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Stress and the Offspring

— Adaptive Transgenerational Effects of —
— Unpredictability on Behaviour and Gene —
Expression in Chickens (*Gallus gallus*).

A licentiate thesis by

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Front cover: W98 HyLine leghorn male, photo by the author.

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List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

Paper I.

Lindqvist, C., Janczak, A.M., Nätt, D., Baranowska, I., Lindqvist, N., Wichman, A., Lundeberg, J., Lindberg, J., Torjesen, P.A. and Jensen P. 2007. Transmission of Stress-Induced Learning Impairment and Associated Brain Gene Expression from Parents to Offspring in Chickens. *PLoS one*, e364:1-7

Paper II.

Nätt, D., Lindqvist, N., Stranneheim, H., Lundeberg, J., Torjensen, P.A. and Jensen, P. Transgenerational Phenotypic Tuning of Offspring: Adaptive Responses to a Prenatal Environmental Challenge in Chickens. (Submitted manuscript).

Abstract

Environmental stress has shown to affect both the exposed individuals and the development of their offspring. Generally, it is thought that the stressed organism responds to stress by trying to adapt to it. This thesis investigates possible evolutionary consequences of cross-generational transmissions of stress, where the parent has been stressed but the offspring has not. In two studies we have exposed chicken parents of different breeds to an unpredictable circadian light rhythm, to investigate the influence of genetic background on the transmission of behaviour and patterns of genome-wide gene expression across generations. In Paper I, we can show that the domesticated chicken, by means of epigenetic factors, transmit their behaviours as well as their gene expression profiles to their offspring to a higher extent than their wild ancestor, the red junglefowl. Furthermore, in Paper II, even though the offspring never experienced the stress or had any contact with their stressed parents, they seemed to have adapted to it, which suggests that the parents might have prepared (or pre-adapted) them for living in the unpredictable environment. Additionally, eggs of stressed hens showed increased levels of estradiol that might have affected gene expression of specific immune genes, which were up-regulated in the offspring of stressed parents. It is possible that the traditional distinction between stress responses and evolutionary adaptation may be reevaluated, since our results indicate that they could be parts of the same evolutionary event.

Sammanfattning (Swedish abstract)

Miljömässig stress har visat sig ha konsekvenser inte bara för individen som blir utsatt för den, utan även dess avkomma. Generellt sett verkar organismen svara på stressen genom att försöka anpassa sig till den. Denna uppsats utforskar möjliga evolutionära konsekvenser av transgenerationell överföring av stress, där föräldrarna utsatts för stress men inte avkomman. I två studier har vår forskargrupp utsatt hönsföräldrar för en oförutsägbar dygnsrytm genom att slumpmässigt ändra ljusförhållandena, med avsikten att studera överföringen av beteende och genanvändningsmönster till avkomman. I artikel I visar vi att den domesticerade hönsföräldern, med hjälp av s.k. epigenetiska faktorer, överför sitt beteende och sin genanvändningsprofil till sina kycklingar i större omfattning än den vilda ursprungsfågeln, det röda djungelhönset. Vidare visar vi i artikel II att även om avkomman aldrig utsattes för någon stress, verkade de ha anpassats till den, vilket tyder på att föräldern kan ha förberett sin avkomma på den oregelbundna miljön. Dessutom visade sig äggen från stressade föräldrar innehålla högre nivåer av estradiol (en steroid könshormon) som kan ha orsakat en uppreglering av vissa typer av immunogener hos avkomman. Sammanfattningsvis pekar resultaten mot att den traditionella åtskillnaden mellan stress respons och evolutionär anpassning måste tänkas över, eftersom de mycket väl kan utgöra två sidor av samma mynt.

Introduction

The adaptive stress response

Selye (1973), who is sometimes called the father of stress research, described the stress response as the general adaptation syndrome (GAS), which in basic terms means that an organism facing an environmental challenge will recruit a non-specific (General) physiological response (Syndrome) that works in resistance to that challenge (Adaptation). By this definition Selye was focusing on the short-term physiological aspects of adaptation, but as a biological term it can be involved in long-term evolutionary processes and developmental processes as well (Lasker 1969; Garruto *et al.* 2004).

From Selye's proximate aspect of stress, a stressor is an external or internal stimulus that threatens an animals' internal stability, or homeostasis, and is therefore the causative agent of stress (Selye 1955;1965; Goldstein and McEwen 2002). To resist, or cope, with a stressor, the body adjusts through a process called allostasis (Goldstein and McEwen 2002). Sterling and Eyer (1988) described allostasis as a critical principle of physiology: “/.../ to maintain stability an organism must vary all the parameters of its *internal milieu* and match them appropriately to environmental demands”. Evolutionary biologists have defined stress as any environmental change that acts to reduce fitness of organisms (Koehn & Bayne 1989). From such a view, the main function of allostasis would be to adjust an organism's physiology or behaviour to cope better with the changing environment, hence acting to maintain fitness.

Altogether, if an allostatic response should be judged as adaptive from a biologist view, it should potentially increase the fitness of the stressed individual, which means that it should be relevant with respect to the type of stressor (Korte *et al* 2005). Recent findings have rejected the non-specificity of GAS, which gives support to the idea that the stress response may often be more specific in relation to the stressors than has commonly been believed (Goldstein and Kopin 2007; Pacak *et al.* 1998).

In accordance with the above citations, *adaptation* will hereby be referred to as the physiological response that acts to maintain fitness in a changing environment.

Unpredictability in the wild and as a stressor

Wild animals face temporal occurrence of food, water, predators, conspecifics *etc.* which animals in laboratories or on farms will never face (Price 1984). Selection for behavioural traits that are vital in an unpredictable environment are therefore likely to be relaxed or selected against in artificial breeding. In numerous studies (Schütz *et al.* 2001; Schütz and Jensen 2001; Väisänen and Jensen 2003; 2004; Väisänen *et al.* 2005; Lindqvist *et al.* 2002) the group of Per Jensen has used chickens as a case of domestication, where they have compared the red junglefowl, the wild type and main ancestor to all domesticated chicken breeds (Fumihito *et al.* 1996; Eriksson *et al.* 2008), and White Leghorn, a domesticated breed selected for high egg mass (Jensen and Andersson 2005). From

several aspects they found that these breeds differ adaptively in relation to their natural environment (Asian forest *versus* captivity) in behavioural traits like fear, sociality and feeding strategy. Previously though, adaptability to unpredictability has not been studied.

Even though the aversive effect of unpredictability is very complex, and sometimes inconsistent, the inability to predict future unpleasant events (*e.g.* tail and foot shock) has in many cases shown to induce stress (reviewed by Weinberg and Levine 1979; Abbott *et al.* 1984). Furthermore, considering food predictability, Pravosudov *et al.* (2001) showed that great tits (*Parus major*) have higher stress symptoms, like increased glucocorticoid levels in the blood, when exposed to unpredictable food availability, than when exposed to predictable. Similar examples can be seen in laying hens (Janczak *et al.* 2007) and in brown capuchins (*Cebus paella*) (Ulyan *et al.* 2006). Generally the trend in many species is a preference for predictable over unpredictable food rewards (Bateson 2003; Bateson and Kacelnik 1995). It must be noted that very few attempts have been made to mimic natural unpredictability in the laboratory, where multiple factors (*e.g.* food, water, conspecifics, climate *etc.*) are stochastically presented.

Interval timing, which is necessary for predictive/anticipatory behaviours to occur, can maximise fitness through increased feeding efficiency under predictable conditions (Bateson 2003), and is thought to be controlled by endogenous biological circadian clocks. These clocks is mainly entrained to the light-dark cycle, and are thought to be organised in a hierarchical matter, where the suprachiasmatic nucleus of the hypothalamus (SCN) is considered to be the master clock, and where the periodic oscillation of the expression of self-regulating clock genes sustains the timing mechanism (Reppert and Weaver 2002). For a long time it has been known that rodents can anticipate a regularly occurring meal without a functional SCN, hence suggesting an alternative clock entrained by food availability and independent of light as a *Zeitgeber* (Stephan 2002). *PER2* is a clock gene that recently has shown to be necessary for food anticipatory behaviour in rodents (Feillet *et al.* 2006). In avian species *PER2* expression in the SCN has been confirmed (Yasuo *et al.* 2003; Okabayashi *et al.* 2003), but so far no one has investigated its role in avian feeding behaviours.

One possible way to increase fitness for animals that experience unpredictable environments is to use more conservative foraging strategies (Bateson 2003), like feeding more at known food sites, spend less time exploring for new sites and aim for high-energy food. It is also possible that increased dominance would be adaptive, because as in the previous examples such changes in behaviour would increase the feeding efficiency in situations where food availability is stochastic.

Transgenerational epigenetics

As diverged groups as plants (Molinier *et al.* 2006), birds (Naguib and Gil 2005), mammals (Kapoor and Matthews 2005) and humans (Yehuda *et al.* 2005) has in common that stress may affect phenotypic traits not only in the individual exposed to it, but also in its offspring. In animal research this is often referred to as prenatal stress.

Non-genetic transmissions across generations can broadly be divided into animal tradition and epigenetic inheritance (Jablonka and Lamb 2007). For the purpose of the studies of this thesis, transgenerational epigenetic inheritance can further be divided into indirect and direct gene regulatory effects. Indirect gene regulatory effects, or foetal programming, involve modifications in gene expression patterns caused by variations in the embryonic chemical and endocrine environment, where typical mediators are different kinds of steroid hormones (Seckl 2004). Direct gene regulatory effects, on the other hand, are mediated by embryonic survival of epigenetic modifications in germ cells, like DNA-methylation or variations in chromatin structure (Rakyan and Beck 2006). An important difference between indirect and direct regulatory effects is that the former only shows a maternal effect, while the latter can originate from both parents (Chong *et al* 2007).

Downing and Bryden (2008) showed that stress causes increased levels of corticosterone in the egg albumen and that these levels correlates to a mean blood value of several hours, which makes it an ideal way to measure physiological response to chronic stress in chickens. Interestingly, maternally derived deposits of steroid hormones in the yolk have shown to affect an array of phenotypic traits in the hatched offspring (reviewed by Groothuis *et al.* 2005). Broadly speaking, many steroid hormones play important roles not only in stress, but simultaneously in development, metabolic processes and biological rhythms. For example, studies on ovariectomized rats have shown that estradiol affects feeding behaviours (Eckel 2004) and clock gene expression in the SCN (Nakamura *et al.* 2005), as well as the ability to cope with stress (Bowman *et al.* 2002).

Whether an allostatic stress response in a parent can be transferred, and mirrored, in the offspring has not been investigated, but at least in egg laying species the embryonic environmental conditions seem to have the necessary properties to adaptively modify the offspring phenotype (Pilz *et al.* 2004; Eising *et al.* 2006; Sockman and Schwabl 2000; Meylan and Clobert 2005). Potentially, such kinds of reflective epigenetic inheritance could allow parents to prepare (or pre-adapt) their offspring to a hostile environment (Dufty *et al.* 2002). Additionally, recent results give reasons to believe that a mismatch between the environment of the pregnant mother and the environment of her adult offspring can lead to health problems like diabetes, cardiovascular diseases and obesity (Bateson *et al* 2004; Pike *et al.* 2008), which may indicate that this offspring have coping problems to their own environment. If pre-adaptation is real the relationship between the more proximate allostatic adaptation and the distant evolutionary adaptation must be reconsidered, because this puts all into an evolutionary process with global consequences.

Aims

The overall aim of this thesis was to study transgenerational effects of unpredictability and how this stressor affects behaviour and gene expression in chickens.

Paper I.

The aim was to investigate behavioural and genetic differences, as well as differences in transgenerational effects, between the wild type red junglefowl (RJF) and the White Leghorn (WL) domesticated breed in their response to an unpredictable environment.

Paper II.

The aim was to investigate the adaptive basis of the transgenerational effects seen in the domesticated breed in Paper I, by hypothesising that the unpredictable environment should promote conservative feeding strategies, increased dominance and preference for high energy food in birds adapting to it.

Concise summary of methods and results

Paper I

Groups of RJF and WL were exposed either to an unpredictable (stress) or a predictable light rhythm (control) (Fig. 1). Stress responses were recorded behaviourally for spatial learning in a T-maze and physiologically as corticosterone concentrations in their eggs.

Regardless of stress treatment and without any parental contact, offspring of each breed was raised in the same group under predictable light rhythm. All offspring were tested in a similar spatial learning test as their parents, and was additionally tested in a dominance test for their competitive ability.

A 14k transcript cDNA microarray was used to study stress effects, as well as effects of having a stressed parent, on brain gene expression. Furthermore, to investigate if there was any genetic transgenerational effect, expression profiles of parents and offspring were compared.

Stressed parents of both RJF and WL showed lower spatial learning capabilities than the controls. Between breeds, RJF was faster in solving the spatial learning task than WL. There was no effect of stress on the corticosterone levels in the eggs.

Offspring of stressed WL, but not RJF, showed similar spatial learning impairments as their parents. Additionally, offspring of stressed WL were more dominant and was heavier at day 8 than offspring of controls. The only transgenerational effect of the unpredictable environment in the RJF was higher hatch weights in offspring of stressed parents.

Several genes were significantly differentially expressed between stressed and control WL parents, and between their respective offspring. Stress and control RJF parents also had significantly differentially expressed genes, but none were found in the offspring comparison. Additionally, there was a significant correlation of gene expression profiles between parents and offspring in WL, meaning that if a certain gene was up-regulated in the stressed parents in relation to the control, it was also likely to be up-regulated in the offspring of stressed parents in relation to the offspring of controls. This relationship was not found in RJF.

Paper II

A commercial WL hybrid breed was exposed to the same unpredictable (stress) and predictable (control) treatments as in Paper I (Fig. 1). The adaptive stress response was tested in a foraging test where birds had to forage for either readily available regular food or hidden attractive food. A similar dominance test as in Paper I was also conducted. Five steroid hormones (corticosterone, testosterone, dihydrotestosterone, androstendion and estradiol) were measured in the eggs.

To investigate if parents had prepared (or pre-adapted) their offspring to the unpredictable environment, all offspring were tested in a similar foraging and dominance test as their parents. Additionally offspring was tested for high energy food preference.

The same cDNA microarray used in Paper I, was used to investigate transgenerational effects of unpredictability on brain gene expression. Specific gene candidates were studied by comparing ranked expression profiles of parents and offspring.

In the foraging test, stressed parents pecked more at the readily available food and also showed higher tendencies to peck in general. There was no effect of the stress treatment in the dominance test of the parents. Additionally, stressed parents showed higher growth rate right after stressor introduction, and the stressed hens also laid eggs with higher levels of estradiol, but not of the other four steroids tested.

Female offspring of stressed parents peck more at the readily available food in the foraging test than female offspring of controls. Both sexes of the offspring from stressed parents were more dominant at adult age and had higher preference for energy rich food, than offspring from controls. Furthermore, offspring of stressed parents were heavier than offspring of controls, especially at adult age, and had higher survival rate.

Some genes were significantly differentially expressed between stressed and control parents, but none was found between their respective offspring. By qualitatively comparing the top ranked differentially expressed genes in each sex and generation, many genes showed a suggestive transgenerational effect. The immunoglobulin light chain gene was of particular interest because of its' high ranks in both sexes and generations.

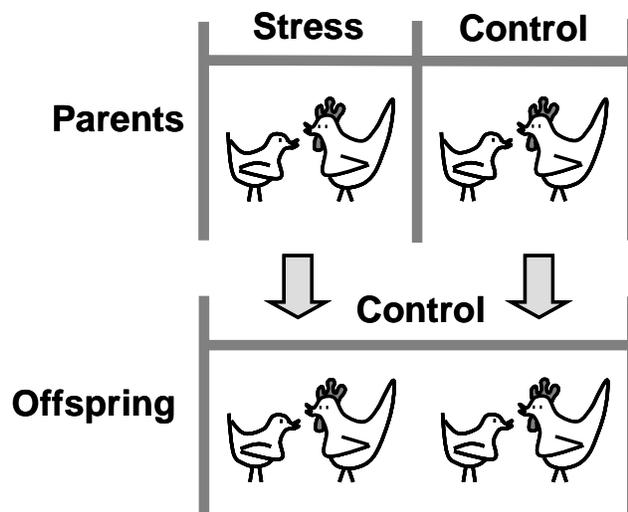


Figure 1. Treatment design. Parents were exposed either to an unpredictable light/dark rhythm (stress) or a predictable 12:12 light/dark rhythm (control).

General discussion

The experiment in Paper I showed, as predicted, that the wild type breed was better in coping with the unpredictable environment. More interestingly, the results illustrate the possibility of an acquired trait being inherited, both when it comes to behavioural phenotype and genome-wide gene expression profiles. These findings are most likely explained by epigenetic factors. Furthermore, the domesticated breed showed higher propensity to inherit the acquired character, which indicate that domestication might have increased the genetic potential of this kind of inheritance.

Many of the results in Paper I were confirmed in Paper II. Again we showed that acquired traits were inherited, and, in addition, that the particular traits in question seemed to be adaptive from a parental view. This suggests the possibility that parents can pre-adapt their offspring for living in a challenging environment. Interestingly, steroid hormone regulation in combination with different kinds of immune genes turned up as possible candidates for the underlying epigenetic mechanism.

Adaptability and transgenerational effects

In Paper I there were an effect of the unpredictable environment on both treatment and breed, meaning that the learning capacity of birds from both populations was affected, and that the wild type breed seemed to be overall better in solving the spatial learning task. This is not surprising, given that the domesticated breed has been selected in human husbandry, with low environmental complexity, whereas the complex and unpredictable natural environment of the wild type breed probably has favoured animals with better abilities to locate and remember food sites.

Considering transgenerational effects, to this date clear reflective inheritance of stress responses, where the stressed phenotype of the parent is mirrored in the unstressed offspring, have only been described in plant species (Molinier *et al.* 2006). Prenatal stress is commonly studied in vertebrates, but in most cases the questions raised is considering the proximate aspects of stress; how the stressor could imply a disadvantage to the parent or the offspring. But when Selye (1973) defined stress as the general adaptation syndrome, which was later developed into the concept of allostasis, it described an organism's ability to adapt to environmental challenges. If the offspring would inherit the same stress response that the parent had, this would imply that the offspring is adapted to the same environmental challenge as their parents.

Already the results in Paper I indicated a reflective transmission of a stress response from parents to offspring, because both generations of the domesticated breed showed similar spatial learning impairments and global brain gene expression profiles. It could be argued from the energy budget rule (Bateson 2003) that decreasing the capacity of spatial memory could be adaptive in a very stochastic environment, where the most efficient foraging strategy might be to concentrate on known predictable food resources instead of searching for new unpredictable ones. This hypothesis was tested in Paper II and it

showed that stressed birds did choose the most readily available food, hence used the most conservative foraging strategy.

The reflective transmission of behaviours in Paper II was not as clear as in Paper I, because only female offspring of stressed domesticated birds showed a similar foraging strategy as their parents. On the other hand, the offspring of stressed parents, which had never been exposed to the unpredictable light rhythm, followed our hypotheses of adapting to the stressor even better than the parents; not only did the female offspring show more conservative foraging strategy, but both sexes were more dominant and showed higher preference for high energy food than offspring of controls.

Adaptation in the parents is crucial for being able to say that the offspring reflectively have inherited their parents stress response. In addition to the strong effect of the unpredictable light rhythm on the parents spatial ability in Paper I and foraging behaviour in Paper II, there is one more factor that indicate adaptation to the stressor in the parents. Right after stressor introduction, the stressed birds increased their growth rate significantly in relation to the controls, but is later compensating for this so that a significant difference in weights between stressed and control birds never appear. Previously, it has shown that many species might increase their food intake and choose the most readily available, as well as the most energetic food, when stressed with certain stressors, which in the long run might result in obesity (Dallman *et al.* 2003; Rowland and Antelman 1976; Solomon *et al.* 2007; Oliver *et al.* 2000). In the present study it seems like the stressed parents over-adjust their food intake in the acute phase after stressor introduction which increase their growth rate, but is later adjusting their food intake when adapting to the stressor, so that obesity never emerge. In contrary, the offspring of stressed parents seem less able to adapt to their own environment because they show higher weights than the controls, which might indicate an obese state. It is striking how well this follows the mismatch concept of developmental origin, which has been demonstrated with several stressors and in numerous species (Gluckman and Hanson 2004). The offspring in Paper II might have been pre-adapted to the parental environment, but when this adaptation is a mismatch to their own environment, this leads to coping difficulties due to a too high food intake, and therefore lower fitness. This would indicate that the adult offspring have a different allostatic state than their parents, which might result in allostatic load problems like obesity (McEwen 1998).

Paradoxical, Paper II shows that offspring of stressed parents have increased survival rates that would indicate higher fitness. But carefully considered, this might be a side effect to an up-regulation of immune genes that could have increased disease resistance in offspring of stressed parents (as discussed below).

Continuing the discussion of adaptability, Paper I showed that domesticated offspring with stressed parent were more dominant than offspring of the same breed but with control parents. To be able to investigate reflective inheritance of stress responses it was necessary to measure the dominance in the parents, which was not done in Paper I. Paper II, on the other hand, did so, but even though the offspring effect was more or less replicated, neither the stressed nor the control parents dominated the other. It could be

argued that this indicates a false reflective transmission and that our hypothesis that unpredictability should increase competitive abilities towards food should be rejected. Alternatively, one possible explanation is that the dominance test, that measured food competition, was not suited for the parents, simply because stressed and control birds lived in two separate groups. Dominance will always be limited in a stable group living under hierarchical rules, because a group can never exclusively inhabit dominant individuals. As in the parents, two established groups with equal numbers of animals should therefore have equal amount of dominant and subdominant individuals. In the offspring, on the other hand, where all birds were living in the same hierarchy, those with stressed parents were mostly located in the dominant classes of the hierarchy in relation to the offspring of controls. This can be explained if the offspring were adapted to their parents' environment, but not restrained in their development by the parental social condition.

Gene expression profiles

In Paper I there was a correlation of global gene expression profiles between stressed parents and their offspring, in relation to the controls and their offspring. Considering this finding critically it might seem unlikely that the genome-wide gene expression profile of the parents is inherited by the offspring, when brain sampling was done in adult parents and pre-adolescence offspring. The variation brought upon by development must be significant. Nevertheless, this was what we found. In contrast, Paper II showed no genome-wide correlation. Instead specific genes appeared as likely candidates for the transgenerational effects seen in the phenotype. The difference between Paper I and II is interesting for further investigation since the two breeds used in the different studies originates from the same lineage, which was diverged not long ago (Liljedahl *et al.*, 1979) (see also further discussion below).

In Paper II many genes showed possible transgenerational effects. One challenge of using microarray data is the grand number of genes tested at the same time without any apparent hypothesis, which makes it very difficult to calculate probabilities. Using microarray data in transgenerational studies is not widespread and in the near future it is important to develop statistical tools to interpret the results correctly.

Anyhow, by using qualitative assessment of the gene expression results in Paper II several immune genes seem to be up-regulated in stressed parents as well as in their offspring, and in particular the transcripts for the immunoglobulin light chain. Interestingly, mothers and sons are mostly affected. In addition, the offspring in Paper I, both from the wild type and the domesticated breed, shows the same trend (unpublished data). Other proteins of the immunoglobulin superfamily, like MHC class I and Thy-1, have shown to be important in neural development (Huh *et al.* 2000; Feng *et al.* 1993). Boulanger *et al.* (2001) have even claimed that there is no surprise that immune genes are involved in the development of neural networks, because the complicated plasticity of these systems must have gene regulatory mechanisms that can deal with such complexity. Immune genes are single genes with the capacity to produce millions of different transcripts (often translated into the variable regions of antibodies), which might provide

the basis of this complexity. It is possible that immune genes might play a role in the cross-generational phenotypic tuning of the behaviour in our study, which struggle with the same problem, but clearly more research is needed on this subject.

Mechanisms for inheritance of acquired characters

In both Paper I and II our chickens have shown an inheritance of an acquired behavioural trait. Indirect and direct epigenetic mechanisms (as defined in the introduction) have previously shown to give the mechanisms for this sort of inheritance (Richards 2006). Even though the evolutionary significance of these processes has been realised (Rando and Verstrepen 2007; Jablonka and Lamb 2007), it have not yet been proved, at least not in vertebrates (see Molinier *et al.* 2006 for an example in plants). Our results show that these processes might play a significant role in evolution, because especially in Paper II we can see adaptive responses to a parental environmental challenge already in the next generation offspring.

Considering indirect epigenetic mechanisms, neither Paper I nor II showed any differences in corticosterone levels in the eggs in response to the stressor. It is interesting that the adaptation to the chronic unpredictable light rhythm and the epigenetic transmission does not seem to be mediated by albumen corticosterone, which previously has been correlated with high stress levels in chickens (Downing and Bryden 2008). Yolk estradiol on the other hand is elevated in eggs from stressed birds, which indicates that this hormone might be a mediator of changes in gene regulation, since steroid receptors is known to have high impact on gene expression (Beato 1989). Furthermore, estradiol has previously shown to modulate feeding behaviours in numerous studies (Eckel 2004), and showed to specifically affect expression of clock genes like *PER1* and *PER2* in the rat brain (Nakamura *et al.* 2005). This is especially interesting because *PER2* was significantly differentially expressed between stressed and control parents in Paper II.

Altogether, Paper I and II shows specific responses in each breed. While transgenerational effects more or less are lacking in the red junglefowl, the two domesticated breeds do show behavioural transmissions, but appear to have distinct gene regulatory patterns; the genome-wide correlation of gene expression profiles between parents and offspring in the experimental inbred line (Paper I), was gone in the commercial hybrid line (Paper II). Interestingly, the breeds are expected to differ in their degree of inbreeding, where the experimental line is the most inbred, followed by the commercial hybrid and then the red junglefowl. It is tempting to speculate that loss of genetic variation might recruit epigenetic inheritance. Both inbreeding and stress are in nature connected with challenges to cope with the environment. Therefore it would be beneficial to increase phenotypic variation in times when populations experience for example bottlenecks and environmental stress, to increase the possibility that a new phenotype will emerge, which can cope better with the environmental challenges. Future studies should further investigate how epigenetic phenotypic tuning might be expressed in relation to the genetic background, because this might expand our understanding of how populations adapt to environmental challenges *e.g.* climate change, when they are on the edge of extinction.

Continuing with speculations, the large difference in transmission of the acquired traits between the two domesticated breeds and the wild type, suggest that there might be a factor brought upon by domestication. Loss of genetic variation and environmental stress (as discussed in the context of the above paragraph) is likely to have influenced populations undergoing domestication. Additionally, intense commercial breeding is focusing on selection for extreme traits. It appears logical to assume that extremes are individuals that use epigenetic factors to increase their variation. So, both in early and late domestication there could be selective pressures on traits that use epigenetic mechanisms to increase phenotypic diversity in the next generation. It could even be possible for future studies to locate genes that inhibit or stimulate epigenetic transgenerational inheritance, by studying genetic differences between wild type and domesticated breeds.

It gets more complicated to explain the more human related mismatch concept of developmental origins of health and disease, which also have been suggested to have transgenerational epigenetic mechanisms (Godfrey *et al.* 2007). The foundation of the mismatch concept is built upon the established phenomena that if mothers undergo starvation during pregnancy, their offspring will have low birth weights, which later in life if they encounter nutritional prosperity, will give them higher risk of for example diabetes and cardiovascular diseases (Pike *et al.* 2008). It is thought that the offspring in embryonic stages or as early neonates pre-adapts to low nutritional needs and that this gives coping problems in nutritional wealth, which possibly leads to higher allostatic load and in the long run disease. One crucial point here is the lag between birth and the diseased symptom. The phenotype seems to accumulate the effects of the allostatic load, or in common words, will experience more “wear and tear” (Goldstein and Kopin 2007). This can explain what is seen in Paper II, that there seem to be a lag between hatch and the appearance of differences in behaviour and weights of the offspring from stressed and control parents. But than again, more research is needed in this area.

Conclusions

By transmitting adaptive behavioural traits, chickens living under unpredictable stress seem to prepare their offspring, which have never been exposed to that specific stressor, for living under the parental stressful conditions. The underlying epigenetic mechanisms for this are very complicated, possibly involving sex specific transmissions of gene expression that are highly dependent on the genetic background. It seems possible that domestication, as well as inbreeding, might affect such inheritance of acquired characters. Conclusively, the results from the papers presented in this thesis stress the importance of reevaluating the division between stress responses and evolutionary adaptations, because according to our findings stress and evolution may be two sides of same event.

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