covered with a mirror, the other sides were covered with brown cardboard. The birds were placed in the far side of the arena from the mirror, facing the mirror. The lights were turned on and the test run for 5 minutes. The arena was divided into two sections, close to the mirror and far away from the mirror. The test was filmed from above using digital Handycams and afterwards it was analysed with 1/0 sampling every 15 seconds. All aggressive behaviours towards the reflection in the mirror were counted (attack, ruffling feathers and waltzing) as well as which zone the bird was in (aggressive zone or away zone).

4.2.4 Open field

When the birds were between 30-39 weeks they were tested in an open field test. The test arena measured 150x120 cm, and the walls were covered with cardboard. The birds were picked out of their home pens and carried in darkness to the test arena. They were placed in one corner of the arena in the dark, and the test started when the lights were turned back on. The test period was 10 min and each test was filmed and analyzed using Ethovision software package. Ethovision detects objects that differ from a background image obtained when the arena was empty. The total distance each bird moved in the arena (cm) the total meander (degrees cm^{-1}) and the mean value of meander for each bird (degrees cm^{-1}) were calculated. Meander is a variable that describes the mean angular turn rate during locomotion. A high meander indicates that the bird moved in a zig-zag fashion during the test, rather than in a straight line (Schütz et al 2004). Birds that are inactive in the OF are usually considered to be more fearful (Schütz et al. 2004).

Fig 2. F7 chickens in the sociality/explorative arena.

4.2.5 Social test

The last test in the test series was a social test, the birds were between 33-42 weeks when they were tested. The arena measured 153x125 cm, the boxes for the companion birds measured 40x50cm. The arena was divided into three equally big zones; the asocial zone (furthest away from the companion birds), the middle zone and the social zone (closest to the companion birds). This test was done to measure the birds' social vs. explorative tendencies (Väisänen et.al, 2004). One test bird was placed in the test arena, and three randomly chosen birds, familiar to the test bird, was placed in the companion boxes covering one side of the arena (fig 2). The other side of the test arena was covered with cardboard. The test bird was placed in the asocial part of the arena in darkness and the test began when the lights where switched on. The companion birds had visual contact with the test bird through a wire mesh, but had no visual contact with the other companion birds since the other sides of the boxes were covered with cardboard. Each bird was tested individually and once only. The tests were carried out in randomly chosen single sex blocks of four birds, which all originated from the same pen. While one bird in a block was being tested the other three were kept individually in the companion bird boxes, acting as social stimuli to the test animal, which in turn was used as a companion bird when the other three birds were tested. At the event of uneven number of birds of the same sex in a pen, already tested birds were used as companion birds to complete a quadruplet. The birds were tested during 10 min. After the test the lights were switched off and the test bird changed to a new one in darkness. The test was analysed using Ethovision software package, for each of the zones total duration (% and seconds) was calculated, as well as the latency of first occurrence for each bird in the social zone and the middle zone, and the total distance each bird moved in the arena (cm) the total meander (degrees cm^{-1}) and the mean value of meander for each bird (degrees cm^{-1}).

4.5 Data analysis

For behavioural analysis of the videos recorded during the tests either a VLC movie player were used for manual analysis, or Ethovision XT, Noldus Information Technology for digital analysis of the recorded videos.

4.6 Statistics

All data were analysed with principal components analysis (PCA). The 10 variables with the highest factor scores in the PCA were then used in a Pearson correlation analysis, an independent sample t-test and a linear regression analysis. SPSS for windows version 17.0 were used for all statistical analysis.

5 Results

5.1 Results from the PCA

The result from the PCA analysis is displayed in table 1a, b and c. The PCA was done to decide which variables that were the most important ones in each of the tests. In a) is the result from the PCA with the social behaviours, in b) results from the TI test and in c) the results from the fear tests. The variables that were chosen to continue on in the statistical analysis were the variables with the highest factor score, either in factor 1 or 2, two variables from each of the behavioural tests were chosen.

Table 1a. Result from the PCA of the sociality related behaviours.

	Factor score	
Variable	**Component 1**	**Component 2**
ST Asocial zone, tot dur (s)	0.827	0.191
ST Middle zone, tot dur (s)	0.641	-0,092
ST Social zone, tot dur (s)	-0.912	-0.088
ST Social zone, lat of first occ (s)	0.692	0.155
Agg Attack (nrs)	0.222	0.690
Agg Ruffling feathers (nrs)	0.323	0.813
Agg Waltz display (nrs)	-0.110	0.423
Agg, in agg zone (nrs)	-0.487	0.685
Agg, in away zone (nrs)	0.440	0.732
% total variance explained	*33. 3*	*26. 6*

Table 1b. Result from the PCA of the variables from the TI test.

Variable	Factor score Component 1	Component 2
TI Latency to head movement (s)	0.879	0.037
TI Righting (s)	0.864	0.127
TI Induction attempts (nrs)	-0.299	0.677
TI Difficulty to catch (nrs)	0.076	0.791
% total variance explained	*40. 4*	*27. 5*

Table 1c. Result from the PCA of fear related behaviours.

Variable	Factor score Component 1	Component 2
FOH Pecks (nrs)	-0.647	0.276
FOH Vocalizations (nrs)	-0.570	-0.402
FOH Latency to leave cage (s)	0.762	0.215
FOH Latency approach human (s)	0.884	0.148
FOH Latency to start eating (s)	0.831	-0.230
OF Distance moved, total (cm)	-0.377	0.135
OF Meander, total (degrees cm^{-1})	-0.120	0.863
% total variance explained	*42. 2*	*16. 0*

5.2 Correlations in behavioural responses

5.2.1 Correlations in all animals

Table 2 shows the correlations in behavioural responses for males and females together. There was strong correlations (p-value <0.01) between variables paired with another variable from the same test; birds with a long latency to head movement in the TI test, also had a long latency to righting in the same test, birds with a long latency to approach in the fear of human test also had a long latency to start eating, a strong negative correlation in the social test where birds that spent a lot of time in the social zone, spent less time in the asocial zone, and lastly, a negative correlation (p-value <0.05) between the variables in the aggression test showing that birds with more ruffling feathers (aggressive) behaviour spent less time in the away zone. When looking at correlations between behaviours displayed in different tests it shows that birds with a long latency to head movement in the TI test had a long latency to start eating in the fear of human test, and they were less inclined to ruffle their feathers towards the mirror in the aggression test (p-value <0.05); birds with a long latency to approach the human moved a shorter distance in the open-field; correlations with a p-value of <0.05 saying that birds with a longer latency to start eating spent less time in the asocial zone and more time in the social zone; and birds with a high amount of ruffling feathers behaviour in the aggression test, spent less time in the social zone and more in the asocial zone in the social test. (n=78-80)

Table 2. Correlation values for tested behavioural variables including all tested animals, displaying Pearson correlation coefficient.
** sig <0.05, **sig. <0.01*

	TI Righting (s)	FOH Lat approach human (s)	FOH Lat start eating (s)	OF Distance moved, total (cm)	OF Meander, total (degrees cm^{-1})	ST Asocial zone, tot dur (s)	ST Social zone, tot dur (s)	Agg Ruffl feathers	Agg, in away zone
TI Lat head mov (s)	.571**	.076	.221*	-.092	-.144	-.141	.144	-.238*	-.010
TI Righting (s)		.188	.125	-.184	-.096	-.055	.133	-.069	-.202
FOH Lat approach (s)			.662**	-.285*	-.009	-.154	.136	.071	-.026
FOH Lat start eating (s)				-.193	-.145	-.260*	.287*	-.109	-.056
OF Dist moved tot (cm)					.091	-.095	.098	.073	-.159
OF Meander tot (degrees cm^{-1})						.080	-.098	.076	-.128
ST Asocial zone. tot dur (s)							-.926**	.283*	.124
ST Social zone. tot dur (s)								-.283*	-.223
Agg Ruffl feathers									-.279*

5.2.2 Correlations in females

Table 3 shows the correlations for the females. The females show the same results in variables from the same tests as the correlation analysis including all animals did, but they also show many of the correlations for variables from different tests. In addition to this the females that had a long latency to head movement in the TI test also spent a lot of time in the away zone in the aggression test (i.e. they did not display a lot of aggressive behaviour). There was also a negative correlation between latency to start eating and distance moved in the open field test, i.e. females that that had a long latency to start eating did not move around as much in the open field test. (n=38-40)

Table 3. Correlation values for behavioural variables in females, displaying Pearson correlation coefficient.
** sig. <0.05, **sig. <0.01*

	TI Righting (s)	FOH Lat approach human (s)	FOH Lat start eating (s)	OF Distance moved, total (cm)	OF Meander, total (degrees cm^{-1})	ST Asocial zone, tot dur (s)	ST Social zone, tot dur (s)	Agg Ruffl feathers	Agg, in away zone
TI Lat head mov (s)	.422**	-.227	.178	-.194	-.012	-.105	.006	-.300	.423**
TI Righting (s)		-.056	-.057	-.178	-.054	.297	-.246	-.027	.032
FOH Lat appr human (s)			.667**	-.332*	-.218	-.234	.211	-.133	.217
FOH Lat start eating (s)				-.339*	-.295	-.281	.311	-.258	.143
OF Dist. moved, total (cm)					-.130	-.030	.032	-.070	-.194
OF Meander, tot (degrees cm^{-1})						.084	-.069	.041	-.035
ST Asocial zone, tot dur (s)							-.923**	.336*	.057
ST Social zone, tot dur (s)								-.352*	-.175
Agg Ruffl feathers									-.361*

5.2.3 Correlations in males

In males the intra-test correlations between the two TI variables and between the two variables in the fear of human test, as well as the negative correlation for which zone the bird spent time in, in the social test, were still significant (p-value < 0. 01) (Table 4). The negative correlation between latency to approach the human and distance moved in the open field test, which was shown in both previous correlation analysis as well, could also be seen in males. (n=40)

Table 4. Correlation values for tested behavioural variables in males, displaying Pearson correlation coefficient.
** sig. <0.05, **sig. <0.01*

	TI Righting (s)	FOH Lat approach human (s)	FOH Lat start eating (s)	OF Distance moved, total (cm)	OF Meander, total (degrees cm^{-1})	ST Asocial zone, tot dur (s)	ST Social zone, tot dur (s)	Agg Ruffl feathers	Agg, in away zone
TI Lat head mov (s)	.542**	.068	.144	-.149	-.179	-.106	.116	-.256	-.039
TI Righting (s)		.174	.026	-.301	-.104	-.158	.230	-.087	-.216
FOH Lat appr human (s)			.614**	-.355*	.135	.246	-.240	.241	-.121
FOH Lat start eating (s)				-.235	-.014	.193	-.157	.053	-.060
OF Dist moved, total (cm)					.234	-.114	.066	.187	-.026
OF Meander, tot (degrees cm^{-1})						.090	-.150	.100	-.270
ST Asocial zone, tot dur (s)							-.935**	.309	-.072
ST Social zone, tot dur (s)								-.252	.024
Agg Ruffl feathers									-.237

5.3 Differences between males and females in their behavioural response

The difference between means in the 10 test variables is shown in table 5. In 7 of the tests there was a significant difference in the mean between males and female. Males had a longer time until head movement and righting in the TI test, they also had longer latencies in both variables in the fear of human test (latency to approach human and to start eating). In the social test females spent more time in the asocial zone than males, whereas males spent more time in the social zone than females. In the aggression test males spent less time in the away zone than females. No significant differences were found in the open field variables (distance moved and total meander) or in the ruffling feathers behaviour in the aggression test.

Table 5 Means and standard errors of the means for males and females, also displaying t-value and p-value from the t-test. (n=40).

	females	males	t	p
TI Latency to head movement (s)	38.6±4.8	77.9±14.5	2.577	0.012
TI Righting (s)	84.0±9.8	168.1±24.9	3.150	0.002
FOH Latency to approach (s)	230.6±31.0	345.2±37.0	2.373	0.020
FOH Latency to start eating (s)	355.3±33.7	511.2±28.0	3.558	0.001

OF Distance moved, total (cm)	1786.1±224.1	2341.1±273.0	1.567	0.121
OF Meander, total (degrees cm^{-1})	-391.2±52.3	-418.2±68.9	-0.312	0.756
ST Asocial zone, total duration (s)	79.9±20.9	17.9±6.9	-2.873	0.005
ST Social zone, total duration (s)	469.4±24.3	561.9±11.1	3.521	0.001
Agg Ruffling feathers (nrs)	2.87±0.76	2.68±0.80	-0.179	0.859
Agg In away zone (nrs)	6.95±1.13	2.95±0.80	-2.904	0.005

5.4. Weight and behaviour

In table 6 and 7 is the regression analysis examining the relationship between weight and the 10 variables with the highest factor scores from the PCA. The p-value in females for variables and weight were not significant in any variable, although there was a tendency towards lighter birds having a shorter latency to start eating in the fear of humans test. In males the p-value was significant for latency to approach human and the weight of the bird (p-value< 0.05), and for total distance moved in the open field. Heavier males had a shorter latency to approach the human (figure 3), and also moved more in the open field (figure 4).

Table 6. Linear regression analysis on the variables in the study and weight for females, f-value and significance is displayed.

			Degrees of freedom	
	f-value	**Sign**	**regression**	**total**
TI Latency to head movement	0.332	0.568	1	39
TI Righting	0.062	0.805	1	39
FOH Latency to approach human	0.751	0.392	1	39
FOH Latency to start eating	3.454	0.071	1	39
OF Distance moved	0.025	0.875	1	38
OF Meander, total	0.315	0.578	1	38
ST Asocial zone, total duration	0.011	0.919	1	37
ST Social zone, total duration	0.285	0.596	1	37
Agg Ruffling feathers	0.489	0.489	1	38
Agg In away zone	0.823	0.370	1	38

Table 7. Linear regression analysis on the variables in the study and weight for males, f-value and significance is displayed. Regression degrees of freedom: 1, Total degrees of freedom: 39.

	f-value	**Sign**
TI Latency to head movement	0.007	0.933
TI Righting	0.248	0.622
FOH Latency to approach human	4.683	0.037
FOH Latency to start eating	2.806	0.102
OF Distance moved, total	6.667	0.014
OF Meander, total	0.395	0.533
ST Asocial zone total duration	0.920	0.344
ST Social zone total duration	1.808	0.187
Agg Ruffling feathers	0.575	0.453
Agg In away zone	0.607	0.441

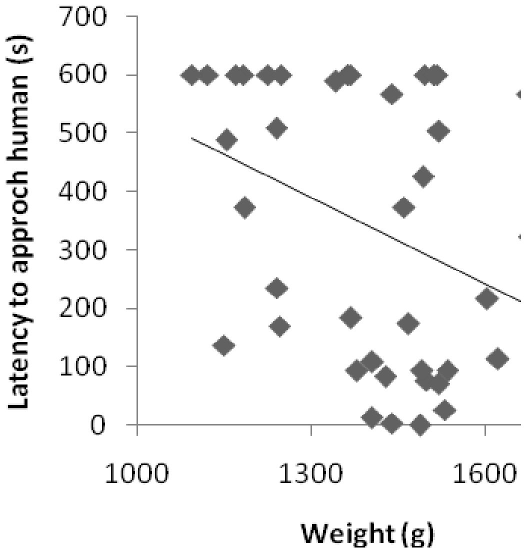


Figure 3. Relationship between latency to approach the human in the fear of human test and weight in male chickens.

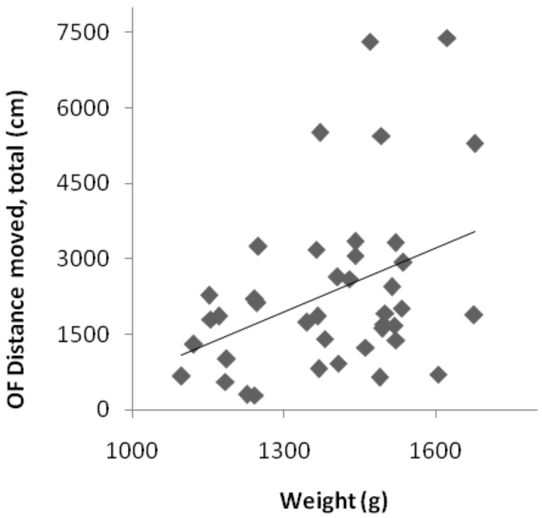


Fig 4.Relationship between total distance moved in the open field test and weight, in male chickens.

6 Discussion

The results from this study do not show enough correlations to draw any definite conclusions, but some correlations in behaviour could be found, which does make genetic correlations possible. We could see marked differences between males and females, both correlation wise, and behavioural response wise, as well as a difference in how weight might affect behaviour; in our study weight only seemed to matter for males, and only when looking into fear of humans and behaviour in an open field.

6.1 Correlations between traits

Mechanisms behind correlations in behaviour when selecting for a specific trait, such as various production traits, can cause problems in the selected line of animal. This is the main reason why correlations in this F7 intercross of Red Junglefowl and White Leghorn layers could be of interest; it could give background information for future studies in this area. Two of the mechanisms that are plausible explanations for why risks are involved when breeding with production gain in mind are pleiotropy and epistasis. Pleiotropy is when several traits are influenced by the same genes, and epistasis is when the action of one gene may be influenced by interaction with other genes (Jensen and Andersson, 2005). The results from the intercross (both the F7, and later and earlier in the generation) gives you a hint to which traits that could be interesting to look further into to see which genes control which traits.

From the PCA we chose two variables from each of the tests. These variables had the highest factor scores in either component 1 or 2 for the test in question, i.e. they explained most of the variation in that PCA. In the PCA of the sociality related behaviours the asocial variables (positive factor score for total duration in asocial zone, and negative for total duration in social zone) explained most of the variance in component 1, and the aggressive variables (ruffling feathers and negative factor score for in away zone) most of component 2. In the PCA for the TI test latency to head movement and latency to righting had the highest factor scores, both in component 1. The variables from the TI test got a PCA of their own since they seemed to be divided from the rest of the fear variables, and the dividing generated the highest factor scores and explained most of the variance. That the TI response is separated from the other variables is supported by the results from Schütz et al. 2004, in which TI was shown to be controlled by one QTL, different from the QTL that have been found to control responses in an open field. In the last PCA examining the other fear related behaviours three of the chosen variables had high factor scores in component 1; latency to approach human and latency to start eating in the fear of human test, and the total distance moved in the open field (negative factor score). That component one can be interpreted as a general fear component in the PCA of the fear tests is in line with previous studies (Campler et al.2009). In that study one component which could explain most of the general fear in the chickens were found. They also found that component two could be associated with locomotion. This is somewhat supported by the results in this study, were the total meander in the open field had the highest factor score in component two, also a trait based on locomotion.

In the results from this study we could see some significant correlations between traits in the tests, both intra-test and inter-test, which could give support to a hypothesis of genetic linkage between different behavioural traits affected by domestication. In the TI test the strong correlation ($p<0.001$) between the two analyzed variables supports the results previously shown by Schütz et.al, 2004 which, as stated above, suggests that the responses in TI tests are controlled by one QTL. The TI test is considered to be acutely frightening to the birds exposed to it, because of it being composed of several frightful events such as capturing, social isolation and novelty (Schütz et al., 2004) which make it a good test to use when measuring fearfulness in chicken. Ghareeb et al., 2008 even suggested that the TI test could be used as part of a breeding programme to assess individual chickens fearfulness, since the behaviour of one individual in the TI test could be used to predict the same individuals' behaviour in other fear-tests. Further on, the correlation shown between a long tonic immobility and less ruffling of feathers towards the mirror in the aggression test could indicate a possibility that there is a genetic linkage between fear and aggression, more specifically that there could be a linkage between the genes controlling the chickens' response during the TI test and its aggressiveness. We could also see an indication that fear responses and sociality could be linked in chickens, based on the correlations between the results in the fear of humans test and the social test. Chickens with a long duration to start eating in the fear of human test spent shorter time in the asocial zone and longer time in the social zone. The correlation between fearfulness and social behaviour has been suggested in a previous study (Hauser et al. 2004). Hauser et al., (2004) suggest that fearful birds might show greater tendencies to aggregate with familiar birds when confronted with an unknown situation, although their results do not support this suggestion. They looked for correlations between fearfulness measured in a TI test and sociality, and their results show that no such correlations could be found, which is supported by the results in this study and other studies (Hocking et al., 2001), however the explanation can instead be used for the correlation between latency to start eating and sociality. The correlations shown in this study could therefore give support for the hypothesis that chickens fear of human and sociality traits have a genetic linkage, which could be affected by domestication. Of course, no conclusions can be drawn about the genes controlling the different traits based on the results from this study, but it indicates an interesting area to look further into.

The results from this study also show a correlation between how quick the bird is to approach the human and to start eating in the fear of human test and how much they move about in the open-field test; the longer the latency to approach the human and to start eating, the less distance moved in the open-field. This supports previous results showing that the more frightened the bird is, the more it freezes, i.e. stands still, in the open-field test (Campler et.al, 2009). These variables are both measures of fearfulness and therefore the results only indicate the stability of the birds' behaviour between different tests, a bird that is highly frightened in one test, will continue to be frightened in the next.

The strong correlation between the two variables analyzed in the social test (time spent in the asocial zone and time spent in the social zone) with a Pearson correlation coefficient of -0.926 indicates that chickens are either social, or they are not. Chickens that spent a long time in the asocial zone in the social test also displayed more ruffling feathers behaviour in the aggression test, i.e. asocial chickens also tend to be more aggressive towards conspecifics. These results seem straight forward and in line with what could be predicted; the birds that are highly social do not show aggressive behaviours towards strangers. The correlation shown between two variables in the aggression test (ruffling feathers and in away zone) is not surprising either; the aggressive chickens spend more time close to the supposed intruder than away from it.

6.2 Differences between males and females

In this study we saw a clear difference when comparing males and females, both in their behaviour in the different tests and in the correlations between different variables. This is somewhat supported by results from a study on the F3 generation of the same intercross (Väisänen, et.al, 2005). More pronounced breed differences were found when comparing red junglefowl females to white leghorn females, than when comparing males of the breeds. The same effect was found in the F3 progeny; birds with higher levels of production traits behaved in a fashion more like the leghorns than the junglefowls, and this was more pronounced in females. So, there appears to be gender differences in traits in chicken, and domestication seem to affect the genders differently.

The males only show correlations between variables from the same test. The correlation in males between the variables in the TI test (the longer the latency to head movement, the longer the latency to righting) further supports the findings in Schütz et al. 2004 which suggests that some of the variation in TI tests are explained by one QTL. That there is a correlation between how quickly the bird approaches the human and how soon it starts eating is not surprising either. Several of the males were brave enough to approach, but then had a longer latency to start eating, compared to the females. This can probably be explained by males generally being more fearful than females **(**Jones and Faure, 1981**).** The negative correlation between time spent in the social zone and time spent in the asocial zone in the social test is present also when looking only at the males, which suggests that the sociality of chickens is one way or the other, independent of the birds' gender. Either they are highly social, or they are not.

The females, on the other hand, show all the correlations previously discussed. This could lead to the suggestion that fear and sociality traits are controlled by genes situated on the chickens W-chromosome, although this is a very speculative suggestion. In chickens the males are homozygote (ZZ) whereas females are heterozygote (ZW). All female chickens inherit the W-chromosome from their mothers, which means that no recombinations occur on the W-chromosome, and therefore traits controlled by genes situated on that chromosome will show correlations, and only in the females. Since the difference between the genders can be found this is a plausible explanation. This is further supported by the results from the t-tests between males and females which show a significant difference between the genders for all but three variables; total meander and distance moved in the open field and ruffling feathers in the aggression test. The open field meander variable did not show any correlations in the correlation analysis either, which probably can be traced back to the factor analysis in which it got an high factor score in component two, instead of component one, which would point to it being more independent from the other variables in that factor analysis. The total distance moved in the open field, on the other hand, had its highest factor score in component one, although it had a low negative score compared to the other variables, which could explain it being more separated as well. It did show correlations with latency to approach human in the fear of humans test, for all animals, even when separating the genders, which also supports the results from the t-test saying that there is no difference between the genders in that specific test. That there is no difference between males and females in ruffling feathers in the aggression test could be a bit surprising. There was a correlation between ruffling feathers and the variables from the social test for females, but not for males, which could be a predictor for a difference, but as stated, no such difference between the genders could be found in the t-test analysis in this study. This is not in line with previous studies (Vallortigara, 1992, as cited by Queiroz and Cromberg, 2006) were differences in behaviour between males and females towards an intruder could be found. Both genders displayed aggressive behaviour towards the intruder, but the males more frequently than the females.

6.3. Weight and behaviour

Kerje et al. (2003) suggested that the same QTL of about 200 genes located on chromosome 1 (growth 1) controls growth and TI-responses in chicken. The regression analysis done in this study, however, did not show any significant p-values between the behavioural traits and weight, which suggests that the traits are not controlled by the same genes. The genes controlling the traits are probably located within the growth 1 QTL, but separated from each other. These results do give a plausible working hypothesis for the F8 generation; that the QTL: s for growth and TI-responses will be divided from each other. Further on the regression analysis pointed at a significant p-value between latency to approach human in the fear of human test and weight, in males. This significance was negative, which suggests that heavier males have a higher motivation than the lighter to approach a human for food. The larger male's higher motivation to feed could be explained by their higher metabolic rate, it could also point at these males being more dominant, since there is thought to be a correlation between weight and dominance; heavier males are believed to be more dominant (Cloutier and Newberry, 2000). The results from the regression analysis also showed that the heavier males moved about more in the open field test, which would indicate them being less fearful also in that test, and thereby suggesting stability in their fear reactions in those tests.

6.4 Conclusion

A few correlations were found in this study, as was hypothesized, although not so many as to draw any conclusions about the genetic mechanisms involved. The correlation between the variables in the TI test supports earlier findings (Schütz et al., 2004) of a QTL that controls chickens response in that test. The correlation found between response in the TI test and aggression suggests a genetic linkage between fear and aggression, as well as a genetic link between fear and sociality, based on the correlation found between response in the fear of human test and the sociality test. A clear difference between males and females in several variables could be seen, which suggest that domestication affects male and female chickens differently. The correlations found in all animals, still exists when looking at only the females, but not for the males. This could give rise to a speculative suggestion that these traits can be partly controlled by genes situated on the chickens W-chromosome. Also found was that the heavier males seem to be the less fearful ones when it comes to fear for humans and fear measured in an open field, whereas no relationship between weight and behavioural traits could be found in females.

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