Examensarbete

Social behaviour responses in red junglefowl (*Gallus gallus*) selected for tameness

Maria Ericsson

4/6-10

LITH-IFM-A-EX--10/2303—SE
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1. **Abstract**

Historically during domestication of animals, tameness towards humans was likely the most desired trait and therefore bred on. The red junglefowl (Gallus gallus) is the wild progenitor of all domestic chicken breeds and earlier studies present clear morphological, physiological and behavioural differences between domesticated breeds and the non-domesticated red junglefowl. These changes may be the result of altered gene expression - pleiotropy or linked genes. The aim of this thesis was to evaluate (1) effects of tameness selection on social behaviour towards conspecifics and (2) social behaviour differences between the sexes. Two generations of red junglefowl, P0 and its offspring F1, were studied. Both generations were divided into three selection lines (tame, intermediate or fearful), depending on their results in a fear of humans test. A novel type of social reinstatement test was set up, containing a non-social area without stimulus, and a social area containing a mirror serving as stimulus animals. The social and aggressive behaviours performed towards the mirror were recorded, so was the time spent in the social versus non-social part. An undisturbed behaviour test was performed, as well as a standardized social reinstatement test. The P0-females performed significantly more social behaviour (p=0.008) at 26 weeks than males in the mirror test, and females also displayed significantly more social (p=0.04) and agonistic behaviour (p<0.001) than males in the undisturbed behaviour test. The social reinstatement test displayed a significant effect between the selection lines with regards to sociality. This suggests that there are immediate selection responses in early domestication.

Keywords: domestication, red junglefowl, sociality, tameness selection

2. **List of abbreviations**

- F1 – First selected generation of red junglefowl
- P0 – Unselected parental generation of red junglefowl
- RJF – red junglefowl
- WL – White Leghorn

3. **Introduction**

The domestication process of an animal can be defined as the process in which captive animals are adapted to an environment provided by humans (Price 2002). Historically, during domestication of different species, reduced fearfulness towards humans were likely to be the most important and desired trait. Thus, the animals displaying reduced fearfulness were easier to handle and were thereby intentionally bred on, consequently leading to a first step in domestication and thereby a change in selection pressure followed.

The nature of the domestication processes includes major differences in the living conditions of the animals when comparing wild and captive individuals. As stated by Price (1999), captive animals are provided feed and shelter by humans, where limited space is common. Predator pressure is often minimized and the captive animals must also often tolerate larger population densities than their wild relatives. Under those living conditions, man induces a forced change in behaviour and an altered selection pressure in the captive animals. These changes in selection pressure have all contributed to the numerous domesticated species we see today.

The change in selection pressure is often associated with a cascade of responses in traits not intentionally selected for. Those additional selection responses have been well-known for a long time; this fact was for instance discussed by Darwin (1859) although the mechanisms for the traits appearing in addition to the changed selection pressure were at that point
unknown. In the late 1950’s, this area was more extensively investigated when Belyaev et al. (1984) initiated a domestication experiment on silver foxes, known as “the farm-fox experiment”. Selection on foxes selected exclusively for tameness was performed. The change in selection pressure resulted in a number of morphological changes; after 8 to 10 generations of breeding, pigment loss resulting in white spots in the fur were ascertained. Additional morphological changes were curled tails, floppy ears, and changes in body size and body proportions (Trut, 1999). Belyaev’s results have been foundational in the domestication research field. Similar domestication experiments have been performed in other non-domesticated species, for instance rats (Albert et al. 2008). The study demonstrates physiological changes which can be connected to reduced fearfulness in animals selected for tameness. The tame rats were reported to have smaller adrenal glands, larger spleens and lower levels of serum corticosterone - changes that indicate lower levels of hormones associates with stress. Similar findings were presented by Plyusnina & Oskina (1996), where grey rats (Rattus norvegicus) selected on reduced aggression towards humans had lower plasma corticosterone level compared to their wild conspecifics in open-field tests, indicating reduced fearfulness in this particular situation due to selection towards aggression to humans. Selection for tame or dolce behaviour also result in earlier sexual maturation (see for example Shishkina et al. 1993, Klotchkov et al. 1998, Trut, 1999)

In addition to morphological and physiological changes, also changes in behaviour have been proven in animals undergoing domestication. Foxes selected for tameness (Trut, 1999) displayed after a number of generations behaviours similar to those of dogs, e.g. seeking human attention and licking hands. Behaviour changes towards conspecifics when selecting on tameness towards humans have also been studied; domesticated Guinea pigs display less aggression and more sociopositive behaviour towards conspecifics (Künzl & Sachser, 1998) and other studies demonstrate decreased fearfulness in domesticated animals (Plyusnina & Oskina, 1996; Campler et al., 2009; Schütz et al, 2001a). One can also see a change in foraging strategies in domesticated animals, for example Schütz et al (2001) and Schütz & Jensen (2001) found that domesticated chickens use more energy conserving foraging strategies (the resource allocation theory) compared to a non-domesticated breed.

Due to the wide range of findings mentioned above, we can draw the conclusion that changes in selection pressure and breeding on specific traits affects morphology, physiology and behaviour. We see a different phenotype compared to the wild progenitor. This phenotype that has arisen on account of domestication effects is referred to as “the domesticated phenotype” (Jensen, 2006). The correlated responses in the domesticated phenotype must logically be a consequence of either pleiotropic or linked genes, probably affecting many different genetic pathways and resulting in effects on gene expression. Pleiotropy is when several phenotypes are affected by one gene. Thereby, when selecting on one particular trait, selection for additional characters may occur. There can also be a tendency for several traits to be inherited together, which is called genetic linkage. The traits are then located on different but adjacent genes. (Lewin, 2008). To investigate these connections between traits, work in our research group has been conducted over several years to map the genetic differences associated with domestication, using chickens as model animals (Schütz et al. 2002; Schütz et al. 2004; Wirén et al., 2009; Wright et al. 2008).

Chickens are used as model animals in a variety of fields of research, for example immunology, embryology and behaviour studies. The genome of the chicken is smaller but has a higher gene density compared with humans, and was completely mapped in 2004, (International Chicken Genome Sequencing Consortium, 2004) which contributes to the attractiveness of using chickens as model animals in research.

The wild-derived progenitor of the domestic chicken is considered to be the red junglefowl (RJF) (Gallus gallus) (Fumihito et al, 1996) and several domesticated populations
seem to have arisen independently in different parts of Asia (Kanginakudru et al., 2008) when the domestication process was initiated around 8000 years ago. Wild specimens of Red jungle fowl can still be found in India, China, Java, Malaysia and the Philippine (Al-Nasser et al, 2007). The wild RJF are known to live in social groups with a strict hierarchy, or pecking order. (Schjelderup-Ebbe, 1922, cited by Guhl, 1968). The chickens display a range of social and aggressive behaviour, from friendly pecks to agonistic behaviours, such as aggressive pecks, threats and fights, which most likely are performed to maintain the pecking order from dominant to submissive individual. Males are commonly regarded as the more aggressive sex (see for example Guhl, 1968; Wirén et al, 2009) however Krujt (1967) did not observe any obvious qualitative differences between the sexes in agonistic behaviours.

The domesticated breeds of chickens are often bred for production purposes beneficial for human consumption, such as high egg production or body weight. Both behaviourally and morphologically, the domesticated breeds differ from their wild relatives. The White Leghorn (WL) is bred on production traits and lays larger and about ten times more eggs during its lifetime compared to the RJF. The RJF have wild-type plumage colour, while the WL breed is entirely white (Figure 1). The WL are also almost double in size compared to the RJF, and does not normally brood. Due to these differences, the WL is a common breed to observe when describing differences between a domesticated breed and its wild ancestor.

![Figure 1](image1.png)

*Figure 1. Top photos display a domesticated White Leghorn male (left) and female. Bottom photos show a male (left) and a female of the wild progenitor to all domesticated breeds, the red junglefowl.*

Numerous studies have been conducted to assess differences connected to effects of domestication between the breeds. For example, it has been demonstrated that the WL redistribute their energy costs to higher egg production and perform less foraging and social behaviour, compared to the RJF, who also displayed a more active behaviour in general, compared to the WL (Schütz & Jensen, 2001). Moreover, WL have a reduced overall fearfulness compared to RJF (Campler et al, 2009) and RJF also seem to display a more active response to frightening stimulus, such as tonic immobility test and restraint test (Schütz et al.
Studies also indicate that there is a difference in ability to imprint on different stimuli, where RJF chicks seem to be more flexible than WL (Kirkden et al., 2008). Several studies thereby assess the theory that when breeding animals on specific traits, we also see different levels of behavioural changes.

In order to measure sociality and group cohesion tendency, a standardized test and stimulus is required. The use of mirrors may be one solution. The chicken has no mirror self-recognition; thereby mirrors can be used when evaluating their social behaviour since they view their mirror reflection as a conspecific. Mirrors have been used as social stimulus in earlier experiments (Feltenstein et al., 2002; Schütz et al., 2001; Anna Wirén, unpublished manuscript) and seem to be a functional alternative to live stimulus birds. By using a mirror you avoid interference or disturbance from a live stimulus bird and it will be an unfamiliar bird of the same size and sex. For example, Montecucchi and Noel (1978) concluded that mirror image-simulation is attractive to chicks, independent of rearing condition (solitary, pairs or groups), although the group-reared chicks displayed a shorter latency in approaching the mirror compared to the solitary reared chicks. It is also proposed that mirrors can be used to reduce isolation-related stress in laboratory birds (Henry et al., 2008). Thereby there is little doubt that birds accept a mirror image as company.

To investigate potential domestication effects in chickens, a PhD-project with the duration of five years was initiated in our Applied Ethology research group. At the age of 12 weeks the first, unselected generation of red junglefowl were exposed to a fear for humans test and divided into three selection lines – low (non-fearful) intermediate (control group) and high (fearful). Upon this, numerous behaviour tests of different character were performed by others in the research group, for example open field test, fear of predators, novel object test and foraging strategies test to evaluate potential behaviour differences between the three selection lines and later, to evaluate differences in behaviour between the upcoming generations when selecting on tameness towards man. After culling, weight measurements from brain, liver, heart, reproduction organs and spleen were taken and tissue samples were extracted for genetic analysis with the purpose to detect possible differences in gene expression between the selection lines and also for future comparisons between the selected generations.

The aim of this project was to study immediate correlated selection responses that might arise during early domestication with regards social behaviour and aggression. The observations were performed using an unselected generation (P0) of red junglefowl and the first generation of offspring (F1) solely selected on tameness. This was carried out by utilizing a novel sociality test/social reinstatement test using mirrors to simulate a flock. By constructing a test arena with two sections – one “social area” with a mirror and one “non-social area” with no mirror, it was possible to measure social motivation, as well as social and aggressive behaviours performed towards the mirror. A test for observing undisturbed behaviour under more natural circumstances was also performed as a complement. To further evaluate social behaviour the distance to the nearest neighbor was measured.

The F1-generation also underwent a runway test measuring social reinstatement. Runway tests are widely used for assessing social reinstatement and thereby social motivation (Charmichael et al., 1998; Vallortigara et al., 1990; Väisinen & Jensen, 2003).

I hypothesized that fearfulness selection for reduced fearfulness will have effect on sociality and aggression against conspecifics and that there will be a significant difference in behaviour between the selection lines with regards to social and aggressive behaviour.

The second hypothesis was that the sexes differ in the way they retain their social structure and thereby, I expected a difference in social and aggressive behaviour when comparing the sexes.
4. Materials & Methods

4.1. Animals and husbandry

To investigate the initiation of a domestication process in chicken, Red jungle fowl (Gallus gallus) was used as model organisms.

The unselected P0-generation that was used in this experiment consisted of 42 females and 33 males. They originate from an intercross between two different populations of Red jungle fowl (the P00-generation); one population came from Copenhagen Zoo (Cop), Denmark and one from Götala research station (Go), Skara, Sweden. The Cop birds had been kept in Copenhagen Zoo for numerous generations since the 1950’s, freely roaming around the park on an area of seven hectares. There were also occasions of predator contact, mainly from foxes. The Go birds, which originated from a population kept at the University of Agricultural Science, Sweden, had lived under controlled, captive circumstances since 1993. Due to different research projects, these birds were used to being handled by humans (Håkansson & Jensen, 2004). These two populations have been proven to have different genetic backgrounds (Håkansson et al, 2006) and they also differ in behaviour, especially in behaviours connected to fear of predators.

Due to the difference in genetic background between the original populations that parented the P00-generation (Håkansson et al, 2006), strict controlled breeding was administered in order to retrieve as high genetic diversity as possible in the P0-generation (Figure 2).

As recently mentioned, two generations of birds were used in this experiment. The unselected P0-generation was hatched 9/2-09 and consisted of 42 females and 32 males. The treatment of the animals has earlier been described by Campler et al (2008), and was briefly performed as follows: The hatching took place automatic incubators (Marsalles) in the hatchery “Krujt” at the University of Linköping, Sweden. The animals were wing-marked and vaccinated against Marek’s disease at hatching. At this point the chickens were moved from the incubators into a room with a 12/12 hour cycle of light and dark and a fixed temperature of 25 degrees Celsius. The chicken pens in the room had the size 1.4 m x 0.7 m, the floor was covered with wood shavings and the chicks had unlimited access to water and commercial

![Figure 2. Schematic drawing of the intercross between the Copenhagen and Götala populations resulting in ultimate outbreeding of the $P_0$-generation.](image-url)
chicken feed. The pen was also equipped with a heat lamp. At the age of 28 days the chickens were moved from the hatchery to the research chicken house “Wood-Gush” at the High School for Agriculture in Vreta Kloster. They were at arrival placed in a pen with a perch and had unlimited access to water and conventional chicken feed. At the age of approximately 60 days, the birds were sex separated and moved into two identical pens, located next to each other with a mesh separating the pens. The groups had full visual and auditory contact. The pens measured 3.1m x 2.5m x 3.0m (LxWxH). The birds had unlimited access to water and conventional chicken feed and the floor was covered with wood shavings. The light in the room followed a 12h/12h light/dark cycle.

The P0-generation was tested in the novel social reinstatement during May 2009, when the birds were 13-14 weeks old. The test was repeated in August the same year when most of the birds had reached maturity, at the age of 26-27 weeks. The undisturbed behaviour test was performed when the birds were 27 weeks of age.

Numerous tests took place to validate different aspects of the behaviour in the P0-generation (not part of this study), starting at the age of 21 days. These tests included fear of humans test, standardised social reinstatement test, open field test, novel object test and air predator test. To generate the next, selected F1-generation, the performance in the fear of humans test in the P0-generation was validated and scored on a scale from 1-3 where 1 = tame and 3 = untame. The scores were summarized and an average value gave a selection index score (SI), which was used to determine which birds to breed on. The relatedness between the birds was taken into consideration to avoid family effects when setting up the bird pairs for breeding. The 10 males and the 10 females with the lowest SI were bred, and the same procedure was done with the high SI birds. Six females and six males with intermediate SI were paired to serve as a control group (Figure 3).

![Figure 3](image)

Figure 3. Schematic illustration presenting number of birds included in the selection and the selection procedures in the P0 generation to generate the F1-generation.

All animals in this experiment were treated identically and there was no change in routines between the P0 and the F1-generations concerning housing conditions, tests and handling. The F1-generation (selected for tameness) was hatched 25/11-09. It consisted of 49 females and 55 males. They underwent the same mirror-tests as the P0-generation at 13 weeks of age.
Neither the repetition of the mirror test at 26 weeks nor the undisturbed behaviour test at 26 weeks was performed due to lack of time. Both generations underwent a social reinstatement test at the age of 20 days, and the results from the F1-generation are included in this study to assess any directly inherited traits.

4.2. Test 1 – Mirror test

To be able to validate and compare social and aggressive behaviour between the P0-generation and the F1-generation, a novel kind of social reinstatement test was performed. Each bird were recorded individually in a test arena, which first was developed and tested on a subset of birds from another group of RJF (not part of the experiment).

4.2.1. Test arena

The test arena (Figure 4a, b) was 1.8 x 1.8 m in total and was in the middle divided by a wall, creating two 0.9 x 1.8m compartments. The floor was covered with wood shavings. In the middle wall a hatch was built, which could be opened and closed by the observer by pulling a string which was attached to the top of the hatch. Thereby the observer was able to start the test when the bird had adapted to the environment without further human disturbance. The arena was covered with a metal mesh to avoid escape attempts.

![Figure 4a. Schematic picture of mirror test arena](image1)

![Figure 4b. Bird performing social inspection in the mirror test arena](image2)

The first compartment was empty and was considered as the non-social area (NSA). The second compartment was to be considered as the social area (SA). A mirror with flexible ends for angle adjustment was placed along the wall opposite to the hatch and was adjusted to covering the corners. This gave the impression of a flock when the tested chicken moved around in the arena. Depending on the position in the arena of the test animal, two mirror reflections simulating conspecifics were visualized.

4.2.2. Method

The birds were initially habituated in the test arena in groups of five individuals. Each group were habituated for 10 minutes in the test arena with the hatch open and the mirrors covered
with cardboard. The stop watch used in the experiment was switched on during the habituation period, so that the birds also got used to a beeping watch. The animals were then removed from the arena and each individual was tested individually. The test subject was placed in the non-social area in the arena with the hatch closed. After a short habituation time, lasting approximately one minute, the hatch was opened, allowing the bird to enter the social area with the mirror and the test started. The different behaviours (see section “Ethogram”) were recorded using 1/0 sampling (Martin & Bateson, 2007) with 10 seconds interval. A stop watch that was set to beep every 10 seconds was used for keeping track in the intervals. Time spent in social vs. non-social area was also recorded, using instantaneous sampling with 10 seconds interval.
4.2.3. Ethogram

The following behaviours were defined and recorded during the mirror test (Table 1).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Foraging</strong></td>
<td></td>
</tr>
<tr>
<td>Ground peck</td>
<td>Pecks at items (visible or not) on ground</td>
</tr>
<tr>
<td>Ground scratch</td>
<td>Scratching at ground, often intermittent during bouts of ground peck, often followed by one-two steps backwards after ground scratch</td>
</tr>
<tr>
<td>Peck objects</td>
<td>Peck at object of interest, including fittings in environment, except on mirror reflection</td>
</tr>
<tr>
<td><strong>Self directed</strong></td>
<td></td>
</tr>
<tr>
<td>Preening</td>
<td>Uses beak to trim and arrange feathers</td>
</tr>
<tr>
<td>Dust bathing</td>
<td>Db corresponds to vertical wing shake, and rubbing phase. Usually preceded by scratching and bill raking, and followed by Fr and Pr.</td>
</tr>
<tr>
<td>Scratching</td>
<td>Uses feet to scratch, clean and preen feathers</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>Stand alert</td>
<td>Stands (legs erect) with open eyes, attending to the surrounding</td>
</tr>
<tr>
<td>Stand relaxed</td>
<td>Standing (legs erect) with reduced attention, eyes may be partly closed, neck short, no alert head movements</td>
</tr>
<tr>
<td>Walking</td>
<td>Taking two or more steps</td>
</tr>
<tr>
<td>Lying</td>
<td>Body touching the ground, either chest or side. Legs bent under body</td>
</tr>
<tr>
<td><strong>Aggressive/social</strong></td>
<td></td>
</tr>
<tr>
<td>Social inspection</td>
<td>Observing mirror reflection</td>
</tr>
<tr>
<td>Raised hackles</td>
<td>Neck feathers erected</td>
</tr>
<tr>
<td>Wing flapping</td>
<td>Flapping wings while standing on ground or perch</td>
</tr>
<tr>
<td>Feather ruffle</td>
<td>Erects feathers, ruffles, and shakes body</td>
</tr>
<tr>
<td>Attack</td>
<td>Running, flying or jumping towards mirror</td>
</tr>
<tr>
<td>Mirror peck</td>
<td>Peck on mirror image</td>
</tr>
<tr>
<td>Waltzing</td>
<td>One bird (usually male) circles around another bird (same sex) with distance &lt;0.5m, with outer wing lowered.</td>
</tr>
<tr>
<td><strong>Vocal</strong></td>
<td></td>
</tr>
<tr>
<td>Gackel</td>
<td>Long, deep sound “krrrooaaaaarrkkrrroooarr</td>
</tr>
<tr>
<td>Crow</td>
<td>Cockerel crowing</td>
</tr>
<tr>
<td>Social klucks</td>
<td>Short klucks</td>
</tr>
<tr>
<td>Other vocals</td>
<td>Any unspecified vocalization</td>
</tr>
<tr>
<td><strong>Fear</strong></td>
<td></td>
</tr>
<tr>
<td>Freeze</td>
<td>Stiff posture, stand, sit or lie motionless, vigilant, open eyes</td>
</tr>
<tr>
<td>Flight</td>
<td>Flying or jumping exaggerated against wall or fence</td>
</tr>
</tbody>
</table>
4.2.4. Data treatment and analysis

The defined behaviours were pooled into six different categories:

- Foraging
- Self directed
- Other
- Aggressive/Social
- Vocal
- Fear

(See Table 1 for details) The behaviours were tested for normal distribution and visually validated, testing males and females separately. A correlation analysis was performed to evaluate whether there was a change in behaviour within the sexes when comparing the both test sessions (May vs. August). As a result from multiple fearfulness towards humans’ tests (not part of this experiment) the animals were given a selection index score:

- High (H) – Fearful behaviour towards humans (12 males, 13 females)
- Intermediate (I) – Control group (11 males, 15 females)
- Low (L) – Non-fearful behaviour towards humans (10 males, 14 females)

To assess any significant difference in behaviour in the P0-generation, an ANOVA (general linear model) was used to determine sex differences and/or selection index effect (n=3) on the observed behaviours. The significance level was set to (p<0.05). The displayed deviations of mean values are shown in standard error of mean (S.E.). All the statistic calculations were performed in MiniTab 15.

The evaluation of the potential effects of the selection in the F1-generation was performed in the same manner as in the P0-generation. The behaviours were tested for normal distribution and an ANOVA were performed to evaluate potential behaviour differences as a result of the tameness selection in their parents. The F1 birds included in the ANOVA were the 12 of each sex who scored either highest or lowest from their respective selection group and all the intermediate birds, see Figure 5.

4.3. Test 2 – Undisturbed behaviour test and Nearest Neighbor test

General behaviour studies were performed of the P0-generation in a more undisturbed manner, in order to quantify other aspects of the social behaviour which may be correlated to the selection test results.
Figure 5. Schematic drawing of the undisturbed behaviour test arena.

4.3.1. Methods

The test was performed in a pen measuring 2.5 x 3.0 m. A perch was built inside the pen and the animals had access to feed and water ad libitum. When the tests took place, the animals lived sex separated and thereby the birds were separately tested with regards to sex.

The birds were observed during a total of eight days. Four days were spent testing females and four days for testing males. The animals were divided into smaller groups to make observations and handling easier. The females were 42 individuals in total, divided into groups of 10, 10, 11, 11. The males were 32 in total, and were tested in groups of 9, 9, 8, 7. One full day divided into four observation sessions were dedicated to each group. The animals were inserted into the test arena in the afternoon the day before testing and were habituated over night, whereupon the test was initiated at 9 am the day after. In order to distinguish between the animals, when moving the animals from their home pen to the test arena, the animals were marked with colour tags which were attached around the right leg. They were marked with pre-decided colours - the first animal picked up in the home pen was given a yellow colour tag and were the first individual in the group to be observed in the test. The second bird picked up was marked with a green tag and was the second individual to be tested and so on. This marking routine were the same during all test days. Certain defined behaviours (listed in section 2.3.3. (Table 2)), were studied using focal animal observations with 1/0-sampling (explained in section 2.2.2) with 10 seconds interval. Each animal was observed for five minutes per session and thereby observed for 4x5 minutes in total. The order in which the animals in one test group were observed were in accordance to a predefined rotating scheme where the colour tags on the leg decided what animal to observe.

In addition to the undisturbed behaviour test, the distance to the nearest neighbour from the focal animal was also measured. By using instantaneous sampling every 10\textsuperscript{th} second, the distance to the nearest neighbour (NN) was estimated. The focal animal was given a score (1, 2 or 3) depending on the distance to the NN where

- 1= distance <0.1 m
- 2= distance 0.1-0.5 m
- 3= distance >0.5 m
The distance was estimated looking at the nearest body part but excluding the tail feathers in the males.

### 4.3.2. Ethogram

The following behaviours were defined and recorded (Table 2).

#### Table 2. Behaviours observed during the undisturbed test

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Social pecks</strong></td>
<td></td>
</tr>
<tr>
<td>Severe feather pecking</td>
<td>Pulling feathers</td>
</tr>
<tr>
<td>Non aggressive peck</td>
<td>Pecking or manipulating gently at other</td>
</tr>
<tr>
<td><strong>Aggressive behaviour</strong></td>
<td></td>
</tr>
<tr>
<td>Threat and receive threat</td>
<td>One bird walks after the other with head held high, the other bird</td>
</tr>
<tr>
<td></td>
<td>walking/running/jumping/flying away</td>
</tr>
<tr>
<td>Threat with wing flap</td>
<td>Bird stands in an upright position and flaps its wings more than once</td>
</tr>
<tr>
<td></td>
<td>in front of another bird at &lt;0.5 m distance.</td>
</tr>
<tr>
<td>Waltzing agonistic</td>
<td>One bird (usually male) circles around another bird (same sex) with</td>
</tr>
<tr>
<td></td>
<td>distance &lt;0.5m, with outer wing lowered.</td>
</tr>
<tr>
<td><strong>Intense agonistic behaviour</strong></td>
<td></td>
</tr>
<tr>
<td>Chase</td>
<td>Bird follows another bird, both running, jumping or flying</td>
</tr>
<tr>
<td>Attack</td>
<td>Bird runs, jumps or flies when approaching another bird in order to give</td>
</tr>
<tr>
<td></td>
<td>one or more aggressive peck. The head is kept above the receivers head.</td>
</tr>
<tr>
<td>Aggressive peck</td>
<td>Bird gives a fast peck, directed to an anterior part of another birds</td>
</tr>
<tr>
<td></td>
<td>body</td>
</tr>
<tr>
<td>Fight</td>
<td>Bird being involved in an aggressive fight, more than just one single</td>
</tr>
<tr>
<td></td>
<td>peck. Both birds are running, jumping or flying towards each other.</td>
</tr>
</tbody>
</table>

#### 4.3.3. Data treatment and analysis

The defined behaviours were pooled into three different categories:
- Social behaviour
- Aggressive behaviour
- Intense agonistic behaviour

(See Table 2 for details).

The behaviours were tested for normal distribution and visually validated, testing males and females separately. To assess any significant difference in behaviour between (1) males and females and (2) between the selection groups and (3) interaction between selection and sex, an ANOVA (general linear model) was used. The confidence interval was set to (p<0.05). The displayed deviations of mean values are shown in standard error of mean (S.E.).

The nearest neighbour results were analyzed by performing ANOVA to assess any effects on behaviour with regards to sex and/or selection index (n=3).
4.4. Test 3 - Standardized social reinstatement test

Using a runway arena, the social reinstatement was assessed. This test makes it possible to measure latency for approaching conspecifics and the motivation for the bird to stay close to other birds.

4.4.1. Experimental design

The F1-generation was tested at the age of 20 days in the standardized social reinstatement test. The arena had the measures 0.38 m x 1.14 m. The arena was divided into 4 different, equally sized parts; the start zone, the far zone, the near zone and the social zone. (see fig 6). “X” indicates the area where the test animal was placed when the test started. The “SB”-area was separated with a metal mesh from the rest of the arena, creating a separate compartment for the stimulus birds. The mesh allowed maintenance of visual and auditory contact between the test bird and the stimulus birds. The arena was covered with a mesh. A video camera attached to a rail was sitting above the arena, recording the test birds’ movements. The division of the arena was pre-programmed into the EthoVision software. Four identical runway arenas were built next to each other, creating the possibility to test four animals in parallel.

![Schematic drawing of social reinstatement test arena](image)

**Figure 6. Schematic drawing of social reinstatement test arena. Lines between stimulus bird zone and social zone indicate the mesh wall.**

4.4.2. Methods

After 30 minutes of habituation in the test room, two stimulus animals were placed in the SB-compartment. The lights were turned out and the test animal was placed in the start zone (“X”) in the runway arena. The test started when the lights were turned on. The birds were tested individually during five minutes with one repetition two days later. Two variables of interest was measured; (1) the social latency, i.e. the latency before entering the social zone and (2) the social duration, i.e. the time spent in the social zone. A camera above the arena recorded the movement of the chick and the data was treated in EthoVision software by Noldus. The stimulus animals were individuals unfamiliar to the test bird and were changed after every fourth test.
4.4.3. Statistical analysis

The mean value from the two tests was calculated for each bird. The behaviours were tested for normal distribution and visually validated. To assess any significant difference in behaviour between (1) males and females and (2) between the selection groups and (3) interaction between selection and sex, an ANOVA (general linear model) was used. The confidence interval was set to (p<0.05).

The birds included in the ANOVA were the 12 of each sex who scored either highest or lowest from their respective selection group, and all the intermediate birds (n=28), see Figure 5.

5. Results

The general results in the test concerning the P0-generation display no difference in behaviour between the selection lines, however more obvious is the sex differences.

5.1. Mirror test P0

When performing a correlation analysis of the mirror test on the results from the two test sessions in May and August, a majority of the behaviour frequencies were not correlated in neither of the sexes. Due to this fact, one has to consider the two tests rounds (May/August) as differentiated and considered as two separate tests even though it is the same animals that have been tested. However, some behaviours did correlate between the two test sessions, the results display a significance in the behaviour categories “Self directed” (r= 0.3, p<0.05) and “Other behaviours” (r= 0.4, p<0.005) in females.

| Table 3. Correlation analysis of mirror tests at 13 weeks vs. 26 weeks, displaying correlation coefficient (r) and p-value. |
|---|---|---|---|
| Behaviour | Females | | Males |
| | r | p-value | r | p-value |
| FORAGING | 0.2 | 0.1 | 0.09 | 0.6 |
| SELF-DIRECTED | 0.3 | 0.05 | 0.2 | 0.2 |
| OTHER | 0.4 | 0.003 | 0.2 | 0.1 |
| AGGRESSIVE/SOCIAL | 0.3 | 0.09 | -0.1 | 0.5 |
| VOCALIZATION | 0.1 | 0.3 | 0.3 | 0.2 |
| FEAR | No data | No data | No data | No data |

A general linear model ANOVA was performed to calculate selection index and sex effects on behaviour, and also for analyzing differences between the sexes. No significant results were found when analyzing selection index and effects on behaviour, although effects on sex and behaviour displayed significant differences, mainly in the August test session results...
where females perform significantly more self directed (p=0.01) and aggressive/social behaviour (p=0.008), and males perform significantly more vocalization (p<0.001). The results from May display that females tend to vocalize (p=0.09) more and display more fearful (p=0.1) and other (p=0.1) behaviours more than males.

Table 4. P-values from the ANOVA, analyzing the mirror test.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>May</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sex</td>
<td>Selection index</td>
</tr>
<tr>
<td>FORAGING</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>SELF DIRECTED</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>OTHER</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>AGGRESSIVE/SOCIAL</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>VOCAL</td>
<td>0.09</td>
<td>0.7</td>
</tr>
<tr>
<td>FEAR</td>
<td>0.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Figure 7. Behaviour differences between P0 males (n=32) and females (n=42) from the two test sessions (13 weeks (May) and 26 weeks, (August)). Each behaviour is displayed as a proportion of the maximum potential observations (%) with S.E. Significant differences between the sexes are indicated (*: p=0.05-0.01, **: p=0.01-0.001, ***: p<0.001). (*) in brackets indicate a trend (p=0.05-0.1)

5.2. Mirror test F1-generation

By performing an ANOVA, the data from the F1-generation was analysed. The results display no effect on the behaviours with regards to selection group or to sex and selection group, except for the behaviour category “fear” where high selected birds display a trend for
performing more fearful behaviour (p=0.08). Again, behaviour differences between the sexes were more frequently seen.

Table 5. Mirror test F1-generation ANOVA, P-values

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Sex</th>
<th>Selection index</th>
<th>Sex x selection index</th>
</tr>
</thead>
<tbody>
<tr>
<td>FORAGING</td>
<td>0.03</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>SELF DIRECTED</td>
<td>0.9</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>OTHER</td>
<td>0.2</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>AGGRESSIVE/SOCIAL</td>
<td>0.1</td>
<td>0.6</td>
<td>0.08</td>
</tr>
<tr>
<td>VOCAL</td>
<td>0.02</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>FEAR</td>
<td>0.2</td>
<td>0.08</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Figure 8. Behaviour differences between F1 males (n=32) and females (n=42). Each behaviour is displayed as a proportion of the maximal potential observations (%). Error bars display standard error of mean (S.E). Significant differences between the sexes are indicated (*: p=0.05-0.01, **: p=0.01-0.001, ***: p<0.001). (*) indicates tendency for significance (p=0.05-0.1).

5.3. Undisturbed test P0

When comparing males and females in the undisturbed test using an ANOVA, the result show a significant difference in intense agonistic behaviour, where females perform these behaviours at a higher rate (p<0.05). The females also gave and received a higher numbers of social pecks compared to the males (p<0.005), and numerically, females also perform more aggressive behaviour than males.
Table 6. P-values from ANOVA; undisturbed behaviour test

<table>
<thead>
<tr>
<th>Behaviour category</th>
<th>Sex</th>
<th>Selection index</th>
<th>Sex x selection index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social pecks</td>
<td>0.04</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Aggressive behaviour</td>
<td>0.2</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Intense agonistic behaviour</td>
<td>&lt;0.001</td>
<td>0.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Figure 9. Results of the undisturbed behaviour test comparing males (n=32) and females (n=42). Each behaviour is displayed as a proportion of the maximal potential observations (%) and displayed with S.E. Significant differences between the sexes are indicated (*: p=0.05-0.01, **: p=0.01-0.001, ***: p<0.001).

When looking at selection index we see no significance in either social pecks or aggressive behaviour. A significant interaction was seen between sex and selection index in intense agonistic behaviour.

Further, a trend was seen in selection lines, where low selected (tame) individuals displayed more aggressive behaviour and high selected (untame) displayed intense agonistic behaviour to a larger extent than the other two selection groups.

Figure 10. Results undisturbed behaviour test (P0), sex and selection line. Each behaviour is displayed as a proportion of the maximal potential observations (%), displayed with S.E. A significant interaction is seen in intense agonistic behaviour (*: p=0.05-0.01, **: p=0.01-0.001, ***: p<0.001).
5.4. Nearest neighbour test

The Nearest Neighbour test was performed on the P0-generation only, and displays no significant difference when comparing the neither the sexes nor the selection index. One can numerically see that both males and females prefer to keep the distance 0.1-0.5 m to the closest individual (Figure 11a). When looking at the selection index, no significant differences appear in the results.

![Figure 11a. Mean time spent in the different intervals of distance to the nearest neighbour with regards to sex; males (n=32) and females (n=42) (% of observations with S.E).](image_url)

![Figure 11b. Mean time spent in the different intervals of distance to the nearest neighbour with regards to selection index; high (n=25), intermediate (n=25) and low (n=24) (% of observations with S.E).](image_url)

5.5. Social reinstatement test

The ANOVA-analysis from the social reinstatement test performed on the F1-birds display significant difference between the selection lines (p=0.002), where the high selected birds (n=23) had the longest social duration and the low-selected birds (n=24) had the shortest. The intermediate group (n=28) ended up in between the high and the low lines. (Figure 12a).
There was also a significant difference between the selection lines in the social latency analysis (p=0.022). The low-selected birds had the longest latency before entering the social zone, while the intermediate group’s results were in between the other two groups (Figure 12b).

![Social latency and duration graph](image)

*Figure 12a, b. Results from the social reinstatement test; percent of time spent (12 a) in the social zone of the test arena and (12 b) latency until entering the social zone of the test arena (seconds) displayed with S.E. A significant effect is seen between the selection lines in both test variables (high: n=23, intermediate: n=28, low: n=24) (*: p=0.05-0.01; **: p=0.01-0.001).*

6. Discussion

The results in this study reveal clear behaviour differences between the sexes in both the mirror test and in the undisturbed behaviour test. Significant social behaviour differences between the selection lines were seen in the social reinstatement test, where high selected (fearful) birds displayed a higher degree of sociality towards conspecifics, and low selected (non-fearful) birds display a lower degree of sociality. No significant behaviour differences between the selection lines were found when analyzing the results from the mirror test, in neither of the generations.

A fear of humans test (not part of this study) classified the birds in the P0-generation into three different selection lines. Low score on the test indicated a tame bird, while high scores classified a bird as fearful towards humans. In this thesis, it was expected that the selection for tameness would change the gene expression and induce social behaviour changes between the selection lines.

One initial problem that potentially could limit the genetic diversity in our test birds is the possibility of a loss of genetic variation due to isolation in captivity in the two origin (P00) populations. However, the problem of inbreeding was considered and a controlled, extended outbreeding during two generations was performed between the two P00 populations to minimize the risk mentioned above.
6.1. Mirror test
In the mirror test, almost all birds spent all their time in the social part of the arena, very few birds were willing to stay in or re-enter the non-social part of the arena and preferred to keep a close distance to the mirror. This indicate that both the unselected line of RJF and the F1-generation have a high motivation for sociality, which is also supported by the nearest neighbour test which did not display any significant differences, but numerically one could see that even though the animals were provided a fairly large area, they most frequently stayed within a distance of 0.5 m to the nearest individual.

Flaws in the construction of the mirror-test arena could of course be discussed. No significant difference was seen in social behaviour between the selection lines; however differences were assessed in the standardized social reinstatement test. This might suggest that the standardized type of test is of better quality or more reliable, or that the variables in the test are easier to define. A change in division of the mirror test arena is therefore suggested. The non-social area contains nothing that is desirable for a social animal like the chicken, unless it is a highly unsociable individual. The suggested would therefore be to divide the social area of the arena into two parts – one “direct social” part, which would be in immediate vicinity to the mirror, while the remaining part of the social area would be “indirect social area”. One can draw the conclusion though, that the mirror in the novel social reinstatement arena worked as a suitable live bird stimulus, which is in line with previous studies measuring sociality in chickens (for example Schütz et al, 2001).

6.2. Correlation analysis
Very few behaviours correlated in the mirror test when comparing the first test sessions at 13 weeks of age (May) and the second test session at 26 weeks (August) of the P0-generation, especially among the males. This could indicate that the test has bad repeatability. On the other hand, one must consider that the repetition took place 13 weeks after the initial test. During this time, most individuals most likely reached maturity, which could be a plausible explanation to the inconsistent behaviour pattern. The behaviours that actually correlated were mainly seen among the females, the correlated behaviour categories were “self directed” (Scratching, preening, dust bathing), “other” (stand alert, stand relaxed, walking, lying) and a tendency was seen in “aggressive/social” (social inspection, raised hackles, wing flapping, feather ruffle, attack, mirror peck). Specific behaviour that significantly correlated among the females was “ground peck”, “walking” and “pecking mirror”. Those three behaviours could be related to exploratory behaviour. Thereby, one can speculate in the possibility that exploratory behaviour and self directed behaviour are constant over time in female chickens while other behaviours, such as vocalization, aggression and social behaviours change over time, possibly due to sexual maturity.

When analyzing the results of the males it reveals only significant correlation in the specific behaviour “walking”. As mentioned when discussing the females, walking can be regarded as an exploratory behaviour which might be stable over time. Logically though, a change in at least certain behaviours should be in its place due to the difference in age between the tests. One can also see that numerically, females performed more aggressive and social behaviour in all three test sessions (P0 May and August, and the F1 test session, and also significantly more in two out of three behaviour in the undisturbed test), which points at an overall higher incidence of social and aggressive behaviour in females. In the mirror test, a higher number of significant differences between the sexes were seen in the August test session when the birds were 26 weeks of age. It is therefore unfortunate that lack of time made it impossible to test the F1-generation a second time. It would have been interesting to see if the results would have revealed the same behaviour differences as in the P0-generation.
6.3. Tameness selection responses
In line with the initial hypothesis, the selection for reduced fearfulness had an effect on sociality towards conspecifics in the first selected generation, F1. The fearful birds had a shorter latency and a longer social duration period than the non-fearful birds, with the intermediate group in between those two. It appears as if social behaviour is a trait that has an immediate selection response in early domestication.

Studies that directly compare RJF with domesticated breeds have revealed differences in several behaviours, implying relatedness between tame behaviour and other behaviour traits, such as general fearfulness (Campler et al., 2008) and foraging and exploratory behaviour (Lindqvist, 2008). A behavioural change also in social behaviour could thereby be expected, since the behaviour changes mentioned above is an effect of domestication and selection for low fearfulness, however over an extended period. Given the length of selection on social behaviour (thousands of generations) it is unsurprising that such large breed differences exist. This implies that the traits are controlled by the same genes (pleiotropy) or are genetically linked. Similar findings has also been observed in other species of birds, for example in Japanese Quail selected for stress response, where the low stress birds had a longer social duration than the birds selected for high stress in a social reinstatement test (Jones et al., 2001).

Relating to selection for tame behaviour, most studies which do exist have only reported results for changes after many generations rather than during the initial generations of selection. Even then, such studies have seldom focused on social behaviour (see for example Trut, 1999; Albert et al., 2008), but rather on morphology and physiology. Those studies that do focus on early behavioural changes mainly evaluated stress response, revealing that stress is reduced in tame individuals due to changes in various hormone producing organs (see for example Albert et al., 2008; Plyusina & Oksina, 1996). The present study has therefore attempted to address the lack of knowledge concerning the early effects of selection for tameness and its correlated responses to social behaviour.

The underlying genetic mechanisms explaining the behavioural changes potentially affects hormonal production, but as no laboratory analyses have been performed during this experiment, it was not possible to verify such a mechanism. However, given the observed correlated response, it would appear that tameness and social behaviour are either at least partly controlled by the same genes (pleiotropy) or the genes affecting these separate behaviours are closely physically linked to one another. Since no obvious morphological changes appeared from the first generation to the second, it might be suggested that social behavior and/or aggression towards conspecifics may be one of the first traits affected by tameness selection.

There are also theories suggesting that changes in social behaviour are a secondary effect of selection for production traits, rather than a consequence of tameness selection. For example, Schütz and Jensen (2001) demonstrated that the WL performs less social behaviour due to a change in resource allocation where energy is redistributed to production. In the study, the authors investigated three chicken breeds, one domesticated breed (WL), a non-domesticated breed (RJF), and the Swedish bantam, which is a domesticated breed not selected for production traits. It was found that the social behaviour in the Swedish bantam was similar to the social behaviour of RJF, but significantly differed from the social behaviour of WL.

When comparing RJF, WL and an intercross between the two breeds with regards to genotype and behaviour, Wirén et al. (2009) suggested that social behaviour has been modified due to domestication, which in males could be explained by variations in genotype at a major growth quantitative trait loci (QTL). The study suggests differences in social
behaviour not only between domesticated and non-domesticated breeds but also between sexes.

6.4. Sex differences

The sex differences are consistent with the common opinion that there are behavioural differences between the sexes in most animal species mainly differences in hormone varieties and production. In chickens, males are considered to display aggressive behaviour to a higher extent than females (see for example Guhl, 1968; Wirén et al, 2009; Anna Wirén, unpublished manuscript) however Krujt (1964) observed no clear cut qualitative differences in agonistic behaviour between the sexes. A higher frequency of aggressive and agonistic behaviour among males compared to females was expected and hypothesized in this study, but this was seemingly not the case, neither in the mirror test nor in the undisturbed behaviour test. Overall, the number of aggressive and agonistic behaviours performed among both sexes was lower than expected, possibly due to the fact that the habituation over night was enough to re-establish a hierarchy in the group.

In the undisturbed behaviour test performed on the P0-generation, the females displayed significantly more intense agonistic behaviour (chase, attack aggressive peck, fight), and also numerically more aggressive behaviour (threat and receive threat, threat with wing flap, waltzing agonistic, proposing that females are generally more aggressive than males. The females also performed significantly more social pecks (severe feather pecking, non aggressive peck). The same trend was seen when analyzing the results from the mirror test. The P0-females performed significantly more aggressive and social behaviour than males at 26 weeks of age and at the age of 13 weeks, both the P0-females and the F1-females performed numerically more aggressive and social behaviour. Thereby the results propose that females are both more social and aggressive towards each other than the males, and this significant difference is seen in two different test situations, both in the mirror test and in the undisturbed behaviour test. Previous studies demonstrate that the presence of males affect aggressive behaviour among females. Odén et al. (1999), observed significantly higher levels of agonistic behaviour (aggression and avoidance) in large, single-sexed groups of domesticated chickens compared to mixed-sex flocks. This suggest that the presence of males reduces female aggression, at least in large population densities and that the presence of males may be important to maintain a stable hierarchy and minimize aggressive and agonistic encounters among females, possibly also in smaller populations as the one in the present study.

A plausible explanation for the low frequency of social and aggressive encounters among the males in the undisturbed test could be that the test arena provided larger space per animal compared to the home pen. They had the possibility to maintain a larger distance to the pen mates, which is in accordance to the findings of Odén et al (2004), who found that dominance hierarchies in large mixed-sex groups of laying hens are mainly maintained by submissive males avoiding contact with high ranked males. It has also been found that aggression among laying hens kept in cages increase with group size (Al-Rawi and Craig, 1975), which again can explain the low total number of aggressive behaviour during the test, due to the large space provided per bird in the test arena. Interestingly, the three most severely pecked males had a very few aggressive encounters directed towards them in the undisturbed behaviour test. Further unexpected observations were made both in the mirror test and in the undisturbed behaviour test which all included heavily pecked males. During all the mirror test sessions (three in total), a large majority of animals spent all of their time on the social part of the test arena and entered the social part of the arena as fast as they noticed the simulated company. Only a total number of 8 males out of 121 spent a majority of their time in the non-social area. When looking only at the F1-generation, five males spent a majority of the time in the non-
social area. Out of these five males, four were low-selected (tame), and three of them were severely feather pecked. It was obvious that those individuals were aware of the mirror and the simulated company, but stayed on the non-social part of the arena during the whole test. An explanation could be that the males simply have bad experience from conspecifics and avoids them if there is an opportunity.

During the undisturbed behaviour test, one heavily feather pecked male was sitting in the perch together with the other males and even occasionally crowed, without any subsequent aggressive onsets. This is in opposite to previous findings; crowing is suggested to be a signal of status and it has been demonstrated that dominant males crow more frequently and at a higher frequency than subordinate males, and that subordinate males who crow often gets attacked by higher-ranked individuals (Leonard & Horn, 1994). Besides the habituation time, the increased amount of space could possibly be a contributing factor to the crowing of the heavily pecked and assumed low-ranked male. The over-all low number of social behaviour recorded in the males, the increased space possibly contributed to an opportunity for the pecked individuals to keep distance to dominant males and thereby avoids aggressive encounters.

As seen when directly comparing a domesticated breed of chicken with non-domesticated breeds, the level of aggressive and agonistic encounters is lower in the domesticated breed. Thereby, it is going to be intriguing to see if the red junglefowl will develop a change in social behaviour towards conspecifics in the future tameness-bred generations.

7. Conclusions
This study displayed clear behaviour differences between the sexes in red jungle fowl, especially in aggressive and social behaviours, which were more frequent in females. A significant effect on social behaviour was seen when comparing the selection lines in the social reinstatement test. This implies that the tameness selection have immediate effects on non-selected behaviour traits during early domestication.

8. Acknowledgements
I would like to direct a warm thank you to my supportive supervisor, Prof. Per Jensen. I would also like to thank the rest of the Applied Ethology research group for all encouragement and help; Beatrix Eklund, Marcus Jöngren, Dominic Wright, Ida Gustafsson, Anna-Carin Karlsson, Lina Roth, Annelie Andersson, Daniel Nätt, Anna Wirén and Viviann Goerlich. Thank you also my fellow students Sara Rydmell and Emma Walett.

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