DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

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IMMUNE FUNCTION, PARASITES, AND CAROTENOID-BASED ORNAMENTS IN GREENFINCHES

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

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

I Hõrak, P., Saks, L., Ots, I. & Kollist, H. (2002) Repeatability of condition indices in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology* 80: 636–643.

II Hõrak, P., Saks, L., Ots, I., Kullissaar, T., Kollist, H. & Zilmer, M. (2003) Physiological effects of immune challenge in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology* 81: 371–379.

III Saks, L., McGraw, K.J. & Hõrak, P. (2003) How feather colour reflects its carotenoid content. *Functional Ecology* 17: 555–561.

IV Saks, L., Ots, I. & Hõrak, P. (2003) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* 134: 301–307.

V Hõrak, P., Saks, L., Karu, U., Ots, I., Surai, P.F. & McGraw, K.J. (2004) How coccidian parasites affect health and appearance of greenfinches. *Journal of Animal Ecology* 73: 935–947.

VI Saks, L., Karu, U., Ots, I., & Hõrak, P. Host resistance and parasite virulence: concurrent variation and relations with immunocompetence and carotenoid signals in greenfinch coccidiosis. (submitted manuscript)

The author’s involvement in all the studies was substantial including data gathering, data analyses and writing process.
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INTRODUCTION

To explain the evolution of extravagant male ornaments, Darwin (1871) proposed the theory of sexual selection, which he separated from the general theory of natural selection. He suggested that the disadvantages to male survival induced by such ornaments are compensated for by more or better females preferring that individual to other potential mates. Darwin did not explain, however, how such female preferences could be developed and persisted. This question remained largely unanswered until the advancement of Amotz Zahavis' handicap principle in 1975. According to this, female preferences for elaborate male ornaments could evolve, if these ornaments would serve as indicators revealing male quality. The honesty of the indicator traits would be assured by the high cost (behavioural, developmental, maintenance etc.) of the signals, so the individuals of inferior quality would not be able to cheat. This idea was developed further by William Hamilton and Marlene Zuk (1982), who proposed that parasites could be of fundamental importance in the evolution of female preferences to extravagant male sexual characters. They hypothesised that the quality revealed by individual's ornamentation is its ability to resist currently prevailing parasites. This idea led to the development of the concept of parasite-mediated sexual selection (PMSS), according to which, males who are more resistant to parasites and thereby in better condition are able to invest more resources into sexual display. Females would benefit from preferring more elaborately ornamented males as mates, as they would obtain direct benefits and/or parasite resistance genes for their offspring (reviewed in Andersson 1994).

Although the majority of living organisms are probably parasitic and their omnipresence is testimony to their success (Hudson & Dobson 1997), parasites were of little interest to ecologists and evolutionary biologists until the 1970s. Then, just in a couple of decades, a ground swell of interest has stimulated intensive research on the role of parasites in the life-histories of their hosts (reviewed e.g. in Clayton & Moore 1997, Zuk & Stoehr 2002). To date, it is widely accepted that although the immune system is essential for individual's survival in a parasite-rich environment, it also imposes costs and is therefore likely to be involved in trade-offs with life-history traits (Sheldon & Verhulst 1996; Lochmiller & Deerenberg 2000; Norris & Evans 2000; Zuk & Stoehr 2002). Several studies have suggested that immune function may be directly integrated with the evolution of sexual selection, reproductive costs or population dynamics (e.g. Apanius *et al.* 1994; Gustafsson *et al.* 1994, 1995; Ebert 1995; Richner *et al.* 1995; Saino & Møller 1996; Sheldon & Verhulst 1996; Verhulst *et al.* 1999; Lochmiller & Deerenberg 2000; Råberg *et al.* 2000; Zuk & Stoehr 2002; Hanssen *et al.* 2004). However, the physiological pathways behind these relationships are still poorly understood. For instance, despite the extensive research focused on the PMSS, the question about the mechanisms, linking parasite resistance to expression of sexual ornamentation, have remained largely unanswered.

Carotenoids have been suggested to play a major role in these relationships (Lozano 1994; Olson & Owens 1998), especially among birds where carotenoid-based ornaments are disproportionately common (reviewed in Gray 1996; Møller *et al.* 2000). Carotenoids are essential for various aspects of immune function, as they participate in immuno-regualtion and -stimulation, lymphocyte proliferation and free-radical scavenging (e.g. Machlin & Bendich 1987; Bendich 1989; Burton 1989; Chew 1993; Lozano 1994; Olson & Owens 1998; Møller *et al.* 2000). Because animals cannot synthesize carotenoids *de novo* and have to acquire them from food (e.g. Fox 1979), a trade-off between investment of carotenoids in maintenance and ornamentation can be predicted. Individuals who are forced to fight infections during the formation of sexual ornaments are expected to have less carotenoids available for developing colourful traits (e.g. Lozano 1994; Olson & Owens 1998; Møller *et al.* 2000). However, despite the accumulating evidence on carotenoid-based sexual signalling, the exact pathways generating trade-offs between individual health status and signal expression are not completely clear (e.g. Olson & Owens 1998; Hill 1999; Zuk & Stoehr 2002; Hartley & Kennedy 2004). The aim of the current thesis is to investigate how the immune function is connected to individual condition and expression of carotenoid-based sexual ornamentation, and to study the proximate mechanisms responsible for these relationships, using captive greenfinches (*Carduelis chloris*) as a model.

In order to understand how parasite resistance is linked to the expression of sexual ornaments we have to understand the physiological mechanisms behind these relationships. The costs of immune defences have been proposed to form the basis of the resource allocation trade-offs linking the immune function to life-history traits and sexual advertisement (e.g. Sheldon & Verhulst 1996; Owens & Wilson 1999; Zuk & Stoehr 2002; Schmid-Hempel 2003). Although several recent studies have confirmed the involvement of immune function in life-history trade-offs (e.g. Deerenberg *et al.* 1997; Nordling 1998; Moreno *et al.* 1999; Cichoń 2000; Råberg *et al.* 2000; Ilmonen *et al.* 2000; Hanssen *et al.* 2004), the physiological mechanisms that create these costs of immunity remain more poorly understood. For instance, some recent studies aiming to clarify the physiological effects of immune challenge have yielded conflicting results (e.g. Svensson *et al.* 1998; Ots *et al.* 2001; Fair & Ricklefs 2002). Therefore, more knowledge is needed about the physiological mechanisms responsible for the connections between immune system and other vital functions of the organism. The question about possible physiological costs accompanying immune response is addressed in the Paper **II**.

Before we can observe and quantify the effects of immune challenge to individual's physiology, we have to use reliable indices, describing these processes. A wide range of simple haematological condition indices have been used to test the relationships between individuals' health state and life-history traits during recent years (e.g. Dufva & Allander 1995; Saino *et al.* 1997; Møller *et al.* 1998; Ots *et al.* 1998; Figuerola *et al.* 1999; Hõrak *et al.* 1999; Nunn *et al.* 2000). However, different condition indices may describe different aspects of individual health, which may be variable in time. Thus, the condition indices used in the studies testing the hypotheses relating inter-individual variation in life-history or ornamental traits to individual phenotypic quality have to be consistent in time. For example, establishing effects of brood size manipulation upon individuals' physiology requires condition indices to be stable in one or a few weeks, while the hypotheses regarding sexual selection assume that the expression of signal traits reliably reflects some more persistent component of individual's health state. Still, most reports about the consistency of condition indices have concentrated on short time periods (Ots *et al.* 1998; Gosler & Harper 2000) or have focused on a limited number of traits (Chappell *et al.* 1995, 1996; Potti & Merino 1997; Bech *et al.* 1999; Dawson & Bortolotti 1999; Potti *et al.* 1999; Koteja *et al.* 2000). Thus, to determine the time periods for which different condition indices are valid, more studies estimating the repeatability of different physiological variables under standard conditions are needed. How consistent are differential leukocyte counts, serum protein and triglyceride concentrations, basal metabolic rate, body mass, and spontaneous locomotion activity over short and long time periods in captive greenfinches, is described in Paper **I**.

During the recent decades, the idea that carotenoid pigments may play a major role in the mechanisms linking parasite resistance to the expression of sexual ornaments has been in the focus of intensive research (reviewed e.g. in Møller *et al.* 2000). However, in order to test whether the trade-off between investing carotenoids into sexual signals and into immune function is involved in these mechanisms, we need to reliably measure the amount of carotenoids deposited into ornamentation. Moreover, if the trade-off between investing carotenoids into sexual signals and into immune function is involved in these mechanisms, the colouration of the ornaments has to reflect the amount of carotenoids deposited into them. Surprisingly, despite the considerable amount of work, concentrated on the relationships between carotenoid-based plumage colour and individual quality (reviewed in Møller *et al.* 2000), the question whether and how the measured plumage colouration does reflect the amount of carotenoids in plumes has remained virtually untested. This is investigated in Paper **III** by describing the relationships between spectrophotometrically measured plumage colour variables and feather carotenoid content.

To date, several studies have demonstrated that carotenoid-based ornamentation reflects individual's ability to mount an immune response to novel antigens. However, all these studies have concentrated on ornaments, based on metabolically active tissues (Zuk *et al.* 1995; Zuk & Johnsen 1998; Verhulst *et al.* 1999; Saino *et al.* 2000; Blount *et al.* 2003; Faivre *et al.* 2003). Surprisingly, despite the fact that feather ornaments are probably the most widespread form of display amongs birds (e.g. Andersson 1994), the relationships between carotenoid deposition into feather ornaments and individual immunocompetence have not been investigated. Yet, the feather ornaments are of particular interest in the context of PMSS because once the carotenoids are deposited into such metabolically inactive tissues like feathers, they became unavailable for use in other vital purposes, like immune function (Lozano 1994; Olson & Owens 1998). Furthermore, as in many bird species moult occurs long (often several months) before the feathers are used in sexual display, carotenoid-based plumage colouration may be predicted to signal especially long-term aspects of individual's quality. Whether the plumage colouration of male greenfinches reflects their general health state and ability to rise an immune response to novel antigens is investigated in Paper **IV**.

Although the important role of carotenoids in PMSS has been supported by numerous evidence (reviewed e.g. in Møller *et al.* 2000), the exact mechanisms how the expression of ornaments reflects parasite resistance are still not clear (e.g. Olsson & Owens 1998; Hill 1999). A way to solve this problem is to simultaneously investigate the effects of parasites on individual physiology and the development of carotenoid-based sexual signals. Avian coccidiosis seems to be a potentially promising model for such experiments. Coccidia from the genus *Isospora* (Protozoa, Apicomplexa, Sporozoa, Coccidia) are widely distributed (reviewed e.g. in Giacomo *et al.* 1997; McGraw & Hill 2000; Duszynsky *et al.* 2004) and can cause severe disease, even death among passerine birds (Box 1977; Sironi 1994; Giacomo *et al.* 1997). Moreover, it is known from the studies on domestic chicken that coccidians from the genus *Eimeria* inhibit the absorption of several essential dietary components, including carotenoids in the intestine (e.g. Allen 1987; 1997; Allen & Fetterer 2002) and can cause depression of carotenoid-based pigmentation (Tyczkowsky *et al.* 1991). How the physiology and ornament expression of greenfinches is affected by the infection with intestinal coccidian parasite, *Isospora lacazei*, is studied in Paper **V**.

The existence of concurrent polymorphism among the host resistance and parasite virulence is one of the main assumptions of the models of host-parasite coevolution (reviewed e.g. in Clayton & Moore 1997; Little 2002; Summers *et al.* 2003). However, this important assumption has not been tested in wild bird-parasite model systems. The issue of variation in the host resistance and parasite virulence, and the possible relationships of these phenomena with the measures of general immunocompetence and plumage colouration in the wild-caught greenfinches and their Isosporan parasites is addressed in Paper **VI**.

STUDY SPECIES

The greenfinch

Greenfinch is medium-sized (ca 30 g) gregarious seed-eating passerine native to the western Palearctic region (Cramp & Perrins 1994). Males are larger and more colourful (Svensson 1992; Merilä *et al.* 1999), old males being olive-green on their back side, with bright yellow breast (greenish-yellow in some geographic regions), less so on the belly and rump and yellow markings on primaries, primary coverts and the sides of the tail feathers (Cramp & Perrins 1994). Females are smaller, more olive-brown and yellowish buff, having faint brown streaks on back and lacking full yellow tints in their plumage. Moult duration is 13–15 weeks from late July to early November (Cramp & Perrins 1994). The mating system is mainly monogamous, but a significant degree of polygamy occurs, at least in some populations (*ca*. 24% of males in a population in southern England; Eley 1991). During the mating season, males perform conspicuous "song-flights", "butterfly-flights" and other kinds of display to the females (Cramp & Perrins 1994). Several clutches are laid each year, and hence, the period of sexual activity is longer than in most other northern temperate-zone passerine birds. Further, the testis size is larger than expected from body weight, suggesting that sperm competition may be relatively intense in this species (Møller 1991; Merilä & Sheldon 1999). The male plumage brightness (measured by visual scoring) has been shown to be sexually selected trait as more brightly coloured male greenfinches are favoured by females as mates (Eley 1991). It has also been shown that males with more yellow ornamental feathers are less likely to be heavily infected with haemoparasites (Merilä *et al.* 1999) and have higher Sindbis virus clearance rates (Lindström & Lundström 2000).

Coccidia

Coccidia of the genus *Isospora* are obligate intracellular parasitic protozoa, infecting a wide range of songbird species in the wild (reviewed by Giacomo *et al.* 1997; McGraw & Hill 2000; Duszynski *et al.* 2004). A host becomes infected when it ingests oocysts that have been passed in the feces of another host. The oocyst excysts in the epithelial cells of intestinal mucosa and liberates sporozoites from its contents. The sporozoites penetrate the cells of the host's small intestine and reproduce asexually. In case of passerine birds, the first-generation sporozoites may also invade liver, spleen and lungs (atoxoplasmosis, e.g. Giacomo *et al.* 1997). In the epithelial cells of intestine, each generation of asexual reproduction produces multiple merozoites that infect new cells. This stage of the infection can result in destruction of massive numbers of cells in the host's small intestine and, ultimately, lead to the host's death (e.g. Box 1977; Sironi 1994). Some of the merozoites that enter the host's cells transform into gametocytes. The gametocytes transform into gametes, the gametes fuse, and the resulting zygote begins to develop into an oocyst. The developing oocyst escapes from the host's cell, and it is passed in the host's feces. The destruction of epithelial cells of small intestine during the reproduction of the parasite is probably the main pathological effect of the Isosporan infections, which can cause a drastic reduction in digestive and absorptive capacity of mucosa (e.g. Ruff & Fuller 1975; Hoste 2001). The pathogeneicity (which can ultimately lead to the host's death) of Isosporan coccidiosis is well documented (Box 1977; Sironi 1994; Giacomo *et al.* 1997; **V**) and therefore, it is likely that these parasites can appear to be an important evolutionary force for passerine birds.

RESULTS AND DISCUSSION

Are the clinical condition indices reliable measures of individual performance? (I)

To investigate whether widely used clinical health state indices are suitable for exploring the relationships between phenotypic quality and other individual characters, repeatabilities of 17 condition indices (Table 1) were measured in captive greenfinches over short (4–8 days) and long (over 4 months) periods. All monitored condition indices were significantly repeatable over the periods of four and eight days (Table 1). However, only leukocytic immune parameters, basal metabolic rate (BMR) and body mass expressed significant consistencies over four-month period (Table 1). This is not surprising, as different condition indices describe different aspects of individual condition, with different durations. It has been shown that PHA-response reflects short-term variation in individual nutritional state (reviewed in Alonzo-Alvarez & Tella 2001; Lifjeld *et al.* 2002). Also serum albumin and triglyceride concentrations are expected to reflect current catabolic processes (e.g. Jenni-Eiermann & Jenni 1998) and increase in serum globulin fraction is often characteristic to acute infections and inflammation (e.g. Coles 1997). Interestingly, evidence for the short-term individual consistency in nutritional condition was present in this data set, as revealed by significant repeatabilities of serum triglyceride and albumin levels (Table 1). Since all birds were fed *ad libitum*, this result probably reflects the significant inter-individual variation of the catabolic processes or nutrient absorption. At the same time, body mass, leukocytic immune parameters and BMR seem to be more influenced by factors that contribute to long-term differences between individual greenfinches. Leukocytes form the basis of the immune system of an organism, and their main function is protection against various pathogenic antigens. Hence, it is possible that high individual consistency of leukocytic variables in this study reflects between-individual differences in the prevalence of chronic infections. Also, elevated BMR might reflect activation of immune system (Demas *et al.* 1997; Ots *et al.* 2001). To summarise, while all the 17 considered condition indices are reliable measures of individual physiological performance if used for detecting the effects of short-term experimental manipulations, only leukocytic immune parameters, BMR and body mass appear suitable for describing long-term components of individual condition.

Table 1. Repeatabilities (*r*) of condition indices of captive male greenfinches between different time periods.

Period	4 days		8 days		4 months	
Trait	*r* (SE)	*n*	*r* (SE)	*n*	*r* (SE)	*n*
WBC	0.36 (0.16)*	31	0.45 (0.14)†	32	0.88 (0.06)‡	14
Lymp. count	0.46 (0.14)†	31	0.51 (0.13)‡	32	0.62 (0.17)†	14
Het. count	0.50 (0.13)†	31	0.40 (0.15)†	32	0.76 (0.12)†	14
H/L ratio	0.58 (0.12)†	31	0.30 (0.16)*	32	0.54 (0.20)†	14
Total protein	0.56 (0.15)†	23	0.52 (0.16)†	23	–0.10 (0.29)	12
Albumin	0.62 (0.13)‡	23	0.56 (0.15)‡	23	0.06 (0.30)	12
β-globulin	0.36 (0.19)*	23	0.52 (0.16)†	23	–0.24 (0.28)	12
γ-globulin	0.69 (0.11)‡	23	0.66 (0.12)‡	23	0.26 (0.28)	12
Alb/Glo	0.74 (0.09)‡	23	0.60 (0.14)‡	23	0.26 (0.28)	12
Triglycerides	0.47 (0.17)†	23	0.43 (0.17)*	23	–0.26 (0.29)	11
Evening mass	0.80 (0.06)‡	32	0.49 (0.14)‡	32	0.70 (0.15)†	13
Morning mass	0.83 (0.06)‡	31	0.63 (0.11)‡	31	0.62 (0.18)†	13
BMR	0.86 (0.05)‡	28	0.87 (0.05)‡	28	0.63 (0.17)†	14
BMR/mass	0.89 (0.04)‡	28	0.84 (0.06)‡	28	0.65 (0.16)†	13
PHA-response					–0.08 (0.33)	10
Jumping activity	0.45 (0.12)†	32	0.30 (0.17)*	31		
Total activity	0.54 (0.13)‡	32	0.38 (0.15)†	32		

Note: Four-day repeatabilities (and corresponding SE-s) are averages of two estimates (day 1 *vs* day 4 and day 4 *vs* day 8). Sample sizes differ due to measurement failures of some variables. Lymp. count and Het. count stand for haemoconcentrations of lymphocytes and heterophils, respectively. For a four-month period, individual values of all leukocyte counts, total protein, albumin, triglycerides and basal metabolic reate (BMR) are standardized within autumn and spring measurement sessions to a mean of zero and variance of unity. *, $p<0.05$; †, $p<0.01$; ‡, $p<0.001$; otherwise ns.

Is the immune response costly? (II)

In order to determine the effect of an immune challenge to individual’s physiology, 17 condition indices of male captive greenfinches were recorded in the period of one, four and eight days subsequent to injection (p.i.) with physiological saline or non-pathogenic antigen (sheep red blood cells, SRBC). Measured variables included estimates for total and differential leukocyte counts, serum protein concentrations, serum triglyceride concentration, BMR, body mass, spontaneous locomotion activity, and total serum antioxidant capacity. Fourteen hours subsequent to SRBC injection, a transient increase in serum beta-globulin concentration was recorded (Fig. 1), indicating an acute phase response to the antigen. None of the other condition indices was affected by the treatment (all $p>$ 0.05; see Tables 1 and 2 in Paper **II**), suggesting

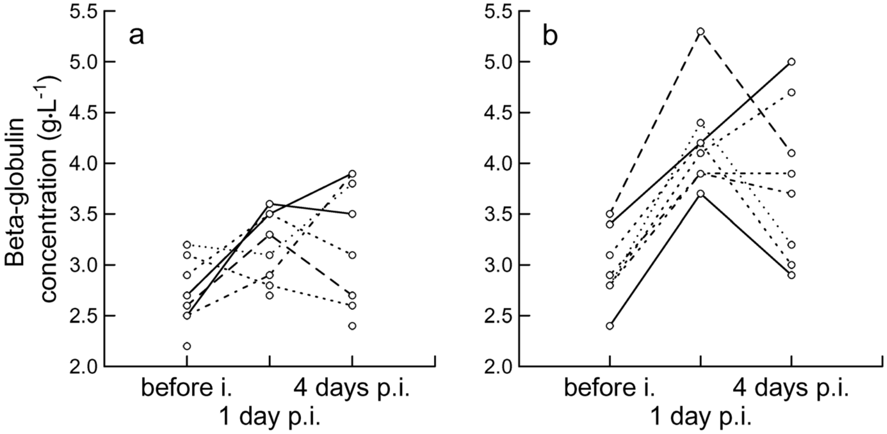

Fig. 1. Serum beta-globulin concentrations of saline-injected (a) and SRBC-injected (b) greenfinches. Statistics are given in Table 2 in the Paper **II**.

that mounting an immune response against SRBC did not incur any serious physiological impact for the birds maintained in affluent feeding regimen in captivity. These results contrast with previous studies, which have demonstrated that the activation of the immune system is costly (e.g. Lochmiller & Deerenberg 2000) as, for instance, remarkable increase in basal metabolism due to humoral immune challenge has been documented in laboratory mice (Demas *et al.* 1997) and in wild great tits (Ots *et al.* 2001). However, as exception from the general pattern of the result, in days 4 and 8 p.i., immune challenged greenfinches reduced significantly their spontaneous locomotion activity (Fig. 2). Such reduction of activity is the typical part of “sickness syndrome”

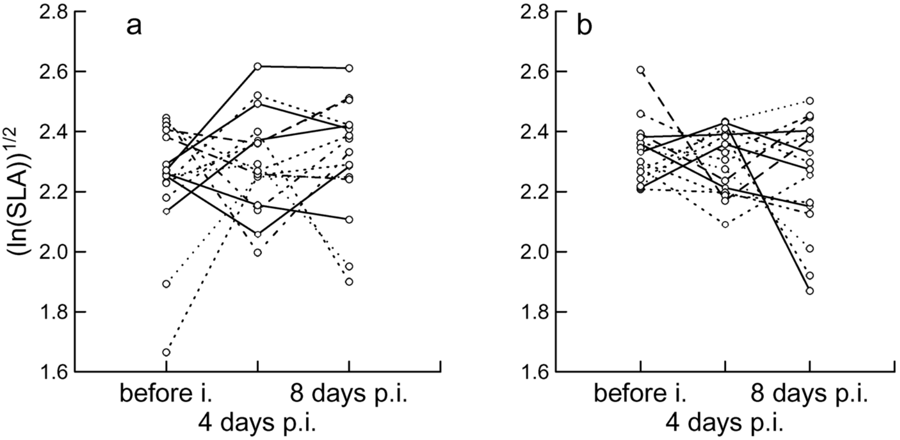

Fig. 2. Spontaneous locomotion activity of saline-injected (a) and SRBC-injected (b) greenfinches. Statistics are given in Table 1 in the Paper **II**.

accompanying host responses to infection and inflammation (e.g. Goodman *et al.* 1990; Aubert 1999; Lindström 2003). This result indicates that the activation of immune system may have caused greenfinches to reduce general energy expenditure. Hence, it is possible that immune response incurred at least some sort of expenses for the birds that could be finally converted to energetic currency.

How does feather colour reflect its carotenoid content? (III)

In this paper it was tested, whether the carotenoid-based colouration of ornamental feathers is a signal about the amount of carotenoids invested into them, as often expected (e.g. Lozano 1994; Olson & Owens 1998; Møller *et al.* 2000). For this purpose, the spectrophotometrically measured colour estimates of hue, chroma and brightness (*sensu* Endler 1990), as well as carotenoid pigment contents were determined from the yellow parts of the tail feathers of male greenfinches. The feathers with higher values of chroma and hue had higher total concentrations of carotenoid pigments (Fig. 3). This study presents the first direct evidence that spectrophotometric measurements of plumage colouration can be used to estimate the amount of carotenoids deposited in bird feathers. At the same time, relationships between colour measurements and carotenoid content were slightly different for the lab-grown and wild-grown feathers (Fig. 3). This suggests that the degree to which different colour parameters reflect feather carotenoid content is not necessarily similar or even linear within the range of observable variation. Considerable amount of variation in plumage colour could be potentially ascribed to other factors in this study, as the proportion of variance in plumage colour explained by feather carotenoid content was not particularly high (32–51%). It has been suggested that the plumage colour is based not only upon the pigments but also on the feather structure (e.g. Auber 1957; Fox & Vevers 1960; Vevers 1982). Feather structure, in turn, has been shown to be very sensitive to developmental stress and abrasion. Thus, feather colouration may also indicate individual qualities complementary or different from those conveyed by the carotenoid pigments (reviewed in Fitzpatrick 1998). For instance, one might expect that abraded or worn feathers are less glossy and therefore reflect less light, completely independent of their carotenoid content (Fitzpatrick 1998).

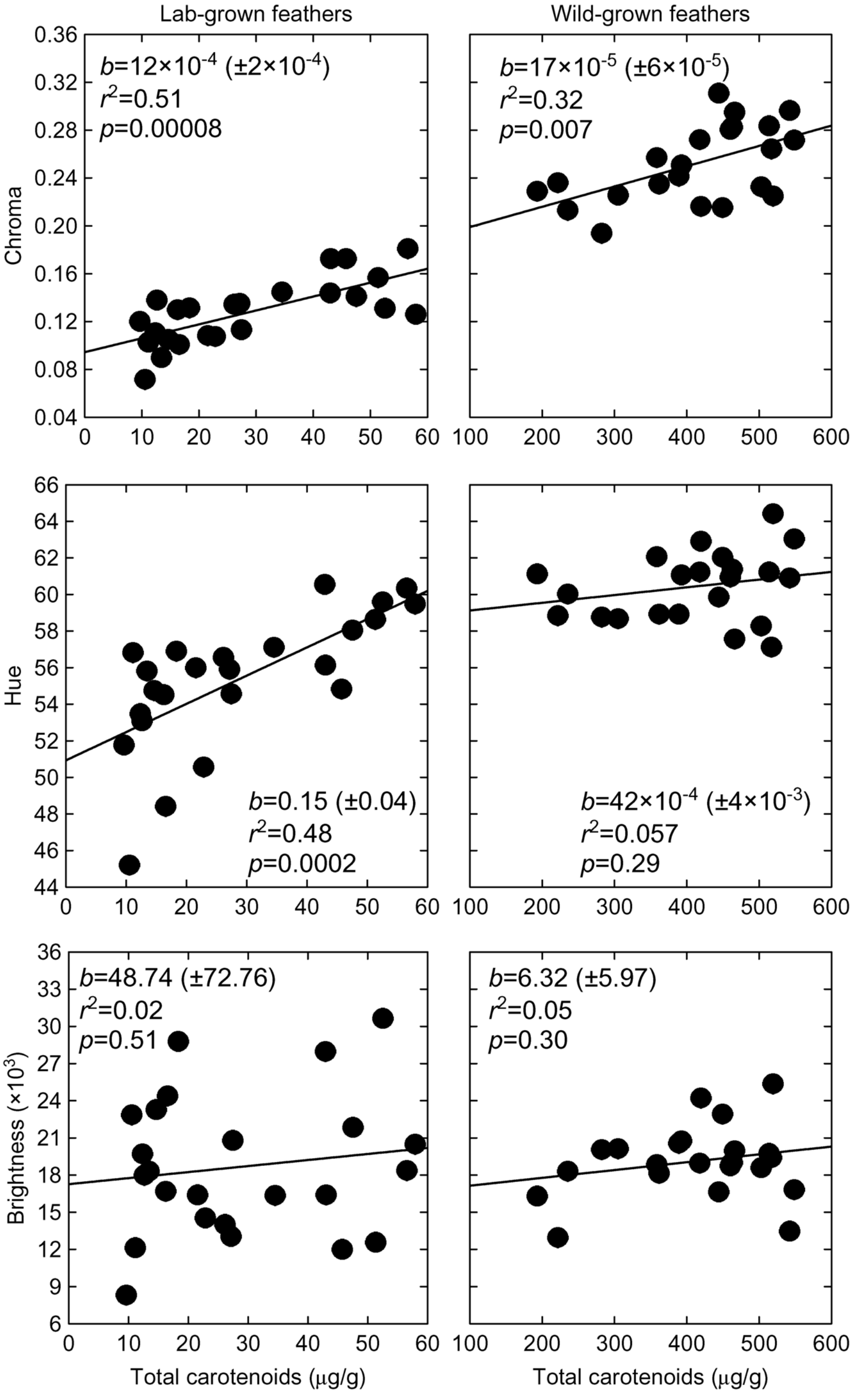

Fig. 3. Relationships between total carotenoid content and colour measurements of the feathers (slope ± SE).

Does the plumage colouration reflect health? (IV)

Carotenoid-based colouration of birds has been hypothesised to function as an honest signal of individual's health due to trade-off between allocation of carotenoids into maintenance and signalling (reviewed e.g. in Lozano 1994; Olson & Owens 1998; Møller *et al.* 2000). An important prediction of this hypothesis, that more brightly ornamented individuals are able to mount stronger immune responses against novel antigens, has so far been tested only on metabolically active ornamental tissues like combs (Zuk *et al.* 1995; Zuk & Johnsen 1998; Verhulst *et al.* 1999), gapes (Saino *et al.* 2000), or peaks (Blount *et al.* 2003; Faivre *et al.* 2003). However, the feather ornaments are probably the most widespread form of display amongst birds (e.g. Andersson 1994). The question, whether male greenfinches display their immunological superiority by yellower plumage was tested by investigating the correlations between individual's colouration and general health state and ability to mount an immune response to a novel antigen. Male greenfinches who had brighter yellow breast feathers mounted significantly stronger humoral immune response to SRBC ($r = 0.45$, $p = 0.022$, $n = 22$; Fig. 4) and had less circulating heterophils ($r = –0.41$, $p = 0.016$, $n = 30$; Fig. 5) than duller individuals. To my knowledge, this is the first direct evidence that carotenoid-based colouration of metabolically inactive ornamental tissues reflects humoral immunocompetence. Heterophils form the first line of cellular defence of an organism and their

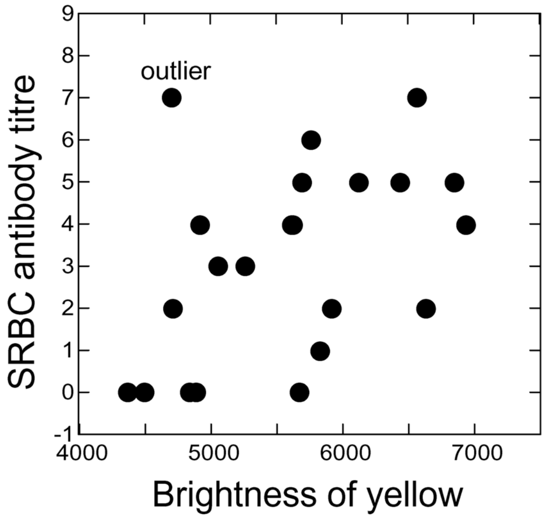

Fig. 4. Relationship between the brightness of yellow feathers and the SRBC antibody titre. If the marked outlier (standardized residual = 2.39) is excluded from the analysis then $r = 0.61$, $p = 0.002$, $n = 21$.

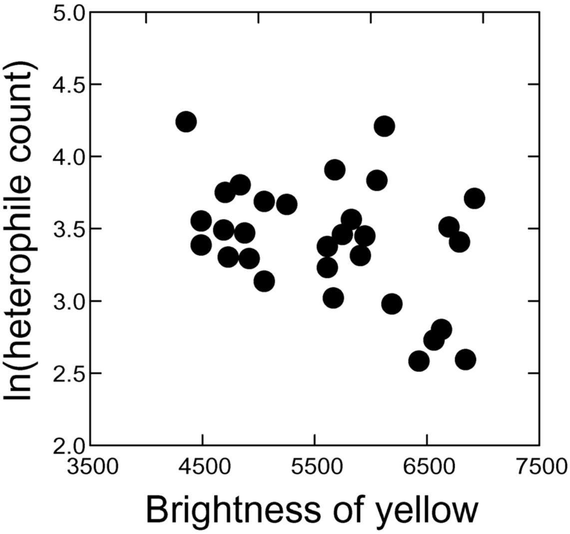

Fig. 5. Relationship between the brightness of yellow feathers and heterophile count (per *ca*. 10 000 erythrocytes).

concentration in the blood stream is known to rise in response to microbial pathogens (reviewed in Harmon 1998). Heterophile counts have been also shown to describe relatively long-term components of individuals' general health state (**I**). Hence, it is possible that the correlations between heterophile concentration and plumage colour arose because individuals suffering microbial infections during moult had to use more carotenoids for immunostimulation and/or repair functions and had therefore less carotenoids available for deposition into plumage. Thus, so far, these results suggest that the carotenoid-based ornamental plumage of male greenfinches might indeed have evolved as an indicator of individual's general health state and its ability to resist parasites as predicted by the PMSS (but see next chapter and Paper **VI**).

Could the coccidian parasites provide the mechanism for PMSS in greenfinches? (V and VI)

According to the concept of the PMSS, successful parasite invasion should decrease the expression of ornamental traits, as the use of limited resources for sexual advertisement is traded off against the need to fight off the parasites (Hamilton & Zuk 1982; Grafen 1990; Andersson 1994). Whether the coccidian intestinal parasites have such an effect on carotenoid-based ornamental plumage colouration of male greenfinches is investigated in Paper **V**. After suppressing the Isosporan infection in the captive population in greenfinches, half of the birds were infected with a mixture of different *Isospora* strains, while the infection in the second half of the birds was continually suppressed by medication. Experimental inoculation of the experimental group resulted in over 200-fold difference in oocyst output between medicated and infected birds. Infection intensity of the infected group rose 16 times over the pre-experimental level (Fig. 6A). This change in infection intensities was mirrored in a sudden decrease of body mass, serum albumin, triglyceride, vitamin E and carotenoid concentrations and in the increased concentration of heterophils in blood stream in infected birds, when compared to medicated individuals (Fig. 6B-H). Experimentally inoculated birds also deposited less carotenoids into their tail and breast feathers, which resulted in greater reduction in the colouration of these feathers, compared to the medicated birds (Fig. 6I-L).

The main pathological effect of the Isosporan infection is probably the destruction of epithelial cells of small intestine during the reproduction of the parasite, causing a drastic reduction in digestive and absorptive capacity of mucosa (e.g. Ruff & Fuller 1975; Hoste 2001). In the case of cardueline finches, Isosporan parasites have been shown to damage most extensively the duodenal and jejunal part of the intestine (Giacomo *et al.* 1997). In domestic chicken, these intestinal compartments have been shown to be responsible for absorption of proteins (*jejunum*) and fats (*duodenum*) (Turk 1974), as well as vitamin E and carotenoids (Surai 2002). Moreover, the increase in peripheral heterophile numbers in infected birds may be indicating an active immune response against the introduced coccidia in the intestinal mucosa, where these phagocytozing cells participate in the removal of destroyed host tissues or dead parasites (e.g. Rose *et al.* 1979). Such phagocygotic processes are often linked to the tissue damage caused by the production of proteolytic enzymes and free radicals (e.g. Klasing & Leshchinsky 1999). Thus, the usage in antioxidant defence against free radicals produced during the immune response could be an additional reason for the drop of serum carotenoids and vitamin E during peak infection phase (e.g. Allen 1997). These results suggest that the bright ornamental colouration of male greenfinches may be signalling individual's ability to resist the currently prevailing Isoporan parasites (Fig. 7). This would be consistent with the results that females of several bird species base their mate choice decisions on ornamental traits, affected by coccidian infection (e.g. ring-necked pheasants, Hillgarth 1990; wild turkeys, Buchholz 1995; American goldfinches, Johnson *et al.* 1993; house finches, reviewed by Hill *et al.* 2002).

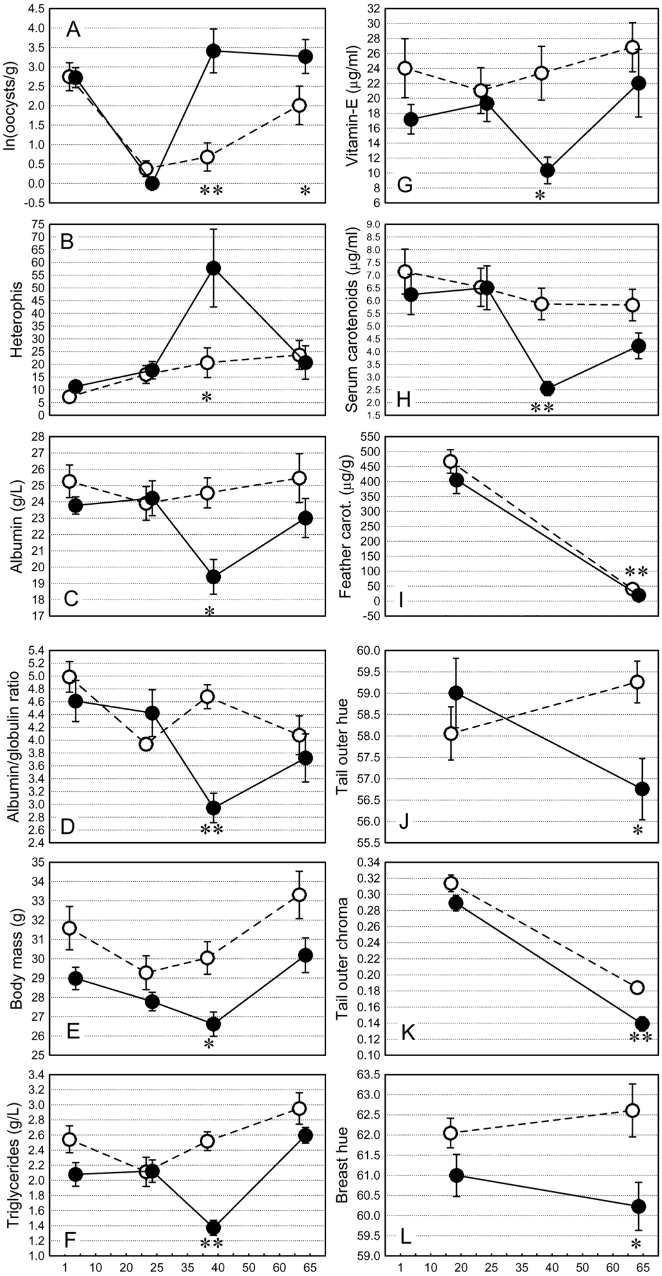

Fig. 6. Effect of the manipulation of parasite load upon the condition indices and feather colouration of greenfinches. Filled circles – infected group; empty circles – medicated group. Asterisks denote significance of pairwise contrasts between infected and medicated group (* $p < 0.05$; ** $p < 0.001$). The course of experiment in days is noted in x-axes of the lowest graphs. Sample sizes and statistics are given in Tables 1 and 2 in the Paper **V**.

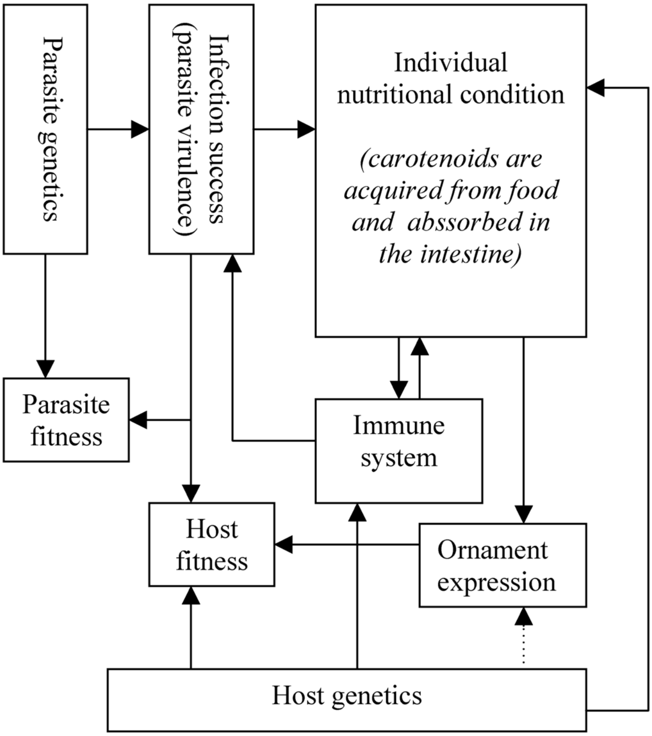

Fig. 7. An hypothetical mechanism connecting immune function to the expression of carotenoid-based ornamentation in greenfinches. Ingested carotenoids have to be absorbed in order to be used in the organism. Individual's ability to acquire carotenoids from food depends on the efficiency of its digestive system. This quality can be drastically influenced by intestinal parasites (e.g. Isosporan coccidia) who diminish organism's ability to absorb vital nutrients, including carotenoids. Organism's ability to resist these parasites is in turn determined by its genetic characteristics and the availability of essential resources for the immune function. Thus, hosts who are more able to neutralise the pathological effects of intestinal parasites have more carotenoids available for ornament development as they have more efficient digestive systems and do not have to use so much carotenoids for their immune function. However, immunologically relevant genetic diversity within a pathogen population allows different strains of a pathogen to infect hosts that are immune to other antigenic variants. The dashed arrow indicating the relationship between host genetics and ornament expression is predicted in arbitrary models of the evolution of sexual signalling but is not required in the context of parasite mediated sexual selection.

Do Isosporan parasites appear an evolutionary force, responsible for the maintenance of individual variation in carotenoid-based sexual ornamentation? To my knowledge, this important assumption of the PMSS has not been tested in wild birds, which are common objects of the studies of parasite-mediated selection. The few previous experimental tests of the assumption of concurrent polymorphism among host resistance and parasite virulence in natural vertebrate models originate from studies of fish (e.g. López 1998; Wegner *et al.* 2003; Kurtz *et al.* 2004) and lizards (Oppliger *et al.* 1999). In Paper **VI** it was investigated, whether different Isosporan strains vary in virulence, whether different host individuals vary in resistance to infection, and whether the variation in host resistance is genetically determined. In order to test these hypotheses, wild-caught captive male greenfinches were infected with Isosporan parasites, originating either from single or multiple hosts.

The experiment revealed that different Isosporan strains varied in their ability to invade hosts. Infection with multiple novel strains resulted in higher virulence than infection with a single novel strain (Fig. 8C). Thus, birds were more likely to encounter novel virulent strains of coccidia from heterologous than from homologous inoculations. Greenfinches also varied in their ability to resist novel infection, as infection intensities of "initially resistant" birds remained low throughout the experiment, compared to other birds, infected with the same inoculum (Fig. 8D). This implies that natural infection intensities confer information about the ability of individuals to resist also novel strains.

In order to be a target of microevolutionary processes, individual variation in resistance to coccidiosis has to be genetically determined. Average infection intensity did not decrease after the second infection among birds who were infected with the same heterologous parasite strains (Fig. 8A). Moreover, although inoculated with the same heterologous strain, "initially resistant" birds had lower infection intensities than "initially susceptible" birds throughout the experiment (Fig. 8D). Thus, primary infection with mixture of Isosporan strains did not result in the development of acquired immunity against the subsequent infections with the same parasite strains. This result strongly argues for the genetic origin of the between-individual differences in infection intensities. An alternative scenario would suggest that some individuals express low infection intensities just because they have not encountered truly pathogenic parasite strains yet. This scenario assumes, that after any encounter with a new parasite strain, the birds are able to acquire immunological memory, which helps to suppress infection efficiently at subsequent encounters with the same parasite strain. This was not the case, which suggests that the variation in resistance to different *Isospora* strains can be caused by genetic differences between individual greenfinches. Thus the results of the current experiment indicate that the outcome of coccidian infection in greenfinches depends on concurrent variation in host resistance, parasite virulence and their interaction, and indirect evidence suggests that this variation has a genetic basis. This hints that coccidian parasites in greenfinches can contribute to the maintenance of variation in host's resistance genes and therefore may be involved in the mechanisms, linking immune function to the carotenoid-based signalling (Fig. 7).

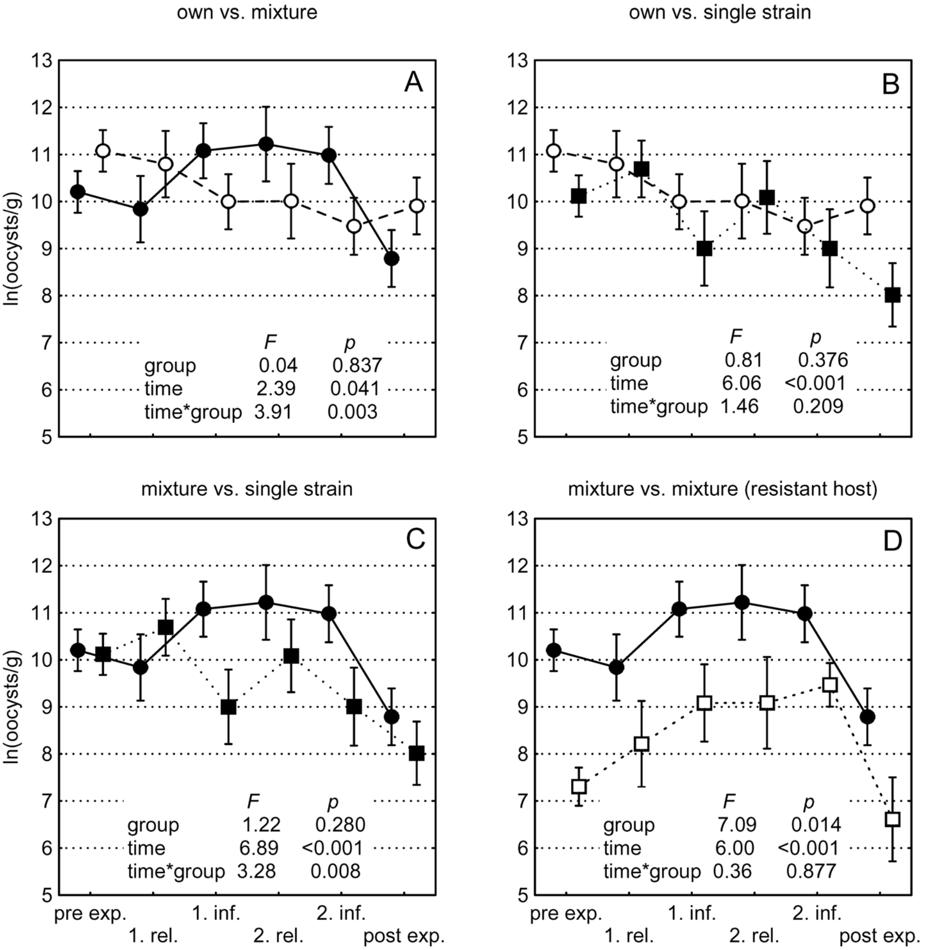

Fig. 8. Effect of experimental infections upon the coccidian oocyst shedding (per gram of feces) in different treatment groups. Open circles – double infection with own strain; filled circles – double infection with a mixture of strains in "susceptible" hosts; open squares – infection with a mixture of strains in "resistant" hosts (second time treated with water); filled squares – infection with a single external strain (second time infected with own strain). Coccidian reproduction was completely arrested both before the first and second infection (not shown in the figure). *n* = 12–13 birds per group. *F*- and *p*-values are from the repeated measures ANOVA. Vertical bars are SE.

It was shown in Paper **IV** that plumage colouration of male greenfinches can signal individual's health and ability to rise a humoral immune response to SRBCs. It has been proposed that such tests of individual's general immunocompetence are a more relevant measure of individual's ability to deal with the invading pathogens than the actual parasite counts (Apanius 1998; Møller *et al.* 1999; 2000). However, the usefulness of such immune tests for evaluation of host ability to resist the currently prevailing parasites is seldom verified in controlled infection experiments (e.g. Owens & Wilson 1999; Ryder 2003). This was also investigated in Paper **VI**. Among the birds who responded to the experimental infection with declined infection intensities, pre-experimental infection intensities correlated negatively with SRBC antibody titres, while no such pattern emerged among the birds whose infection intensity increased after first infection (see Fig. 6 in Paper **VI**). At the same time, SRBC titres also clearly reflected the nutritional state of individuals (see Fig. 7 in Paper **VI**). Therefore, it seems that birds who were less inflicted by or more resistant to coccidian infection had less damaged intestine, and were therefore capable to rise a stronger humoral immune response against a novel antigen. This suggests that high SRBC antibody titres indeed reflect individual's ability to resist an important parasite species. The causality of this relationship, however, cannot be currently inferred from this experiment. One possibility is that better general immunocompetence (indicated by high SRBC titres) enabled some birds to suppress the challenge infections more efficiently, which also resulted in higher body mass and plasma triglyceride levels due to less damaged intestinal mucosa. Alternatively, genetic resistance would enable some individuals to manage their challenge infections so efficiently that this results in better nutritional condition. Better condition, in turn, would enable to mount stronger antibody response against SRBC.

Birds who were more susceptible to the first experimental infection mounted stronger swelling response to PHA injection (r = 0.49, p = 0.008, n =38). This result suggests exactly the opposite, when compared to the relationship between infection intensities and SRBC antibody titres. If the magnitude of the swelling response to PHA indeed reflects the animal's general potential for cell-mediated immunity (e.g. Smits *et al.* 1999; Martin *et al.* 2001), then it would appear that most immunocompetent individuals are least resistant to a real infections in this study! However, such an interpretation would be overly simplistic, given the integrated and extremely complex structure of the vertebrate immune system. It is important to note here that the immune response against coccidiosis is mainly based on the same processes, which are also responsible for the swelling response to PHA injection (e.g. Lillehoj 1998). It is therefore likely that birds who were not resistant to the experimental infection were actively fighting against it and had therefore up-regulated their T-cell mediated immune function. Similar enhancing effect of the parasites on T-cell mediated immune function has been also previously shown in house martins (Christe *et al.* 2000) and in European starlings (Gwinner *et al.* 2000). These results imply that strong PHA-responses do not always need to indicate individual's ability to resist the currently prevailing parasites, but can also be reflecting the effects of current infections. This calls for caution in interpreting the results of the standard immune tests in the context of parasite resistance (see also Tella *et al.* 2001 and Ryder 2003).

The results, presented so far (**III**; **IV**; this Paper) suggested that the carotenoid-based ornamental plumage of male greenfinches might have evolved as an indicator of individual's general health state and ability to resist coccidian parasites as predicted by the PMSS (Fig. 7). Therefore, it was expected that individuals' ability to resist coccidian infections would be mirrored in their plumage colouration. However, no significant correlations between colour variables and infection parameters were detected (all *p*-values 0.1…1). Moreover, on the contrary to the previous results (Fig. 4), the greenfinches who showed weak SRBC antibody titres grew tail feathers with higher values of chroma ($r_s = -0.33$, $p = 0.030$, $n = 45$) and brightness of yellow ($r_s = -0.32$, $p = 0.034$, $n = 45$) than the birds who were able to produce higher SRBC antibody titres during the experiment. Previous results showed that the value of chroma of the yellow parts of the tail feathers correlate positively with the feather carotenoid content (**III**) and that Isosporan infection reduces the amount of the carotenoids deposited into the feathers (**V**). These findings suggested that a rather straightforward mechanism could be responsible for the connections between individual's immune function and the carotenoid-based ornament expression in greenfinches (Fig. 7). Given the immunostimulatory role of carotenoids, it was expected that the birds whose digestive system is less damaged by the coccidian infection are able to deposit more carotenoids into the feathers growing during the experiment, and also mount stronger immune response against foreign antigens due to their better condition. The fact that actually the opposite pattern was observed indicates that the relationships between individual sexual ornamentation and immune function appear much more complicated. One of the reasons for the discordance between the previous results (**IV**) and this experiment may be that the individual responses to the artificial antigens appear very sensitive to the current experimental conditions.

The lack of positive correlations between the ornament quality, parasite resistance and immunocompetence appears surprising in the context of previous work on parasitism, immunity and plumage colouration in greenfinches. Previously observed correlations between the plumage colouration and blood parasites loads (Merilä *et al.* 1999), Sindbis virus clearance rates (Lindstöm & Lundstrom 2000) and the indices of general health state and immunocompetence (**IV**), together with the drastic effect of coccidian infection on the condition and appearance of male greenfinches (**V**) have suggested that parasites can play a role in the evolution of ornamental plumage in greenfinches. However, the results of this experiment imply that the physiological mechanisms by which the competence of the immune system is expressed in sexual ornaments, are more complicated than predicted in the figure 7. As a possible explanation to this controversy, I could imagine that individual's ability to suppress efficiently the challenge infections may not necessarily be the trait maximised by selection. For instance, it has been suggested that the lack of an immune response may sometimes also be an adaptation of the host rather than a failure to fight off the parasites (e.g. Boots & Bowers 2004). When the costs, inflicted by the immune system activation, outweigh the costs inflicted by parasite invasion, the tolerance of the infection should result in greater fitness benefit for the host than the suppression of the infection (Råberg *et al.* 1998; Roy & Kirchner 2000). Therefore, the possibility that under certain circumstances, condition-dependent ornaments may be used also as an indicator of individual's ability to tolerate infection, cannot be totally excluded.

In conclusion, this study has demonstrated the great potential of the avian coccidiosis models for microevolutionary research, but has also revealed novel and somewhat unexpected aspects of the relationships between infection resistance, immunocompetence and the expression of signal traits. These questions, especially the ones related to the potential costs and benefits of infection resistance and tolerance certainly call for further experiments in this area.

SUMMARY

The aim of the current thesis was to investigate how immune function is connected to the individual condition and the expression of carotenoid-based sexual ornamentation, and to describe the proximate mechanisms responsible for these relationships, using captive greenfinches (*Carduelis chloris*) as a model.

First: To quantify the effects of immune challenge to individual physiology, reliable indices describing these processes have to be used. How consistent are differential leukocyte counts, serum protein and triglyceride concentrations, basal metabolic rate, body mass, spontaneous locomotion activity, and total serum antioxidant capacity over short and long time periods in captive greenfinches, were investigated in Paper **I**. All the studied traits appeared suitable for the detection of short-term experimental effects upon individual's physiology. Leukocytic parameters, basal metabolic rate and body mass were significantly consistent over four months' period and may therefore appear suitable for exploring the relationships between individual phenotypic quality, life-history and signal traits.

Second: To determine the effect of an immune challenge to individual's physiology, the above-mentioned condition indices of male captive greenfinches were monitored subsequent to injection with physiological saline or sheep red blood cells (SRBC; **II**). Despite to the fact that acute-phase response to the antigen was detected, most of the condition indices were not affected by the immune challenge. However, immune challenged individuals reduced their spontaneous locomotion activity, suggesting a switch to energy conservation. Unfortunately, these results did not enable an insight into the nature of the costs accompanying immune challenge.

Third: Carotenoid-based colouration of birds has been hypothesised to function as an honest signal of individual's health due to trade-off between the allocation of carotenoids into maintenance and signalling. This hypothesis relies on the assumption, that the colouration of the ornaments reflects the amount of carotenoids deposited into them. This was tested, by describing the relationships between spectrophotometrically measured plumage colour estimates of hue, chroma and brightness and feather carotenoid content (**III**). It appeared that the estimates of chroma and hue are reliable indices of feather carotenoid content.

Fourth: Although the importance of carotenoids in sexual signalling has been in the scope of extensive research, the relationships between individual immunocompetence and carotenoid-based plumage colouration have not been investigated. However, the feather ornaments are the most widespread form of display among birds and can be predicted to signal especially long-term aspects of individual's quality. Whether the plumage colouration of male greenfinches reflects their general health state and immunocompetence was investigated in Paper **IV**. Individuals with brighter yellow breast feathers were in better general

health state and were able to mount stronger humoral immune response against a novel antigen (SRBC).

Fifth: Whether the coccidian infections could provide the mechanism linking parasite resistance to carotenoid-based sexual ornamentation in greenfinches was investigated in Papers **V** and **VI**. The experimental infection with *Isospora lacazei* resulted in drastic effects upon the physiology and expression of carotenoid-based plumage colouration in greenfinches, probably due to the reduced absorption of nutrients (including carotenoids and vitamin E) in infected birds (**V**). This suggests a rather straightforward mechanism for the connections between the immune function and carotenoid-based ornament expression in greenfinches – the birds whose digestive system is less damaged by the coccidian infection are able to deposit more carotenoids into the feathers.

The existence of concurrent polymorphism among host resistance and parasite virulence is one of the main assumptions of the models of parasite mediated sexual selection. It was thus predicted that greenfinches should vary in genetic resistance to coccidian infection while different parasite strains should vary in virulence. This was tested in Paper **VI**. Additionally, the correlations between parasite resistance, immunocompetence and carotenoid-based plumage colouration were examined. The outcome of experimental infection with different Isosporan strains depended on concurrent variation in parasite virulence and host resistance, which was most likely genetically determined. This suggests the great potential of avian coccidiosis models for micro-evolutionary research. However, the lack of positive correlations between plumage colour, parasite resistance and immunocompetence implies that the physiological mechanisms, connecting immune function and ornament expression in greenfinches are more complicated than could be predicted by the results presented in Papers **V** and **IV**.

KOKKUVÕTE

Parasiidid, immuunfunktsioon ja karotinoididel põhinevad ornamendid rohevintidel

Charles Darwini poolt esitatud sugulise valiku teooria on olnud teadusliku debati teemaks juba enam kui sajand. Darwin väitis, et silmatorkavad sekundaarsed sootunnused võivad olla sugulise valiku poolt soositud, kui emased loomad eelistaksid partneri valikul ornamenteeritumaid isaseid. Põhjused, miks emased sääraseid tunnuseid eelistavad, on aga siiani ebaselged. Viimastel aastakümnetel on suurt tähelepanu pööratud parasiitide rollile peremehe elukäigu evolutsioonis ja sugulise valiku mehhanismides. Ehkki parasiitide vastu võitlemine on ilmselgelt adaptiivne, nõuab selleks vajaliku immuunsüsteemi ülesehitamine ja rakendamine ressursse. Seega peab immuunfunktsioon konkureerima ühiste ressursside pärast ning seeläbi interakteeruma kõigi teiste organismi funktsioonidega, sealhulgas sugulise valiku signaaltunnuste väljaarendamisega. Parasiitide poolt vahendatud sugulise valiku hüpoteesi kohaselt suudaksid antud hetkel levivate parasiitide suhtes vastupanuvõimelisemad isendid investeerida enam sugulisse signaliseerimisse, kuna nad ei pea kulutama piiratud ressursse parasiitide vastu võitlemiseks. Emastel tasuks seega eelistada enam ornamenteeritud isaseid, kes oleksid võimelised rohkem investeerima sigimisse ja/või oleksid väiksema tõenäosusega mõne nakkuse edasikandjad. Lisaks, kui eeldada, et immuunvõime on päritav, tagaksid emased ornamenteeritumate isastega paaritudes kõrge immuunvõime ka oma järglastele. Küsimus, missugused füsioloogilised mehhanismid seovad sekundaarsete sootunnuste avaldumist parasiidiresistentsusega, on aga seni jäänud konkreetse vastuseta. Käesoleva dissertatsiooni eesmärgiks oli selgitada, millised on seosed immuunfunktsiooni ning indiviidi üldise konditsiooni ja karotinoidsetel pigmentidel põhinevate sekundaarsete sootunnuste ekspressiooni vahel. Uurimisobjektina kasutati vangistuses peetavaid rohevinte (*Carduelis chloris*), kelle karotinoidsetel pigmentidel põhinev sulestik on teadaolevalt sugulise valiku indikaatortunnus – eredamalt värvunud isased on emaste poolt eelistatumad sigimispartnerid.

Esiteks: Et adekvaatselt hinnata immuunreaktsioonide mõju indiviidi füsioloogilistele parameetritele, peavad kasutatavad konditsiooniindeksid neid protsesse piisava täpsusega kirjeldama. Artiklis **I** vaadeldi, kui püsivad on erinevad leukotsüütsed immuunparameetrid, vereseerumi valkude ning triglütseriidide sisaldus, põhiainevahetustase, kehamass, spontaanne liikumisaktiivsus ning vereseerumi üldine antioksüdatiivne potentsiaal erinevate ajavahemike vältel. Selgus, et kõik vaadeldud konditsiooniindeksid on sobivad kirjeldamaks lühiajalisi füsioloogilisi muutusi isendi seisundis. Samas, leukotsüütsed immuunparameetrid, põhiainevahetustase ning kehamass püsisid suhteliselt stabiilsetena nelja kuu vältel. Järelikult võivad need konditsiooniindeksid peegeldada ka pikaajalisi isenditevahelisi kvaliteedierinevusi ning olla seega sobivad konditsiooni, elukäiguomaduste ning sugulise valiku indikaatortunnuste vaheliste seoste uurimiseks.

Teiseks: Immuunfunktsiooni osalemine erinevate elukäiguomaduste vahelistes lõivsuhetes eeldab, et immuunsüsteemi ülesehitus ja rakendamine on kulukas. Selgitamaks, kas humoraalne immuunväljakutse on kulukas, süstiti vangistuses peetavaid isaseid rohevinte mittepatogeense antigeeni (lamba erütrotsüüdid) või füsioloogilise lahusega (**II**). Kuigi võõrantigeeniga süstitud lindudel kutsuti esile lühiajaline akuutse faasi vastus, ei kutsunud humoraalne immunuväljakutse esile konditsiooniindeksite väärtuste olulisi muutusi. Siiski vähenes võõrantigeeniga süstitud lindude spontaanne liikumisaktiivsus. See osutab kaudselt immuunreaktsioonide kulukusele, kuna võõrantigeeniga süstitud linnud olid sunnitud energeetilisi kulutusi piirama.

Kolmandaks: Lindude karotinoididel põhinevat sulestiku värvust peetakse usaldusväärseks signaaliks isendi tervisliku seisundi kohta, kuna eeldatakse, et esineb lõivsuhe karotinoidide suunamisel elutähtsatesse füsioloogilistesse protsessidesse või signaliseerimisse. See hüpotees põhineb eeldusel, et sulgede värv peegeldab neisse paigutatud karotinoidide hulka. Selle eelduse paikapidavuse kontrollimiseks kirjeldati seoseid isaste rohevintide sabasulgede karotinoidide sisalduse ja neilt spektrofotomeetriliselt mõõdetud värviparameetrite (värvi toon – *hue*, puhtus – *chroma* ja ülderedus – *brightness*) vahel (**III**). Selgus, et suled, millelt mõõdeti kõrgemad värvi tooni ja puhtuse väärtused, sisaldasid ka enam karotinoide.

Neljandaks: Ehkki karotinoidsete pigmentide roll sugulise valiku indikaatormehhanismides on pälvinud suurt tähelepanu, ei ole siiani uuritud, kas karotinoididel põhinev sulgede värvus peegeldab linnu immuunvõimet. Nimetatud hüpoteesi testiti artiklis **IV.** Eredamate rinnasulgedega linnud tekitasid tugevamat humoraalset immuunvastust võõrantigeenile ning nende verest mõõdeti oluliselt madalamad heterofiilide kontsentratsioonid kui tuhmimalt värvunud isenditel. Need tulemused viitavad eredamalt värvunud lindude tugevamale immuunvõimele.

Viiendaks: Artiklites **V** ja **VI** selgitati, kas ainuraksed sooleparasiidid, koktsiidid, võivad olla seotud parasiitide poolt vahendatud sugulise valiku mehhanismidega. Eksperimentaalselt esile kutsutud koktsiidinakkuse mõju vangistuses peetavate isaste rohevintide tervislikule seisundile ning ornamentaalsete sulepartiide värvusele vaadeldi artiklis **V**. Koktsiididega nakatamine põhjustas lindude toitumusliku ning tervisliku seisundi ja ornamentaalsete sulgede karotinoidide sisalduse ning värviparameetrite drastilise languse. Saadud andmetele tuginedes võib väita, et koktsiidid võivad mängida olulist rolli parasiitide vahendatud sugulise valiku mehhanismides. Linnud, kelle soolestik on koktsiidinakkuse tagajärjel enam kahjustatud, ei ole võimelised piisavalt karotinoide omastama ja on seetõttu sunnitud vähendama sulgedesse paigutatava pigmendi hulka.

Peremehe ja parasiidi koevolutsiooni mudelid eeldavad, et ühe populatsiooni indiviidid erinevad üksteisest nakkusresistentsuse poolest, ning samaaegselt varieerub ka erinevate parasiiditüvede virulentsus. Selle eelduse kehtivust rohevindi koktsiidinakkuse mudelis kontrolliti artiklis **VI**. Lisaks testiti, kas rohevintide vastupanuvõime eksperimentaalsele koktsiidinakkusele korreleerub nende immuunvõime (mõõdetud kui immuunvastus mittepatogeensetele antigeenidele) ja karotinoididel põhineva sulestiku värvusega. Selgus, et erinevad koktsiiditüved põhjustasid erinevates peremeestes erineva virulentsusega nakkust. Kaudsed tõendid viitasid, et vaadeldud isenditevahelised erinevused nakkusresistentsuse osas võivad olla geneetiliselt determineeritud. Need tulemused näitavad selle mudelsüsteemi sobivust parasiitide vahelise sugulise valiku ning peremehe nakkusresistentsuse ja parasiidi virulentsuse koevolutsiooni uurimiseks. Siiski ei ilmnenud käesolevas katses ennustatud positiivseid seoseid sulgede värvuse, immuunvõime ja koktsiidiresistentsuse vahel. Need tulemused osutavad, et füsioloogilised mehhanismid, mis seovad sekundaarsete sootunnuste avaldumisel parasiidiresistentsusega, on palju keerukamad, kui võis oletada artiklites **IV** ja **V** kirjeldatud tulemuste põhjal.

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PUBLICATIONS

Hõrak, P., Saks, L., Ots, I. & Kollist, H. (2002)
Repeatability of condition indices in captive greenfinches (*Carduelis chloris*).
Canadian Journal of Zoology 80: 636–643.

Hõrak, P., Saks, L., Ots, I., Kullissaar, T., Kollist, H. & Zilmer, M. (2003)
Physiological effects of immune challenge in captive greenfinches
(*Carduelis chloris*). *Canadian Journal of Zoology* 81: 371–379.

Saks, L., McGraw, K.J. & Hõrak, P. (2003)
How feather colour reflects its carotenoid content.
Functional Ecology 17: 555–561.

Saks, L., Ots, I. & Hõrak, P. (2003) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* 134: 301–307.

Hõrak, P., Saks, L., Karu, U., Ots, I., Surai, P.F. & McGraw, K.J. (2004)
How coccidian parasites affect health and appearance of greenfinches.
Journal of Animal Ecology 73: 935–947.

Saks, L., Karu, U., Ots, I., & Hõrak, P. Host resistance and parasite virulence: concurrent variation and relations with immunocompetence and carotenoid signals in greenfinch coccidiosis. (submitted manuscript)

CURRICULUM VITAE

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II. Research history

1. Research interests

   Immunological ecology and sexual selection

2. Publications
   1. Hõrak, P., Saks, L., Ots, I. & Kollist, H. (2002) Repeatability of condition indices in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology* 80: 636–643.
   2. Saks, L., Ots, I. & Hõrak, P. (2003) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* 134: 301–307.
   3. Hõrak, P., Saks, L., Ots, I., Kullissaar, T., Kollist, H. & Zilmer, M. (2003) Physiological effects of immune challenge in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology* 81: 371-379.

    4. Saks, L., McGraw, K.J. & Hõrak, P. (2003) How feather colour reflects its carotenoid content. *Functional Ecology* 17: 555–561.
    5. Hõrak, P & Saks, L. (2003) Animal allure and health linked by plant pigments. *BioEssays* 25: 746–747.
    6. Hõrak, P., Saks, L., Karu, U., Ots, I., Surai, P.F. & McGraw, K.J. (2004) How coccidian parasites affect health and appearance of greenfinches. *Journal of Animal Ecology* 73: 935-947.

3. Conference theses
    1. **Eighth Congress of the European Society for Evolutionary Biology.** 20–25 August 2001. Århus, Denmark. Symposia of evolution and development of colour patterns. **Oral presentation: "Carotenoid-based plumage coloration reflects humoral immunoresponsiveness in male Greenfinches".** Authors: Lauri Saks, Peeter Hõrak and Indrek Ots. Presenting author: Lauri Saks.
    2. **International Workshop on Evolutionary Ecology of Resistance and Defence.** 12–14 December 2001. Konnevesi, Finland. **Oral presentation: "Condition indices in captive greenfinches: repeatability in time and relations to plumage coloration".** Authors: Lauri Saks, Peeter Hõrak and Indrek Ots. Presenting author: Lauri Saks.
    3. **Ninth Congress of the European Society for Evolutionary Biology.** 18–24 August 2003. Leeds, UK. Symposia of evolution of host defence against parasites. **Poster presentation: "How coccidian parasites affect health and appearance of greenfinches (*Carduelis chloris*)".** Authors: Peeter Hõrak, Lauri Saks, Ulvi Karu, Indrek Ots, Peter F. Surai and Kevin J. McGraw. Presenting author: Lauri Saks.
    4. **10th Congress of the International Society for Behavioral Ecology.** 10–15 August 2004. Jyväskylä, Finland. Symposia of parasites and immune function. **Poster presentation: "Parasite resistance and immunocompetence in greenfinch-coccidiosis model"** Lauri Saks, Ulvi Karu, Indrek Ots and Peeter Hõrak. Presenting author: Lauri Saks.

4. Review work

    Reviewed manuscripts for following journals: *Oecologia* (3), *Functional ecology* (1), *Biological Journal of the Linnean Society* (1).

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I. Üldandmed

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9. Teenistuskäik	Studio Viridis ltd., taksidermist-butafoor, juuni-september. 1996. Tartu Ülikool, Zooloogia ja Hüdrobioloogia inst., tööline, mai – juuli 2000. a., laborant, august 2001. a., erakorralin teadur, alates septembrist 2004. a.

II. TEADUSLIK JA ARENDUSTEGEVUS

1. Peamised uurimisvaldkonnad

   Immunoloogiline ökoloogia ja suguline valik.

2. Publikatsioonide loetelu

   7. Hõrak, P., Saks, L., Ots, I. & Kollist, H. (2002) Repeatability of condition indices in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology* 80: 636–643.
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4. Muu teaduslik organisatsiooniline ja erialane tegevus
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