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The domesticated phenotype in Red
Junglefowl selected for tameness

Rebecca Katajamaa

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Rebecca Katajamaa

IFM Biology
Department of Physics, Chemistry and Biology
Linköpings universitet, SE-581 83 Linköping, Sweden
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Survival of the Tamest: The Domesticated Phenotype in Red Junglefowl Selected for Tameness

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Ukille ja Mummolle, minustakin tuli lopulta herra.

Kiitos kaikesta.

Abstract

Early animal domestication was probably associated with reduced fear of humans. Domestication is a process in which animals adapt to humans and the captive environment provided by humans. Selection on tameness as the only trait has previously been found to generate changes in other phenotypes in different animal species. These changes in the traits correspond to the domesticated phenotype, a set of traits that are common to domesticated species. In this thesis, I have focused on measuring a number of phenotypic traits in two Red Junglefowl lines selected for low and high levels of fear of humans in a number of generations. Furthermore, I have studied the correlations between fear of human score, and other traits in an F3 intercross of the selected lines. In total, the thesis consists of four papers investigating the effects of fear of human score on other phenotypic traits.

In **paper I**, we found that basal metabolic rate (BMR), serotonin levels, feed conversion efficiency and boldness were affected by selection on tameness. Chickens from the low fear line had a higher BMR, tended to have a higher feed conversion efficiency and were bolder in the novel object test. Peripheral serotonin levels were also higher in the low fear males. **Paper II** investigated effects on other behaviours, besides tameness, in both chicks and adults. We found that low fear was associated with a general higher activity level and that high fear individuals were more intensive in their courtship behaviour compared to the low fear individuals. **Paper III** found effects of the selection on fear towards humans on both brain size and fear habituation. Brain size relative to body size was significantly smaller in the low fear line, with changes in specific brain regions likely as the main cause. Fear habituation, measured in a test with exposure to a novel frightening stimulus over two consecutive days, was more effective in chicks from the low fear line compared to the high fear line. We found no evidence of effects on conditioned place preference learning.

In the final paper, **paper IV**, we generated an intercross line using our eighth generation of selected high- and low fear chickens. The paper compares fear of human score with behavioural as well as physical phenotypes. We found that low fear of human score was associated with a higher body weight, faster growth and less fearful behaviour in the open field test as well faster habituation in a test measuring fear habituation to a novel fearful stimulus. Brain size was also measured, and we found that low fear of human score was associated with a smaller brain relative to body weight in females and that this change in brain size was due to changes in specific brain regions, rather than changes in the brain in a concerted fashion.

In summary, selection on tameness in Red Junglefowl has changed other phenotypes that were not intentionally selected upon. These changes correspond to the domesticated phenotype and are consistent with changes that have been suggested as happening in early domestication. Furthermore, the level of fear towards humans is correlated with a number of other phenotypes, showing that there is a possibility that pleiotropy or linkage may be behind these changes. Taken together, the results suggest that tameness could have been a driving factor of the domesticated phenotype in chickens.

Populärvetenskaplig sammanfattning

Domesticeringen är en process genom vilken djur adapterar till ett liv i fångenskap och till människan. Den tidiga domesticeringen lär ha drivits av en selektion på tamhet, eller också känt som lägre rädsla för människor. Vid selektion för tamhet förändras även andra egenskaper som inte direkt utsatts för selektionstryck. Dessa går i riktningen mot en domesticerad fenotyp, ett begrepp som används för att beskriva de fenotyper som många domesticerade djurarter har gemensamt. Dessa kan till exempel vara ökad socialitet, ökad kroppsvikt och en högre kapacitet för reproduktion.

I den här avhandlingen har jag undersökt ett antal fenotyper hos röda djungelhöns, anfadern till våra domesticerade hönsraser, selekterade på låg och hög rädsla för människor i flera generationer. Jag har även undersökt sambandet mellan nivån av rädsla för människor och andra fenotyper i en korsning av våra selektionslinjer. Avhandlingen består totalt av fyra artiklar som tillsammans ger en bild av hur selektion på tamhet påverkar andra fenotyper hos röda djungelhöns.

I **artikel I**, undersökte vi basalmetabolism (den energiåtgång, räknat genom mängden konsumerad syre, som används i vila), perifer (i blodet) serotoninhalt, födointag i förhållande till tillväxt och djärvhet. Höns från den lågrädda selektionslinjen hade en högre basalmetabolism som kycklingar, men använde också födan mer effektivt då de gick upp mer i vikt i förhållande till mängden de åt jämfört med höns från den högrädda selektionslinjen. De lågrädda hönsen var även djärvare i ett beteendetest där de blev exponerade för ett främmande objekt samtidigt som de lockades av en aptitlig belöning. **Artikel II** undersökte hur andra beteenden påverkats av selektionen för tamhet i kycklingar och vuxna höns. Lägre rädsla för människor var associerat med en generellt högre aktivitet men lägre intensitet i uppvaktningsbeteende av honor hos tuppar. I **artikel III** hittade vi effekter av selektionen både på hjärnstorlek och habitueringsinläring. Hjärnstorleken i förhållande till kroppens storlek var mindre i de lågrädda individerna. Skillnaden beror på förändringar i olika hjärnregioner, snarare än en samlad förändring i hela hjärnan. Habitueringsinläringen testades i en situation där vi exponerade kycklingar för ett tidigare okänt och skrämmande stimulus, i det här fallet ett starkt blått ljus. Kycklingar från den lågrädda selektionslinjen reagerade mindre starkt dag två när testet genomfördes, medan det inte fanns någon skillnad i reaktionerna mellan de två selektionslinjerna dag ett. Vi testade även minnet i ett test där kycklingarna skulle lära sig associera en specifik plats (ett rum med olika mönster på väggarna) med en belöning, men fann inga effekter på denna inläring.

I den sista artikeln, **artikel IV**, beskrivs resultaten från ett korsningsexperiment, där vi korsat de två selektionslinjerna i tre generationer för att kunna undersöka fenotyper som möjligen är genetiskt korrelerade med nivån av rädsla för människor. Vi undersökte nivån av rädsla för människor och korrelationen mellan olika fenotyper. Kroppsstorleken var generellt större hos de individer som hade en lägre rädsla för människor och likaså tillväxten. Vi testade även hjärnstorleken igen och fann återigen att den relativa hjärnstorleken var mindre och att detta berodde på skillnader i enskilda regioner i hjärnan. Habitueringsinläringen var också den effektivare med lägre rädsla för människor, mätt med samma test som i **artikel III** och vi kunde se en högre aktivitet i ett beteendetest där djuren får undersöka en öppen arena.

Sammanfattningsvis fann jag att selektion på tamhet hos röda djungelhöns genererar skillnader i andra fenotyper som inte medvetet selekterats på. Dessa skillnader hör samman med den domesticerade fenotypen och visar att selektion på tamhet kan ha varit viktigt i ett tidigt skede av domesticeringen. Dessutom fann jag att nivån av rädsla för människor korrelerade med ett antal

olika fenotyper i en korsningslinje. Detta indikerar att det kan finnas en gemensam genetisk komponent eller mekanism mellan nivån av rädsla för människor och dessa fenotyper. Sammantaget pekar resultaten på att tamhet kan ha varit en egenskap som drivit den tidiga domesticeringen av höns.

Acknowledgements

A couple weeks before my thesis deadline I complained that I was probably expecting too much of myself and that I should try to just be content with whatever I had produced in order to get on with my life. Someone wise responded by saying that the best way to deal with high expectations is to set high expectations and then live up to them. I'm sure that it was at least partly a joke, or so I hoped, but at the time I was not very encouraged by these words. However, I feel that I have gotten more than enough encouragement through my time as a PhD student, especially from this wise person who happens to be my supervisor, **Pelle**. Thank you for believing in me from start to finish and for not giving up on me when I wavered halfway through. You helped me see my results from a fresh point of view so many times and made me realise that I am not entirely useless. It's not a coincidence that I chose you as my supervisor three (!) times.

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List of publications included in this thesis

This thesis is based on the listed papers. Their roman numerals will be used whenever they are referred to in the text.

- I. Agnvall B, Katajamaa R, Altimiras J and Jensen P. 2015. Is domestication driven by reduced fear of humans? Boldness, metabolism and serotonin levels in divergently selected red junglefowl (*Gallus gallus*). *Biology Letters* 11 (9), 20150509.
<https://doi.org/10.1098/rsbl.2015.0509>
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- II. Katajamaa R, Larsson LH, Lundberg P, Sörensen I and Jensen P. 2018. Activity, social and sexual behaviour in Red Junglefowl selected for divergent levels of fear of humans. *PLoS One* 13 (9), e0204303.
<https://doi.org/10.1371/journal.pone.0204303>
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- III. Katajamaa R and Jensen P. 2020. Selection for reduced fear in Red Junglefowl changes brain composition and affects fear memory. *Royal Society Open Science* 7, 200628.
<https://doi.org/10.1098/rsos.200628>
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- IV. Katajamaa R and Jensen P. 2020. Tameness correlates with domestication related traits in a Red Junglefowl intercross. *Genes, Brain & Behavior*, e12704.
<https://doi.org/10.1111/gbb.12704>
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Not included in this thesis

- I. Agnvall B, Bélteky J, Katajamaa R and Jensen P. 2018. Is evolution of domestication driven by tameness? A selective review with focus on chickens. *Applied Animal Behaviour Science* 205, 227-233.
<https://doi.org/10.1016/j.applanim.2017.09.006>

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Introduction

The domestication of animals has provided us humans with a range of domesticated animal species, mostly consisting of mammalian species and a few species of birds. Not only do they function as producers of food as well as raw materials, subjects in research and as grazers to help keep our landscapes open, they are also kept as loved pets all over the globe. It is clear that humans benefit immensely from domesticated animals. However, they benefit from living with us too. We provide them with shelter, food, protection from predation and sometimes with tasks that could be perceived as rewarding (e.g. working dogs or grazing sheep and cows).

A question that has been asked a few times is how animals did become domesticated in the first place. A few of the ancestral species to our first domesticates have gone extinct, which means that only the domesticates themselves remain. This makes direct comparisons between domesticates and ancestors difficult for those species. There are, however, exceptions to this, such as the Red Junglefowl, ancestor to modern chicken breeds (Tixier-Boichard et al. 2011). Studying the differences between modern domesticates and ancestral individuals provides valuable insight into the domestication process.

We are not restricted to comparisons between wild and domesticated species in order to study the process of domestication. Some research groups have attempted to recreate domestication in a variety species (e.g. silver fox (Trut et al. 2009), mink (Malmkvist and Hansen 2002) and rat (Albert et al. 2008)). This thesis is based on similar work in the chicken, which in contrast is one of the traditional domesticates (Agnvall et al. 2012). Something that these studies have in common is the selection on tameness, or otherwise known as reduced fear towards humans. It seems as though selection solely on tameness can produce changes in additional phenotypes. These changes correspond to the domesticated phenotype, a central concept in this thesis, which I will describe in more detail later.

Animal domestication

Domestication of animals has been studied for a long time. Even Charles Darwin used domestication as a proof of principle of natural selection, where human selection on desirable traits in both animals and plants brought about a large variety of phenotypes that were different from the wildtype ancestors (Darwin 1868). Domestication has been defined by Jensen and Wright (2013) as an evolutionary process that entails changes in both genetics as well as phenotypes that aid animal populations in coping with the captive environment that humans provide for them. It is important to note that, according to the definition, the challenges of the captive environment, of which a major part is controlled by humans, serve as the selection pressures that drive these changes. Domestication is an evolutionary process, of which the product is a domesticated population. Domesticated populations are characterised by what is commonly called the domesticated phenotype (Price 1999; Jensen 2014). This is a suite of phenotypic traits that are common to domesticated species, e.g. floppy ears, increased reproductive ability and changes in body size.

Zeder (2015) describes domestication as a mutualistic relationship between humans and animals where humans are in control over resources and reproduction of the animals. The animals in turn benefit from this relationship by gaining access to these resources that are under human control, which could be e.g. access to food and protection from predators. I agree with the description of domestication as a mutualistic relationship between humans and animals. However, her definition of domestication I believe could be applied to any population of captive animals, but I would not

go as far as to claim that wild animals kept in zoo's are necessarily undergoing domestication. The same reasoning could be applied to the most commonly used definition offered by Price (1999), but I agree more with Jensen (2014) who argues that experiential effects should not be included in the definition of domestication, as these are not transferrable to the next generation. There is of course a caveat here with epigenetic effects that actually can be transferred between generations, however, this is not what either of Zeder or Price refer to when they define domestication. There are several examples of failed domestication attempts made at wild animal species (Diamond 2002). Some characteristics seem to make certain species more suitable to domesticate than others, and in fact most species have not been domesticated. Price (1984) describes these characteristics as preadaptations to domestication, and they include a promiscuous mating system, precocial young and living in social groups organised by a hierarchy, among others.

It is difficult to discuss domestication of animals without mentioning our best friend, the dog, so I want to do this very briefly. The dog, with close to 400 breeds, is an often-used example of the diversity that selection on very specific traits can accomplish. It was the first animal to be domesticated, at least as long as 15,000 years ago (Wang et al. 2016). Dogs are used for different purposes, and as a result of this, not only do they differ in their overall appearance, but there are also differences in behaviour between breeds. One of the most striking characteristics of dogs is that they can and will understand and communicate with humans and they do this in many different ways (Siniscalchi et al. 2018). Humans are likewise adept at understanding dogs. Even humans without much experience of dogs can correctly interpret images showing dogs' emotional expressions (Bloom and Friedman 2013). Feral dog populations retain the social behavioural characteristics of dogs rather than reverting to wolf-like social structures (Boitani and Ciucci 1995). The social groups of feral dogs are much less rigid, and mating occurs between all members of the group, whereas in a wolf pack there is usually just one breeding pair. There are different hypotheses concerning the way in which prehistoric wolves first came into close contact with humans and were subsequently domesticated. A couple leading theories are that either scavenging wolves approached human populations or that humans socialized wild wolf pups (Miklosi 2015). The tamest individuals would probably have been more successful and perhaps they developed some of the traits we categorize as the domesticated phenotype.

Domesticated animals consist mostly of species that are now used for food production or other resources for humans. The sheep was the first domesticated farm animal, domesticated around 11,000 years ago (Meadows 2014). Our beloved cow was domesticated around the same time, but somewhat later (Bollongino et al. 2012). Mice, who are very successful, both as wild dispersers (much thanks to humans) and as domesticated contributors in the lab, were living close to humans with domestication starting already 15,000 years ago (Weissbrod et al. 2017). When it comes to mice, one study found that a population of mice developed white patching and a shortened skull even with passive exposure to humans over a number of generations (Geiger et al. 2018), supporting the idea that animal domestication may in some cases even have started as a commensal or even parasitic relationship between humans and animals. I think it is probable though, that in the vast majority where both animals and humans benefit, i.e. a mutualistic relationship, is where domestication has been most effective. Imagine for instance if tame wolves would not have provided any benefit for humans at all, then it is likely that those individuals would have been hunted down. In the case of mice and rats, it is easier to understand how they could have taken advantage of humans without a major risk at the population level of being subject to hunting.

Chicken domestication

Another animal, the chicken, will be the focus of this thesis. It is the most abundant farm animal on Earth, with more than 90 million chickens just in Sweden alone raised for meat production in the year 2015 (Svenskaägg.se 2015). Globally, there are over 60 billion chickens used for food production each year (Nicol 2015). Despite being so numerous, few people actually seem to be aware of chickens at all as they are usually not kept for everyone to see. It is not uncommon for chicken facilities used for egg production in Sweden to house more than 10,000 chickens together in one stable section, but there are farms with considerably larger groups of chickens housed together, almost 70,000 chickens were housed in the same section on one farm participating in a 2015 survey (Svenskaägg.se 2015). If we compare this to the natural habitat of the Red Junglefowl (*Gallus gallus*) (Figure 1), which is the ancestor of our modern domesticated chicken breeds (Tixier-Boichard et al. 2011), we can easily conclude that they live in very different conditions that present their own challenges. The Red Junglefowl is native to South-East Asia where it inhabits semi-open habitats and forests (Al-Nasser et al. 2007). They live in groups of about 20 individuals that usually consist of a group of females with their chicks and a rooster (Collias and Collias 1996).

Figure 1. Red Junglefowl female with chicks (left) and male (right). Photo: Per Jensen

Much like the dog and other domesticates, there are many different breeds of chickens that have been selected for various purposes. Initially, chickens may have been used for cock fighting and ceremonial purposes and later for food production (Nicol 2015). There are also game breeds, with long legs for running as well as dwarf breeds that nowadays are mostly used as egg producing pets on smaller farms or in people's back yards. Even though the chicken is popular to keep as a pet in your back yard, it is obvious that the chicken is mainly used and known of as a production animal. Selection in the animal production is very specific, with chickens being used either for meat or egg production and there are only a few dominating breeds used in the food production industry. A Red Junglefowl in the wild typically lay about 20 eggs per year (Collias and Collias 1996) while a White Leghorn of the egg production industry lays about 300 (Yue and Duncan 2003). Domesticated White Leghorns also reach sexual maturity seven to eight weeks earlier compared to Red Junglefowl (Schütz et al. 2002). The sex-average weight of an adult Red Junglefowl is about 960 grams (Kerje et al. 2003), which is in contrast to the broiler chicken, a meat producing giant, that weighs in at about 2.0 kg at the time of slaughter as a 35-day-old chick (Zuidhof et al. 2014). Left to grow past its slaughter weight, the broiler will weigh around 4.2 kg at 56 days old (Zuidhof et al. 2014). The fast growth rate contributes to lameness (Williams et al. 2000), which is considered as one of the most pressing welfare issues in broiler chickens (Pedersen and Forkman 2019). Laying hens, on the other hand, are prone to fracturing their keel bone partly perhaps because of the high production rate of eggs for which the calcium needed for the shell is derived directly from the hen's own skeleton (Whitehead 2004), but also because they are heavier and less agile compared to junglefowl (Moinard et al. 2004). Behavioural

problems are also common in chickens, where high stocking density and poor litter condition contributes to feather pecking and cannibalism (Nicol 2015).

Behaviours generally do not seem to change very much during domestication. Important aspects of behaviours are retained, and differences can be attributed to changes in thresholds at which the behaviours are performed (Price 1999). For example, HPA-axis reactivity attenuation in domesticated breeds (Künzl and Sachser 1999; Harri et al. 2003) could explain differences in reactions to fearful stimuli. Increased motivation to feed and a general energy saving behavioural strategy could be a side effect in breeds that have been selected for intense production traits (Schütz and Jensen 2001). Behavioural differences that have been observed between Red Junglefowl and domesticated chickens are e.g. a decrease in explorative behaviour and domesticated chickens are also more tolerant to conspecifics (Lindqvist and Jensen 2009; Schütz et al. 2001). Furthermore, domesticated White Leghorns are less fearful than Red Junglefowl (Campler et al. 2009). These changes correspond to the domesticated phenotype and can be seen in other domesticated species as well. Another interesting aspect is that Red Junglefowl are more prone to perform contrafreeloading (Schütz and Jensen 2001), which means that they will work for access to food even in a situation where there is a freely available food source.

Red Junglefowl possess many of the characteristics of wild animals that are prone to being domesticated. First of all, they live in social groups with a hierarchy where individuals form a pecking order. They will eat just about anything, and leaving leftovers for your backyard chickens, I imagine, is probably as common as bringing your dog a doggy bag. Consuming a diet that is easy for humans to obtain seems to be a road to domestication success (Price 1984). Another feature that speaks in the favour of chickens is that they can reproduce quite freely, and removing the clutch of eggs from a mother hen will induce her to lay more eggs in response. And, as I will properly get into, they can be selected on tameness.

Replicating early domestication

Early domestication has been suggested to be driven by selection on tameness (Jensen 2014). Belyaev (1979) suggested that early selection by humans on “domesticated behaviour”, as he called it, must have been the cause for a general loss in reproductive season in domesticated animals. The domesticated behaviour that he referred to could be summarised as tameness. Belyaev’s hypothesis was tested through a well-known experiment in silver foxes (*Vulpes vulpes*), where not only reproductive season was altered, but other phenotypic traits as well, in response to selection on tameness (Trut et al. 2009). After successive selection on tameness, the foxes started to resemble dogs in both behaviour and appearance. A similar experiment in rats (*Rattus norvegicus*) demonstrated changes in e.g. anxiety related behaviours where tame rats were overall behaving less anxiously and they had smaller adrenal glands but larger spleens (Albert et al. 2008). Mink (*Mustela vison*) selected on tameness seem to generalise their fear response in a battery of different behavioural tests examining both social and non-social contexts (Malmkvist and Hansen 2002).

Tameness and fear

When an animal responds to the perception of actual danger, we call this emotional response fear (Forkman et al. 2007). Many situations in a farm animal’s life may elicit a fear response, e.g. transportation, mixing of social groups and proximity to humans. Fear is a problem in the animal production industry, not only from an ethical standpoint, but also because fearfulness in captive populations is associated with economical losses. In broiler chickens for example, higher fearfulness towards humans is associated with poorer feed conversion efficiency (Hemsworth et al. 1994). Similar effects have been found in layer breeds, where fearfulness towards humans was associated with lower egg productivity (Barnett et al. 1992). Likewise, high fear towards humans

was associated to high mortality at the end of production, and higher fear towards a novel object was associated with lower egg weight in parent stock layer breeds (de Haas et al. 2013). It is common to adjust the light cycle in commercial rearing in order to improve production, generally by increasing the length of perceived daylight. Fear of humans is however lower in broilers housed with decreased daylight length (Bassler et al. 2013). Exposing birds regularly to gentle handling could also help lower the fear of humans (Hemsworth et al. 1994), however this is not feasible in housing systems with thousands of birds.

Behavioural indicators can be used to assess fear. Chickens experiencing fear may react in different ways depending on context (Jones 1996), showing some of the complexity behind fear responses in chickens. In general, freezing, crouching as well as low activity (depending on context) are considered as markers of high fear in chickens. Chickens may also flee from frightening stimuli by either running or flying. In contrast, displaying explorative behaviours and short latencies to reach potentially frightening stimuli are indicative of low fear. The neural circuitry behind fear responses in birds has not been as widely studied as it has for mammals. Fear responses in quail are affected by lesions in the arcopallium and posterior pallial amygdala (Saint-Dizier et al. 2009) and dopaminergic measures were affected in the arcopallium in laying hens selected for low mortality (Kops et al. 2013), where the low mortality hens are less fearful compared to controls (Nordquist et al. 2011). Several quantitative trait loci (QTLs) for fearfulness have been found in chickens, a couple of which correlate with production traits (Schütz et al. 2004). Level of fear of humans in Red Junglefowl shows a heritability of 0.17, although low, it is significant and indicates that the trait can be selected upon (Agnvall et al. 2012).

Selecting solely on increased tameness has resulted in an attenuation of the stress response in the fox (Gulevich et al. 2004) and rat (Albert et al. 2008). The HPA-axis is involved in regulation of the stress response and will be activated by the emotional perception of fear. However, the stress response is activated in other situations as well that do not involve fear. It was defined by Selye (1973) as a non-specific response of the body to any demand. Activation of the HPA-axis helps animals cope with their environment and some of the effects seen in overall fearfulness could be explained by differences in HPA-axis reactivity, which tends to be attenuated in domestic animals (Albert et al. 2008; Künzl and Sachser 1999). An interesting observation in the comparison between the White Leghorn and Red Junglefowl is that the latter responds with higher levels of corticosterone to an acute stressor, but also returns to baseline faster (Ericsson et al. 2014).

Selection experiment

The experiments in this thesis were conducted on a population of Red Junglefowl that had been selectively bred on either low or high fearfulness towards humans for several generations. In order to maximise genetic diversity, two different zoo populations of Red Junglefowl were used to create an outbred parental population (P0). The populations originated from Copenhagen zoo and Götala research station. Individuals from these populations were interbred for two generations, ending up with about 100 P0 birds (Agnvall et al. 2012). From these birds, 27 % of the most fearful, and 27 % of the least fearful individuals, determined by a standardised behaviour test, were chosen to initiate the two selection lines. This test, called the fear of human test, measures the level of fear towards an approaching human (Agnvall et al. 2012). We used the score from each individual in this test for our selection regime. For **papers I, II and III**, we selected the least fearful individuals for breeding pairs in the low fear line and the most fearful individuals in the high fear line for each new generation, taking care not to mate siblings in order to maintain genetic diversity as much as possible. The selected generations studied in this thesis range from generation five up until generation ten.

The test setup consisted of a longitudinal arena that measured 100 × 300 × 210 cm, built out of eight wooden partitions consisting of an opaque 50 cm high section at the bottom and wire mesh section on the top (Figure 2). Three equally sized sections (100 × 100 cm) of the floor were denoted as centre and peripheral zones (invisible to the birds). To avoid escaping birds, the top was covered with plastic mesh. Before the start of the test, a bird was brought into the arena and placed in the centre zone, head facing the long side of the arena. The lights were turned off and windows were covered prior to the test to keep external light out of the room. The experimenter, always wearing the same clothes, placed herself in one of the peripheral zones. Test start was initiated by the lights turning on. From the test start until the end of the test three minutes later, the fear level of each bird was assessed every ten seconds. Each fear level was defined in an ethogram (Table 1). At the end of the test, the experimenter attempted to touch the bird and gave this response a fear score as well. The overall fear score of each bird was determined as the mean of all of the ten-second intervals from the test.

Figure 2. Schematic view of the arena used for the fear of human test. Dotted red line indicates each of the three zones. The arena was built out of eight partitions (pictured in colour to the left in the image), the ones on the long side at the front of the image not shown. Measurements shown in cm.

Table 1. Ethogram with descriptions of the behaviours associated with each fear level in the fear of human test. Previously described in Agnvall et al. (2012).

Fear level	Behaviour
1	Exploring, standing or walking, with short neck
2	Standing or walking with eyes open and neck stretched. Head flicks and vocalising 1 to 5 / 10 s.
3	Standing or walking with eyes open and neck stretched. Head flicks and vocalising 6 to 15 / 10 s.
4	Standing or walking with eyes open and neck stretched. Head flicks and vocalising > 5 / 10 s.
5	Escape attempts and vocalising loudly alt. the bird is completely still (freezing behaviour)

Advanced intercross line

Genetic mechanisms that cause seemingly unrelated traits to correlate with each other, i.e. correlated selection responses, can be e.g. pleiotropy or linkage. Pleiotropy is a mechanism where one gene, or locus, controls several traits, and linkage refers to the situation where two loci are in close proximity to each other and end up being inherited together as a result (Stearns 2010).

Differentiating between pleiotropy and linkage is not a trivial task, but it can be done. However, that is outside the scope of this thesis. Another mechanism that could be responsible for selection effects in the selection lines is genetic drift. It is the random loss of genetic variation that cannot be attributable to natural selection or other evolutionary processes. Genes that are not truly linked, but are close to a region that we select upon, will hitchhike with the selected region. This will end up looking like there is a correlation with selection on tameness.

Two inbred lines that differ from each other in quantitative traits, such as fearfulness towards humans that we selected on here, can be intercrossed to generate an F2 intercross line. If two traits are determined by the same loci, then the differences in these traits should correlate in an F2 intercross. Since behaviours as quantitative traits are almost always determined by several genes that each exert small effects, it is an advantage to continue the intercrossing beyond the F2 generation, a so-called advanced intercross line. In this way we increase the recombination events (Figure 3), and therefore the resolution due to smaller haplotype blocks, and thus have a higher probability of finding traits that are actually controlled by the same loci (Darvasi and Soller 1995). A haplotype block is a group of genes on a chromosome that are inherited together because they are linked. Recombination during meiosis can break up haplotype blocks into smaller fragments. The recombination rate in chickens is quite high, with an average of 2.5 to 21 cM per Mb, depending on the size of the chromosome (International Chicken Polymorphism Map Consortium 2004). For **paper IV**, we generated an advanced intercross line from the two divergent selection lines in order to determine if phenotypes that seem to have been affected by selection on tameness have any correlation to this trait. The intercross line generated in **paper IV** is an important control that gives insight into whether the observed correlated selection responses are due to genetic drift or whether they are products of selection on tameness.

Figure 3. Schematic representation of the effect that crossing over has on haplotype block length over several generations of intercross breeding. Blue and red colours indicate selection line origin.

Aim of this thesis

The aim of this thesis was to measure behavioural and physical traits in Red Junglefowl selected for diverging levels of fear towards humans in a standardised behavioural test. One selection line was selected for low fear towards humans, or tameness, and the other was selected for high fear towards humans. First, we measured a number of traits in the selection lines, to finally move on with measurements in an advanced intercross line between the two selection lines. The trait measurements in the advanced intercross line were compared to the individual fear of human

scores that were obtained for each individual using the standardised behavioural test. We hypothesised that selection on tameness would affect other phenotypes to resemble the domesticated phenotype and that these phenotypes would be correlated to level of fearfulness towards humans in the intercross line.

Paper summaries

Paper I

Is domestication driven by reduced fear of humans? Boldness, metabolism and serotonin levels in divergently selected Red Junglefowl (*Gallus gallus*)

Previous research on our divergent selection lines has found that low fear chickens are heavier both at hatch and later in life compared to high fear chickens. Since there is likely a trade-off to other energy demanding processes, we wanted to test whether basal metabolic rate, measured through open flow respirometry, was affected through our selection regime. In **paper I**, we set out to do this in chickens of the fifth selected generation, as well as measure feed efficiency and boldness in a novel object situation with a food reward as the motivator to approach the novel object.

The results from **paper I** show that basal metabolic rate was higher in young chicks (5-6 weeks old) while feed efficiency also tended to be higher in these birds when they were older (19 weeks old). In the same time period as the feed efficiency measurements, we tested the chicks in a novel object test. Both males and females of the low fear line were faster in reaching a novel object compared to the high fear line. Overall, the results from **paper I** show that selection on tameness can cause correlated selection responses in traits that seem unrelated.

Paper II

Activity, social and sexual behaviour in Red Junglefowl selected for divergent levels of fear of humans

Based in part on our findings in **paper I**, where low fear chickens had a lower basal metabolic rate and were faster in a novel object test, we wanted to find out about their behaviours in a more naturalistic setting. We measured a range of different behaviours but were especially interested in activity behaviours in young and adult Red Junglefowl in **paper II**. While the young birds did not differ in the amount of activity behaviours seen, there was an effect on feather preening, possibly as an effect of poorer plumage condition or higher flock cohesion in the low fear line. The same pattern was seen in the adult birds. In the adult birds, on the other hand, the low fear individuals spent more time on locomotion and vocalisations. We concluded in **paper II** that this could be due to higher fear in the high fear line, rendering them less active and vocal as a response to an uncertain environment (outdoor pen).

An additional experiment was included in **paper II**, in which we observed male courtship behaviour in a test where the males were suddenly presented with a female after being socially isolated. We found that males of the low fear line ground pecked less in the presence of a female, a behaviour that is usually done to attract females together with food calls and presenting appealing food items.

Paper III

Selection for reduced fear in Red Junglefowl changes brain composition and affects fear memory

An earlier study in our selection lines revealed that brain size and the sizes of brain regions had been affected by the selection on lower fear. The study was performed on adult birds and we wanted to test if the changes could be seen already in chicks. We performed a five-piece dissection where the brains were divided into distinct regions. In **paper III**, we found that brain size relative to body weight has been affected by selection on low fear. Especially interesting was that the brain size had not been selected on as a whole, as we observed that brainstem region size relative to the size of the whole brain was also smaller in the low fear chickens. This further strengthens the notion that the brain regions could be selected on independently of each other. The differences in brain size are principally an allometric response to body size increase in the low fear line.

Together with brain size measurements, we performed some tests to measure cognitive traits. The tests were a fear habituation test and a modified conditioned place preference test. While conditioned place preference learning had not been affected by selection on level of fear towards humans, fear memory seemed to have been. Chicks from the low fear line habituated more effectively towards a startling cue, in our experiment a flash of blue light emitted every 30 seconds, five times in a row. The test was performed on two consecutive days. Fear reactions did not differ between the selection lines on the first day of the test, but on the second day of the test, the low fear chicks responded less to the light cues. This suggests that the low fear chickens could be more effective at habituating to fearful stimuli over time.

Paper IV

Tameness correlates with domestication related traits in a Red Junglefowl intercross

From our earlier papers, we can conclude that selection on tameness has affected other traits as well. In **paper IV**, we sought to find out if the level of fear towards humans could be genetically correlated to any of the traits that changed as a response to the selection. We also wanted to control for genetic drift. To do this, we interbred the two selection lines to create an F3 advanced intercross line. Traits that were significantly affected by fear score from the standardised fear of human test were body weight as well as growth rate at different ages. We also tested the birds in open field-, fear habituation- and social behaviour tests. The behaviours from those tests did generally not differ, with the exception being in distance moved by females in the open field test as well as latency to utter food calls by males in the social behaviour test. In fear habituation, there was an interaction effect between sex and fear of human score, showing that females with lower fear scores were better at habituating to the fearful stimulus.

We also measured brain as well as brain region weights and found that there was an effect on brain size relative to body size, such that individuals with lower fear scores had smaller relative brains. Females with low fear of human scores also had a larger cerebrum in relation to the rest of the brain.

The results from **paper IV** further strengthen the idea that tameness may have been an important driving factor for early domestication in chickens. As an important control for genetic drift it also shows that many of the trait changes seen in the selection lines are probably not an effect of genetic drift, but rather genetic mechanisms such as pleiotropy or linkage.

Discussion

With this thesis, I set out with a general aim of finding out more about the early domestication process using the Red Junglefowl as my study species. One central question has been on my mind for years: Could early animal domestication have been driven by a primary selection on tameness? As an ethologist I am mainly interested in the effects that domestication has had on behaviours. However, I have also investigated some effects that our selection regime has had on physiological and morphological traits. I finished off with an advanced intercross line in order to begin untangling the genetic mechanisms behind the correlated selection responses of selection for tameness, and to control for genetic drift, the random loss of genetic variation. The results from the advanced intercross line will be discussed together with the relevant phenotypes in the following text. Our selection experiment is unique in the sense that it is an attempt at domesticating an old domesticate. In this way, we have been able to observe whether the changes in Red Junglefowl resemble those of domesticated chicken breeds.

Behavioural changes

Throughout this project, we have done various behaviour tests and observations to assess the effects of level of fear of humans on behaviours. Throughout domestication, the repertoire of behaviours generally does not change very much. What can change is the threshold at which certain behaviours are expressed (Price 1999), as opposed to there being changes such as loss of behaviours altogether from the behavioural repertoire of an animal species.

Fear, novelty and learning behaviour

Fear can be difficult to measure reliably by only behavioural observation. A study comparing fearfulness in layer and broiler breeds highlighted this difficulty, showing that the fear level induced by a particular test and/or strain background, could affect the outcome of the behavioural fear response (Keer-Keer et al. 1996). Physiological measurements, such as plasma corticosterone levels and heart rate variability can give additional information to the assessment of fear and other stress reactions (Jones 1996). However, depending on the situation, they do not give enough information on their own and therefore behavioural observations are necessary for an accurate assessment of fear. As a measurement of overall fearfulness it seems to be an advantage to combine several behavioural tests (Campbell et al. 2019). This project is based on the fear reaction towards humans using a single behavioural test. The selection criteria has a significant heritability (Agnvall et al. 2012), which means that there is a genetic component that can be selected upon.

Additionally, I have done some behaviour tests that assess other types of fear and learning. The novel object test is widely used in a range of species as an assessment of fearfulness or boldness and it correlates with other types of fear as well and is not affected by human handling (Forkman et al. 2007). We used the novel object test in **paper I** on adult chickens and used the latency to reach a food reward in the presence of a novel object as a measure of boldness in order to add another variable of fear in addition to the ones previously measured by Agnvall and Jensen (2016). A fast approach towards a novel object is usually interpreted as boldness or low fearfulness (Forkman et al. 2007). Chickens from both sexes of the low fear line were faster at reaching the novel object in our test, which is what we expected. This is in line with an earlier study comparing Red Junglefowl with White Leghorns that found the domesticated White Leghorns had a shorter latency to reach the novel object (Schütz et al. 2004). In the same chickens used in **paper I**, the fear response to an aerial predator was lower in the low fear line and there was an interaction effect between selection line and sex in the open field and social reinstatement tests, showing that the sexes were affected differently (Agnvall and Jensen 2016).

Tonic immobility, another fear test, was not affected. In **paper IV**, we tested behaviour in the open field on our advanced intercross line, finding that low fear score was associated with lower fearfulness in females in the open field test. The open field test is, similar to the novel object test, also commonly used and well validated as a general fear test for chickens (Forkman et al. 2007). For the open field test, the latency to move as well as the distance moved are more indicative of fear, whereas leaping and vocalisations reflect motivation to reach social companions (Suarez and Gallup 1983; Forkman et al. 2007). Open field test behaviour of chickens selected for five generations on high and low fear indicates that there has been no main effect of selection in this trait, although there was an interaction between selection on tameness and sex, indicating that the sexes are not affected in the same way (Agnvall and Jensen 2016). In an earlier generation, the open field test showed that chickens from the low fear line were less fearful (Agnvall et al. 2012). We decided to add the open field test in the battery of tests that were performed in **paper IV**. There was a significant correlation between distance moved in the females, corroborating the results from generation five where an interaction between selection and sex was found (Agnvall and Jensen 2016). An intercross between tame rats and an aggressive control yielded two QTL regions that were associated with tameness and that overlapped with QTL for anxiety related behaviours (Albert et al. 2009).

The relationship between brain size and cognitive abilities has still not been completely understood, despite efforts from many researchers. Most studies supporting a relationship between cognitive abilities and brain size have been in interspecies comparisons, e.g. innovativeness in different bird species is positively correlated with brain size (Overington et al. 2009). I would like to argue that we should instead focus more efforts on intraspecies comparisons in order to fully understand these links, if they even exist. This is because each species has different characteristics that are specific and could act as confounders. In an effort to shed some light on this, I did a conditioned place preference test and constructed a fear habituation test for **paper III** with the well-established fear extinction test in mind. The fear extinction test is used in rodent studies (Chang et al. 2009). Domesticated animals have adapted to the captive environment, which includes startling stimuli and sometimes unexpected events that could induce fear, making this a relevant test. We found that chickens from the low fear line were more effective at habituating to artificial stimuli that produced a fear response. Habituation learning is distinct from both effector fatigue as well as sensory adaptation (Rankin et al. 2009). Previous studies in the selection lines have found that the low fear chickens were less fearful in some other contexts as well (Agnvall et al. 2012; Agnvall and Jensen 2016). The intriguing aspect in our experiment in **paper III** is, however, that both selection lines reacted equally much on the first day of testing in the fear habituation test, whereas the low fear chickens reacted less on the second day. This indicates that some form of learning has happened in order to elicit this response. These results make sense in the way that animals in the wild need to be vigilant and cannot habituate as freely to frightening stimuli since there is greater risk of predation. In captivity, high fearfulness to repeated frightening but non-harmful stimuli is energetically very costly. Furthermore, we found in **paper IV** that there was a significant correlation between habituation learning and fear of humans in females, showing that this is in fact a correlated selection response to the selection on tameness.

The conditioned place preference test in **paper III** did not indicate any changes in associative learning in response to the selection on fear of humans. This test is based on associative learning, where a conditioned stimulus is paired with an unconditioned stimulus. The unconditioned stimulus is usually something frightening or attractive, in this case we used mealworms since they are a known attractive reward for chickens and since chickens can form a conditioned place preference for food rewards (Jones et al. 2012). For my test, I used two different patterns as conditioned stimuli. It can take time for chickens to create associations (Zidar et al. 2018) and the

outcome is affected by affective state (de Haas et al. 2017). The test was performed in young chicks who are very motivated to reinstate contact with conspecifics when isolated. To try to alleviate this problem, I added a mirror into the test arena in the learning stage. Upon reflection, I should probably have kept the mirror for the test days as well since I cannot rule out the fact that motivation for social reinstatement was higher than the motivation to gain access to the food reward in the test situation. The results from the test suggest that the chicks did not manage to create an association to the reward so I would like to be cautious about making any conclusions about possible effects the selection on tameness may have had on associative learning. In no way do these results indicate that further research on associative learning in the selection lines should be discontinued, however, they provide a lesson for future experimental design.

The behavioural observations in this section show that some aspects of behaviour have changed in response to selection on tameness but that overall these changes are small and mostly related to fear as measured by other fear tests. One interesting finding was that fear habituation learning was improved in chickens with low fear, showing that they may have an increased ability to adapt to the captive environment.

General behavioural budgets

In **paper II**, we wanted to do some behavioural observations on both juvenile and adult birds in the selection lines in semi-natural environments. Domesticated chickens adopt a more energy saving behavioural strategy, possibly to compensate for other energetically expensive phenotypes (Schütz and Jensen 2001). We know that the low fear chickens spend more energy on reproductive traits and grow larger (Agnvall et al. 2014), therefore I wanted to investigate behavioural budgets in an environment similar to the home pen. Up until the sixth generation, no such observations had been done in the selection lines. We found that chickens from the high fear line spent more time feather preening both as juveniles as well as adults. Agnvall et al. (2014) found that the feather condition was poorer in the high fear line, a possible suggestion for the increase in this behaviour compared to the low fear line. The poorer plumage condition could be a consequence of increased susceptibility to feather pecking. Feather preening also seems to be more common in groups with a high flock cohesion (Keeling and Duncan 1991), and domesticated chickens have a less rigid group structure (Eklund and Jensen 2011). Unfortunately, we did not measure the feather condition in the tested generations in **paper II**.

Other behaviours that we found increased in the low fear line were locomotion and vocalisations in the adults, and the number of pecks at the feeder in juveniles. The environments used for our observations between the juveniles and the adults differed quite a bit, even though the idea was to make them semi-natural. The juveniles were housed in an indoor arena in pairs with one of each sex whenever possible, whereas the adults were housed in an outdoor arena in groups of four, with two males and two females in each group. In both settings we housed the birds within selection line. The outdoor arena used for the adults was considerably more challenging and complex than the indoor arena used for the juveniles. Wild birds of prey are known to inhabit the surrounding area and there is considerable activity in the vicinity with humans, farming vehicles, livestock as well as dogs. These factors contribute with considerable environmental disturbances that could have induced a less conspicuous behaviour in the adults of the high fear line, whereas the juveniles were not disturbed to the same extent. Comparisons between Red Junglefowl and domesticated White Leghorns showed that the domesticated variant adopts a more energy conserving behaviour (Schütz and Jensen 2001). Although fear of humans is genetically correlated to other measures of fearfulness in our selection lines (Agnvall et al. 2012), the correlated selection responses to other measures of fear are only minor (Agnvall and Jensen 2016). The results from **paper II** do not show that chickens selected on tameness adopt a more energy conserving behaviour, since they spend more time on locomotion and vocalisations as

adults. However, these behaviours may be a reflection of an environment that is perceived as more threatening by the high fear birds, therefore inducing a less conspicuous behaviour. Furthermore, observations in the adults were made with two visible human experimenters which could be a drawback considering that the birds were selected on fear of humans.

Courtship and social behaviour

The third experiment in **paper II** concerned courtship behaviour in males. We constructed an arena directed at measuring courtship behaviours towards females performed by males that were housed individually, but with a visible social partner of the same sex in an adjacent arena. Although most behaviours associated with courtship did not differ, males from the high fear line performed more ground pecking when presented with females. Males often perform food calls, with the intent of attracting females, when they have found attractive food items (van Kampen 1997; Marino 2017), who prefer frequently food calling males for mating (Pizzari 2003). Sometimes, males produce dishonest food calls in the absence of true valuable food sources (Gyger and Marler 1988). Food calling is usually accompanied by a characteristic behaviour in which a male picks up and drops a food item, called tid-biting, which happens after an initial bout of intense ground pecking behaviour. The differences in ground pecking could be related to social status since subdominant males avoid vocalisation when courting females in order to lower the risk of confrontation with dominant males, focusing on just tid-bit signalling instead (Smith et al. 2011), therefore high fear males were perhaps focusing their efforts on finding an actual food item instead of food calling. It would have been interesting to add a palatable food item into the male enclosure at some point during the observations in order to gain insight into how food calls, tid-biting and ground pecking are affected in these birds.

Agnvall et al. (2014) found that females from the low fear line were socially dominant compared to females from the high fear line, but males were not tested. Her tests were performed in groups of birds that were given a score together. In order to obtain individual scores on social behaviour for each bird that could be compared to fear of human score in the advanced intercross line in **paper IV**, I constructed the mirror test. The intent was to measure the behaviour of both males and females individually faced with an unknown matched social opponent (mirror), which was presented together with a small bowl containing a food reward placed directly in front of the mirror. The expectation was that dominant birds would behave aggressively towards the mirror. This only happened in a few instances. Food calls emitted by males, usually to attract females (Marino 2017), were more frequent with lower fear score. Although it was ground pecks, and not food calls, that differed between males in **paper II**, I think there may have been some effects on the courtship and/or social dominance behaviour of males, but they need more careful investigation. The dominance relationship in males in relation to selection lines still deserve further examination and the results on females are inconclusive from the mirror test.

Physiological and morphological changes

The phenotype that has shown the most consistent change throughout the breeding project has been body size. Already early on it was evident that low fear line chickens grew larger and that they produced larger offspring (Agnvall et al. 2014). Given that increased growth and reproduction are the main exploits in today's production industry, it is possible that these were desirable traits already early on in the domestication of chickens. We were further interested in this body size increase, and in **paper I** we decided to measure basal metabolic rate (BMR) and food conversion ratio in the 5th selected generation to try to find possible explanations for the increase. Even though the differences in BMR were not very large, they were significantly higher in the low fear chickens measured by amount of oxygen consumed per grams of body mass as chicks. Feed conversion ratio was also higher. This means that they managed to grow larger with a more efficient food conversion ratio while having a slightly higher BMR. Consuming less food

for the same amount of output, in this case body weight, must have been an advantage for the Red Junglefowl undergoing early domestication even though cock fighting was probably the first use (Nicol 2015). We show with **paper IV** that increased body size is probably a central trait in the selection for tameness in chickens. Similarly, on the chicken chromosome 1, there is a small region that affects traits such as body size and fear behaviour (Schütz et al. 2004).

Similar to an investment in body size, investment in increased brain size is energetically costly (Mink et al. 1981; Isler and van Schaik 2006) and therefore we decided to measure possible changes here as well. Brain size and composition was affected in adults of the fifth selected generation, previously reported by Agnvall et al. (2017). In **paper III**, our aim was to study whether changes in brain size could be seen already in juveniles. Brain size changed allometrically with increased body weight, but the relative brain size in relation to body size decreased in the low fear line, similar to what happens during domestication. A comparison between White Leghorns and Red Junglefowl found that the domesticated Leghorn had a smaller brain (Henriksen et al. 2016). Spatial learning performance was impaired in the White Leghorn in another study (Lindqvist and Jensen 2009). As I wrote earlier, we also measured some cognitive traits, finding that fear habituation was more effective in the low fear line, a relationship which was confirmed in females in **paper IV**. While we were not able to directly test effects of brain size on fear habituation learning or associative learning in the conditioned place preference test, it remains a possibility for future research. Brain size is related to cognition in guppies (*Poecilia reticulata*) (Kotrschal et al. 2013) and selection on maternal investment is related to brain size in the Japanese quail (*Coturnix japonica*) (Ebneter et al. 2016). However, a large relative brain size in the White Crested Polish chicken was not associated with any advantage cognitively (Mehlhorn and Rehkämper 2013).

There is a debate concerning whether the domestic phenotype has emerged due to direct selection on desirable traits (Rubin et al. 2012) or if they emerge as correlated selection responses due to selection on a key trait that is either linked or pleiotropically determined (Trut et al. 2009). Whereas recent breed formation in e.g. dogs is indisputably a result of directed selection, I show with this thesis that it is possible that early domestication in chickens could have been driven by selection for tameness. This is because the phenotypic changes that we have observed are in line with this hypothesis. The phenotypic correlations in the intercross line further strengthen this idea, showing that some of these traits could be determined by linked or pleiotropic genes connected to tameness. Exact mechanisms behind these changes need in-depth analysis, through e.g. QTL analysis and the candidate gene approach.

Effects of sex

For many of the measured phenotypes, the selection responses were significantly affected by sex. Previous studies in chickens have shown the same pattern with sex specific domestication effects both in comparisons between Red Junglefowl and domesticated chickens (Nätt et al. 2014; Schütz et al. 2004) as well as in the selection lines used here (Agnvall and Jensen 2016). Overall, the changes seem more frequent in females, which could reflect the importance of female specific traits related to fecundity during domestication. Females investment in reproduction is higher and perhaps this investment is possible through compromises in other phenotypes.

Methodological concerns

Studies on animal behaviour are difficult in many ways. One of the greatest difficulties begins with the definition of behaviours. How we define our behaviours impacts the reliability of our recordings. A thorough knowledge of the behaviour of the study species is important. Several standardised behavioural arenas exist for rodent studies, and attempts have been made to adapt

these to other species as well. I have discussed the concerns of those tests with each relevant test in the discussion above. In this section, I want to discuss the fear of human test more closely as it is central to our selection regime. After that, I will tie off with a discussion of some concerns regarding the breeding scheme itself.

Fear of human test

The fear of human test used for selection in this thesis measures the behavioural reaction towards an approaching human. Other tests in similar experiments have used the exposure to a human hand or glove to measure fear of humans instead (Hansen 1996; Belyaev 1979; Albert et al. 2008). Since chickens imprint on larger moving objects and are handled within the first 24 hours of hatching, the use of a human hand to assess fear after the imprinting window is probably not feasible. Because of this, Agnvall et al. (2012) chose to measure the response towards the whole body of the human instead. There are, however, other concerns as well. The heritability of the fear of human trait was 0.17 (Agnvall et al. 2012), which means that most of the variation in the trait can be explained by differences in environment. The chickens were reared under identical conditions to minimise the effect of environment. As the fear habituation test showed, fear habituation to a startling artificial stimulus was more effective in the low fear line (**paper III**). If the same is true for fear of humans, it is possible that daily exposure to humans, although brief, could cause a better habituation in the low fear chickens. It should not be forgotten that although environmental conditions as assessed by us humans may be identical, the perception of each individual bird could be very different. For this reason, we do our best to limit human contact with the birds during rearing and keep handling to the lowest possible amount during experiments. It would be interesting to assess fear of humans already in chicks to see how the fear response to humans develops over time.

Breeding scheme

It is generally agreed upon that selection experiments should include several identical selection lines in order to ascertain that the selection on a given trait gives rise to the same changes. In this case, we only had one of each selection line, high and low. The reason for this is that chickens are quite large compared to e.g. fruit flies and mice where selection studies are often made. In other words, there were resource limitation forcing this regimen. In order to alleviate some of the problems, such as genetic drift, that arise from keeping small populations, the selection lines were derived from two populations of zoo fowl that were interbred for two generations in order to increase genetic diversity (Agnvall et al. 2012). Furthermore, throughout each generation, we took care not to mate siblings to maintain the genetic diversity as much as possible, and family representation was of a higher priority than fear score. For some generations, the sample size was low, due to problems with hatchability and unfortunate loss of animal material due to unforeseen events. Despite our efforts, genetic drift cannot be excluded. However, the other phenotypes measured are in the expected direction and the advanced intercross between the two selection lines shows that several phenotypes are in fact correlated to tameness, indicating that genetic drift may not be the primary cause of those phenotypic differences.

Conclusions

Traits corresponding to the domesticated phenotype emerge in animal populations that are experimentally selected for tameness. We found overall that selection on tameness in Red Junglefowl did induce changes similar to the domesticated phenotype in other traits that were not the primary focus of selection. How this happened, whether it was conscious or not, is still an open question. In **paper I, II and III**, we show that some behaviours, mostly related to fear or novelty, change when selecting for tameness. Females especially seem to become less fearful in other situations as well, whereas males show some signs of increased courtship behaviour. Both sexes are better at habituating to a fearful artificial stimulus when selected for tameness, showing that some aspects of memory and cognition may have been affected. BMR was measured in **paper I** and was higher in the low fear chickens even though they grow faster and larger. In **paper IV**, we made an intercross between our two divergently selected Red Junglefowl lines. These birds were given individual fear of human scores that were compared to other traits, both behavioural and physical. Given that some of the measured traits, such as body weight, correlated with fear of human score, we have an indication that these traits are genetically correlated, i.e. could be determined by either linked or pleiotropic genes and that they are likely not a result of genetic drift. Taken together, our results from these four works strengthen the idea that early domestication may have been driven by selection on tameness. Future work should focus on the genetics behind the selection on tameness, through e.g. QTL studies.

Future directions

In order to truly find the genetic mechanisms behind the changes that we have seen in traits as a response to selection on tameness, the first step in continuing this work would be to conduct a QTL analysis. To do this, you genotype each individual in the advanced intercross line using a suitable number of Single Nucleotide Polymorphisms (SNPs). These are then compared to the phenotypes. When a locus correlates with a phenotype, it is an indication of there being a gene, or set of genes, at that locus that are important in determining the phenotype. In most cases you will not be able to pinpoint the specific gene. This is especially true for behaviours as they are, with a few exceptions, usually determined by several genes that each have a very small effect. However, a QTL analysis could yield candidate genes that you can investigate further with other methods. Genome editing with e.g. CRISPR-Cas9 is a method that has not been used extensively in chickens yet but could perhaps give some interesting answers to questions about domestication in the future. Another topic that deserves further investigation is the role of tameness in cognitive abilities and possibly an investigation into the links between brain size, as well as composition, and cognition. It would be interesting to find out whether possible differences in cognitive abilities are mainly due to diverging fear in the selection lines, or if they are inherent differences connected to e.g. brain size or brain neurochemistry. I suggest measuring cognitive bias as a first step, perhaps in conjunction with different treatments aimed at inducing environmental stress.

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Linköping Studies in Science and Technology, Dissertation No. 2099, 2020
Department of Physics, Chemistry and Biology

Linköping University
SE-581 83 Linköping, Sweden

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