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	I	II	III	IV	V
Original idea	*				*
Study design	*			*	*
Data collection	*	*	*	*	*
Data analysis	*	*			*
Manuscript preparation	*	*	*	*	*

LIST OF ABBREVIATIONS

HPA-axis – hypothalamus-pituitary-adrenal-axis
CORT – corticosterone
MC1R – melanocortin 1 receptor
DEX – dexamethasone
PHA – phytohaemagglutinin
SEM – structural equation modeling
H/L – heterophil/lymphocyte

1. INTRODUCTION

1.1. Physiological ecology and neuro-endocrine-immune communication

Physiological ecology is a hybrid field integrating disciplines of physiology and ecology. Research in this field has two major goals: to understand how the interaction between an organism and its environment determine characteristics of individuals that are relevant to ecology, and to understand how these individual characteristics affect population and interspecific dynamics (Kingsolver 1989). In response to interaction with the environment organisms have developed highly complex nervous, endocrine and immunological systems to elicit appropriate responses to various biotic and abiotic stimuli. Up until recently, however, these systems were regarded as separate entities, especially in the case of immunity. Realization that nervous, endocrine and immunological systems are closely interconnected and are involved in a bidirectional communication gave rise to the field of immunoecology (ecoimmunology) in the 1990s (Martin *et al.* 2011) and psychoneuroimmunology in the early 1970s (Ader 2001).

Immunoecology is a newly emergent, interdisciplinary research field that examines interactions among host physiology and disease ecology in a wide range of environmentally relevant contexts (Demas & Nelson 2011). Immuno-ecological studies give great attention to extrinsic and intrinsic factors that explain why organisms vary in their immune responses to pathogens in different environments and time points. To ensure ultimate protection, it would make sense to use one's immune system maximally all the time. Yet organisms exhibit remarkable variability and seasonality in their immune function (Nelson & Demas 1996; Martin *et al.* 2008) and in capacity of defence against pathogens and parasites (Graham *et al.* 2011). Immunoecology proposes that susceptibility persists because immune defense is just one element of a context-dependent, integrated, whole-organism response to parasitism (Graham *et al.* 2011; Martin *et al.* 2011). Immunoecological research also assumes trade-offs between investment of resources into life-history traits (Ricklefs & Wikelski 2002) and various physiological processes (Moore & Hopkins 2009) that integrate the organism to form a whole. In order to understand these trade-offs, it is imperative to quantify the costs and benefits that accompany immune defenses and other vital physiological functions of an organism.

Among the many factors that can affect disease outcome is organism's stress response. In response to an acute stressor, the stress response prepares the nervous, cardiovascular and neuroendocrine systems for fight or flight response; it may also prepare the immune system for wounding or infection which may be imposed by the stressor (Dhabhar 2002). As the link between stress and immune function is bidirectional, the activation of immune system can also cause a rise in stress hormone levels (Adamo 2010). Although the general principles of

interaction between the stress response and immune system are known, the adaptive explanations are often incomplete (Adamo 2012). For instance, the question about why chronic stress in general appears immunosuppressive lacks definite answers (Miller *et al.* 2002; Segerstrom 2007; Martin 2009). E.g., assuming that the CORT response serves to "turn off" heightened immune responses that could result in autoimmune damage if sustained over long periods (Sapolsky *et al.* 2000; Martin 2009), still leaves the question about whether the benefits of avoidance of immunopathology would normally outweigh the costs of infection in immunocompromised organisms under natural conditions (e.g. Graham *et al.* 2005).

Brain-immune communication necessitates integrating behavior into the study of stress and immunity. Psychoneuroimmunology is a research field that combines the studies of the relationships among behavioral, neural, endocrine and immune processes (Ader 2001). Recently, researchers from a broad array of ecological sub-disciplines (e.g. population biology, ecophysiology, behavioral ecology) have begun to consider between-individual variation in behavior as an important ecological and evolutionary characteristic of wild populations (Careau *et al.* 2008). Consistent between-individual differences in behavior have been found in many animal species (Van Oers *et al.* 2004). Correlated repeatable and heritable behavioral traits form individually consistent behavior that have been referred to as temperament, personality, behavioral syndrome or coping style. The notion of behavioral syndrome or coping style suggests that behavior is not completely flexible and different behavioral traits do not vary independently of each other (Carere *et al.* 2010). For instance, aggressive individuals tend to be aggressive across different contexts, i.e. they cannot display optimal behavior in all situations. Aggressive behavior should be advantageous in contexts like territory defense or maintaining dominance in social hierarchy, but not in parental care. Studying correlations between different behavioral traits is a key to fully comprehend complex behavioral responses (Sih *et al.* 2004).

The extremes in individually consistent behavior and physiological characteristics in coping with stressful situations can be described as reactive versus proactive coping styles (Koolhaas *et al.* 1999). Individuals with proactive coping style are aggressive, bold and relatively inflexible in their behavioral responses, on the other hand, individuals with reactive coping style can be described as shy, precautious and behaviorally flexible. As there are individually consistent differences in behavior there are also respective consistent differences in stress responsiveness (Cockrem 2007; Bonier *et al.* 2009). These differences are related to differentially activated hypothalamus-pituitary-adrenal-axis (HPA-axis) and sympathetic nervous system, which could link stress responsiveness with personality (Koolhaas *et al.* 1999; Carere *et al.* 2010). Typically proactive, cue-independent, risk-prone individuals try to prevent or manipulate stressors and react to stressful situations with activation of the sympathetic nervous system with the increase of noradrenergic

stimulation. Reactive individuals on the contrary react with activation of the HPA-axis with the consequent rise in blood glucocorticoid (GC) levels (Carere *et al.* 2010). However little is known about the exact nature of causal relationship between differential behavioral and neuroendocrine characteristics (Koolhaas *et al.* 1999).

An excellent example of a mechanism how different physiological systems can be interconnected is provided by pleiotropic effects of genes that bind together physiological, morphological and behavioral traits. For example, genes regulating the synthesis of the main animal coloration pigment – melanin – are also involved in modulation of immune system, stress responses and behavior (Ducrest *et al.* 2008; Gangoso *et al.* 2011). Different animal color morphs have been associated with exploitation of alternative ecological niches, differential responses to immune activation, distinct wing, tail, tarsus, bill length and alternative mating strategies (reviewed by Roulin 2004). The information content of melanotic signals in birds is very diverse. Melanotic color signals are involved in sexual-selection, indicative of social rank or involved in inconspicuous appearance (e.g. camouflage) (Jawor & Breitwisch 2003). Already in 1982, William Hamilton and Marlene Zuk proposed that individual's appearance could reveal cues about organism's ability to successfully deal with infections (Hamilton & Zuk 1982). Now the evidence has started to emerge that melanin-based plumage color could be associated with organism's immune defenses (Ducrest *et al.* 2008; Gangoso *et al.* 2011). Differences in immune system responses could be reflected in melanotic plumage coloration which would thus eventually indicate disease susceptibility (Keyser & Siefferman 2005). Considering the enormous importance of parasites and pathogens as selective agents in the wild, one would also expect that melanotic pigmentation would provide cues predicting morbidity and mortality due to infections.

The second most widespread class of pigments – carotenoids – is fundamentally different from melanins as carotenoids cannot be synthesized by animals and must be acquired from food. As carotenoid pigments are thought to play important role not only in integument coloration but also in other vital physiological functions of the body, the carotenoid pigments have received a lot more attention in immunological and medical research. Therefore the number of studies documenting direct associations between melanotic plumage coloration and infection-induced mortality or morbidity in birds is far more limited (Keyser & Siefferman 2005) than there is for carotenoid-based plumage coloration (Nolan *et al.* 1998; Lindström & Lundström 2000; Hill & Farmer 2005; Van Oort & Dawson 2005; Svensson & Wong 2011; Simons *et al.* 2012).

In addition to pigmentation, other feather properties, such as size, integrity and hormonal content, are potentially valuable sources of information about physiological processes experienced by the organism during feather growth. Avian plumage could thus appear to be a powerful tool for studying the nexus between immune and neuroendocrine systems. Birds are regularly used for studying trade-offs between costly physiological systems, e.g. in the context of

survival, reproduction and development (Bennett & Owens 2002; De La Hera *et al.* 2009). However, lots of information that is decoded in the external morphology of birds appears still underused (Telleria *et al.* 2013) and researches have just started to realize the full potential of information gained from feathers and incorporating it to the main research areas in physiological ecology.

1.2. Information content of feathers

Feathers are the most conspicuous, complex, and varied (both in structure and color pattern) integumentary derivatives of birds (Stettenheim 2000; Prum & Williamson 2002). Essentially, a feather is a tubular integumentary appendage that grows at its base from a feather follicle (Prum & Williamson 2002). A developing feather is sensitive to energetic, nutritional and hormonal milieu (Murphy 1996; Stettenheim 2000; Hill & McGraw 2006). Therefore, various feather properties can reflect intrinsic and extrinsic conditions experienced by an organism during feather growth (Hill & McGraw 2006). The sensitivity to various internal and external stimuli is one of many qualities that makes feathers a unique and exceptional research material (Bortolotti 2010).

1.2.1. Feather size

Feathers are imperative for bird's survival, therefore all factors affecting molt have direct impact on bird's fitness. Reduction in plumage quality can influence survival by decreasing flight performance and increasing thermoregulatory costs (Nilsson & Svensson 1996; Swaddle *et al.* 1996; Dawson *et al.* 2000; DesRochers *et al.* 2009). Indirectly, plumage quality can impinge on fitness by mate choice (e.g. (Pryke & Andersson 2005). The functional relationships between different measures of feather size are diverse. Feather mass has been shown to correlate with the number of barbs, and hence the tightness of feather surface (Butler *et al.* 2008). Feather tightness enhances heat absorption, sustained flight (Butler *et al.* 2008) and resistance to abrasion (Dawson *et al.* 2000). Feather length affects take-off speed and maneuverability (Swaddle *et al.* 1996; Matyjasiak *et al.* 2004), and hence predator escape performance. Rachis diameter affects rigidity of feathers (Dawson *et al.* 2000). Fault bars are translucent areas across feathers that can decrease aerial performance due to brakeage of feathers at these sites (Møller *et al.* 2009). Fault bars form when feathers grow under stressful conditions. The incidence of fault bars have been associated with increased mortality in different bird species (Bortolotti *et al.* 2002; Møller *et al.* 2009). In captive birds, an interesting plumage characteristic that conveys information about individual behavior is a tendency to accrue damage to tail feathers in cages. It has been proposed that this trait could be indicative of the ability of bird to cope with captive conditions and is associated

with different immune parameters (Sild *et al.* 2011) and stress responsiveness (Krams *et al.* 2013). All these feather parameters could be potentially affected by physical, physiological or psychological stressors. Feather parameters could thus implicate its bearer's stress responsiveness, amount of encountered stressors or the ability to avoid stressful situations altogether. However, the information about the effects of pathogens and parasites on feather growth has only recently started to emerge (Pap *et al.* 2013), so it would be important to find out how stress- and immunity-related factors affect feather growth or quality and how it associates with fitness.

1.2.2. Feather corticosterone content

In vertebrates, the brain and immune system are linked by two primary pathways: the sympatho-adrenal system and the HPA-axis with the subsequent release of glucocorticoids (e.g. cortisol, corticosterone (CORT)) from the adrenal cortex (Demas *et al.* 2011). Glucocorticoid hormones are the end-product, primary effectors and principal negative regulators of the HPA-axis (Sapolsky *et al.* 2000). The main glucocorticoid to be released in response to stressful stimuli in birds is CORT. It has been proposed that CORT could be the most important hormone in the mediation of environmental effects on physiology and behavior (Demas & Nelson 2011). CORT has many different physiological and behavioral effects aimed to promote fitness at times of stressful events that include suppression of reproductive behavior, regulation of the immune system, increased gluconeogenesis and proteolysis, energy reallocation from fat stores and promotion of escape behavior (Sapolsky *et al.* 2000; Wingfield & Kitaysky 2002).

Due to its sensitivity to environmental perturbations, increases in CORT levels are often used to evaluate individual stress levels (Romero 2004) in ecological, physiological and conservation studies of wild animals. Stress is defined here as a physiological and behavioral state engaged to endure, avoid or recover from an aversive stimulus or condition (Martin 2009). The most common practice is to measure CORT from blood, either to evaluate baseline CORT levels or rise in CORT levels in response to stressor. Measuring CORT from blood though proves to be problematic due to rapid rise of CORT levels caused by investigator-induced stress of capture and handling (Romero & Reed 2005). Measuring CORT from urine or feces has also its own obstacles: problematic sample collection, species specificity and representation of relatively short time frame (Bortolotti *et al.* 2008).

A new method – measuring CORT from feathers – is expected to overcome all above-listed problems. Feather CORT measurement does not necessarily require capture of individuals, it is adjustable to all avian species and most of all, represents longer time periods as CORT is incorporated into feathers during feather growth (from days to weeks) (Bortolotti *et al.* 2008). Due to cumulative nature of CORT deposition into feathers, the feather CORT measurement has

the potential to integrate different aspects of HPA activity, including variation in baseline levels, magnitude and duration of the stimulated stress response and the number of stressors experienced (Bortolotti *et al.* 2008; Lattin *et al.* 2011). The most important advantage in assessing CORT levels from feathers is long-term retrospective measure of stress (Bortolotti *et al.* 2008), which has the potential to add new insights to avian ecology and stress physiology research. For example, feather CORT measurement can be used to tease apart long-term costs on feather growth relating to immune activation or suppression, a question that has been previously overlooked. Feather CORT measurement can also be implemented in conservational biology, avian ecology studies or for example in long-term monitoring of populations or historical trends in CORT using museum specimens, as CORT is stable over long time and in different environmental conditions (Bortolotti *et al.* 2009).

However, several basic aspects of information content of feather CORT measurement are still unknown. For example, it is not known exactly how and when CORT is deposited into feathers and to what extent the variation in feather CORT represents variation found in blood baseline vs induced CORT levels (Bortolotti *et al.* 2008; Lattin *et al.* 2011). Furthermore, virtually nothing is known of the impact of immune system activation on feather CORT or how it relates to other physiological and behavioral measures of stress. Addressing these issues is important as it would facilitate understanding and interpretation of the information content of feather CORT levels.

1.2.3. Eumelanotic coloration

Melanin is the most abundant and widespread pigment in birds and other animals (Hill & McGraw 2006). Melanins consist of two main pigment types-eu- and phaeomelanins. Eumelanin forms grey and black colors whereas phaeomelanin forms brown, red or buff colors (McGraw 2006). Melanin-based coloration is frequently implicated in social communication (Hill & McGraw 2006a) and covaries with many other physiological, morphological and behavioral traits (Roulin 2004). Ducrest and coauthors (2008) suggest that pleiotropic effects of key regulators of melanogenesis might be responsible for the widespread association between melanin-based coloration and other phenotypic traits in wild vertebrates (Emaresi *et al.* 2013). Melanocortins are posttranslational products of the proopiomelanocortin gene. In addition to binding to melanocortin 1 receptor (MC1R) that triggers production of melanin pigments, melanocortins also bind to four other melanocortin receptors (MC2-5R) which have very different functions. These functions include, among others, anti-inflammatory activity, HPA stress response, energy expenditure, sexual activity and aggressiveness (Ducrest *et al.* 2008). By binding to MC4R, melanocortins increase organism's resistance to stressors. By binding to MC1R and MC3-5R, melanocortins reduce acute, allergic and systemic inflammation, septic shock and increase antipyretic activity (Gangoso *et al.* 2011). Based on

this, Ducrest and coauthors (2008) proposed that darker eumelanotic individuals should be less sensitive to stressful factors and have better anti-inflammatory, antipyretic and anti-oxidative responses than lighter individuals. These predictions need to be tested with special attention directed to ecological, social and physiological factors that influence and maintain inter-individual variation in melanin-based coloration and associated phenotypic traits.

1.3. Aims of the thesis

Some feather characteristics have been used in ecological research for a long time, e.g. ptilochronology (the study of growth rates of feathers by the measuring of growth bars (Grubb 1989)) has been used in the field of nutritional ecology for several decades. Interest in feather coloration dates back even further to Darwin and Wallace, with the initial focus on evolution and function of feather coloration (Hill & McGraw 2006). Although thoroughly studied, morphological and color characteristics can still provide novel information about organism's endogenous and exogenous processes in conjunction with the advances in methods used in ecological physiology and with emergence of new fields, such as immunoecology and psychoneuroimmunology. One of such new methods is measuring glucocorticoid hormones from feathers which I will employ in my thesis in collaboration with feather size, integrity and color parameters.

Three papers of this thesis address the question about effects of experimental manipulation of physiology of greenfinches on different parameters of feathers. In the first study, I asked whether a single administration of synthetic glucocorticoid, dexamethasone (DEX) and induction of inflammatory response by administration of plant toxin (phytohaemagglutinin, PHA) affects feather growth in adult birds **(Paper I)**. Elevation of glucocorticoid hormone levels is an integral part of stress response (as well as its termination) and immunomodulation (Sapolsky *et al.* 2000; Perretti & D'Acquisto 2009). These hormones are also responsible for mobilizing energy stores by stimulation of gluconeogenesis and inhibition of protein synthesis (Remage-Healey & Romero 2000). Elevation of glucocorticoids is thus incompatible with other protein-demanding processes like molt. Previous studies have shown that chronic elevation of GC hormones suppresses feather growth (Romero *et al.* 2005; Strochlic & Romero 2008; DesRochers *et al.* 2009). Here I asked whether similar effect would also occur in the case of acute GC elevation and induction of an inflammatory response by foreign antigen and whether the potential somatic cost of elevated CORT levels is of comparable magnitude with the one induced by the immune system activation. The latter question is interesting because both immune and stress responses deplete somatic resources; however, the question about which of those is more costly has never been directly assessed. I measured three different parameters of feather size in order to find

out which of these are most sensitive to treatments, and hence most indicative of physiological insults experienced during feather growth. This information is of methodological interest for studies aiming to quantify somatic impacts of physiological manipulations in birds.

To add further ecological dimension to the study, I asked whether supplementation of dietary carotenoids affects the expenditures imposed by immune activation and elevated CORT levels. Carotenoids are dietary pigments with proposed immunomodulatory and antioxidative properties (Chew & Park 2004). Few previous studies have shown that carotenoid supplementation can attenuate body mass loss imposed by immune challenge (Hõrak *et al.* 2006; Pap *et al.* 2009) and that CORT administration can interfere with carotenoid sequestration to integument (Loiseau *et al.* 2008; Cote *et al.* 2010, but see Costantini *et al.* 2008). Therefore, I tested whether carotenoid supplementation will positively affect feather growth of birds receiving immune activation and GC challenges.

In the second study, I tested the effects of immune activation and suppression on feather CORT content **(Paper II)**. Until recently (Meitern *et al.* 2013), there was no information about whether and how activation or suppression of immune system affects feather CORT levels and how feather CORT relates to other physiological measures of stress, such as differential leukocyte counts and an individual's ability to mount immune responses. Explaining these issues is important for interpretation and understanding the information content of feather CORT levels in studies using this marker for characterizing stress history of individuals and populations. For instance, both the acute phase response and avian stress response share some of the same neuroendocrine control mechanisms such as the HPA-axis (Owen-Ashley & Wingfield 2007) and inflammatory agents can be considered as 'immunological stressors' (e.g. Laugero & Moberg 2000; Xie *et al.* 2000). Because inflammatory responses are usually accompanied by the rise in blood CORT levels (Sapolsky *et al.* 2000), I was interested in testing whether induction of inflammation will affect deposition of CORT in feathers.

In the **Paper III** I continued with measuring the effects of immune system manipulation on feather CORT by asking whether experimental infection with a natural pathogen (coccidian intestinal parasites) affects feather CORT levels. Additionally I asked whether and how are feather CORT levels of greenfinches related to resistance to experimental infection and the measure of captivity-intolerance, i.e., the amount of damage accrued to tail feathers in captivity. Extent of tail damage is expected to reflect captivity-intolerance in greenfinches, because this trait correlates with the frequency of flapping bouts against cage walls (Sild *et al.* 2011). I therefore predicted that the extent of tail damage accrued in captivity correlates positively with feather CORT content as indicator of susceptibility to stress.

In the **Paper IV** I tested whether feather CORT levels are indicative of personalities of greenfinches assuming the existence of a "fearfulness

syndrome", based on expected covariation of behavioral responses to different fear-eliciting situations. For this purpose I recorded individual variation and consistency of five behavioral traits that were presumably indicative of responses to fear-eliciting and stressful situations. Because individual differences in behavioral responses to stressful situations are at least partly mediated by differences in neuroendocrine activity (Koolhaas *et al.* 1999; Carere *et al.* 2003; Kralj-Fiser *et al.* 2007), I expected to discover a covariation between the amount of CORT deposited into feathers grown in captivity and at least some of the behavioral measures of fearfulness.

In the **Paper V** I studied whether feather size and color parameters predict survival in greenfinches facing epidemic of emerging infectious disease, finch trichomonosis. Plumage characteristics have been long thought to reveal individual's ability to deal successfully with infections (Hamilton & Zuk 1982; Lozano 1994; Moreno & Møller 2006; Simons *et al.* 2012). However, despite the relatively well-documented evidence about covariation between feather characteristics and survival (see Jennions *et al.* 2001; Roulin & Altwegg 2007; Saino *et al.* 2013), the number of studies documenting direct associations between plumage characteristics and infection-induced mortality or morbidity has remained low. Here I report about associations between plumage characteristics and trichomonosis-induced mortality in wild-caught greenfinches in captivity. Trichomonosis is caused by the flagellate protozoan parasite *Trichomonas gallinae*, which is well known historically in pigeons and raptors and can cause high mortality. The parasite inhabits the upper digestive tract of the bird and spreads through saliva. Spread of trichomonosis since 2005 has coincided with extensive reductions in breeding passerine (especially greenfinch) populations in Great Britain and Northern Europe. In 2013, 11 % of female wild-caught greenfinches brought into aviary died of trichomonosis, which provided a unique opportunity to measure survival selection in relation to feather parameters. I first asked whether the variation in parameters of feather size that were sensitive to glucocorticoid administration and immune system activation predict survival of epidemics. In addition, I tested whether survival could be predicted on the basis of the occurrence of fault bars and eumelanin-based coloration of black feather tips.

2. METHODS

Using wild-caught captive greenfinches (*Carduelis chloris*) in research of physiological ecology has many advantages. First, they tolerate captivity stress well (Sepp *et al.* 2010) and are easy to maintain in laboratory conditions. Second, their biology is well known as greenfinches have been widely used as a model species in ecological studies (Lilliendahl 2000; Lindström *et al.* 2001; Peters *et al.* 2008; Samas *et al.* 2012; Minias & Iciek 2013). Third, they are naturally infected with coccidian parasites that are relatively easy to trace and manipulate.

Greenfinches are medium-sized (ca. 28 g) sexually dichromatic gregarious seed-eating passerines native to the western Palearctic region. Males are larger and more colorful than females, with older males developing olive-green plumage on the back, bright or greenish yellow color on the breast, and striking bright yellow markings on the primaries, primary coverts and sides of the tail feathers (Cramp & Perrins 1994). Females are more olive-brown and yellowish-buff, having faint brown streaks on back and lacking full yellow tints in their plumage.

All the birds (N = 45–93) participating in our studies were captured in mist-nets at bird feeders either in the Sõrve Birds Station in Saaremaa or in a garden in the city of Tartu in December or January and released in early March. The birds were housed indoors in individual cages (27 × 51 × 55 cm) with sand-covered floors. The birds were supplied *ad libitum* with sunflower seeds and tap water. Birds were held on the natural day-length cycle on artificial lighting by luminophore tubes.

Feathers of birds have the distinct advantage of being periodically replaced in a predictable fashion and time scale that allows for repeated assessment of developmental history of birds (Bortolotti *et al.* 2002). Another useful property of feathers is that they provide information about organism's physiology in a non-invasive way, for example as compared to blood sampling or killing of birds. In addition, feather size, integrity and color are easy to measure. I used feather mass, length and rachis diameter as a proxy to feather quality (**Papers I, V**). Direct measures of feather strength, durability or aerodynamic properties are more difficult to obtain and may be less reliably measured (than using high-resolution scale, simple ruler or spessimeter) (De La Hera *et al.* 2009). Several studies have also shown that rachis width and feather mass explain a large part of variation in mechanical performance of feathers within populations (Dawson *et al.* 2000; De La Hera *et al.* 2009). Feather integrity (**Papers III, IV**) and fault bars (**Paper V**) are directly observable and assessable; the color of feathers can be measured spectrophotometrically (**Paper V**).

Mortality by infectious diseases enables to observe natural selection in action, as well as consequences of these selection pressures on the appearance of birds. Differential survival of infected birds is also potentially informative about how coloration and immune processes are interconnected and how feather

coloration has evolved (**Paper V**). Many infectious diseases, however, are not highly virulent and hosts are able to tolerate chronic infections. One of such infections is coccidiosis, which is caused by obligate intracellular parasite of the genus Isospora that infect a number of songbird species (Greiner 2008). All greenfinches that have been caught and examined in our laboratory have been naturally chronically infected with coccidians. Coccidiosis of wild birds is becoming a popular model of parasite-mediated selection that enables to avoid assessment of immune function on the basis of responses to artificial antigens that may not appear optimal predictors of infection resistance or disease outcome (Adamo 2004; Saks *et al.* 2006). High prevalence of coccidiosis in our research model enabled us to test whether chronic infections relate to feather CORT content and how feather CORT levels are related to resistance to experimental infection (**Paper III**).

In order to find out more about information content of feather CORT levels, I tested the hypothesis that feather CORT levels reflect variation in avian personalities (**Paper IV**). This hypothesis relies on the assumption that feather CORT is indicative of consistent between-individual differences in responsiveness to fear-eliciting situations. To test this hypothesis I had first to quantify behavioral responses to different stimuli and test whether between-individual differences in such responses are repeatable in time. The variables that included behavioral responses to fear-eliciting stimuli were propensity to give distress calls at handling, latency to feed at the presence of a predator image, and changes in locomotor activity in response to distress calls of conspecifics. Additionally, I measured the frequency of flapping flight movements against cage bars and the amount of damage accrued to tail feathers in captivity as a measure of captivity-intolerance. The next step was to test whether and to what extent the measured behavioral traits and feather CORT covary and if this covariation reveals the existence of general "fearfulness syndrome". This was analysed by structural equation modelling (SEM).

3. RESULTS AND DISCUSSION

3.1. How do immune activation and glucocorticoid administration affect feather growth?

Immune defenses are considered extremely complicated and resource demanding organismal functions (Lochmiller & Deerenberg 2000; Demas 2004; Schulenburg *et al.* 2009). Activated immune responses impose costs, which eventually can lead to physiological trade-offs in allocation of resources between immunity and other vital functions. Glucocorticoid hormones are important down-regulators and terminators of immune (Perretti & D'Acquisto 2009) and also stress responses (Sapolsky *et al.* 2000). Rise in baseline, as well as in stress-induced levels of CORT has been shown to suppress feather growth (Strochlic & Romero 2008; Müller *et al.* 2009). This occurs via inhibition of protein synthesis and mobilization of endogenous glucose stores (Remage-Healey & Romero 2000). Quality of feathers can in turn affect thermoregulation, flight (Butler *et al.* 2008), resistance to abrasion, rigidity of feathers (Dawson *et al.* 2000), take-off speed and maneuverability (Swaddle *et al.* 1996; Matyjasiak *et al.* 2004) with potential effects on fitness. In the **Paper I**, I asked whether a single, short-term elevation of CORT levels can also affect feather growth in adult birds. To put this information into wider ecophysiological context, I tested whether such potential somatic cost is of comparable magnitude with the one induced by immune system activation. Both immune and stress responses deplete somatic resources; however, the question about which of those is more expensive has never been directly assessed. To add further ecological dimension to the study, I also asked whether supplementation of dietary carotenoids affects the possible somatic cost imposed by immune activation and immune suppression. Carotenoid-based signals have been extensively studied by animal ecologists since recognition of their possible role in health maintenance and signaling. Carotenoids can modulate immune function (Chew & Park 2004) and may also act as antioxidants (Krinsky 1989, but see Costantini & Møller 2008). So far, few studies have shown that carotenoid supplementation can attenuate body mass loss imposed by immune challenge (Hõrak *et al.* 2006; Pap *et al.* 2009; Sepp *et al.* 2011) and that CORT administration can interfere with carotenoid sequestration to integument (Loiseau *et al.* 2008; Cote *et al.* 2010).

93 male greenfinches were assigned to groups of immune activation and glucocorticoid administration in a factorial manner. The experiment involved induction of immune response by subcutaneous injection of phytohaemagglutinin (PHA), a plant lectin known to induce localized swelling response which is used as an indicator of strength of immune response (Tella *et al.* 2008). Glucocorticoid administration consisted of a single injection of synthetic glucocorticoid dexamethasone (DEX). DEX is a CORT agonist which, binding to the same receptors, decrease endogenous CORT production via

negative feedback on the hypothalamic-pituitary cascade (reviewed by Holberton *et al.* 2007). DEX injections are routinely used for immune suppression (Huff *et al.* 1999) and manipulation of CORT levels (Rich & Romero 2005). Carotenoid supplementation consisted of birds receiving high and low doses of carotenoids or water. Upon arrival to the laboratory one tail feather (rectrix) from all the birds was removed. Feather mass, length and rachis diameter of wild-grown as well as replacement feathers were recorded.

This study clearly showed that a single boost of glucocorticoid hormone is sufficient to restrict feather growth of wild birds, despite the benign conditions and *ad libitum* access to food in captivity. This raises the possibility that experiencing an acute stress or termination of immune responses by elevation of glucocorticoid hormones may induce long-term somatic costs with a potential impact on fitness. DEX injection significantly reduced all three measures of feather size. The most probable way how DEX could affect feather growth is through its property of suppressing protein synthesis (Sapolsky *et al.* 2000). This indicates that feather size could contain information about the ability of its bearer to avoid stressful situations or block the catabolic effects of CORT during stress. As DEX injection suppressed feather growth, it has practical implications for studies using DEX to study stress physiology, where possible somatic costs, accompanying the physiological effects of DEX, need to be considered. In further studies it would be most interesting to evaluate whether a natural downregulation of HPA-axis by negative feedback mechanism can also induce similar somatic costs to an exogenous glucocorticoid administration.

Immunostimulation by PHA reduced feather length, but did not affect feather mass or rachis diameter. This implies, that in face of resource limitation, investment into feather mass (which increases feather strength) is prioritized over investment into feather length. This study adds another piece of evidence to what we know about somatic costs of induced immune responses in terms of feather growth. Importantly, this study also exemplifies the value of measuring several parameters of feather size. For example, I would not have detected the impact of immune activation by measuring only feather mass.

Previous studies in greenfinches have shown that carotenoid supplementation can incline birds to fattening and alleviate body mass loss induced by immune challenge with sheep erythrocytes (Hõrak *et al.* 2006) or PHA (Sepp *et al.* 2011). Current experiment showed that dietary carotenoids had no effect on feather growth, suggesting that any anabolic effects of carotenoids could involve lipid rather than protein metabolism. Altogether these results demonstrate the sensitivity of feather growth to manipulations of immune and adrenal functions. These findings reinforce the potential utility of feather parameters for assessment of somatic impacts of physiological manipulations in birds.

3.2. Synthetic glucocorticoid inhibits CORT deposition into feathers

Assessment of individual well-being on the basis of glucocorticoid hormone levels has become a standard tool in ecological, physiological and conservation studies of wild animals. In birds, CORT is the primary glucocorticoid hormone that has been linked to the promotion of an emergency life-history stage (Wingfield & Sapolsky 2003), and which stimulates behaviors and physiological processes aimed at recovering from disruption of homeostasis caused by external stressors (reviewed by Johnstone *et al.* 2012). Feather CORT content is thus a potent source of information relating to activation of the HPA-axis during feather growth which can be used for assessment of well-being and stress history of individuals. However, despite increasing popularity of feather CORT measurement in avian studies, the research aimed at understanding the processes underlying CORT deposition into feathers and their interconnections to other physiological processes is still only in its infancy.

My objective in the **Paper II** was to study different factors affecting CORT deposition into feathers and how feather CORT covaries with other markers of stress imposed upon individuals during feather growth. I used the same dataset as in the **Paper I**, consisting of 93 male greenfinches that were assigned to treatment groups in a factorial manner. To mimic acute stress half of the birds were injected with DEX, half with PHA to induce inflammation. Upon arrival to the laboratory one tail feather from all the birds was removed. CORT levels of wild-grown as well as replacement feathers grown in the captivity were recorded. Birds were blood sampled three times during the period of feather growth, which enabled to test whether leukocytic markers of stress (concentrations of total leukocytes, heterophils, lymphocytes and heterophil/lymphocyte (H/L) ratio) correlate with feather CORT levels. Heterophils and lymphocytes comprise the majority of circulating immune cells in birds and H/L ratios are specifically sensitive to either natural stressors or administration of stress hormones, so that relatively high heterophil counts in relation to lymphocytes reliably indicate high glucocorticoid levels (Ots *et al.* 1998; Davis *et al.* 2008). Assessment of swelling response to PHA enabled to test whether feather CORT levels relate to the magnitude of cutaneous inflammatory response to the antigen.

From the two experimental treatments applied, only DEX injection had significant effect on feather CORT levels. DEX-injected birds had on average 37% lower level of CORT in their feathers than saline-injected birds. Because the physiological effects of DEX are mediated by glucocorticoid receptors and DEX, like endogenous glucocorticoids, ultimately reduces CORT concentrations by forming the negative feedback loop that controls CORT secretion (Bennett *et al.* 2013), the DEX-induced down-regulation of circulating CORT levels provides the most parsimonious explanation for the observed pattern. This finding provides novel evidence that feather CORT levels are

sensitive to manipulation of hormonal balance of birds, thereby supporting the diagnostic value of feather CORT measurements. This result compares favorably with those of Lattin and coauthors (2011) who used CORT implants to increase feather CORT levels and with a recent study by Fairhurst and coauthors (2013a) showing that feather CORT correlated with circulating CORT levels in some (but not all) stages of feather growth.

Absence of the effect of PHA injection on feather CORT is noteworthy as inflammatory responses generally increase circulating CORT levels (Sapolsky *et al.* 2000; Owen-Ashley & Wingfield 2007). Therefore, I expected that PHA-injected birds deposit more CORT into their feathers than saline-injected birds. One possible explanation is that PHA injection induced negative feedback loop that would down-regulate inflammation-induced surge of CORT, so that the eventual amount of CORT secreted during feather growth would equal that of control group. Alternative explanation would be that although PHA injection caused mild stress, increase in chronic stress level and CORT concentration was not severe enough to increase feather CORT. Cutaneous swelling response to PHA and feather CORT levels did not correlate either. This suggests that measuring feather CORT would give information about more severely increased chronic stress level and would appear a relatively "noise-free" method, not affected by smaller stressors.

Surprisingly there were no significant correlations between feather CORT levels and circulating leukocyte concentrations, measured at three stages of feather growth. Assessment of stress on the basis of H/L ratio is considered as an indicator of stress over longer period of time than on the basis of glucocorticoid hormone levels in the blood (Davis *et al.* 2008; Müller *et al.* 2010). Therefore I expected that birds with high heterophil haemoconcentrations and H/L ratios deposit more CORT into feathers grown in captivity, especially given that PHA injection in the current experiment resulted in elevated heterophil haemoconcentration and H/L ratios for at least 30 days (Sarv & Hõrak 2009). Absence of such correlations indicates that circulating leukocyte counts and feather CORT levels convey differential information about HPA-axis activation in birds. Hence, these markers cannot substitute each other, but appear rather complimentary indicators of stress status of birds (Davis *et al.* 2008; Müller *et al.* 2010). In conclusion, this study suggest that assessment of health state and stress history of birds on the basis of feather CORT levels appears more complicated than previously thought and calls for further research on information content of feather CORT.

3.3. High feather CORT indicates better resistance to experimental coccidian infection

Even when exposed to the same parasites and pathogens, some individuals manage to cope with their infections better than others. The questions about the causes and consequences of these processes have remained largely unclear, particularly as regards wild animals and their natural pathogens. Differential exposure or sensitivity to stressors may have substantial effects on the variation in immune responsiveness of animals. Furthermore, there is a reason to expect that interrelationships between stress and immune responses relate to animal personalities or coping styles (Segerstrom 2007; Koolhaas 2008). Proactive coping styles tend to be coupled with efforts to prevent or manipulate stressors, high sympathetic nervous system activity and a Th1 biased (inflammatory) immune system. Reactive coping styles entail high HPA-axis reactivity and a Th2 biased (humoral) immune responses (Koolhaas 2008; Martin 2009, but see Martins *et al.* 2007). One might thus expect that individual differences in disease resistance relate to behavioral phenotypes. In the **Paper III**, I ask whether infection with a natural pathogen affects feather CORT levels and how a potential marker of stress responsiveness, the feather CORT content, reflects the resistance to an experimental infection with natural coccidian parasites. To study the link with behavior, I measured the captivity-intolerance (as a potential marker of individual susceptibility to stress on the basis of the extent of tail damage accrued in captivity) of birds and predicted that it will correlate positively with feather CORT content.

All 56 male greenfinches that were involved in the study appeared naturally infected with isosporan coccidians at capture. The birds were subsequently divided to control group and experimental infection group. At the beginning of the experiment all birds were subjected to anticoccidian medication with Toltrazuril in order to standardize their infection status. Toltrazuril specifically reacts to apicoplast of coccidians, which is unique to the Apicomplexan parasites (see Sepp *et al.* 2012). Subsequently, infection group was infected orally with sporulated heterologous coccidian oocysts. At the same time control group received another round of medication in order to increase differences in infection intensity between infected and control birds. In order to monitor the course of infection, fecal samples were collected at different time points of the study. Upon arrival to the laboratory one tail feather from all the birds was removed and CORT levels of wild-grown as well as replacement captivity-grown feathers were recorded. Before the birds were released their tail feathers were photographed under standard conditions in order to assess tail damage.

As expected, I found that extent of tail damage accrued in captivity correlated positively with captivity-grown feather CORT content. The most likely explanation for this pattern would be that some greenfinches, i.e., those with the most damaged tails, possessed more stress-prone personalities than others and that these differences were mirrored in feather CORT content. This

explanation is consistent with finding of Sild and coauthors (2011), showing that the propensity to perform flapping flight bouts against cage walls (which correlated with tail damage scores) was individually consistent over 57 days; qualifying thus as a personality trait according to Reale and others (2007).

This study demonstrated that coccidian infection (or it's suppression) is associated with CORT secretion in wild bird species. Birds subjected to experimental infection had on average 23% higher CORT content in their captivity-grown tail feathers than sham-inoculated individuals. Unexpectedly, the pre-infection medication had long-term effects, so that post-infection levels of oocyst shedding among experimental birds never reached pre-experimental levels. Thus, I cannot distinguish whether differences in feather CORT between infected and un-infected birds were primarily caused by enhanced CORT secretion due to novel infection or due to reduction of CORT levels among control birds that were relieved from chronic infection.

The most surprising result of this study was positive correlation between feather CORT content and measures of resistance to an experimental infection. Experimental birds with lowest feather CORT levels developed highest infection intensities at peak infection phase as well as highest increase in average infection levels. The most likely explanation for such pattern is that birds that invest more into fighting novel coccidian infections have higher stress responsiveness or that birds that experience captivity as more stressful have higher resistance to novel infections. On the basis of current data these explanations are difficult to discern. Interpretation of these findings is further complicated by conjunctions between chronic and acute stress responses and CORT secretion. Captivity can cause both acute and chronic stress; in turn, chronic stress can suppress baseline and acute-stress-induced CORT levels in birds (Rich & Romero 2005; Cyr & Romero 2008; Cyr *et al.* 2009). Hence, it would be difficult to distinguish whether elevated CORT levels in more resistant birds reflect unhealthy chronic stress or a healthy coping response (Cyr & Romero 2007). Assuming that feather CORT is affected more by stress-induced stimulation than baseline levels of CORT (Bortolotti *et al.* 2008), our results would support an idea that sustaining higher stress responsiveness in captivity is beneficial. Under this scenario, birds that were experiencing less stressful events, or perceiving their environment least stressful, appeared most susceptible to novel infection with heterologous parasite strain. Finally, I cannot exclude the possibility that high feather CORT levels could primarily reflect the importance of this hormone as a mediator of energy-allocation processes, rather than stress responsiveness *per se*. Thus, high feather CORT among the most resistant individuals could have reflected high energetic demand/expenditure associated with efficient immune responses within the normal stress reactive scope (see (Romero *et al.* 2009).

Coccidians are the most prevalent avian parasites (Greiner 2008) with well documented pathological effects (reviewed by Pap *et al.* 2011; Sepp *et al.* 2012), so the benefits of increased resistance in the wild is obvious. Findings of

this study suggests that further research on CORT levels in relation to resistance to coccidiosis might increase our understanding about the mechanisms linking individual variation in CORT levels to fitness (Breuner *et al.* 2008; Angelier *et al.* 2009).

3.4. Is feather CORT indicative of avian personalities?

Animal behavior is not completely flexible and different behavioral traits do not vary independently of each other (reviewed by Sih *et al.* 2004; Carere *et al.* 2010). Research on consistent between-individual differences in suites of correlated traits, known as behavioral syndromes or personalities is becoming increasingly popular in behavioral ecology (Sih *et al.* 2004; Dingemanse & Réale 2005; Careau *et al.* 2008).

Individual differences in behavior are particularly apparent when individuals face mildly stressful situations (Koolhaas *et al.* 1999). Challenging conditions, for example the presence of a predator in the environment, seem to induce correlations between behavioral traits (Bell & Sih 2007; Dingemanse *et al.* 2007). Fear is prompted by imminent and real danger, and galvanizes active defensive responses (Davis *et al.* 2010). Fearfulness can thus be defined as a basic psychological characteristic of the individual that predisposes it to perceive and react in a similar manner to a wide range of potentially frightening events (Boissy & Bouissou 1995).

Individual differences in behavioral responses to stressful situations are at least partly mediated by differences in neuroendocrine activity (Koolhaas *et al.* 1999; Carere *et al.* 2003; Kralj-Fiser *et al.* 2007). Individuals with proactive, risk-prone, aggressive and bold personalities respond to stress with a strong sympathetic activation and increase in noradrenergic stimulation. In contrast, individuals with a passive, risk-averse, non-aggressive, shy personality respond to stress with a strong HPA stimulation and a consequent increase in circulating glucocorticoids (Carere *et al.* 2010, but see Kralj-Fiser *et al.* 2010 and Koolhaas *et al.* 2011 for opposite results). Such patterns indicate that individual variation in stress responsiveness is linked to behavioral syndromes (Carere *et al.* 2010; Garamszegi *et al.* 2012). In this context, the covariation between stress hormone secretion and behavioral fear responses is of particular interest. Aim of the **Paper IV** was (1) to test for the presence of "fearfulness syndrome" in captive greenfinches and (2) to determine whether stress responiveness (or suceptibility) of individuals, assessed on the basis of feather CORT is a part of this syndrome.

In 45 male greenfinches, altogether five behavioral traits were recorded that were expected to contribute to the general "fearfulness syndrome" on the basis of previous observations in greenfinches (Sild *et al.* 2011) and great tits (*Parus major*) (Krams *et al.* 2013). CORT levels of wild-grown and replacement tail feathers grown in captivity during the study were measured. Measured

behavioral variables included latency to feed at the presence of a predator image, the propensity to give distress calls at handling, changes in locomotor activity in response to distress calls of conspecifics, frequency of flapping flight movements against cage bars and tendency to damage tail feathers in captivity. Associations between these traits and repeatability in time were assessed in correlation analyses. The presence of a behavioral syndrome was tested by structural equation modelling (SEM).

All behavioral traits were repeatable in time. In an unprovoked situation, without any outside disturbance, flapping frequency against cage bars correlated with damage accrued to tail feathers. This confirms the findings of Sild and coauthors (2011) and indicates that in this model system, damage to tail feathers describes the behavior of birds in captivity and can be used as a proxy of captivity tolerance.

None of the behavioral parameters correlated with feather CORT levels. This result was unexpected but compares favorably to a study of collared flycatchers (*Ficedula hypoleuca*), where individuals with higher levels of fecal CORT metabolites did not show consistently higher or lower behavioral scores along the shy/bold spectrum, and where environmental challenges owing to the presence of a novel object, territorial intruder and a potential predator caused no elevation in the level of fecal CORT metabolites (Garamszegi *et al.* 2012). The authors of this study emphasise that the relationship between CORT levels and behavior, if it exists at all, is likely to be of small magnitude, a conclusion that is also supported by our data. Generality of this conclusion, however, awaits for further confirmation because feather CORT levels correlated with problem-solving performance in a recent study of house sparrows (*Passer domesticus*) (Bókony *et al.* 2014). It is also possible that feather CORT levels did not correlate with behavioral measures of fear because the behaviors are taking place on a different time scale than the growth of captivity-grown feathers. For instance, Fairhurst and coauthors (2013a) recently showed that feather CORT correlated with blood hormone levels only in some days of feather growth in nestling tree swallows (*Tachycineta bicolor*).

I was particularly surprised about the absence of correlation between CORT deposited to captivity-grown feathers and flapping frequency. This finding contradicts that of the previous study (**Paper III**) where a positive association between feather CORT and tail damage was detected. This contradiction between the findings of two studies in the identical model system is difficult to explain. One might argue that exposure of birds to different potentially fear-eliciting stimuli in the current study could have obscured the relationship between HPA-axis activity and tail damage that appeared in the previous study, where such stimuli were not present. On the other hand, we also failed to find any evidence that behavioral responses to fear-eliciting stimuli had correlated with feather CORT or tail damage scores. A conclusion from this part of the study is that tail damage does not appear a universal correlate of stress responsiveness in captive greenfinches.

None of the measured behavioral parameters correlated with each other. This is an intriguing result, considering that all of these traits (latency to feed in a presence of a predator/novel object, propensity to give distress calls, response to stress calls of conspecifics, flapping frequency in cages) can be intuitively associated with fearfulness or anxiety. However, no "fearfulness syndrome" emerged from our data, as shown also in the SEM analysis. None of the a priori composed hypothesis about causal relationships between measured traits proved to be better than the null model assuming independence of traits. This indicates that responses to different fear eliciting situations might appear more uncoupled than expected and could be condition- and context-dependent. At the same time, all the behavioral variables showed individual consistency in time, which hints that these traits might reflect some aspect of personality (Réale *et al.* 2007; Dingemanse & Wolf 2010).

According to Boissy and Boissou (1995), consistency of individual responses to different fear-eliciting situations reflects the existence of an underlying psychological profile, currently labelled fearfulness. However, the question of fear as a single variable has been challenged by the recognition of its multidimensionality: very diverse events may generate fear responses, and it is likely that these events are not perceived equally by animals in diverse internal states (Boissy 1995; Saint-Dizier *et al.* 2008). The findings of this study challenge the concept of a single internal variable responsible for fearfulness and support the proposed multidimensional nature of fear responses.

3.5. Emerging infectious disease selects for darker plumage coloration

Parasites exert persistent selection pressure on all organisms. Hosts differ in their vulnerability to infections and these differences could be mirrored in individual's appearance as immune system is integrated with almost any physiological function of an organism. Color and morphology of feathers provide ample cues about the physiological condition of individual birds during feather growth. For instance, most common feather pigments – melanins and carotenoids – can be indicative of the individual's developmental and hormonal milieu, their ability to find and process specific micronutrients and of immunological and oxidative challenges experienced during feather growth (reviewed by Hill & McGraw 2006). Additional information about the individual's state during feather growth is provided by the physical properties of the feathers such as different parameters of size and structure (e.g. DesRochers *et al.* 2009, **Paper I**). All of these parameters have a potential to affect organism's fitness. However, despite the relatively well-documented evidence about covariation between feather characteristics and survival (Jennions *et al.* 2001; Roulin & Altwegg 2007), the number of studies documenting direct associations between plumage characteristics and infection-induced mortality or

morbidity is limited. The spread of trichomonosis epidemics from Great Britain over Fennoscandia to Estonia provided an excellent opportunity to study associations between plumage characteristics and trichomonosis-induced mortality. The protozoan parasite *Trichomonas gallinae* causes necrotic inflammation of the oropharynx that eventually inhibits swallowing and leads to starvation and dehydration (Atkinson *et al.* 2008). Given the extreme pathogenicity and recent emergence of trichomonosis in greenfinches, it has been suggested that infection may have been recently spilled over from another host species, leaving little or no time for host adaptations to evolve (Neimanis *et al.* 2010).

The aim of the **Paper V** was to establish a connection between plumage characteristics of greenfinches and mortality caused by trichomonosis. Therefore melanin-based black plumage patches and three parameters of feather size (mass, length and rachis diameter) of greenfinch's tail feathers were measured. Additionally I was interested if the occurrence of fault bars can predict mortality as fault bars have been associated with increased mortality in different bird species (Bortolotti *et al.* 2002; Møller *et al.* 2009).

Out of 46 wild female greenfinches, five birds died during the first 11 days in captivity. Three of them displayed clear symptoms of trichomonosis (ingluvitis), while the remaining two exhibited similar pattern of total depletion of bodily reserves. Feathers of survivors were on average 22 % darker than feathers of dead birds. There are two alternative explanations why birds with higher plumage reflectance were selected against.

First explanation involves covariation between melanin-based coloration and some relevant aspect of immune function. Accumulating evidence indicates that melanin-based coloration is often correlated with other physiological, morphological and behavioral traits as an outcome of pleiotropic effects of key regulators of melanogenesis (Ducrest *et al.* 2008). Melanocortin peptide hormones, that trigger the production of melanin pigments (by binding to MC1R), also bind to four other melanocortin receptors, causing the reduction of acute, allergic and systemic inflammation and septic shock (Catania *et al.* ; Ducrest *et al.* 2008). In addition, activation of MC1R causes a collective reduction in the pro-inflammatory cytokines and prostaglandins and an increase in the anti-inflammatory mediators involved in the inflammatory process (Gangoso *et al.* 2011). Under such mechanism, one would predict enhanced inflammatory responses in paler, less-eumelanotic individuals (which has been established in at least one avian species (Gangoso *et al.* 2011, but see Vinkler *et al.* 2014). Inflammation, leading to terminal septicemia is a typical hallmark of *Trichomonas gallinae* infection (Neimanis *et al.* 2010), so one might hypothesize that paler greenfinches (i.e. the ones with suppressed expression of genes involved in eumelanin production) were more likely to die due to their over-responsive inflammatory defenses.

Another possible mechanism relates to pleiotropic covariation between melanin production and stress susceptibility. Association between melanin-

based pigmentation and behavioral-physiological aspects of animal personality seems to be widespread in vertebrates, with more distinctly pigmented individuals typically being more proactive, stress-resistant, and socially dominant (Kittilsen *et al.* 2009; Kittilsen *et al.* 2012). It is possible that birds with paler eumelanotic tail coloration appear to be more susceptible to stress and consequently stress-induced immune suppression would turn pale individuals more susceptible to trichomonosis. Higher stress-susceptibility of pale greenfinches is consistent with significantly paler plumage coloration among birds with fault bars.

Prevalence of fault bars was five times higher among the feathers of dead birds than among survivors. In addition, feathers which contained fault bars were significantly paler. Fault bars are developmental irregularities in feathers that result from a variety of stressors that occur during feather growth (Bortolotti *et al.* 2002; Møller *et al.* 2009). High prevalence of fault bars in dead birds thus suggests that those individuals either experienced more stressors or appeared more susceptible to stress already in the preceding autumn when those feathers were growing. Therefore, it seems unlikely that trichomonosis caused fault bars in finches, but instead, birds that were more stressed during preceding autumn molt appear to be more susceptible to trichomonosis in winter. The finding about associations between occurrence of fault bars and mortality is consistent with those of two previous studies. In female American kestrels (*Falco sparverius*), high number of fault bars was associated with lower survival probabilities (Bortolotti *et al.* 2002). More specifically, Møller and colleagues (2009) demonstrated that prey items of the goshawk (*Accipiter gentilis*) had nearly three times higher occurrence of fault bars than the general population of different prey species. The suggested mechanism involves decrease of aerial performance of prey due to breakage of feathers at fault bars. My results corroborate the diagnostic value of fault bars in predicting mortality as found in two above-cited studies. However, they also show that reduction of aerial performance is not the single mechanism connecting occurrence of fault bars with mortality. This study also shows that fault bars and feather size parameters convey different information about the stressful experiences during feather growth as feather size parameters did not differ between dead birds and survivors.

Current study is the first one to detect trichomonosis-induced viability selection on plumage characteristics. Detection of extremely strong selection for lower plumage reflectance is particularly interesting. Assuming non-zero heritability for tail feather coloration, one would predict that the recent outbreak of trichomonosis leads to evolution of darker melanin-based coloration in greenfinches.

SUMMARY

There are many characteristics that make birds perfect model organisms for evolutionary ecology research. Among the most notable of these are feathers that are considered the most complex integumentary structures of vertebrates (Prum & Williamson 2002). The interplay of feather growth and pigmentation produces tremendous diversity of within- and across-taxa coloration patterns, from elaborated sexual ornaments to exceptional camouflage matching (Landeen & Badyaev 2012). Not less importantly for ecologists, coloration, size, integrity and hormone content of feathers provide information about specific endogenous or exogenous processes and events of interest in the life of individuals or ecology of species, as well as the environment from which they came (Bortolotti 2010). The full potential of this approach has been realized only recently.

In this thesis, I relied on experiments and correlational data on wild-caught captive greenfinches to explore the applicability of different feather parameters for immunoecological and behavioral research. The first three papers are experimental. They aim at explaining whether and how artificial and natural immune challenge and manipulation of hormonal milieu by administration of synthetic stress hormone dexamethasone affect feather growth and corticosterone (CORT) content. However, in addition to specifying diagnostic value of feather parameters, these experiments also provided valuable information about somatic costs and correlates of up- and down-regulation of immunity and stress responses, which belong to the central research questions of physiological ecology. Remaining two papers are correlational. One of these asks whether feather CORT content provides any information about fear-related personality traits. The final paper takes an advantage of an outbreak of emerging infectious disease – trichomonosis – that enabled me to study associations between feather parameters and infection-induced mortality under controlled laboratory conditions.

One of the main aims of my thesis was to test the suitability of newly developed feather CORT measurement method for studying the nexus between neural, endocrine and immunological functions. Despite increasing popularity of integrated measures of glucocorticoid hormones, the potential for feather CORT measurements to advance avian ecology has been hindered by a lack of experimental research into what CORT values represent physiologically (Fairhurst *et al.* 2013b). For example, we still do not exactly know what impact frequently encountered physiological (immune activation or rise in blood CORT levels) or psychological (encounters with predators) stressors have on feather CORT content. Therefore, I tested the effects of immune activation and glucocorticoid administration on feather CORT content. In addition, I recorded the effects of these manipulations on feather mass, length and rachis diameter. These morphological characteristics are responsible for the mechanical performance of feathers and can therefore directly affect fitness of birds.

Glucocorticoid administration significantly decreased feather CORT levels. This evidence that feather CORT levels are sensitive to hormonal manipulations supports the suitability of feather CORT for characterization of stress history of birds. Surprisingly, feather CORT levels did not correlate with other well-established measures of stress such as leukocyte differentials. Neither did I found that immune activation with artificial antigen had affected feather CORT levels. This finding, along with those of Meitern (2013) and Fairhurst (2013a) suggests that assessment of health state and stress history of birds on the basis of feather CORT levels is not as straightforward as previously thought and calls for further research on information content of feather CORT.

In addition to reducing CORT content of feathers, glucocorticoid administration also reduced all three measures of feather size. This points to the possibility that experiencing an acute stress or termination of immune responses by rising the concentration of circulating glucocorticoid hormones may induce long-term somatic costs with potential impact on fitness. Immunostimulation experiment showed that in the face of resource limitation investment into feather mass (which increases feather strength) is prioritized over investment into feather length. These results add another piece of evidence about somatic costs of induced immune responses in terms of feather growth.

Taking a step further from artificial antigens, I asked whether infection with a natural pathogen affects feather CORT level and how feather CORT content, a potential marker of stress responsiveness, reflects the resistance to an experimental infection with natural coccidian parasites. I also asked whether individual differences in disease resistance relate to behavioral phenotypes, as can be expected on the basis of previous work on animal personalities and endocrine activity (Martins *et al.* 2007; Koolhaas 2008). To study the link with behaviour, I assessed the extent of damage to tail feathers accrued in captivity. Extent of tail damage was suggested to reflect the captivity-intolerance in greenfinches (Sild *et al.* 2011). The same study also showed that greenfinches that inflicted greater extent of damage to their tail feathers exhibited compromised immune responsiveness in terms of reduced capability for producing antibody response to a novel *Brucella abortus* antigen and reduced oxidative burst capability of phagocytes, which suggests a possibility of CORT-induced immune suppression in birds with most damaged tail feathers.

Coccidian infection (or it's suppression) indeed was associated with CORT secretion as birds subjected to experimental infection had on average 23% higher feather CORT content than control birds. In addition we found that experimental birds with lowest feather CORT levels developed highest infection intensities at the peak infection phase as well as highest increase in average infection levels. The most likely explanation for such pattern is that birds that invest more into fighting coccidiosis have higher stress responsiveness or that birds that experience captivity as more stressful have higher coccidian resistance. The benefits of increased resistance to coccidian parasites in the wild are obvious as coccidians are the most prevalent avian parasites (Greiner 2008)

with well documented pathological effects (Pap *et al.* 2011; Sepp *et al.* 2012). As predicted, I found that extent of tail damage accrued in captivity correlated positively with feather CORT content. The most likely explanation for this pattern would be that some greenfinches possessed more stress-prone personalities than others, consequently depositing more CORT in their feathers. These findings suggest that further research on CORT levels in relation to resistance of coccidiosis might increase our understanding about the mechanisms linking individual variation in CORT levels to fitness (see Breuner *et al.* 2008; Angelier *et al.* 2009).

Confirming that feather CORT content and morphological characteristics of feathers are sensitive to manipulations of organism's hormonal status and immune function, I proceeded with the second aim of my study, namely, with the investigation of whether and how feather parameters relate to behavioral traits. Between-individual variation in behavior is an important ecological and evolutionary characteristic of wild populations (Careau *et al.* 2008). Ample evidence suggests that behavior is not completely flexible and different behavioral traits do not vary independently of each other, thus giving rise to behavioral syndromes or personalities (Carere *et al.* 2010). I recorded five behavioral traits that are associated with different immune parameters (Sild *et al.* 2011) and stress responsiveness (Krams *et al.* 2013), and asked whether and which of these traits covary and manifest in general "fearfulness syndrome". The measured traits included latency to feed at the presence of a predator, the propensity to give distress calls at handling, changes in locomotor activity in response to distress calls of conspecifics, frequency of flapping flight bouts against cage bars and tendency to damage tail feathers in captivity. All of these traits show a response to an acute stressor and can therefore indicate different sides of a fear response. To study the associations between endocrine activity and behavior I recorded CORT levels of feathers. To my surprise, none of the behavioral parameters correlated with each other. These findings challenge the concept of a single internal variable responsible of fearfulness and support the proposed multidimensional nature of fear responses. Similarly, none of the behavioral parameters correlated with feather CORT levels. Furthermore, tail damage accrued in captivity did not correlate with the amount of CORT deposited into feathers grown in captivity. This finding contradicts my previous study where I detected a positive association between feather CORT and tail damage. This contradiction between the findings of two studies in the identical model system is difficult to explain and it calls for caution of generalizing the interpretations of feather CORT measurements obtained in different settings. Importantly, this study also showed that tail damage does not appear a universal correlate of stress responsiveness in captive greenfinches.

In the last part of my thesis I asked whether feather size and color parameters predict survival in greenfinches facing epidemics of emerging infectious disease, finch trichomonosis. Animals differ in their vulnerability to infections and these differences could be mirrored in their appearance as

immune system is integrated with almost any physiological function of an organism. The spread of trichomonosis epidemics to Estonia provided an excellent opportunity to study associations between plumage characteristics and trichomonosis-induced mortality. I measured the reflectance of black eumelanin-based tips of tail feathers because I expected that black coloration may relate to disease resistance. This expectation was based on the information that eumelanotic coloration is linked to expression of pleiotropic pro-opiomelanocortin genes that affect inflammatory and stress responses (Ducrest *et al.* 2008). Additionally, I was interested if the occurrence of fault bars can predict mortality as fault bars have been associated with increased mortality in different bird species (Bortolotti *et al.* 2002; Møller *et al.* 2009).

Out of 46 female greenfinches five birds died in captivity. Three of them displayed symptoms of trichomonosis, and the remaining two died most likely in the same reasons. Feathers of survivors were 22 % darker than feathers of dead birds. One possible explanation would be covariation between melanin-based coloration and some relevant aspect of immune function. Another possible explanation involves pleiotropic covariation between melanin production and stress susceptibility. Feathers of dead birds had significantly higher prevalence of fault bars. In addition, feathers with fault bars were significantly paler. As fault bars result from experiencing stress during feather growth (Bortolotti *et al.* 2002; Møller *et al.* 2009), high prevalence of fault bars in dead birds suggests that those individuals either experienced more stressors or appeared more susceptible to stress already in the preceding autumn when those feathers were growing. My results thus corroborate the findings of Bortolotti (2002) and Moller (2009), showing that fault bars can be used as a diagnostic cue in predicting mortality. Notably, this study also showed that fault bars and feather size parameters convey different information about stressful experiences during feather growth as feather size parameters did not differ between dead and alive birds. This study is the first to detect trichomonosis-induced viability selection on plumage characteristics. It enables to predict that the recent outbreak of trichomonosis leads to evolution of darker melanin-based coloration in greenfinches. Importantly, this study also suggests that melanin-based coloration can evolve via mechanisms that are independent of visual information provided by the pigment. This idea is relevant for understanding the mechanisms of evolution of melanin-based coloration in general. So far, the research in this area has been mainly concentrating on finding the costs of melanin-based display (McGraw, 2006). I suggest that the possibility that melanin-based integument coloration can evolve as a by-product of selection on pleiotropically linked physiological traits (see also Bize *et al.* 2006) deserves serious consideration in future studies.

In conclusion, my study showed that morphological characteristics, as well as CORT content of feathers are sensitive to manipulations of hormonal milieu and immune function, which makes these parameters potentially valuable indicators about the events that occurred in the period of feather growth. In

particular, feather mass, length and rachis diameter can be used to assess somatic costs of different stressors or trade-offs between feather growth and other demanding organismal functions. Feather wear in captivity can reflect behavior of birds and possibly be linked to stress susceptibility. Fault bars can be used as a diagnostic cue in predicting mortality. My study also indirectly suggests that eumelanotic color of feathers is linked to immune and/or stress responses, which helps us to understand the mechanisms of evolution of melanin-based coloration in general. Detected associations between feather CORT content and resistance to coccidiosis are particularly interesting, as they suggest a novel route of selection for higher stress responsiveness.

SUMMARY IN ESTONIAN

Rohevintide füsioloogiline ökoloogia: sulgedes sisalduv informatsioon immuunfunktsiooni ja käitumise kontekstis

Füsioloogiline ökoloogia uurib organismide füsioloogiliste funktsioonide ja keskkonna vahelisi suhteid. Selgroogsete närvi-, endokriin- ning immuunsüsteemid kuuluvad tõenäoliselt kõige keerukamate evolutsiooniliste kohastumuste hulka. Alles hiljuti on jõutud arusaamisele, et nimetatud süsteemid ei toimi üksteisest sõltumatult, vaid on omavahel ühendatud ühtseks tervikuks. Sellest tõdemusest kasvas välja kaks uut teadusharu: immuunökoloogia ja psühhoneuroimmunoloogia. Immuunökoloogia otsib vastuseid küsimustele, miks organismid erinevad vastuvõtlikkuses haigustele ja mis takistab immuunsüsteemi paremini toimimast. Immuunfunktsiooni isenditevahelise varieeruvuse üheks põhjuseks võib olla immuunsüsteemi regulatsioonimehhanismide keerukus ja hävitusmehhanismide ohtlikkus, mis muudab immuunsüsteemi kasutamise kulukaks. Kui organismi mingi funktsioon on kulukas, siis peab see paratamatult teiste funktsioonidega ühise ressursi pärast konkureerima; tulemuseks on erinevate funktsioonide vahelised lõivsuhted (*trade-off*). Närvi-, endokriin- ning immuunsüsteemi tööga kaasnevate lõivsuhete mõistmiseks on oluline nende toimimisega kaasnevate kulude ja tulude kvantifitseerimine.

Haiguse kulgu võivad mõjutada paljud erinevad faktorid; üheks nendest on organismi stressivastus. Vastuseks kogetud stressitekitajale vabanevad hüpotaalamuse-ajuripatsi-neerupealise kaskaadi kaudu stressihormoonid (lindude puhul eelkõige kortikosteroon (KORT)), mis võivad läbi füsioloogiliste ja käitumuslike mehhanismide mõjutada näiteks immuun- ja sigimissüsteemi ning ainevahetust. Kuigi immuun- ning stressivastuse vahelised seosed on üldjoontes teada, ei osata kõiki nähtusi adaptiivselt seletada. Näiteks pole teada, miks pikaajaline stress pärsib immuunsüsteemi funktsionaalsust.

Psühhoneuroimmunoloogia ühendab immuun- ning stressivastuse uuringud käitumise uurimisega. Ajas püsivaid isenditevahelisi erinevusi käitumises on leitud paljudel loomaliikidel. Päritavad, ajas ning erinevates olukordades püsivad ja omavahel korreleeruvad käitumistunnused moodustavad isendi isiksuse või iseloomu. Erineva isiksusega loomad erinevad üksteisest ka füsioloogia poolest, seega on põhjust eeldada, et käitumine ja immuun- ning stressivastused on omavahel seotud.

Üheks füsioloogilisi, morfoloogilisi ja käitumuslikke tunnuseid siduvaks mehhanismiks on geenide pleiotroopne mõju (ühe geeni mõju mitmele tunnusele). Näiteks peamise loomse pigmendi melaniini sünteesi eest vastutavad geenid osalevad nii stressi- ja immuunvastuse kui ka käitumise kujunemisel. Nii on erineva värvusega loomi seostatud alternatiivsete ökoloogiliste niššide, erinevate immuunvastuste, iseloomulike morfoloogiliste tunnuste või sigimisstrateegiatega.

Füsioloogilise ökoloogia peamiseks töömeetodiks on biokeemiliste markerite mõõtmine uurimisobjekti erinevatest kudedest. Lindude suled pakuvad selles kontekstis mitmeid unikaalseid võimalusi. Sulgede kasutamine uurimistöös on väheinvasiivne meetod, mille abil saab uurida looduslikke populatsioone ja sealhulgas ka kaitsealuseid liike. Sulgedes leidub rohkelt teavet organismisiseste ja organismiväliste protsesside, kogetud sündmuste või keskkonnatingimuste kohta. Informatsiooni võivad anda sulgede värv, suurus, terviklikkus ning erinevate hormoonide sisaldus. Selle informatsiooni potentsiaali on füsioloogilise ökoloogia uuringutes hakatud mõistma alles hiljuti.

Käesolevas töös kasutasin mudelorganismina rohevinte (*Carduelis chloris*), et välja selgitada erinevate suleparameetrite rakendatavus immuunökoloogilistes ja käitumisuuringutes. Esimesed kolm artiklit baseeruvad eksperimentaalsetel uuringutel. Nende eesmärgiks oli uurida immuunvastuse ning stressihormooni kunstliku analoogi deksametasooni manustamise mõju sule kasvule ja sule KORTi sisaldusele. Lisaks suleparameetrite diagnostilise väärtuse täpsustamisele võimaldasid need eksperimendid saada väärtuslikku informatsiooni aktiveeritud või alla surutud immuun- ja stressivastuste somaatiliste kulude ning korreleeruvate füsioloogiliste ja käitumuslike parameetrite kohta. Nende küsimuste uurimine on füsioloogilise ökoloogia keskmeks. Järgnevad kaks uurimust toetuvad korrelatiivsetele andmetele. Esimeses neist uurin, kas sule KORTi sisalduse kaudu on võimalik saada uut teavet hirmuga seostuvate käitumistunnuste kohta. Teine uurimus põhineb rohevintide uuel nakkushaigusel trihhomonoosil (põhjustaja algloom *Trichomonas gallinae*), mis võimaldab laboritingimustes uurida suleparameetrite ning nakkushaigusest põhjustatud suremuse seoseid.

Üheks minu töö peamiseks eesmärgiks oli testida, kas uus meetod, KORTi mõõtmine sulgedest, sobib neuro-, endokriin- ning immuunsüsteemi omavahelise seoste uurimiseks. Kuigi see meetod on viimastel aastatel kiiresti populaarsust kogunud, puuduvad eksperimentaalsed uuringud sulgede KORTi sisalduse füsioloogilise tähenduse kohta. Näiteks pole ikka veel täpselt teada, milline on erinevate füsioloogiliste (nt immuunsüsteemi aktiveerumine või vere KORTi sisalduse tõus) ja psühholoogiliste (nt kiskja kohtamine) tegurite mõju sulgede KORTi sisaldusele. Seepärast testisin immuunsüsteemi aktiveerimise ja stressihormooni manustamise mõju sulgede KORTi sisaldusele. Lisaks uurisin, kuidas sellised manipulatsioonid mõjutavad sule massi, pikkust ning suleroo diameetrit. Need sule morfoloogilised tunnused vastutavad sulgede mehhaanilise kvaliteedi eest ja võivad seega otseselt mõjutada lindude kohasust.

Minu töö tulemuse kohaselt vähendas deksametasooni manustamine oluliselt sulgede KORTi sisaldust (artikkel II). Kunstliku stressihormooni lisandumine vereringesse annab organismile signaali vähendada kehaomase stressihormooni tootmist. Sulgede KORTi sisalduse tundlikkus hormoonide taset mõjutavale manipulatsioonile kinnitab selle parameetri sobivust lindude stressivastuse kirjeldamiseks. Huvitav on avastus, et sulgede KORTi sisaldus ei korreleerunud teise linnu-uuringutes laialt kasutusel oleva stressiindikaatoriga, heterofiilide ja

lümfotsüütide suhtega veres. Lisaks ei mõjutanud sulgede KORTi sisaldust immuunsüsteemi aktiveerimine kunstliku antigeeniga (fütohemaglutiniin, PHA) (artikkel I). Need tulemused koos mõnede teiste uurimustega lubavad oletada, et lindude tervisliku seisundi ja stressivastuse hindamine sule KORTi sisalduse põhjal ei ole nii lihtne kui arvatakse.

Lisaks sulgede KORTi sisalduse vähendamisele vähendas kunstliku stressihormooni manustamine kõiki sulgede suuruse parameetreid (artikkel I). See viitab võimalusele, et akuutne stress või vere KORTi sisalduse kunstliku tõstmise tulemusena inhibeeritud stressivastus (negatiivne tagaside) võib esile kutsuda pikaajalisi somaatilisi kulusid, mis võivad mõjutada isendi kohasust. Kui KORT mõjutas nii sulgede pikkust, massi kui roo diameetrit, siis immuunsüsteemi aktiveerimine PHA-ga vähendas vaid sule pikkust, mis näitab seda, et ressursside limiteerituse korral panustatakse rohkem sule massi (mis suurendab sule tugevust). Need tulemused täiendavad meie teadmisi aktiveeritud immuunsüsteemi ja stressivastuse somaatiliste kulude kohta.

Kolmandas artiklis uurisin, kuidas mõjutab sule KORTi sisaldust nakatamine koktsiididega (rohevinte looduslikult nakatavad rakusisesed sooleparasiidid perekonnast *Isospora*) (artikkel III). Lisaks huvitas mind, kas sule KORTi sisaldus (kui potentsiaalne stressivastuse marker) peegeldab resistentsust eksperimentaalsele koktsiidinakkusele. Katse võimaldas välja selgitada, kas erinevused haigusresistentsuses seostuvad lindude isiksusega, nagu on viidanud mitmed varasemad füsioloogia- ja käitumisalased uuringud. Käitumise kvantifitseerimiseks hindasin rohevintidel vangistuses tekkinud sabakahjustusi. Eelnevad uuringud on näidanud, et erinevused sabakahjustuste ulatuses võivad peegeldada lindude erinevat võimet taluda vangistust.

Koktsiidinakkus tõstis sule KORTi sisaldust: eksperimentaalselt nakatatud lindude suled sisaldasid 23% rohkem KORTi kui kontrollgrupi lindude suled. Lisaks leidsin, et eksperimentaalselt nakatatud lindude hulgas oli nakkus kõige intensiivsem nendel, kel oli sulgedes kõige vähem KORTi. Sellel tulemusel on kaks võimalikku seletust. Esiteks võib lindudel, kes panustasid kõige rohkem nakkusega võitlemisse, olla keskmisest kõrgem stressivastus. Teiseks võivad linnud, kellel on tugevam vangistusstress, olla nakkusele resistentsemad. Resistentsus koktsiidinakkusele on looduses ilmselgelt kasulik, sest koktsiidid on levinuimad lindude patogeensed parasiidid. Ootuspäraselt korreleerus sabasulgedele tekkinud kahjustuste määr sule KORTi sisaldusega. Võib arvata, et mõned linnud olid madalama stressitaluvusega kui teised, mille tagajärjel sisaldasid nende lindude suled rohkem KORTi ja nad tekitasid sabasulgedele suuremas ulatuses kahjustusi. Need tulemused aitavad selgitada, kuidas võib sule KORTi sisaldus olla seotud isendi kohasusega.

Töö teises pooles uurisin, kas ja kuidas on suleparameetrid seotud lindude käitumisega (artikkel IV). Isendite erinev käitumine (erinevad isiksused ehk iseloomud) on looduslike populatsioonide tähtis evolutsiooniline ja ökoloogiline omadus. Eelnevad uuringud on näidanud käitumise piiratud paindlikkust, mis tuleneb erinevate käitumistunnuste omavahelistest seostest. Neljandas artiklis

mõõtsin viit käitumistunnust, mis on seotud vastusega akuutsele stressitekitajale. Uurisin, kas need käitumistunnused omavahel korreleeruvad ja moodustavad „hirmu sündroomi“. Mõõdetavad parameetrid olid toitumislatentsus kiskja juuresolekul, kalduvus käsitsemisel hirmukisa kuuldavale tuua, aktiivsuse muutus vastuseks liigikaaslaste hirmukisale, vastu puuriseina raplemise sagedus ning vangistuses tekkinud sabakahjustuste ulatus.

Vastupidiselt oodatule mõõdetud käitumistunnused omavahel ei korreleerunud. See tulemus on vastuolus arusaamaga, et hirmukäitumine on ühedimensiooniline isiksuseomadus, ning näitab, et lindude hirmureaktsioonid on konteksti- ja seisundispetsiifilised.

Ükski käitumistunnus ei korreleerunud sule KORTi sisaldusega. Sule KORTi sisaldus ei korreleerunud ka vangistuses sabasulgedele tekitatud kahjustustega. See tulemus on vastuolus varasema tulemusega (artikkel III), kus sule KORTi sisaldus korreleerus positiivselt sabasulgedele tekitatud kahjustustega. Kahe sarnase katse vastukäivad tulemused seavad kahtluse alla üksikutel sule KORT-i käsitlevatel uurimustel põhinevate järelduste üldistusvõime.

Oma töö viimases osas (artikkel V) uurisin, kas rohevintide sule suurus ja värvus ennustab vastupanuvõimet uudsele ning epideemilisele trihhomonoosinakkusele. Organismide vastuvõtlikkus haigustele on erinev ning need erinevused võivad peegelduda välimuses, sest immuunsüsteem on seotud paljude teiste füsioloogiliste funktsioonidega. Trihhomonoosi hiljutine levik Eestisse andis võimaluse uurida trihhomonoosist põhjustatud suremuse seoseid sule parameetritega. Mõõtsin sulgede musta (eumelaniinse) suletipu heledust, sest eeldasin sule musta värvuse seotust haigusresistentsusega. See eeldus põhineb varasematel uuringutel, kus näidati, et eumelaniini süntees on seotud pro-opiomelanokortiini geeni aktiivsusega, mis omakorda mõjutab ka põletikulisi protsesse ning stressivastust. Lisaks uurisin, kas sulgedel esinevad kasvulüngad ennustavad vintide suremust, kuna seda tunnust on erinevatel linnuliikidel varem suremusega seostatud.

Loodusest püütud 46 rohevindist suri vangistuses viis lindu. Kolmel neist esinesid selged trihhomonoosi sümptomid ning kaks ülejäänut surid tõenäoliselt samal põhjusel. Ellujäänud lindude suled olid võrreldes surnud lindude sulgedega 22% tumedamad. Üheks võimalikuks seletuseks on melaniinil põhineva värvuse füsioloogiline seos immuunsüsteemiga. Teiseks võimalikuks seletuseks on seos melaniini sünteesi ja stressivastuse vahel. Surnud lindude suled sisaldasid rohkem kasvulünki, lisaks olid kasvulünkadega suled ka oluliselt heledamad. Kuna kasvulüngad tekivad sule kasvamise ajal kogetud stressi tulemusena, viitab rohkemate kasvulünkade esinemine surnud lindude sulgedes sellele, et need isendid olid stressile vastuvõtlikumad või puutusid juba eelneval sügisel, sulgede kasvu ajal, kokku tõsisemate stressitekitajatega. See kinnitab varasemaid tulemusi, mille kohaselt kasvulünki on võimalik kasutada suremuse ennustamisel. Märkimist väärib ka järeldus, et kasvulüngad ja sule suuruse parameetrid annavad sule kasvu ajal kogetud stressitekitajate kohta erinevat informatsiooni, kuna sule suuruse parameetrid surnud ja ellujäänud

lindudel ei erinenud. Käesolev uurimus on esimene, mis näitab trihhomonoosi valikusurvet sule parameetritele. Selle põhjal võib ennustada, et hiljutine trihhomonoosipuhang viib rohevintidel tumedama sulevärvuse evolutsioneerumiseni. Uurimus viitab ka sellele, et eumelaniinil põhinev sulevärvus võib evolutsioneeruda sõltumatult värvipigmendi poolt edastatud visuaalsest informatsioonist. See avastus aitab mõista melaniinil põhineva värvuse üldisi evolutsioonimehhanisme. Siiani on peamiselt keskendutud melaniinsete ornamentide kulude kvantifitseerimisele. Võimalus, et melaniinse värvuse kujunemisel on oluline roll värvuse tootmisega pleiotroopselt seotud füsioloogilistele omadustele avalduva valikusurvega, väärib tulevastes uuringutes tõsist tähelepanu.

Kokkuvõttes võib minu töö põhjal järeldada, et suled sisaldavad külluslikult erinevat informatsiooni. Sule erinevad parameetrid on tundlikud organismi hormonaalse sisekeskkonna ja immuunsüsteemi manipulatsioonidele, andes seega väärtuslikku informatsiooni sulgede kasvu ajal kogetud sündmuste kohta. Sule massi, pikkust ja suleroo diameetrit on võimalik kasutada erinevate stressitekitajate somaatiliste kulude hindamiseks või sulekasvu ja organismi teiste oluliste füsioloogiliste protsesside vaheliste lõivsuhete kirjeldamiseks. Vangistuses tekkinud sulekahjustused peegeldavad lindude käitumist ning võivad iseloomustada vastuvõtlikkust stressile. Kasvulünki on võimalik kasutada suremuse ennustamisel. Minu uurimistöö viitab võimalusele, et sulgede melaniinil põhinev värvus on seotud immuun- ja stressivastusega, mis aitab mõista melaniinil põhineva värvuse üldisi evolutsioonimehhanisme. Sule KORTi ja koktsidioosiresistentsuse vaheline seos on eriti huvitav, sest see võib aidata selgitada, miks looduslik valik võib soosida kõrgenenud stressivastust.

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Sild E., Meitern R., **Männiste M.,** Karu U., Hõrak P. (2014). High feather corticosterone indicates better coccidian infection resistance in greenfinches. *General and comparative Endocrinology, 204, 203–210.*

Sepp T., **Männiste M**., Kaasik A., Hõrak P. (2014). Multidimensionality of fear in captive greenfinches (*Carduelis chloris*). *Behavioral Ecology and Sociobiology, 68, 1173–1181.*

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Meitern R., Sild E., Lind M.-A., **Männiste M**., Sepp T., Karu U. & Hõrak P. (2013). Effects of Endotoxin and Psychological Stress on Redox Physiology,

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Sild E., Sepp T., **Männiste M.** & Hõrak P. (2011). Carotenoid intake does not affect immune-stimulated oxidative burst in greenfinches. *The Journal of Experimental Biology*, 214, 3467–3473.

Conference theses:

Männiste M., Hõrak P., poster presentation: "Surm heledatele vintidele: trihhomonoos tapab värvi järgi", XII Ecology conference "Eesti teadus- ja arendustegevuse konkurentsivõime", 18–19 october, Tartu, Estonia.

Männiste M., Sepp T., Hõrak P., poster presentation: "The effects of environmental enrichment on feather corticosterone content in captive wild-caught birds", Conference "Behaviour", 4–8 August 2013, Newcastle Gateshead, Great Britain.

Männiste M., Sild E., Karu U., Meitern R., Hõrak P. poster presentation: „Can feather corticosterone predict behavior under stressful conditions?", Conference on Behavioural Biology to celebrate the 20th anniversary of the Netherlands Society for Behavioural Biology (NVG), 28–30 November 2012, Soesterberg, Netherlands.

Männiste M., oral presentation: „Behavior and plumage quality in greenfinches", International Society for Behavioral Ecology conference (ISBE 2012), 12–18 August 2012, Lund, Sweden.

Männiste M., oral presentation: "Acute glucocorticoid administration suppresses feather growth in greenfinches", 8th Conference of European Ornithology Union, 27–30 August 2011, Riga, Latvia.

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Sild E., Meitern R., **Männiste M.,** Karu U., Hõrak P. (2014). High feather corticosterone indicates better coccidian infection resistance in greenfinches. *General and comparative Endocrinology, 204, 203–210.*

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Sild E., Sepp T., **Männiste M.** & Hõrak P. (2011). Carotenoid intake does not affect immune-stimulated oxidative burst in greenfinches. *The Journal of Experimental Biology*, 214, 3467–3473.

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Männiste M., Hõrak P., posterettekanne: "Surm heledatele vintidele: trihhomonoos tapab värvi järgi", XII Ökoloogiakonverents "Eesti teadus- ja arendustegevuse konkurentsivõime", 18–19 oktoober, Tartu, Eesti.

Männiste M., Sepp T., Hõrak P., posterettekanne: "The effects of environmental enrichment on feather corticosterone content in captive wild-caught birds", konverents "Behaviour", 4–8 august 2013, Newcastle Gateshead, Suurbritannia.

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