JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

128

Janne Kilpimaa

Male Ornamentation and Immune Function in Two Species of Passerines

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2004

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 128

Janne Kilpimaa

Male Ornamentation and Immune Function in Two Species of Passerines

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa (YAA 303)
tammikuun 9. päivänä 2004 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
in the Building Ambiotica, Auditorium YAA 303, on January 9, 2004 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2004

Male Ornamentation and Immune Function in Two Species of Passerines

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 128

Janne Kilpimaa

Male Ornamentation and Immune Function in Two Species of Passerines

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2004

Editors
Jukka Särkkä
Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Marja-Leena Tynkkynen
Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-8843-2
ISBN 978-951-39-8843-2 (PDF)
ISSN 1456-9701

Jyväskylän yliopisto, 2022

ISBN 951-39-1640-5
ISSN 1456-9701

Jyväskylä University Printing House,
Jyväskylä and ER-Paino Ky, Lievestuore 2004

ABSTRACT

Janne Kilpimaa
Male ornamentation and immune function in two species of passerines
Jyväskylä: University of Jyväskylä, 2004, 34 p.
(Jyväskylä Studies in Biological and Environmental Science,
ISSN 1456-9701; 128)
ISBN 951-39-1640-5
Yhteenveto: Koiraan ornamentit ja immuunipuolustus varpuslinnuilla
Diss.

Male ornamentation may have evolved as a result of sexual selection or to signal dominance outside the breeding season. The aim of this thesis was to examine the function of male plumage variability and how immune function would contribute to the honesty of sexual signalling in the great tit and in the pied flycatcher. Immune function may affect sexual signalling because immune function is costly and traded off against sexual advertisement. Alternatively genetic variation in specific immune responsiveness may generate variation in condition that may be reflected in the expression of sexual traits. Our results support both of the hypotheses. We found large antigen specific genetic variation in humoral immune responsivenss suggesting potential for host-parasite coevolution to work as suggested by the Hamilton & Zuk hypothesis. Instead, general immune effectiveness was strongly condition dependent with no significant genetic variation. We also found both correlative and experimental evidence that immune function may be traded off against sexual advertisement. Our results indicate that sex differences in immune function may arise as a consequence of higher mating effort of males. Juvenile dominance in great tits was environmentally determined during the early development with no heritable variation. We found little evidence that black breast stripe would signal dominance in the great tit. Instead the breast stripe was flexible in its expression and may be used to signal aggressive motivation. Flexibility of ornament expression may have evolved to track changes in condition or status and need to be considered in studies of sexual selection and status signalling.

Key words: Dominance; immune function; ornamentation.

Janne Kilpimaa, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FIN-40014 University of Jyväskylä, Finland

Authors's address Janne Kilpimaa
Department of Biological and Environmental Science
University of Jyväskylä
P.O. Box 35
FIN-40014 University of Jyväskylä
Finland
e-mail: kjkilpim@jyu.fi

Supervisor Professor Rauno V. Alatalo
Department of Biological and Environmental Science
University of Jyväskylä
P.O. Box 35
FIN-40014 University of Jyväskylä
Finland

Reviewers Professor Erkki Korpimäki
Department of Biology
Section of Ecology
University of Turku
FIN-20014 University of Turku
Finland

Jukka Jokela
Department of Biology
University of Oulu
P.O. Box 3000
FIN-90014 University of Oulu
Finland

Opponent David F. Westneat
Department of Biology
101 Morgan Building
University of Kentucky
Lexington, KY
USA

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

LIST OF ORIGINAL PUBLICATIONS

I Kilpimaa, J., Van de Casteele, T., Mappes, J. & Alatalo, R.V. Genetic variation in specific and general immune defense in the great tit. Manuscript.

II Kilpimaa, J., Alatalo, R.V. & Siitari, H. 2003. Life history trade-offs and sex differences in immune function in the pied flycatcher. Submitted.

III Kilpimaa, J., Alatalo, R.V. & Siitari, H. 2003. Trade-offs between sexual advertisement and immune function in the pied flycatcher (*Ficedula hypoleuca*). Proc. R. Soc. Lond. B, in press.

IV Kilpimaa, J., Mappes, J. & Alatalo, R.V. Determination of dominance: a cross-fostering experiment in the great tit. Manuscript.

V Kilpimaa, J., Alatalo, R.V., Mappes, J. & Siitari, H. Plumage characters, dominance and winter survival in the great tit. Manuscript.

1 INTRODUCTION

The extreme intraspecific plumage variability in males is a topic of widespread interest. There are two major hypotheses explaining the evolution of male plumage variation. First, sexual selection theory suggests that male plumage ornamentation has evolved as a result of sexual selection through female preference or male-male competition over territories or females (Andersson 1994). Recently, an important role for the immune function in the sexual selection has been suggested. For example, it has been suggested that male ornamentation may reveal the heritable parasite resistance and the host-parasite coevolution may explain how variation in fitness is maintained (Hamilton & Zuk 1982). Secondly, status signalling hypothesis suggests that male plumage variability has evolved to signal fighting ability or general dominance outside the breeding season (Rohwer 1975, 1982). Agonistic signalling between males may be important also during breeding in the context of sexual selection in contests over territories or females.

1.1 The sexual selection theory

Sexual selection is defined as the competition between conspecifics of the same sex in relation to mating success (Darwin 1871). Both female choice and male-male competition over females or territories can drive the evolution of conspicuous plumage signals (Andersson 1994). Females may gain either direct (parental care, territorial quality) or indirect (genetic) benefits by choosing the most conspicuous males.

1.1.1 The Fisher process

The Fisherian self-reinforcing theory assumes that female preference is genetically coupled with the male trait preferred (Fisher 1930, Lande 1981, Kirkpatrick 1982). Hence, females mated with highly ornamented males produce male offspring with a preferred trait and female offspring with a preference on the same trait. As a consequence female preference and a male trait coevolve to ever more extreme expressions in self-reinforcing runaway process. The Fisher process does not assume condition dependence of the male trait. The fitness advantage to the preferred highly ornamented males or females choosing those males will be the increased mating success of their sons. Hence, ornaments that are unconnected to condition or have ceased to be connected to condition are often cited as Fisher traits.

1.1.2 Good genes

Good genes models of sexual selection suggest that female preference for male ornamentation has evolved as a way of acquiring mates of high genetic quality (Trivers 1972, Pomiankowski 1988). The key idea of various handicap models is that ornamentation is costly and the associated costs should be relatively larger for low quality males (conditional handicap) (Zahavi 1975, 1977). Hence male ornamentation would serve as an honest indicator of male heritable viability (Iwasa et al. 1991, Rowe & Houle 1996). The main objection against the idea that ornamentation would serve as a signal of heritable quality is that fitness traits including ornaments would have zero heritability at equilibirium (Falkner & MacKay 1997). Consequently females would not gain any genetic benefits by choosing the most ornamented males (Rowe & Houle 1996). However, a recent meta-analysis suggests that preferred males often produce more viable offspring but the genetic benefits seem to be small (Møller & Alatalo 1999). There are several mechanisms how genetic variation in fitness may be maintained including genotype by environment interaction (Kotiaho et al. 2001), mutation-selection balance (Rowe & Houle 1996) and host parasite coevolution (Hamilton & Zuk 1982).

1.2 Causes of variation in immune defence

1.2.1 Avian immune system

Avian immune system consists of non-specific innate immunity and highly specific acquired immunity that work together during infection (Wakelin & Apanius 1997). Innate immunity is important during the early phase of immune insult. Acquired immunity depends on lymphocytes and is slower but more definitive and highly specific for particular pathogen antigens. Immunological memory of acquired immunity enables rapid responses to pathogens exposed earlier in life.

1.2.2 Specific versus general immune defence

There are two major sources of variation in immune function. First, variation in general immune responsiveness may be a result of costliness of maintenance and functioning of the general immune defence (Sheldon & Verhulst 1996, Zuk & Stoehr 2002, Schmid-Hempel & Ebert 2003, Schmid-Hempel et al. 2003). Hence, the quality of immune function would depend on the condition and hence resources available to immune function and other competing physiological or behavioral traits. As a consequence immune function has also the potential to shape individual life histories. For example general immune efficiency may be traded off against other costly life history traits like reproduction.

Secondly, variation in immune defense may originate from host-parasite interactions that may shape the genetic variation of highly specific immune responses (Hamilton & Zuk 1982, Schmid-Hempel & Ebert 2003, Schmid-Hempel 2003). Parasites adapt to host defence mechanisms. As a consequence rare resistant host genotypes may be favoured and frequency dependent selection would maintain genetic variation in a host population. Host parasite coevolution assumes that immune defence is specific to particular parasite types (Hamilton 1980). In the molecular level antigen recognition of acquired immunity is always specific because particular antigen epitopes bind to specific receptors on the lymphocyte surface (Wakelin & Apanius 1997). However, some antigens may be recognized more easily or elicit stronger responses generating variation in the immune repertoire (functional specificity). Unlimited immune repertoire is not likely, because large immune diversity increases the risk of autoimmunity diseases (Råberg et al. 1998). Hence, genetic architecture of host immune function may reflect the history of parasite infections in the past.

1.2.3 Sex differences in immune function

Male immune function has been suggested to be reduced compared to females (Poulin 1996, Zuk & McKean 1996, Møller et al. 1998). Proximate reason for this may be immunosuppressive effects of androgens (Zuk & McKean 1996). Sex differences in immune function may also be a result of sex differences in interactions between immune function and life history (Zuk & McKean 1996). Male fitness is often limited by the number of offspring fertilized whereas female fitness is limited by the number of offspring reared (Trivers 1972). Hence males are expected to invest relatively more in mate attraction and may have to compromise their immune function during the mating period. Also the mating system of the host species will influence the likelihood of sex differences in parasite infection, because males in monogamous species are subject to weaker sexual selection than males in polygynous species (Zuk & McKean 1996).

1.3 Sexual selection and immune function

Why is immune function important in sexual selection? First there is lots of evidence that females often prefer parasite free males and male ornamentation has been shown to be affected by parasites (Møller 1990, Møller 1991, Zuk et al 1990, von Schantz 1999). Secondly host parasite coevolution may explain why heritable fitness is maintained and hence the evolution of male ornamentation (Hamilton & Zuk 1982). There are two major hypotheses that explain how immune function can contribute to the honesty of sexual signalling, The Hamilton & Zuk-hypothesis (1982) and the Immunocompetence handicap-hypothesis (Folstad & Karter 1992, Wedekind & Folstad 1994).

1.3.1 Hamilton & Zuk (HZ) hypothesis

HZ states that male ornamentation has evolved to signal heritable parasite resistance (Hamilton & Zuk 1982). The model suggests that the expression of male secondary ornaments is condition-dependent and that only individuals with superior genetic disease resistance and vigour can fully express exaggerated secondary ornaments. According to the HZ variation in male quality is maintained as a consequence of highly specific host parasite coevolution. If parasites fluctuate in time and space and hosts show pathogen specific genetic resistance, different genotypes may be favoured at different rounds of selection maintaining variation in individual quality (Hamilton 1980). Because of the specificity of immune function i.e. the efficiency of host parasite resistance would depend not only on the host genotype but also on the genotype of parasites at the given time, it is very difficult to measure the quality of immune function. The best empirical evidence supporting HZ comes from the study on pheasants where male MHC genotype was found to predict both the spur length (male ornament) and the male viability (von Schanz et al. 1996). These data indicate that polymorphic genes with a central role in immune recognition can be associated with viability and the expression of a condition-dependent intersexually selected male trait, thus supporting essential parts of the Hamilton and Zuk model.

1.3.2 Immunocompetence handicap (ICH) hypothesis

The key idea of the ICH hypothesis is costliness of immune function. Because of different costs of immune defence immune function has been suggested to shape host life history (Zuk & Stoehr 2002). According to the ICH hypothesis animals may have to trade off the efficiency of their immune defence against sexual advertisement (Folstad & Karter 1992, Wedekind & Folstad 1994). Originally immunocompetence handicap hypothesis suggested that a trade off between immunocompetence and ornamentation is a direct consequence of the dual effect of testosterone (Folstad & Karter 1992). Androgens are needed to produce sexual ornamentation but at the same androgens suppress immune function. However, recent studies suggest that the physiological link between ornamentation and immune function may be the effects of stress hormones rather than sex hormones (Evans et al. 2000). In addition, the trade off between immunocompetence and ornamentation may also arise in the absence of direct effects of hormones if maintenance and functioning of the immune system and the production and maintenance of secondary sexual characters compete for the same resources (Wedekind & Folstad 1994). Immune function may be suppressed during mating also to avoid autoimmune diseases during stress. To sum up the key idea of ICH-hypothesis is that immune function is an important life history trait that is costly and traded off against sexual advertisement.

1.4 Dominance and plumage variation

1.4.1 Determination of dominance

Social dominance can be defined as the success in antagonistic contests or priority of access to resources. Since dominance depends not only on the properties of the given individual but also on the characters of the opponents, dominance rank is a result of several asymmetries between the contestants (Drews 1993). Asymmetries between individuals that have been suggested to determine the outcome of conflicts are age, sex, body size, prior residency and motivation (Sandell & Smith 1991, Lemel & Wallin 1993). Since dominance is strongly affected by social environment, it is difficult to measure dominance and it has been suggested that dominance can not be inherited (Dewsbury 1990, Drews 1993). However, the general ability to dominate resources or females may be heritable at least in particular environments or ecological context, because many traits that are closely related to dominance are heritable (Horne & Ylönen 1998).

1.4.2 Plumage variation and status signalling

If males exhibit ornamental plumage also outside the breeding season it is possible that ornamentation has also functions other than the sexual display. Status signalling hypothesis suggests that intraspecific plumage variability in some birds have evolved to reduce contest intensity outside the breeding season and plumage variation (patches of status) would signal general dominance and fighting ability (Rohwer 1975, 1982).

Many patches of status in birds are melanin-based. There is evidence that melanin based ornaments are cheap to produce (Hill & Brawner 1998, Gonzales 1999, but see Veiga and Puerta 1996). Because of the low production costs, honesty of melanin based badges is probably maintained through social costs i.e. individuals with a large patch carry a cost of increased aggression by conspecifics (Maynard-Smith & Harper 1988).

Contrary to melanin based ornamentation carotenoid signals are regarded costly to produce and hence honest signals of quality. Carotenoids can not be synthesized by a bird and hence only the most efficient foragers may express the most extreme carotenoid ornamentation. Carotenoids are also immunostimulants and antioxidants (Olson & Owens 1998) and hence carotenoid colouration may reveal nutritional or immunological stress. Dual function of carotenoids as both antioxidants and colour pigments may also provide a physiological mechanism behind the hypothesized trade off between ornamentation and health (von Schantz et al. 1999). Accordingly there is lot of empirical evidence that carotenoid based plumage variation is sensitive to stress and parasitism contrary to melanin based coloration (Hill & Montgomerie 1994, Hill 1996).

Because of the costliness of carotenoid based plumage coloration it has been regarded as a good candidate for honest signal in the context of sexual selection and it has often been assumed that carotenoid based colours would work in status signalling or intra sexual competition only during breeding season when the value of the contested resource (territories or females) is high. There is firm evidence that carotenoid colours signal dominance in male-male contests over territories in red-collared widowbirds (Pryke et al. 2001). Instead the few studies on the relation between carotenoid variation and dominance outside the breeding season have found no evidence that carotenoid colours would signal dominance (Wolfenbarger 1999, McGraw & Hill 2000).

2 STUDY SPECIES

2.1 The great tit

The great tit (*Parus major*) is a small sedentary cavity nesting passerine. The great tit is sexually dimorphic the black breast stripe being the most conspicuous male character. Also females have the breast stripe in a reduced state. Males are also slightly bigger in body size.

2.1.1 Breast stripe

It has been suggested that the black breast stripe may work in sexual selection and females may gain genetic benefits in choosing males with a wide breast stripe (Norris 1993). On the other hand black breast stripe of the great tit has been often cited as a classical example of a badge of status (Järvi & Bakken 1984, Järvi et al 1987, Maynard-Smith & Harper 1988) but see Wilson (1992). Lemel & Wallin (1993) suggested that the breast stripe has impact only in conflicts between individuals of no prior experience of each other.

2.2 The pied flycatcher

The pied flycatcher (*Ficedula hypoleuca*) is a small cavity nesting migratory passerine, in which some males are polygynous in separate territories. The plumage coloration is sexually dimorphic. Males have conspicuous white forehead patch which females in a study population lack (Lundberg & Alatalo 1992). In our study population males are highly polymorphic in dorsal plumage colour some males being conspicuously black and some resembling cryptic brown colour of females.

2.2.1 Forehead patch

The function of variability in the forehead patch is not properly understood although it has been suggested that the white forehead patch is sexually selected as indicated by studies in Spain (Potti & Montalvo 1991). Instead the forehead patch of the sibling species collared flycatcher has been a subject of intensive research. In the collared flycatcher it has been shown experimentally that forehead patch functions as a signal of status in male-male contests over territories during the breeding season (Pärt & Qvarnström 1997). In addition males with large forehead patch enjoy increased success in acquiring secondary females (Gustafsson et al. 1995) and extra-pair copulations (Sheldon & Ellegren 1999).

3 METHODS

3.1 General methods

The study was carried out at the Konnevesi Research Station (62 ° 37′ N, 26 ° 20′ E), Central Finland during years 1999-2002. We applied a cross-fostering experiment for lab-raised great tit offspring to study genetic and environmental sources on variation in immune function (I), dominance and melanin based plumage coloration (breast stripe and throat patch)(IV). At the same time the role of melanin based plumage coloration on dominance was studied in aviary experiments. In a separate study the effects of male plumage variation on winter dominance and survival were studied using aviary experiments and field observations on these individually colour ringed birds (V). In the pied flycatchers we investigated the trade offs between the immune function and sexual advertisement by experimentally manipulating both the male mating effort and the activity of immune function (III). Correlative data on the relationships between ornamentation, mating effort and immune function were collected in two different study years (II).

3.2 Cross-fostering experiment

To separate common environmental from pre-hatching maternal and genetic causes of resemblance, a partial cross-fostering experiment was conducted in a study on great tits (I). Half of the two-day old nestlings were swapped between pairs of nests with the same hatching date. As a result half of the offspring were raised by genetic parents and half by foster parents.

3.3 Measuring immune function

3.3.1 Leucocyte counts

In 2002 blood smears were taken from pied flycatcher males for leucoyte counts. For this a drop of blood from a microcapillary tube was transferred to a glass slide, smeared, air-dried and fixed in absolute ethanol. After fixing the blood smears, they were stained with commercial Giemsa dye for 1 hour in room temperature (1:10 Giemsa dye in 5mM phosphate buffer). After staining, blood smears were inspected under a light microscope (Nikon Eclipse E400). The number of heterophils (H) and lymphocytes (L) was calculated per 1000 erythrocytes in randomly chosen eye fields first under 40X and finally 100 X magnifications with oil immersion. Only heterophil counts are used in the analyses, because heterophil count itself is strongly correlated with a stress index (H/L ratio) (Lebigre, Alatalo, Kilpimaa & Siitari 2003, unpublished).

3.3.2 Immune challenging technique

The rationale behind the challenging technique is that the ability to mount immune response against novel antigens reflects the ability to resist real pathogens. By challenging the immune system with novel harmless antigens one can measure immune efficiency without confounding direct costs of pathogens. Also by using novel antigens one avoids the problem of some individuals being immune.

3.3.2.1 Humoral immune responsiveness

In a study on heritable variation in immune responsiveness (I) immune system of juvenile great tits was challenged with diphtheria-tetanus vaccine. Two months old lab-raised juvenile great tits were blood sampled and were injected in the pectoral muscle with 100 µl diphtheria-tetanus vaccine (Finnish National Public Health Institute, diphtheria 38 Lf (limit of flocculation) and tetanus 10 Lf, mixed with adjuvant aluminum phosphate at 1.0 mg ml-1). This vaccine contains two antigens novel to the birds. Blood samples were taken twelve days after these injections to evaluate the final immune responsiveness (Svensson et al. 1998). Specific antibodies against diphtheria and tetanus were measured using enzyme-linked immunosorbent assay (ELISA). By challenging immune system with two different antigens we were able to investigate also the genetic basis of specificity in immune responsiveness.

In a study on pied flycatchers male immune function was challenged with sheep red blood cells (SRBC) and change in total immunoglobulins was used as a measure of humoral immune responsiveness. Immunoglobulin concentrations were measured using ELISA.

3.3.2.2 Cellular immune response

Phytohaemaglutinin (PHA) is a non specific mitogen that stimulates T-cells to proliferate non specifically without previous immunization and this test provides a general index of cell-mediated immunity. PHA skin test is only challenge method that can be applied in nestling birds whose immune system is not fully developed. We applied this method to thirteen-day old great tit nestlings. Nestlings were injected intradermally in the center of the right wing-web with 0.2 mg of PHA (Sigma, L-8754) in 0.04 ml PBS. The left wing (control) was injected with 0.04 ml PBS (phosphate buffered saline pH 7.4). Thickness of the injection site was measured with an accuracy of 0.01 mm using a spessimeter just prior and 24 hours after injection. The swelling of the wing-web was calculated as the difference in thickness prior to and after injection. The cell-mediated immune response was calculated as the difference in swelling between the PHA-injected wing and the control wing (wing-web index).

3.4 Measuring dominance

Dominance was determined in aviary experiments. In a study on juvenile great tits (IV) at each dominance trial 5 males and 5 females were introduced to an aviary where they were allowed to habituate for 2 hours. During the 2 hours birds were not given food to motivate them to contest for food resources. Dominance index was based on three measures: access to food (the first individual to feed full 1 minute was ranked number 1 and the last to feed during half an hour trial was ranked number 10), the proportion of interactions won and total number of interactions. In a study on male great tits (V) dominance was measured in a similar way but there were five males at each trial. In addition feeding order and aggressiveness were analyzed separately.

3.5 Measuring plumage variation

3.5.1 Melanin based colouration

Breast stripe size and throat patch size was obtained from image analysis of digital photographs (IV). We also measured the breast stripe size during dominance experiment from the video captures (V). Breast stripe size was obtained using image analysis program and known length of the perch was used as a scale to get absolute area in square centimetres.

3.5.2 Carotenoid colours

We measured plumage reflectance of yellow breast feathers from feather samples using a Zeiss MCS 500 spectroradiometer at 135° (90° to illumination). Reflectance was calculated relative to a Spectralon™ 99% reflectance standard for the wavelength range 300 to 700 nm. For every individual, we calculated the average reflectance (brightness; $R_{300\text{-}700nm}$, Andersson et al. 1998) and chroma (R_{700}-R_{450nm}/R_{700nm}, Johnsen et al. 2003).

3.5.3 Forehead patch of the pied flycatcher

At each capture male forehead patch size was measured with a digital calliper to the nearest 0.1 mm. In Central and Northern Europe male forehead patch is highly variable in shape (Lundberg & Alatalo 1992). Three different shapes can be separated: rectangular, a patch divided into two dots or a combination of both (heart shaped). We measured maximum width, maximum height and minimum height of the forehead patch. In the study II forehead patch size was calculated as a first principal component from a PCA analysis of the correlation matrix of three forehead patch dimensions. The first principal component explained 68.76 % (May 2001), 73.14 % (2001 June) and 62.10 % (May 2002) of the total variation in the three measures of forehead patch size. All the three patch dimensions were highly positively correlated with the first principal component. In the study III forehead patch width and height were analyzed separately.

4 RESULTS AND DISCUSSION

4.1 Determination of immune efficiency

We investigated three potential sources of variation in immune efficiency: genetic variation, condition dependence and phenotypic plasticity of immune efficiency. In the study on great tits (I) we applied cross-fostering technique to study genetic sources of variation and condition dependence of immune responsiveness. In the study on pied flycatchers (III) we investigated phenotypic plasticity of immune responsiveness by comparing immune efficiency between males with experimentally prolonged mating effort and control males. We also compared immune function of each sex during different life stages in pied flycatchers (II).

4.1.1 Sex differences in immune function

We found that males had much higher hematocrit during the mating period but there was no difference between sexes during the nestling period. Hematocrit is a proportion of red blood cells of the whole volume of blood. Hematocrit is a measure of locomotory activity (Palomeque & Planas 1978) but it is also under hormonal control and in birds males often have higher hematocrit (Nirmalan & Robinson 1972, Kern et al, 1974). Male immunoglobulin (IgG) levels were highest during the nestling period whereas in females immunoglobulin levels were highest during the mating period especially just prior egg laying. High female immunoglobulin levels during the mating period may be a result of altered immune profile due to transmission of immunoglobulins to eggs. Whereas in males high hematocrit and low IgG during the mating period suggests that males may compromise the efficiency of their immune function during mating period in the line with the predictions of the ICH hypothesis. Hence in polygynous species, in which selection pressure for high male mating effort is relatively strong, differences between sexes in immunocompetence and parasite susceptibility may arise as a result of different reproductive strategies of each sex. Our results further suggest that sex related differences in immune function may arise only during mating season and males may compromise the efficiency of their immune function only temporarily.

4.1.2 Heritability and condition dependence of immune responsiveness

It has been suggested that variation in general and specific immune defence may be maintained by different mechanisms. Variation in general immune responsiveness may be a result of trade off between immune function and other costly life-history traits. Instead variation in specific immune defense may originate from host-parasite interactions. Hence, the relative contribution of genetic and environmental sources on variation in general and specific immune responses may be different. Actually that is exactly what we found in our study. In a cross-fostering experiment in the great tit (I) we found large origin-related variation for humoral immune responsiveness for both novel antigens studied. The origin-related variation was significant both for general immune responsiveness (average response to two antigens) and for specificity of immune responsiveness (significant origin-antigen interaction). Hence, our results provide evidence that genetic variation in humoral immune responsiveness is specific to particular antigens. For cell-mediated immune responsiveness (a measure of general immune responsiveness) variation was strongly affected by the rearing environment but not by the origin.

Our results also suggest that antigen-specific humoral immune responses are not strongly condition-dependent, because the environment during early development had no effect on humoral immune responsiveness against two antigens. This could indicate that maintenance and functioning of humoral immune defense is not energetically or nutritionally costly. However, there might be other costs involved in expressing the immune responses. Immune function may be down-regulated by sex or stress hormones during reproduction (Folstad & Karter 1992) to decrease the risk of autoimmune diseases during stress (Råberg et al. 1998). Instead, cell-mediated immune response was strongly environmentally determined. The PHA skin test we used provides an estimate of nonspecific general immune effectiveness. Our results and earlier studies using a PHA skin test (Saino et al. 1997, Brinkhof et al. 1999, Tella et al. 2000, Christe et al. 2000) suggest that some aspects of general immune effectiveness are strongly condition-dependent, whereas antigen-specific humoral immune responses are strongly genetically determined.

Our results suggest that genetic variation is much higher in immune responses where both specific antigen recognition and processing as well as non specific processes are involved. It is possible that large genetic variation in humoral immune responsiveness in our study is a result of frequency dependent selection suggested by theories of host parasite coevolution. Nevertheless, large antigen specific genetic variation in immune function suggests potential for host-parasite coevolution to operate. Low genetic variation in non-specific immune effectiveness (t-cell proliferation) suggests that genes common for all immune responses irrespective of the given antigen show much less variation. Low heritability and strong condition dependence of general immune responses may suggest that general immune efficiency is under positive directional selection.

Our results have also implications on sexual selection. Hamilton & Zuk hypothesis suggests that good genes that females choose determine health and parasite resistance. However, it is not clear what aspects of immune function 'good genes' that females choose influence. Our results suggest that genes coding for general immunocompetence may not qualify for good genes, because the amount of genetic variation in general immune responsiveness is not large. Instead genes coding for specific immune responses may qualify for the good genes. Interestingly, the relative ranking of some of the families in their capacity to elicit immune responses depended on the particular antibodies measured. This suggests that the quality of immune function would not depend only on the host genotype but also on the genotype of parasites. Our results suggest that studies that ignore specificity of immune defences may fail to measure individual quality. However, the extent to which specificity could reflect quality will depend on the composition, the predictability of the pathogen/parasite fauna and the virulence of the pathogens/parasites an individual and/or its descendants will experience. This will depend on the spatio-temporal variability of pathogens/parasites and on an individual's mobility. Whereas high specificity will be advantageous in a constant, predictable and virulent pathogen/parasite environment, low predictability may favour low specificity as a bethedging strategy.

4.1.3 Trade off between immune function and ornamentation

We investigated the trade offs between the immune function and sexual advertisement by experimentally manipulating both the mating effort and the activity of immune function in male pied flycatchers (III). Experimentally widowed males that were forced to continue their sexual activities suffered reduced humoral immune response compared to control males indicating the trade-off between sexual advertisement and immune function. In addition, males whose immune system was challenged by vaccination with a novel antigen had reduced expression of the forehead patch width compared to males injected with NaCl. Hence, causality behind the trade off between immune function and sexual advertisement may work in both directions; sexual activity suppresses immune function but immune challenge also reduces sexual advertisement.

In a correlative study on male pied flycatchers (II) males with large patch size were more stressed during mating and had reduced immune response during the nestling period. In addition forehead patch size and hematocrit were positively correlated during mating, but the relation was absent during the nestling feeding period, suggesting that highly ornamented males invest relatively more in sexual display. In sum, our results provide evidence that male forehead patch size predicts male mating effort and increased mating effort and male ornamentation (forehead patch expression) are at least temporarily costly.

4.2 Dominance and plumage variability

4.2.1 Heritability and condition dependence of dominance

In a cross-fostering experiment (IV) there was no heritable variation in juvenile dominance in the great tits. Instead common rearing environment (including parental care of the foster parents) during early development had a significant effect on dominance rank. The only condition measure that was phenotypically correlated with dominance was cell mediated immunity before fledging. This suggests that rearing related variation in dominance may be attributable to health.

4.2.2 Plumage variation and status signalling

Breast stripe size or size of the throat patch did not influence the dominance rank of cross-fostered lab raised juvenile great tits (IV). Neither did the breast stripe predict dominance of captive male great tits in winter (V). Instead high level aggression decided the dominance with no impact of less intense display or breast stripe size. However, males with large breast stripe size had lower fat scores at capture. This suggests that also the size of the breast stripe itself may affect dominance in certain conditions, in particular when the value of the contested resource is low. In our experiment two hours starvation may have resulted in the food resource becoming too valuable for contestants to decide the access to food by low cost breast stripe display only.

Plumage brightness of yellow breast feathers correlated positively with both dominance and aggressiveness in male great tits, but plumage chroma did not (V). Only experimental manipulation of plumage brightness would reveal whether plumage brightness is a true signal or whether it is only a correlate of general dominance. In earlier studies carotenoid colours were not related to dominance outside breeding season (Wolfenbarger 1999, McGraw & Hill 2000). Instead carotenoid signals may be more important in antagonistic competition during mating season (Pryke et al. 2001).

4.2.3 Determination of plumage variability

4.2.3.1 Heritability and condition dependence of plumage characters

We investigated genetic and environmental variation in melanin based plumage characters in cross-fostered juvenile great tits IV). Rearing environment had no effect on the size of the throat patch and size of the breast stripe, but there was significant origin related variation in throat patch size suggesting heritable variation. Body condition during moult was phenotypically correlated with both the size of the throat patch and size of the breast stripe. This result suggests that producing melanin based plumage character may be costly or individuals in poor condition may moult less conspicuous plumage characters to avoid conflicts between conspecifics. Latter explanation seems to be more likely since melanin based ornaments have been regarded cheap to produce (Hill & Brawner 1998, Gonzales 1999). In addition if these plumage characters were strongly condition dependent one should expect also significant effects of rearing environment, which was not found in our study.

4.2.3.2 Flexibility of ornament expression

Our results suggest that in the pied flycatcher both forehead patch width and height show plasticity during the breeding season (III). Experimental removal of females affected the patch height but not patch width, whereas activating immune function by vaccinating with novel antigen reduced patch width, and there was also a tendency for patch height to be reduced. Hence patch size is flexible in its expression and may reflect activity and current health.

We found evidence that also the black breast stripe of the great tit is flexible in its expression (V). In the dominance experiment individual breast stripe expression was largest in the beginning of the experiment. Breast stripe width measured in hand correlated with the breast stripe size measured from video only in the second and third video captures after decline in the breast stripe size. Hence breast stripe of the great tit is partly coverable and great tits may adjust the expression of the signal according to their motivation or intent. Indeed, mean breast stripe size measured during interactions from video correlated with aggressiveness but breast stripe measured in hand did not.

Both immune function and ornamentation show phenotypic plasticity. Hence males may adjust the ornament expression to their current health or condition. Flexible ornamentation is probably a poor indicator of male long term condition and may not be very important in female choice. However plasticity of ornament expression may be important in male-male competition where male status strongly depends on social and environmental factors like prior residency, ownership and characters of the opponent or endogenous factors like infection status and health. Flexibility of ornament expression may have evolved to track changes in condition or status and must be considered in studies of sexual selection and status signalling. More fixed ornaments like carotenoid signals or elongated feathers (e.g. barn swallow tail) may be more reliable signals of male viability and hence subject to female choice.

CONCLUSIONS

The aim of this thesis was to examine the function of male plumage variability and how immune function would contribute to the honesty of sexual signalling.

By using cross-fostering design we quantified the relative contribution of genetic and environmental sources on general and specific immune defence. It has been suggested that variation in general and specific immune defence may be maintained by different mechanisms. Our results suggest that genetic variation is much higher in immune responses where both specific antigen recognition and processing as well as non specific processes are involved. Instead general immune effectiveness is strongly condition dependent and shows less genetic variation. It is possible that large genetic variation in humoral immune responsiveness in our study is a result of frequency dependent selection suggested by theories of host parasite coevolution. Low heritability and strong condition dependence of general immune responses may suggest that general immune efficiency is under positive directional selection.

Our results have also implications on sexual selection. Large environmental variation in general immune efficiency suggests that general immune defence is costly and hence has potential to shape host life history. Condition dependence of immune function may contribute to the honesty of sexual signalling because only males in good condition can afford to have both costly elaborate ornamentation and efficient immune function. Large genetic variation in specific immune responsiveness may also generate variation in condition through variation in specific parasite resistance and hence fitness.

In a separate study we found evidence that immune function may be traded off against sexual advertisement. Experimentally increased male mating effort suppressed humoral immune responsiveness. In addition, experimental activation of immune defence by vaccination with novel antigens reduced the expression of male ornament dimensions. Hence, causality behind the trade off between immune function and sexual advertisement may work in both directions: sexual activity suppresses immune function but immune challenge also reduces sexual advertisement. In a correlative data there was a negative correlation between forehead patch size and humoral immune responsiveness suggesting also a trade off between immune function and ornamentation. During mating male hematocrit (a measure of activity) was positively correlated with forehead patch size but the relation was absent during nestling feeding period suggesting that more ornamented males invest relatively more in mating. Thus, our results suggest that in the pied flycatcher immune function is traded off against sexual advertisement and forehead path expression may reflect sexual activity and current health. Our results also indicate that sex differences in immune function may arise as a consequence of higher mating effort of males.

Juvenile dominance in the great tits was environmentally determined during early development with no heritable variation. We found little evidence that black breast stripe would signal dominance in the great tit. Instead we found evidence that the black breast stripe of the great tit is flexible in its expression and may be used to signal aggressive motivation. Males with bright yellow breast feathers were more dominant. Only experimental manipulation of plumage brightness would reveal whether plumage brightness is a true signal or whether it is only a correlate of general dominance.

To sum up, immune function may contribute to the honesty of sexual signalling because immune function is costly and traded off against sexual advertisement. Alternatively large genetic variation in specific immune responsiveness may generate variation in condition that may be reflected in the expression of condition dependent sexual traits. Many apparently fixed plumage characters are flexible and may be used to signal aggressive motivation, sexual activity or current condition. Individual condition is not fixed and may be affected by life-history trade-offs and infections by pathogens. Flexibility of ornament expression may have evolved to track changes in condition or status and must be considered in studies of sexual selection and status signalling.

Acknowledgements

First of all I would like to thank my supervisor Rauno Alatalo who gave me the opportunity to be a part of his research project. I also thank Johanna Mappes and Heli Siitari from our research group. Tom van de Casteele made a big contribution to perhaps the most crucial paper of my thesis. I also thank Anders Pape Møller who gave me the opportunity to work in his barn swallow project in 1998 and 1999 which had a big impact on my future decision to continue with science. Anders also introduced me to the field of immunoecology. Pekka Rintamäki introduced me to Anders and his barn swallow project with his letter while I was in Israel as a volunteer ringer in autumn 1997. Without his letter, perhaps, I would have never started my PhD work. Pekka also introduced me to the wonderful world of Finnish bird stations and bird ringing. Matti Halonen made a big contribution with collecting data in the field during all these years. Other people who helped in the field are Panu Halme, Antti Hannuniemi, Heikki Helle, Laura Häsä, Satu Kuntsi and Annukka Näyhä. I would like to thank also the staff of the Konnevesi Resarch Station, especially Helinä Nisu who took good care of our study birds. Elina Virtanen made most of the laboratory analysis and Ilmari Jokinen helped in developing laboratory methods. I would also like to thank all people in our department especially Markus Rantala, Raine Kortet, Jari Ahtiainen, Seppo Kuukasjärvi, Tero Toivanen and Jouni Toivola. Most importantly, I would like to thank my parents, the only people I can always rely on.

YHTEENVETO

Koiraan ornamentit ja immuunipuolustus varpuslinnuilla

Tämän väitöskirjan tarkoituksena oli selvittää koiraan höyhenpuvun ornamenttien merkitystä. Koiraan ornamentit voivat olla seksuaalivalinnan tulos tai ilmentää dominanssisuhteita lisääntymisajan ulkopuolella. Lisäksi tarkoituksena oli selvittää immuunipuolustuksen osuutta seksuaalisen signaloinnin rehellisyyden takaajana. Koiraan ornamentit voivat olla rehellinen signaali koiraan kunnosta, jos sekä ornamenteista että immuunipuolustuksesta on kustannuksia, ja täten vain hyväkuntoisilla koirailla on varaa tuottaa iso ornamentti immuunipuolustuksen kustannuksella ("immuniteettihaitta" -hypoteesi). Vaihtoehtoisesti geneettinen muuntelu tautispesifisessä immuunipuolustuksessa voi aiheuttaa eroja kunnossa koiraiden välillä, mikä voi heijastua seksuaalisessa signalonnissa ("Hamilton & Zuk" -hypoteesi). Väitöskirjani tutkimukset puoltavat molempia hypoteesejä. Ristiinkasvatuskokeiden perusteella spesifisessä immuunipuolustuksessa on paljon geneettistä muuntelua, ja täten naaraat voivat saada geneettistä hyötyä valitsemalla terveimpiä tai hyväkuntoisimpia koiraita, kuten "Hamilton & Zuk" -hypoteesi ennustaa. Sen sijaan yleinen ei-spesifinen immuunipuolustus oli heikosti periytyvää, mutta se oli sen sijaan voimakkaasti kasvatusympäristöstä ja kunnosta riippuvainen. Lisäksi kun koiraan lisääntymispanosta kokeellisesti lisättiin, koiraiden immuunipuolustus heikkeni. Myös koiraiden immuunipuolustuksen kokeellinen aktivointi vaikutti niiden ornamentin kokoon. Nämä tulokset tukevat "immuniteettihaitta" -hypoteesiä. Myös korrelatiivinen aineisto kirjosiepolla tukee ajatusta, että koiraat voivat joutua tekemään kompromissin seksuaalisen signaloinnin ja immuunipuolustuksen välillä. Tutkimusteni mukaan koirailla voi olla pariutumisaikaan heikentynyt immuunipuolustus verrattuna naaraisiin, ja tämä johtuu sukupuolten välisistä eroista lisääntymisstrategioissa. Ristiinkasvatuskokeessa tutkin dominanssin periytyvyyttä talitiaisella. Kokeen perusteella dominanssi on pitkälti ympäristön, erityisesti pesäpoikasajan kunnon määräämää, ilman periytyvää komponenttia. Talitiaisen keltaisten rintahöyhenten kirkkaus selitti dominanssia nuorilla koirastalitiaisilla. Kahden eri tutkimuksen mukaan talitiaisen rintajuova ei ikä- ja sukupuoliluokan sisällä juuri selitä dominanssia. Sen sijaan rintajuovan koko on joustava ja se mahdollisesti ilmentää aggressiivista motivaatiota. Ornamenttien plastisuus on voinut kehittyä ilmentämään yksilön sisäisiä muutoksia kunnossa tai statuksessa, ja ornamentin mahdollinen koon joustavuus kannattaa ottaa huomioon signaalien evoluutiota käsittelevissä tutkimuksissa.

REFERENCES

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton.

Andersson, S., Örnborg, J. & Andersson, M. 1998 Ultraviolet sexual dimorphism and assortative mating in blue tits. Proceedings of the Royal Society of London B 265, 445-450.

Brinkhof, M.W.G., Heeb, P., Kolliker, M. & Richner, H. 1999. Immunocompetence of nestling great tits in relation to rearing environment and parentage. Proceedings of the Royal Society of London B 266, 2315-2322.

Christe, P., Møller, A.P., Saino, N. & De Lope, F. 2000. Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, Delichon urbica (the house martin). Heredity 85, 75-83.

Darwin, C. 1871. The descent of man, and selection in relation to sex. Murray, London.

Dewsbury, D.A. 1990. Fathers and sons: genetic factors and social dominance in deer mice, Peromyscus maniculatus. Animal Behaviour 39, 284-289.

Drews, C. 1993. The concept and definition of dominance in animal behaviour. Behaviour 125, 3-4.

Evans, M.R., Goldsmith, A.R. & Norris, S.R.A. 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (Passer domesticus). Behavioral Ecology and Sociobiology 47, 156-163.

Fisher, R.A. 1930. The genetical theory of natural selection. Clanrendon Press, Oxford.

Falkner, D.S. & MacKay, T.F.C. 1997. Quantitative Genetics. Wessex, Longman.

Folstad, I. & Karter, A.J. 1992. Parasites, bright males and the immunocompetence handicap. American Naturalist 139, 603-622.

Gonzales, G., Sorci, G., Møller, A.P., Ninni, P., Haussy, C. & de Lope, F. 1999. Immunocompetence and condition-dependent sexual advertisement in male house sparrow (Passer domesticus). Journal of Animal Ecology 68, 1225-1234.

Gustafsson, L, Qvarnström, A. & Sheldon, B. 1995. Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. Nature (London) 375, 311-313.

Hamilton, W.D: 1980. Sex versus non-sex versus parasites. Oikos 35, 282-290.

Hamilton, W.D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? Nature (London) 218, 384-387.

Hill, G.E. 1996. Redness as a measure of the production cost of ornamental coloration. Ethology Ecology and Evolution 8, 157-175.

Hill, G.E. & Brawner, W.B. 1998. Melanin-based plumage colouration in the house finch is unaffected by coccidial infection. Proceedings of the Royal Society of London B 265, 1105-1109.

Hill, G.E. & Montgomerie, R. 1994. Plumage colour signals nutritional condition in the house finch. Proceedings of the Royal Society of London B 258, 47-52.

Horne, T.J. & Ylönen, H. 1998. Heritabilities of dominance-related traits in male bank voles (Clethrionomys glareolus). Evolution 52, 894-899.

Iwasa, Y., Pomiankowski, A. & Nee, S. 1991. The evolution of costly mate preferences. II. The handicap principle. Evolution 45, 1431-1442.

Järvi, T. & Bakken, M. 1984. The function of the variation in the breast stripe of the great (Parus major). Animal Behaviour 32, 590-596.

Järvi, T., WalsØ, Ø. & Bakken, M. 1987. Status signalling by Parus major: an experiment in deception. Ethology 76, 334-342.

Johnsen, A. Delhey, K., Andersson, S. & Kempenaers, B. 2003. Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects. Proceedings of the Royal Society of London B 270, 1263-1270.

Kern, M.G., DeGraw, W.A. & King, J.R. 1972. Effects of gonadal hormones on the blood composition of White crowned sparrow. General and Comparative Endocrinology 18, 43-53.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. Evolution 36: 1-12.

Kotiaho, J.S., Simmons, L.W. & Tomkins, J.L. 2001. Towards a resolution of the lek paradox. Nature 410: 684-686.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. Proceedings of the National Academy of Sciences of the United States of America 78, 3721-3725.

Lemel, J. & Wallin, K. 1993. Status signalling, motivational condition and dominance: an experimental study in the great tit, Parus major L. Animal Behaviour 45, 549-558.

Lundberg, A. & Alatalo, R.V. 1992. The Pied Flycatcher, London: T & AD Poyser.

Maynard-Smith, J. & Harper, D.G.C. 1988. The evolution of aggression: can selection generate variability? Philosophical Transactions: Royal Society Series 319, 557-570.

Mcgraw, K.J. & Hill, G.E. 2000. Carotenoid-based ornamentation and status signalling in the house finch. Behavioral Ecology 11, 520-527.

Møller, A.P. 1990. Effects of an haematophagous mite on the barn swallow (Hirundo rustica): a test of the Hamilton and Zuk hypothesis. Evolution 44, 771-784.

Møller, A.P. 1991. Parasite load reduces song output in a passerine bird. Animal Behaviour 41, 723-730.

Møller,A.P & Alatalo, R.V. 1999. Good-genes effects in sexual selection. Proceedings of the Royal Society of London B 266, 85-91.

Møller, A.P., Sorci, G. & Errizoe, J. 1998. Sexual dimorphism in immune defense. American Naturalist, 152: 605-619.

Nirmalan, G.P. & Robinson, G.A. 1972. Hematology of Japanese quail treated with exogenous stillbestrol dipropionate and testosterone propionate. Poultry Science 51, 920-925.

Olson, V.A. & Owens, I.P.F. 1998. Costly sexual signals: are carotenoids rare, risky or required? Trends in Ecology and Evolution 13, 510-514.

Palomeque, J. & Planas, J. 1978. Blood volume in domestic pigeons. Comparative Biochemistry and Physiology 59A, 413-417.

Pärt, T. & Qvarnström, A. 1997. Badge size in collared flycatchers predict outcome of male competition over territories. Animal Behaviour 54, 893-899.

Pomiankowski, A. 1988. The evolution of female preferences for female male quality. Oxford Surv. Evol. Biol. 5:136-184.

Potti, J. & Montalvo, S. 1991. Male arrival and female mate choice in pied flycatchers Ficedula hypoleuca. Ornis Scandinavica, 22, 45-54.

Poulin, R. 1996. Sexual inequalities in helminth infections: a cost of being male? American Naturalist, 147, 287-295.

Pryke, S.R., Lawes, M.J. & Andersson, S. 2001. Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the colour signal of both the territory owner and model intruder. Animal Behaviour 62, 695-704.

Råberg, L., Grahn, M., Hasselquist, D. & Svensson, E. 1998. On the adaptive significance of stress-induced immunosuppression. Proceedings of the Royal Society of London B 265, 1637-1641.

Rohwer, S.A. 1975. The social significance of avian winter plumage variability. Evolution 29, 593-610.

Rohwer, S.A. 1982. The evolution of reliable and unreliable badges of fighting ability. American Zoologist 22, 531-546.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proceedings of the Royal Society of London B 263, 1415-1421.

Saino, N., Calza, S. & Møller, A.P. 1997. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. Journal of Animal Ecology 66, 827-836.

Sandell, M. & Smith, H.G. 1991. Dominance, prior occupancy and winter residency in the great tit (Parus major). Behavioral Ecology and Sociobiology 29, 147-152.

von Schantz, T., Wittzell, H., Göransson, G. & Grahn, M. 1996. Mate choice, male condition-dependent ornamentation and MHC in the pheasant. Hereditas 127, 133-140.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999. Good-genes, oxidative sress and condition-dependent sexual signals. Proceedings of the Royal Society of London B 266, 1-12.

Sheldon, B. C. & Ellegren, H. 1999. Sexual selection resulting from extra-pair paternity in collared flycatchers. Animal Behaviour 57, 285-298.

Svensson, E., Råberg, L., Koch, C. & Hasselquist, D. 1998. Energetic stress, immunosuppression, and the costs of an antibody response. Functional Ecology 12, 912-919.

Tella, J.L., Bortolotti, G.R., Forero, M.G. & Dawson, R.D. 2000. Evironmental and genetic variation in T-cell mediated immune response of fledgling American kestrels. Oecologia 123, 453- 459.

Wedekind, C. & Folstad, I. 1994. Adaptive or nonadaptive immunosupression by sex hormones? American Naturalist 143, 936-938.

Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. Proceedings of the Royal Society of London B 270, 357-366.

Schmid-Hempel, P. & Ebert, D. 2003. On the evolutionary ecology of specific immune defence. Trends in Ecology and Evolution 18, 27-32.

Sheldon, B.C. & Verhulst, S. 1996. Ecological immunology: costly parasite defence and trade-offs in evolutionary ecology. Trends in Ecology and Evolution 11, 317-321.

Trivers, R.L. 1972. Parental investment and sexual selection. In: B. Cambell (ed.), Sexual selection and the descent of man, 1871-1971: 136-179. London, Heinemann.

Veiga, J.P. & Puerta, M. 1996. Nutritional constraints determine the expression of a sexual trait in the house sparrow. Proceedings of the Royal Society of London B 263, 229-234.

Wakelin, D. & Apanius , V. 1997. In: Clayton, D.H. & Moore, J. (eds), Host-Parasite Evolution, General Principles & Avian Models: 30-58. Oxford University Press, Oxford.

Weatherhead, P.J. & Robertson, R.J. 1979. Offspring quality and the polygyny treshold: " the sexy son hypothesis". American Naturalist 113, 201-208.

Wolfenbarger, L.L. 1999. Is red coloration of male northern cardinals beneficial during the nonbreeding season? : a test of status signalling. Condor 101, 655-663.

Zahavi, A. 1975. Mate selection - a selection for a handicap. Journal of Theoretical Biology 53, 205-214.

Zahavi, A. 1977. The cost of honesty (further remarks on the handicap principle). Journal of Theoretical Biology 67, 603-605.

Zuk, M. & McKean, K.A. 1996. Sex differences in parasite infections: patterns and processes. International Journal of Parasitology 26, 1009-1024.

Zuk, M. & Stoehr, M. 2002. Immune defense and host life history. American Naturalist 160, 9-22.

Zuk, M., Johnsen, K., Thornhill, R. & Ligon, J.D. 1990. Parasites and male ornaments in free-ranging and captive red jungle fowl. Behaviour 114, 232-248.

Zuk, M., Thornhill, R., Johnson, K. & Ligon, J.D. 1990. Parasites and mate choice in red jungle fowl. American Zoologist 30, 235-244.

ORIGINAL PAPERS

I

GENETIC AND ENVIRONMENTAL VARIATION IN SPECIFIC AND GENERAL IMMUNE DEFENCE IN THE GREAT TIT

By

Janne Kilpimaa, Tom Van de Casteele, Johanna Mappes & Rauno V. Alatalo

Manuscript

https://doi.org/10.1111/j.0014-3820.2005.tb00958.x

II

LIFE HISTORY TRADE-OFFS AND SEX DIFFERENCES IN IMMUNE FUNCTION IN THE PIED FLYCATCHER

By

Janne Kilpimaa, Rauno V. Alatalo & Heli Siitari

Manuscript (Submitted)

Life history trade-offs and sex differences in immune function in the pied flycatcher

Janne Kilpimaa, Rauno V. Alatalo & Heli Siitari

Department of Biological and Environmental Science, University of Jyväskylä,
P.O. Box 35, FIN-40014 University of Jyväskylä, Finland

ABSTRACT

The immunocompetence handicap (ICH) hypothesis predicts a negative correlation between male ornamentation and immune function because both sexual advertisement and immune function are expected to be costly. Because males are expected to invest more in mate attraction the trade off between immune function and sexual advertisement may also explain sex differences in immune function during the breeding season. We investigated inter-relationships between male mating effort, ornamentation and immune function in the pied flycatcher. We found that males had much higher hematocrit during the mating period but there was no difference between sexes during the nestling period. Male immunoglobulin levels were highest during the nestling period whereas in females immunoglobulin levels were highest during the mating period especially just prior to egg laying. High female immunoglobulin levels during the mating period may be a result of altered immune profile due to transmission of immunoglobulins to eggs. Whereas in males high hematocrit and low IgG during the mating period suggests that males may compromise the efficiency of their immune function during mating period in the line with the predictions of the ICH hypothesis. Male forehead patch size and hematocrit were positively correlated during the mating period but the relation was absent during the nestling feeding period, suggesting that highly ornamented males invested relatively more in sexual display. Males with large patch size had higher concentration of heterophiles (an indicator of stress) suggesting that there might be a cost to male sexual display. Males with large patch had also reduced immune response after SRBC challenge during the nestling period suggesting longer lasting negative consequences of sexual display. Our results provide evidence that males have reduced immune function during mating season as a result of their higher mating effort. Moreover, male forehead patch size in the pied flycatcher reflects male mating effort and elaborate ornamentation is at least temporarily costly.

INTRODUCTION

Good gene models of sexual selection suggest that to honestly signal heritable quality male ornaments should be more costly to low quality males (Andersson 1994). One important cost of ornamentation may be impairment of the immune function. The immunocompetence handicap-hypothesis (ICH) suggests that sexual ornamentation that has a hormonal basis is an honest indicator of heritable quality, because androgens suppress the immune function and only males in good condition can cope with the associated costs (Folstad & Karter 1992, Wedekind & Folstad 1994). Hence, the ICH hypothesis predicts a negative correlation between male ornamentation and immune function during the breeding season. Accordingly in correlative studies a negative relation between the strength of the immune response and ornamentation during the breeding season has been found (Zuk et al. 1995, Skarstein & Folstad 1996, Zuk & Johnsen 1998). Instead, outside the breeding season the relation between immune function and ornamentation may be reversed (Zuk & Johnsen 1998, Gonzales et al. 1999). Relationships between ornamentation and immune function may also depend on which aspect of immune function is measured (Zuk & Johnsen 1998). For example cell-mediated immune response has been found to be positively correlated with ornamentation also during the breeding season (Zuk & Johnsen 1998, Saino et al 2002). In line with this in the house sparrow (*Passer domesticus*) experimentally increased testosterone during breeding season suppressed humoral immune responsiveness but the manipulation did not affect cell-mediated immune response (Buchanan et al. 2003).

Males have been suggested to be more susceptible to parasites and to have less efficient immune function than females (Poulin 1996, Zuk & McKean 1996, Møller et al. 1998). In the line with ICH hypothesis immunosuppressive effects of androgens may be a physiological mechanism generating differences between sexes in immune function. Alternatively sex differences may arise as a result of differences in life history between sexes (Zuk & Stoehr 2002). Because male fitness is limited by the number of offspring inseminated and female fitness is limited by the number of offspring reared, males are expected to invest more in mate attraction (Trivers 1972). If both immune function and mating effort are costly, higher mating effort of males may be traded off against immune efficiency during mating season. Hence, although the proximate reason for sex differences in immune function during breeding season may be the effects of androgens, the ultimate reason would be different reproductive tactics between sexes. Moreover, sex differences in immune function should be larger in polygynous species where the difference between sexes in mating effort is expected to be large (Zuk & Stoehr 2002).

The pied flycatcher (*Ficedula hypoleuca*) is a small cavity nesting passerine, in which many males attempt to attract secondary females in separate territories. The plumage coloration is sexually dimorphic. Males have conspicuous white forehead patch which females in our study population lack (Lundberg & Alatalo 1992). In our study population males are highly polymorphic in dorsal plumage colour some males being conspicuously black and some resembling the cryptic brown colour of females. Although the function of variability in size of the forehead patch is not yet properly understood it has been suggested that this character is related to sexual selection (Potti & Montalvo 1991). In the sibling species, the collared flycatcher (*Ficedula albicollis*) male forehead patch is sexually selected and it signals fighting ability in male-male competition over territories (Pärt & Qvarnström 1997). It has been suggested that male forehead patch size is not only an indicator of male condition, but it also reflects male mating effort (Qvarnström 1999).

In this study we compared the immune function between sexes during different breeding stages. We also used correlative data to examine whether male forehead patch size is related to male mating effort and experimentally challenged the immune function to investigate whether forehead patch size and mating effort are related to male immune function.

METHODS

The study was carried out in Central Finland, at Konnevesi Research Station (62 ° 37′ N, 26 ° 20′ E) during years 2001 and 2002. In 2001 males were captured both during the mating season in May (N = 32) and during the nestling period in June (N = 54). Some males were captured both during the mating season and the nestling period (N = 17). In 2002 males were captured only during the mating season (N = 60). Females were captured only in 2001 during both the mating period (N = 54) and the nestling period (N = 58). 36 females were captured both during the mating period and the nestling feeding period. In both years, mist nets and traps set inside the nest-box were used.

At each capture male forehead patch size was measured with a digital calliper to the nearest 0.1 mm. In Central and Northern Europe male forehead patch is highly variable in shape (Lundberg & Alatalo 1992). Three different shapes can be separated: rectangular, a patch divided into two dots or a combination of both (heart shaped). We measured maximum width, maximum height and minimum height of the forehead patch. If forehead patch consisted of two separate dots a minimum height was determined as the distance between the spots and the value was set negative. Forehead patch size was calculated as a first principal component from a PCA analysis of the correlation matrix of three forehead patch dimensions. The first principal component explained 68.76 % (May 2001), 73.14 % (2001 June) and 62.10 % (May 2002) of the total variation in the three measures of forehead patch size. All the three patch dimensions were highly positively correlated with the first principal component.

At each capture both males and females were also blood sampled for hematocrit and for immunoglobulin analysis. A drop of blood from branchial vein was collected in a microcapillary tube. Afterwards tubes were centrifuged at 12000 rpm for 5 minutes. Hematocrit was measured in each capillary tube as the percentage of the length of the part of the capillary tube occupied by red blood cells in respect to the total length of the part of the tube occupied by all blood components. Plasma was stored (-20°C) for IgG analysis (see below).

In 2002 for a subset of males blood smears were taken for leucoyte counts. For this a drop of blood from a microcapillary tube was transferred to a glass slide, smeared, air-dried and fixed in absolute ethanol. After fixing the blood smears, they were stained with commercial Giemsa dye for 1 hour in room temperature (1:10 Giemsa dye in 5mM phosphate buffer). After staining, blood smears were inspected under a light microscope (Nikon Eclipse E400). The number of heterophils (H) and lymphocytes (L) was calculated per 1000 erythrocytes in randomly chosen eye fields first under 40X and later 100 X magnification with oil immersion. Hetrophile/lymphocyte ratio is a measure of stress in poultry (Gross & Siegel 1983) and wild birds (Ots et al. 1998). Under stressful conditions birds produce more heterophiles than lymphocytes. Only heterophil counts are used in the analyses, because heterophil count itself is strongly correlated with a stress index (H/L ratio) (Lebigre et al., unpublished).

In 2001 to measure the humoral immune responsiveness males were injected intraperitoneally with 100µl of a PBS solution containing a concentration of 5 x 10^5 sheep red blood cells (SRBC). This was applied during the nestling period when nestlings were 7 days old. Males were recaptured 5 days later to measure their immune response. Blood samples were collected during each capture and change in total plasma immunoglobulins was used as a measure of humoral immune responsiveness.

Immunoglobulin concentrations were measured for total IgG levels and for humoral immune response from plasma samples with ELISA method. In brief, 96-well ELISA-plates (Nunc™ Immunoplate) were first coated with anti-chicken IgG (Sigma C-6409). The plates were incubated in +4°C overnight. After empting the wells, they were masked with 1% BSA-PBS (Roche Diagnostics) for 1 hour and washed 3 x 200µl with PBS-Tween. Samples and their replicates (50µl/well) were diluted with 1% BSA-PBS and added to the wells. For each plate, a standard with different dilutions (50 µl/well; diluted also with 1% BSA-PBS) was added. As a standard we used a mixture of plasma of all individuals measured, that was given an arbitrary concentration of 10^6. Samples and standards with different dilutions were incubated for 3 hours at room temperature. After washing the plates (3 x 200µl with PBS-Tween), an alkaline phosphatase conjugated antibody (Sigma A-971 anti-chicken IgG; diluted with 1% BSA-PBS) was added to the wells and incubated overnight at +4°C. Finally, after washing the wells (3 x 400µ with PBS-Tween) an alkaline phosphatase substrate PNPP (p-nitrophenyl phosphate, Sigma 104® phosphatase substrate) in 1M diethanol amine buffer (1 mg/ml) was applied (50µl/well). The absorbance of the wells was read several times in an ELISA reader at 405 nm for up to 1 hour (or until the highest standard reached the absorbance 2.0).

Because change in total plasma immunoglobulin concentrations after injection with SRBC's (measure of humoral immune responsiveness) was negative for some males we did not log-transform the values and used non-parametric tests in the analysis on immune responsiveness. In the repeated measurement analysis on variation in immune function between sexes during the mating season and the nestling period log-transformed immunoglobulin levels were used.

RESULTS

In a repeated measures analysis where breeding stage was a repeated measures within subjects factor and its levels hematocrit during the mating period and the nestling period, there was a significant breeding stage*sex interaction (Fig. 1; F(1,48) = 52.81, $P < 0.001$) suggesting that the change in hematocrit levels between the mating period and the nestling period was not similar in both sexes. Hence the effect of breeding stage on hematocrit was analyzed also separately for each sex. Male hematocrit levels were much higher during mating than during nestling period (Fig. 1; hematocrit during mating period: mean 0.57, SD = 0.03; hematocrit during nestling period: mean 0.48, SD = 0.03, paired t-test: $t_{14} = 8.367$, $P < 0.001$). There was only a slight decrease in female hematocrit between the mating period and the nestling period (Fig 1; hematocrit during mating period: mean 0.50, SD = 0.03; hematocrit during nestling period: mean 0.49, SD = 0.02, paired t-test: $t_{35} = 2.165$, $P = 0.037$). Moreover, hematocrit was much higher in males than in females during the mating period (Fig. 1.; male hematocrit: mean 0.56, DF = 0.03, N = 32; female hematocrit: mean 0.50, SD = 0.03, N = 54, $t_{84} = 8.996$, $P < 0.001$), whereas during the nestling period there was a tendency for female hematocrit to be higher (Fig 1.; male hematocrit 0.48, SD = 0.02, N = 54; female hematocrit 0.49, SD = 0.03, N = 56, $t_{108} = -1.885$, $P = 0.066$).

Also, in total immunoglobulin levels there was a significant sex*breeding stage interaction in repeated measures analysis (Fig 2; F(1,53) = 21.648, $P < 0.001$). In males total immunoglobulin levels were lower during the mating period than during the nestling feeding period (Fig 2; mating period: mean 8.75, SD = 0.30; nestling period: mean 8.97, SD = 0.30, paired t-test: $t_{16} = -3.485$, $P = 0.003$). Instead, in females immunoglobulin levels decreased between the mating period and the nestling feeding period (Fig 2; mating period: mean 9.24 SD = 0.33; nestling period: mean 9.10, SD = 0.32, paired t-test: $t_{37} = 3.138$, $P = 0.003$.). During the mating period immunoglobulin levels were highest in females caught just before egg laying (Fig. 3.) suggesting that high immunoglobulin levels in females during mating is a partly a result of transmission of immunoglobulins in egg yolk. Females had higher immunoglobulin levels than males during the mating period (Fig 2; male IgG: mean 8.75, SD = 0.30, N = 17; female IgG: mean 9.12, SD = 0.57, N = 43, t_{58} = -2.53, P = 0.014), but there was no difference between sexes during the nestling feeding period (Fig 2; male IgG: mean 9.03, SD = 0.30, N = 48; female IgG: mean 9.10, SD = 0.28, N = 58, t_{104} = -1.123, n.s.) .

Hematocrit was positively correlated with male forehead patch size during the mating period in both study years (Fig. 4.). In a pooled data where both hematocrit and forehead patch size were standardized for each year separately there was also a significant positive correlation between forehead patch size and hematocrit during the mating period (rs = 0.338, N = 90, P = 0.001). There was no significant correlation between forehead patch size and hematocrit during the nestling feeding period (rs = 0.00, N = 49, n.s.). There was a negative relation between forehead patch size and humoral immune response during the nestling period (Fig. 5) suggesting a trade off between ornamentation and immune function. There was also a positive relation between male forehead patch size and the number of heterophiles during the mating period (Fig. 6.), suggesting that more ornamented males are more stressed during mating.

DISCUSSION

Males had much higher hematocrit levels than females during the mating period. After the mating period male hematocrit levels decreased becoming similar with female hematocrit that decreased only slightly. After the mating period male immunoglobulin levels increased whereas in females immunoglobulin levels decreased. High immunoglobulin levels during the mating period in females are probably a result of altered immune profile due to transmission of immunoglobulins to eggs. In a study on barn swallow (*Hirundo rustica*) female plasma immunoglobulin levels peaked just prior laying the first egg, but immunoglobulin levels well before egg laying did not differ from post laying levels (Saino et al. 2001). However, in male pied flycatchers low immunoglobulin levels during the mating period may be a result of trade off between mating effort and immune function.

Hematocrit is a measure of locomotory activity (Palomeque & Planas 1978) but it is also under hormonal control. In birds males often have higher hematocrit (Saino et al. 1997a) and in laboratory animals administration of androgens increases hematocrit (Nirmalan & Robinson 1972; Kern et al, 1974). Hence, a proximate reason for the high male hematocrit during the mating period may be increased testosterone levels. In the pied flycatcher male testosterone levels reach the peak during the nest building period and decrease dramatically already during the egg laying period (Silverin & Wingfield 1982). Irrespective of the physiological causality behind the hematocrit levels, the ultimate reason for high male hematocrit during the mating period is probably high sexual activity. High hematocrit during the mating period soon after arrival may also be related to high oxygen demand during migration (Saino et al. 1997a). However, this is probably not the case in the pied flycatcher because in our study females had much lower hematocrit during the mating period despite the fact that females are the later arriving sex. Moreover, in an experiment where mating effort was manipulated pied flycatcher males with prolonged mating period sustained higher hematocrit (Kilpimaa et al. 2003), suggesting that high male hematocrit indeed is related to sexual behaviour. Hence, higher hematocrit of males during the mating period may suggest that males invest relatively more in mate attraction and their low IgG during the mating period suggests that males may compromise the efficiency of their immune function during this period. Lower immunocompetence and increased susceptibility to parasites of males has often been suggested but rarely demonstrated (Zuk & Stoehr 2002). Our results indicate that in polygynous pied flycatcher, in which selection pressure for high male mating effort is relatively strong, differences between sexes in immunocompetence and parasite susceptibility may arise as a result of different reproductive strategies of each sex.

In both study years, the hematocrit during the mating period correlated positively with the forehead patch size suggesting that more ornamented males make a higher mating effort. This is in concordance with earlier studies on the collared flycatcher. Early-mated males with large forehead patch were in worse condition at the end of nestling period as a result of their larger mating effort, (Qvarnstöm 1999). In our study there was no relation between the hematocrit and the forehead patch size during the nestling period which is expected if the forehead patch functions in female choice or intra sexual competition. In the barn swallow sexually selected male tail length correlates positively with hematocrit (Saino et al. 1997a) and experimental elongation results in increased hematocrit (Saino et al. 1997b). It has been suggested that long tail is handicapping flight performance and hence is a reliable signal of male quality. In the pied flycatcher the role of forehead patch size in sexual selection is unclear. However, the positive relationship between forehead patch size and activity as measured in hematocrit levels during the mating period suggests that male forehead patch may have some function in sexual behaviour. There is no evidence in pied flycatchers that male forehead patch is a signal of heritable quality. It has also been suggested that females choose territorial quality rather than the quality of males (Alatalo et al. 1986). Hence the importance of forehead patch size in sexual selection may be related to male-male competition over territories rather than to female choice.

Males with large forehead patch size had reduced humoral immune response during the nestling period suggesting a trade off between sexual display and immune function as the ICH hypothesis suggests. An important assumption of the ICH hypothesis is that variation in male ornamentation is controlled by testosterone. Therefore it has been suggested that the ICH hypothesis may not explain the evolution of male plumage variability in birds, because plumage ornamentation is usually produced outside the breeding season when the testosterone levels are low (Owens & Short 1995). However, in the collared flycatcher the forehead patch size is flexible within the breeding season being largest during the mating season and becoming smaller during the nestling feeding period (Griffith & Sheldon 2001). Hence, the forehead patch size may be under hormonal control also during the breeding season. Flexibility of the ornament expression allows males also to adjust the ornament expression to their current health and condition. Accordingly, experimental activation of immune function resulted in rapid change in the expression of male forehead patch size in the pied flycatcher (Kilpimaa et al 2003). Moreover males with experimentally prolonged mating period sustained higher expression of forehead patch size compared to control males. We do not know whether males with large forehead patch size had higher testosterone levels in our study. However, higher hematocrit of males with large forehead patch suggests that those males make larger mating effort possibly as a result of higher testosterone levels. In addition since stress hormone levels usually covary with testosterone levels (Evans et al. 2000) higher heterophile concentration of males with large forehead patch may indicate also higher testosterone levels. ICH hypothesis may also work without direct effects of sex hormones if both immune function and sexual display compete for the same resources (Wedekind & Folstad 1994). In the collared flycatcher the white forehead patch functions as an honest signal of male fighting ability where the honesty is controlled by repeated punishment from other males (Pärt & Qvarnström 1997). Hence, the trade off between forehead patch size and immune function may be a result of direct effects of androgens on both forehead patch size and immune function or alternatively forehead patch size may have socially mediated energetic costs that are traded off against the efficiency of the immune function.

To conclude, in females immunoglobulin levels were highest just prior egg laying probably as a result of altered immune profile due to transmission of immunoglobulins to eggs. Instead, in males immunoglobulin levels were lowest and hematocrit levels were highest during the mating period, which may suggest that males invest much in mate attraction and may compromise the efficiency of their immune function during this period. Male forehead patch size and hematocrit were positively correlated during the mating period whereas there was no this relationship during the nestling period. This suggests that males with large forehead patch invest relatively more in mate attraction. Moreover, elaborate ornamentation is at least temporarily costly because male forehead patch size was negatively related to strength of the immune response and was also related to increased stress during the mating period. However, the long term fitness consequences of increased mating effort on survival and mating success remains to be investigated.

Acknowledgments

We thank Matti Halonen, Antti Hannuniemi, Laura Häsä and Satu Kuntsi for assistance in the field work and Elina Virtanen for laboratory analysis. We also thank staff at Konnevesi Research station for technical help during the experiment. The experiment was carried out under permission of Animal Care Committee of the University of Jyväskylä (permission number 11 / 2001 and 33 / 2002). This study was funded by the Academy of Finland under the Finnish Centre of Excellence Programme.

REFERENCES

Alatalo, R., Lundberg, A. and Glynn, C. 1986. Female pied flycatchers choose territory quality and not male characteristics. *Nature*, **323**:152-153.

Andersson, M. 1994. *Sexual selection*, Princeton, NJ: Princeton University Press.

Buchanan, K.L., Evans, M.R. and Goldsmith, A.R. 2003. Testosterone, dominance signalling and immunosuppression in the house sparrow *Passer domesticus*. *Behav. Ecol. Sociobiol.*, **54**: published online.

Duffy, D.L., Bentley, G.E., Drazen, D.L. and Ball, G.F. 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. *Behav. Ecol.*, **11**: 654-662.

Evans, M.R., Goldsmith, A.R. and Norris, S.R.A. 2000. The effects of testosterone on antibody

production and plumage coloration in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.*, **47**: 156-163.

Folstad, I. and Karter, A.J. 1992. Parasites, bright males and the immunocompetence handicap. *Am. Nat.*, **139**: 603-622.

Gonzales, G., Sorci, G. and de Lope, F. 1999. Seasonal variation in the relationship between cellular immune response and badge size in male house sparrows (Passer domesticus). *Behav. Ecol. Sociobiol.*, **46**: 117-122.

Griffith, S.C. and Sheldon, B.C. 2001. Phenotypic plasticity in the expression of sexually selected traits: neglected components of variation. *Anim. Behav.*, **61**: 987-993.

Gross, W.B. and Siegel, H.S. 1983. Evaluation of heterophil lymphocyte ratio as a measure of stress in chickens. *Avian Dis.*, **27**: 972-979.

Hasselquist, D., Marsh, J.A., Sherman, P.W. and Wingfield, J.C. 1999. Is avian humoral immunocompetence suppressed by testosterone? *Behav. Ecol. Sociobiol.*, **45**: 167-175.

Kern, M.G., DeGraw, W.A. and King, J.R. 1972. Effects of gonadal hormones on the blood composition of White crowned sparrow. *Gen. Comp. Endo.*, **18**: 43-53.

Kilpimaa, J., Alatalo, R.V. and Siitari, H. 2003. Trade-offs between sexual advertisement and immune function in the pied flycatcher (*Ficedula hypoleuca*). *Proc. R. Soc. Lond. B.*, in press.

Lundberg, A. and Alatalo, R.V. 1992. *The Pied Flycatcher*, London: T & AD Poyser.

Møller, A.P., Sorci, G. and Errizoe, J. 1998. Sexual dimorphism in immune defense. *Am. Nat.*, **152**: 605-619.

Nirmalan, G.P. and Robinson, G.A. 1972. Hematology of Japanese quail treated with exogenous stillbestrol dipropionate and testosterone propionate. *Poult. Sci.*, **51**: 920-925.

Ots, I., Murumägi, A. and Horak, P. 1998. Haematological health state indices of reproducing Great tits: methodology and sources of natural variation. *Funct. Ecol.*, **12**: 700-707.

Owens, I.P.F. and Short, R.V. 1995. Hormonal basis of sexual dimorphism in birds: implications for the new theories of sexual selection. *Trends. Ecol. Evol.*, **10**: 44-47.

Palomeque, J. and Planas, J. 1978. Blood volume in domestic pigeons. *Comp. Biochem. Physiol.*, **59A**: 413-417.

Potti, J. and Montalvo, S. 1991. Male arrival and female mate choice in pied flycatchers *Ficedula hypoleuca. Ornis Scand.*, **22:** 45-54.

Poulin, R. 1996. Sexual inequalities in helminth infections: a cost of being male? *Am. Nat.*, **147**: 287-295.

Pärt, T. and Qvarnström, A. 1997. Badge size in collared flycatchers predict outcome of male competition over territories. *Anim. Behav.*, **54**: 893-899.

Qvarnsröm, A. 1999. Different reproductive tactics in male collared flycatchers signalled by size of secondary sexual character. *Proc. R. Soc. Lond.* B, **266**: 2089-2093.

Saino, N., Cuervo, J.J., Ninni, P., de Lope F. and Møller, A.P. 1997a. Haematocrit correlates with tail ornament size in three populations of Barn Sallow (*Hirundo rustica*) *Funct. Ecol.*, **11**: 604-610.

Saino, N., Cuervo, J.J., Krivacek, de Lope, F. and Møller, A.P. 1997b. Experimental manipulation of tail ornament size affects the hematocrit of male barn swallows (*Hirundo rustica*). *Oecologia*, **110**: 186-190.

Saino, N., Martinelli, R. and Møller, A.P. 2001. Immunoglobulin plasma concentration in relation to egg laying and mate ornamentation of female barn swallows (*Hirundo rustica*). *J. Evol. Biol.*, **14**: 95-109.

Saino, N., Incagli, M., Martinelli, R. and Møller, A.P. 2002. Immune response of male barn swallows in relation to parental effort, corticosterone plasma levels, and sexual ornamentation. *Behav. Ecol.*, **2**: 169-174.

Skarstein, F. and Folstad, I. 1996. Sexual dichromatism and the immunocompetence handicap: an observational approach using Arctic charr. *Oikos*, **76**: 359-367.

Silverin, B. and Wingfield, J.C. 1982. Patterns of breeding behaviour and plasma levels of hormones in a free-living population of pied flycatchers, *Ficedula hypoleuca. J. Zool.*, **198**: 117-129.

Trivers, R.L. 1972. Parental investment and sexual selection. (B. Cambell, eds), in *Sexual selection and the descent of man, 1871-1971*. pp 136-179. London: Heinemann.

Wedekind, C. and Folstad, I. 1994. Adaptive or nonadaptive immunosupression by sex hormones? *Am. Nat.*, **143:** 936-938.

Zuk, M., Johnsen, T.S. and MacLarty, T. 1995. Endocrine-immune interactions, ornaments and mate choice in red jungle fowl. *Proc. R. Soc. Lond. B*, **260**: 205-210.

Zuk, M. and McKean, K.A. 1996. Sex differences in parasite infections: patterns and processes. *Int. J. Parasitol.*, **26**: 1009-1024.

Zuk, M. and Johnsen, T.S. 1998. Seasonal changes in the relationship between ornamentation and immune response in red jungle fowl. *Proc. R. Soc. Lond. B*, **265**: 1631-1635.

Zuk, M and Stoehr, M. 2002. Immune defense and host life history. *Am. Nat.*, **160**: 9-22.

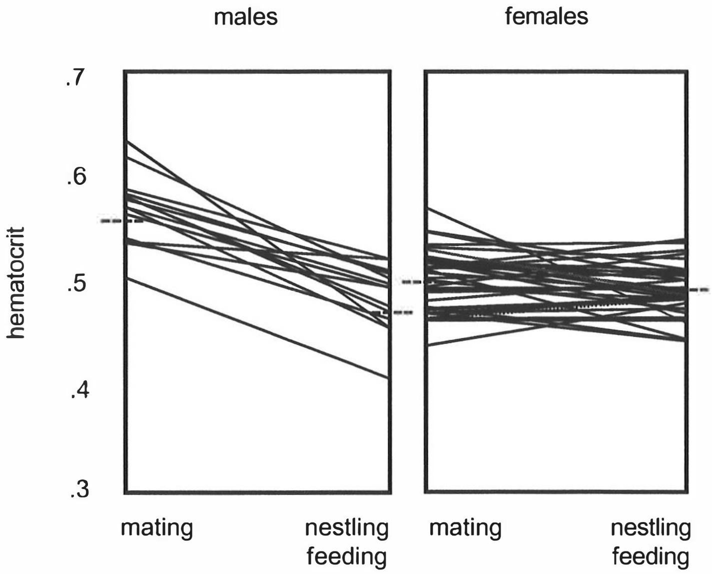

FIGURE 1 Within individual change in hematocrit levels between the mating period and the nestling feeding period for each sex (means given in figures as broken lines in the vertical axis).

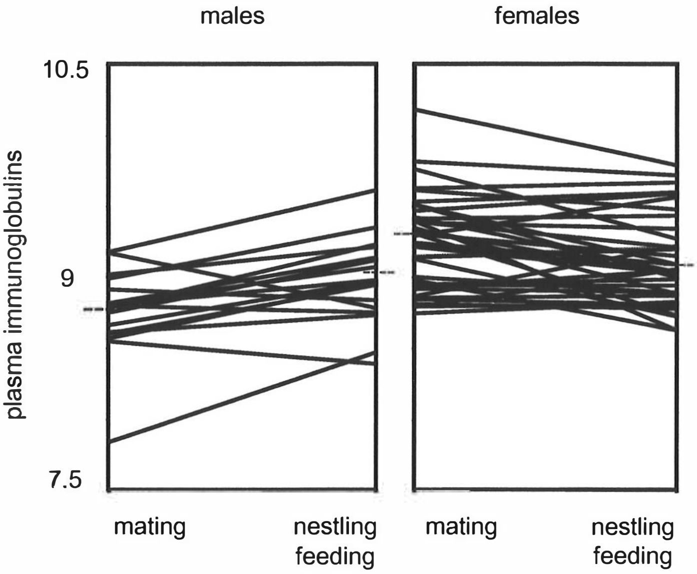

FIGURE 2 Within individual change in immunoglobulin levels between the mating period and the nestling feeding period for each sex (means given in figures as broken lines in the vertical axis).

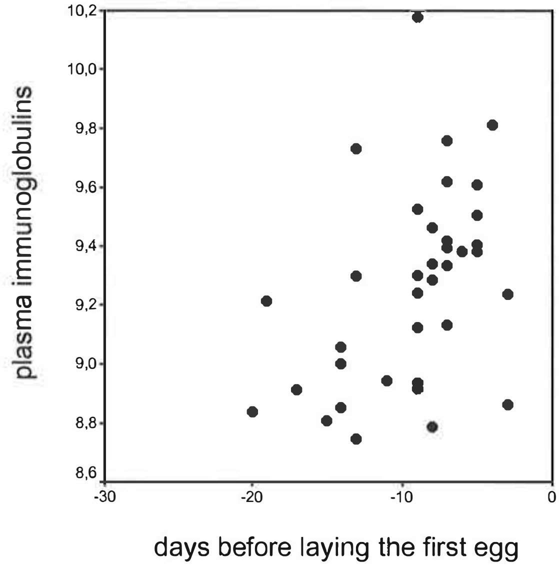

FIGURE 3 Relation between female immunoglobulin levels and number of days between blood sampling and laying the first egg (rs = 0.476, *P* = 0.003, N = 37).

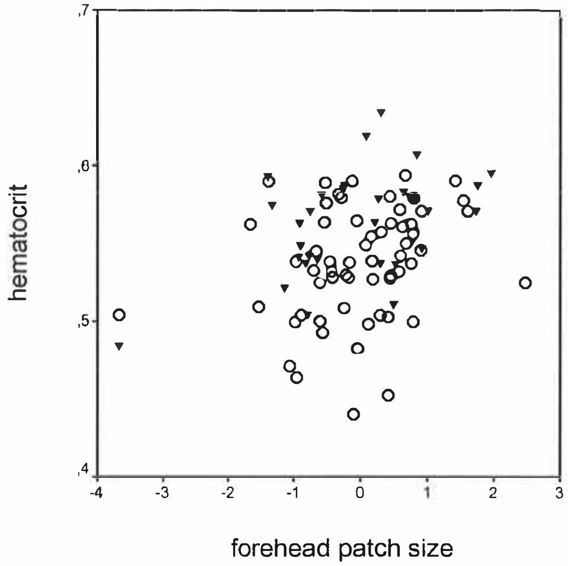

FIGURE 4 Relation between male forehead patch size and the hematocrit during the mating period in year 2001 (▼) (rs = 0.368, *P* = 0.045, N = 30) and year 2002 (o) (rs = 0.296, *P* = 0.022, N = 60).

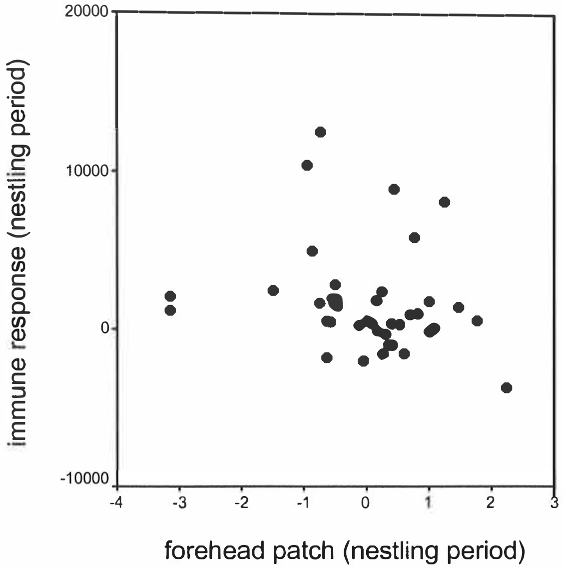

FIGURE 5 Relation between male forehead patch size and humoral immune response against SRBC during the nestling feeding period (rs = -0.328, $P = 0.039$, N = 40).

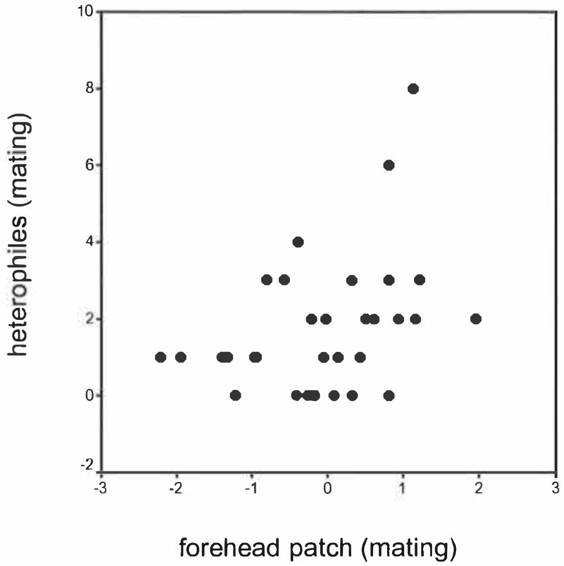

FIGURE 6 Relation between male forehead patch size and heterophile concentration during the mating period (rs = 0.394, $P = 0.023$, N = 33).

III

TRADE-OFFS BETWEEN SEXUAL ADVERTISEMENT AND IMMUNE FUNCTION IN THE PIED FLYCATCHER (*FICEDULA HYPOLEUCA)*

By

Janne Kilpimaa, Rauno V. Alatalo & Heli Siitari

Proceedings of the Royal Society of London, in press

Reproduced with the permission of the Royal Society

https://doi.org/10.1098/rspb.2003.2568

IV

DETERMINATION OF DOMINANCE: A CROSS-FOSTERING EXPERIMENT IN THE GREAT TIT

By

Janne Kilpimaa, Rauno V. Alatalo & Johanna Mappes

Manuscript

Determination of dominance: a cross-fostering experiment in the great tit

Janne Kilpimaa, Rauno V. Alatalo & Johanna Mappes

Department of Biological and Environmental Science, University of Jyväskylä,
P.O. Box 35, FIN-40014 University of Jyväskylä, Finland

ABSTRACT

We conducted a partial cross-fostering experiment to quantify the genetic and the environmental effects on both the social dominance and variation in melanin based coloration (breast stripe and throat patch size) in the great tit. Dominance of juvenile great tits measured as the ability to compete for food resource in a novel environment was affected by the common rearing environment. We found no parentage related variation that would suggest genetic variation in dominance. Instead there was a significant phenotypic correlation between dominance and the cell-mediated immune response at fledging. This suggests that the rearing related variation in dominance may partly be attributable to the health. Surprisingly, breast stripe size had no effect on dominance. Throat patch size was significantly heritable whereas breast stripe size was not. Both the breast stripe size and the throat patch size were positively related to body condition during the post nuptial moult. Rearing related variation in dominance probably due to parental care provides one mechanism for the observed relationship between fledging condition and later survival and subsequent breeding success.

INTRODUCTION

Social dominance can be defined as success in antagonistic contests or priority of access to resources. Dominance has been hypothesized to have several benefits and hence it should be closely correlated with fitness. Dominance has been suggested to affect access to limited food resources (Ekman & Askenmo 1984), survival (Acrese & Smith 1985, Piper & Wiley 1990), dispersal decisions (de Laet 1985, Acrese 1989) and also to be selected through female preference and intrasexual selection (Andersson 1994, Qvarnström & Forsgren 1998).

Genetic basis of dominance is not well understood. Main problem in quantifying genetic variation in dominance is the strong environmental influences on dominance. Since dominance is strongly affected by the social environment i.e. it is not only a character of an individual but depends also on the characters of the opponent it has been suggested that dominance can not be inherited (Dewsbury 1990, Drews 1993). However, the general ability to dominate resources or females may be heritable, because many traits that are closely related to dominance are heritable. In laboratory it has been shown that many behavioural patterns that affect dominance like differences in aggression are heritable (Nol et al. 1996). However the ability to dominate is not purely a behavioural trait and for example the most aggressive animal is not always the most dominant. Also physical characters may determine dominance and can be inherited. In male bank voles both behavioural (urine marking) and physiological (preputial gland size), traits closely related to dominance are heritable (Horne & Ylönen 1998). Which heritable traits contribute to dominance can be very species specific and also depends on the ecological context and the social environment. For example, dominance during the breeding season and outside the breeding season may have different functions and can be also be controlled by different endogenous factors or set of genes. In Japanese quail both juvenile and adult dominance are heritable. However, juvenile dominance ranking was uncorrelated with dominance ranking in adults (Nol et al. 1996). Also hatchling behaviour was uncorrelated with dominance later in life. Hence dominance may be affected by different genes throughout ontogeny and in different social environments the genetic component of dominance is expressed differently.

Controlling all the environmental factors that interact with the genotype and determining dominance is difficult and usually possible only in the laboratory. Hence it is not surprising that there are only a few studies on genetic determination of dominance in wild birds. Moss et al (1982, 1985) found heritable variation in dominance rank in selection experiments in the red grouse. Instead, Westman (1990) found no genetic variation in dominance rank in cross-fostered lab raised great tit offspring, but evidence of maternal effects on dominance. However, the sample size in this study was very small. Maternal effects and state-dependence of dominance is often ignored, although it is known that for example motivation and previous success in antagonistic interaction affect dominance in birds. In primates and spotted hyaenas (Engh et al. 2000) maternal rank is inherited through defensive maternal interventions, coalitionary support and aggression directed by adult females towards offspring of lower-ranking individuals. Genetic heritability of rank-related traits plays a negligible role in rank acquisition in primates and spotted hyaenas (Engh et al. 2000). Also in birds the social experiences during the period of independence after fledging may be important on the future dominance status (Garnett 1981) and the performance during this crucial period may be influenced by non genetic maternal effects. For example in social birds early hatched individuals may become dominant, because birds that start the group usually dominate the newcomers (Velando 2000). Offspring dominance status may also be influenced through parental care, if early dominance is influenced by condition or health status. However, there are very few studies suggesting condition dependence of dominance. In the carrion crow underfed chicks achieved lower social status (Richner et al. 1989) and in captive rooks, dominance was related to fledging weight (Roeskaft 1983). However, these studies did not quantify the relative importance of genetic and environmental causes of dominance. Thus, because of the small number of studies and somewhat conflicting results, genetic and environmental determination of dominance needs further investigation.

Since dominance depends not only on the properties of the given individual but also on the characters of the opponents, dominance rank is a result of several asymmetries between the contestants. In the great tit such asymmetries between individuals that have been suggested to determine the outcome of conflicts are age, sex, body size, motivation (Lemel & Wallin 1993), prior residency (Sandell & Smith 1991) and signals of fighting ability (badges of status). The great tit is sexually dimorphic passerine the black breast stripe being the most conspicuous male character. Also females have the black stripe in a reduced state. Variable plumage characters may function as a 'badge' or signal of status to reduce contest among individuals of different fighting ability (Rohwer 1975). Black breast stripe has been regarded as a classical example of a badge of status (Järvi & Bakken 1984, Järvi et al 1987, Maynard-Smith & Harper 1988). However, Wilson (1992), failed to show any relation between breast stripe size and dominance in free living great tit males. He suggested that if dominance during winter has significance also in territory establishment during breeding season, the value of winning interactions may be too high for low cost patch signalling to be used. Lemel & Wallin (1993) suggested that breast stripe has an impact only in conflicts between individuals of no prior experience of each other. In some species juveniles use plumage coloration to signal their subordinate status to avoid aggression from adult birds but the signal does not work within age classes (delayed plumage maturation). In the great tit Sandell & Smith (1991) found that juveniles became dominant over adults as a result of experimental enlargement of the breast stripe only when their body size was larger than in the adult opponent. It has also been suggested that breast stripe may function in individual recognition rather than in signalling general status

(Collias 1943).

The mechanism how honesty of status signalling is maintained is also controversial. Social punishment of cheaters (large patch, poor fighting ability) may not explain the evolution of status signalling, because badges of status to be useful should be recognized before any real fights take place. Production costs of patches of status could be one mechanism to maintain honesty of status signalling. Melanin based ornaments like black bib of the house sparrow and breast stripe of the great tit have been regarded cheap to produce, because melanin is synthesized by a bird from plentiful tyrosine. Veiga and Puerta (1996) demonstrated a cost of producing a melanin based sexual character in house sparrow whereas Hill & Brawner (1998) and Gonzales et al. (1999) found no condition dependence in similar traits. Condition dependence of melanin based coloration in the great tit, however, has never been demonstrated. Studies on heritability of breast stripe size are also controversial. Norris (1993) found high heritability of male breast stripe and demonstrated fitness advantage of females choosing males with wide breast stripe whereas Lemel (1993) found no heritability but substantial environmental variation to this trait. In house sparrow inheritance of melanin based male ornament has also been suggested to be mainly due to non-genetic sources like parental care (Griffith et al. 1999). Since the empirical evidence on heritability, condition dependence and the function of melanin based plumage characters is not consistent further studies are needed.

The aim of this study was to investigate the genetic and environmental determination of both dominance and size of the breast stripe and throat patch and to test whether breast stripe size would be correlated with dominance in juvenile great tits. To achieve this we raised cross-fostered great tit offspring in the lab after fledging. Dominance was estimated in autumn by the time juvenile great tits disperse and dominance hierarchy is established in wintering flocks.

METHODS

The study was conducted during the 2000 breeding season in a nestbox population in Konnevesi, Finland (62 ° 37′ N, 26 ° 20′ E). Nestboxes were regularly checked to determine the start of egg laying, hatching date and the number of eggs and nestlings.

Cross-fostering experiment

To distinguish between genetic and environmental causes of resemblance, partial cross fostering experiment was conducted during the first clutch. As a result half of the nestlings of each brood were raised by foster parents and half by their biological parents. To achieve this half of the two days old nestlings were swapped between a pair of nests with same hatching date and approximately the same number of nestlings. When the number of nestlings was uneven, a number below one half was transferred. Hatching date was determined by daily examination of nests at the end of the incubation period. Because of the hatching asynchrony hatching date was defined as a day on which at least half of the nestlings were hatched. In order to identify the origin of each nestling, claws of the foster young were painted with nail-polish, which made them identifiable until all nestlings were banded with individually numbered aluminium rings at the age of 8 days.

When nestlings were 13 days old, three foster young and three nestlings of the original brood were randomly picked from each brood and were taken into the laboratory with their putative parents. Remaining nestlings were replaced in extra nests with similar aged young. Birds were captured and kept with the permission of the Central Finland Regional Environmental Centre (permission 0900 L0312/254) and the Animal Care Committee of the University of Jyväskylä (permission 20/29.5.00). In the laboratory each brood was housed family-wise in 1m x 1m x 1m cages in visual isolation from other families. During the course of the study the birds received daily replenished food (mealworms, ant pupae and mixture of juvenile dog food (Purina trade mark), vitamin enriched tallow, calcium, peanuts, oat grains and wheat grains) and vitamin-enriched water *ad libitum*). Two weeks after fledging broods with their putative mothers were joined to form flocks in large indoor aviaries. To exclude age related competition among flocks broods of approximately same hatching date were raised together in four different aviaries.

Measures of condition and immunity

As a measure of body condition we used the residuals of the regression of body mass on tarsus length. Because of the size dimorphism between sexes body condition index was calculated for each sex separately.

Cell mediated immune response of thirteen-day old nestlings was measured using a phytohemagglutinin (PHA) skin test (Goto et al. 1978). PHA is a mitogen that stimulates T-lymphocytes to proliferate non-specifically without previous immunization and this test provides a general index of cell-mediated immunity (McCorkle et al. 1980). Nestlings were injected intradermally in the center of the right wing web with 0.2 mg of PHA (Sigma, L-8754) in 0.04 ml PBS. The left wing (control) was injected with 0.04 ml PBS (phosphate buffered saline pH 7.4). Thickness of the injection site was measured with an accuracy of 0.01 mm using a spessimeter just prior and 24 hours after injection. The swelling of the wing web was calculated as the difference in thickness prior to and after injection. The cell-mediated immune response was calculated as the difference in swelling between the PHA-injected wing and the control wing (wing-web index).

Dominance index

Dominance was determined in aviary experiments in September-October, where five females and five males were randomly picked from different aviaries to each trial. Dominance was measured in an aviary that none of the tested birds had experienced before. After birds were transferred to the study arena they were motivated to feed by two hours food deprivation before the trial began. All trials were videotaped for further analysis. Dominance index was based on three measures: access to food (the first individual to feed full 1 minute was ranked number 1 and the last to feed during half an hour trial was ranked number 10), the proportion of interactions won and total number of interactions. Dominance index was calculated as a first principal component from a PCA analysis of the correlation matrix of three measures of dominance (Table 1.). All birds were tested only once, and hence dominance score does not represent the rank of the given individual over all individuals tested. However, it reflects the ability of an individual to dominate food resource in a standardized environment. In our experimental design we were able to control for many asymmetries that could bias our results including age (all birds were juveniles), motivation (food deprivation) and prior residency (aviary was a novel environment for all birds in the trial). We could not control for the dominance rank in the aviaries where birds were originated or prior knowledge of the opponents. Because of the limited sample size both females and males were included in a same analysis. Males were dominant over females (mean dominance score for males 0.311, $n = 57$; mean dominance score for females -0.361, $n = 47$, $t = 3.646$, $p < 0.001$) and dominance index was standardized for each sex separately to be able to use dominance scores of both sexes in a same analysis. High dominance status may be more important for males who fight also for territories. However, there is assortative mating with respect to dominance status in black-capped chickadee (Otter et al. 1999), which suggests that dominance status may play important role during the breeding season also for females. Dominance depends always on the social environment (that usually includes individuals of both sexes) and the ecological context. We can not say that dominance measured during autumn is a same trait with dominance during the breeding season even within sexes. Our dominance index reflects ability to access for food resources in autumn that should be equally valuable for both sexes especially when competition for food resources occurs also between sexes. Male great tits are dominant over females probably due to the size dimorphism. Hence dominance may not be sexually dimorphic trait in a traditional sense and same heritable traits may constitute dominance rank in both sexes. For example in selection experiment in red grouse both parents contributed equally to the dominance ranks of both sons and daughters (Moss et al. 1985).

Breast stripe and throat patch size

Breast stripe size and throat patch size was obtained from image analysis of digital photographs. The area covered with black feathers for both throat patch and breast stripe were calculated separately. Each bird was placed on its back fully extended and was held by its bill and legs. The back and crown were in contact with the background. The ventral plumage was then photographed with a digital video camera. Each bird was photographed three times and after each shot bird was removed from the background and it was allowed to flap its wings. Feathers were not smoothed by the person who handled the bird. In the images obtained the throat patch was defined as a black area from the base of the bill to the point where throat patch narrows to become a ventral stripe. The ventral stripe area was measured from a distance of 4 centimetres from the throat patch. Both throat patch and breast stripe areas were highly repeatable within both sexes. Repeatability of throat patch area for males: 0.79, df = 55, $p = < 0.001$; for females 0.74. df = 48, $p < 0.001$. Repeatability estimate for breast stripe area for males: 0.92, df = 54, $p < 0.001$, for females: 0.91, df = 48, $p < 0.001$. Both throat patch area (mean throat patch area for males 2.812, SD = 0.442, n = 56; mean throat patch area for females 2.511, SD = 0.311, n = 49, t = 3.979, $p < 0.001$) and breast stripe area (mean breast stripe area for males 5.073, SD = 0.749, n = 56; mean breast stripe area for females 2.917, SD = 0.732, n = 49, t = 14.872, $p < 0.001$) were highly sexually dimorphic. Because of the limited sample size plumage measures of different sexes were used in the same analysis and both throat patch area and breast stripe area were standardized for each sex separately. Even if the sexual dimorphism is large in a secondary sexual trait, there may be high genetic correlation between male and female expression of the trait and many of the genes that affect the expression of a trait that is sexually selected in either sex are the same in males and females. In the cross-fostering study on zebra finch genetic correlation between male and female bill colour (sexually selected trait in males) was large (0.81) suggesting that the same genes affect bill colour expression in males and females, although there is substantial sexual dimorphism for bill colour caused by genetic factors that affect the sex specific expression of red bill colour pigment (Price 1996).

Statistical analysis

Genetic and environmental effects on offspring plumage characteristics were estimated using nested ANOVA, where the term 'pair of nests' is the main nesting factor. Each "pair of nests" consisted of two partially cross-fostered broods. The term "pair of nests" reflects any temporal variation faced by offspring among pairs of nests during breeding season. In each model the term "nest of origin" and the term "nest of rearing" were nested as a factor within the "pair of nests". The term "nest of rearing" estimates the effects of common rearing environment during nestling period. The term "nest of origin" accounts for any pre manipulation effects including additive genetic variation and dominance variation and pre manipulation maternal effects if present.

RESULTS

Determination of dominance

Genetic and environmental variation in dominance was tested using nested ANOVA. We found significant variation caused by the common rearing environment, but there was no origin related variation on dominance (Table 2.). When each sex was analyzed separately foster effect was significant for males despite the small sample size (df = 11, F = 2.508, p = 0.030) but not for females (df = 7, F = 0.979, n.s.). Thus, dominance measured four months after fledging was environmentally determined during early development with no significant heritable variation. There was a significant positive phenotypic correlation between dominance index and cell mediated immunity measured before fledging (Fig. 1; $r_s = 0.232$, n = 106, p = 0.017). However, nestling body condition index or body condition index measured later in life did not correlate with dominance index.

Determination of the throat patch and the breast stripe size

The rearing environment had no effect on the size of the throat patch and size of the breast stripe, but there was significant origin related variation in throat patch size suggesting heritable variation (Table 3.). When variation in throat patch size was analyzed separately for each sex origin effect was significant for females (df = 10, F = 2.767, p = 0.034) but not for males (df = 9, F = 1.613, n.s.). Body condition index during moult was positively correlated with the size of the throat patch and size of the breast stripe (Fig 2.).

Dominance and size of the breast stripe the throat patch

There was no phenotypic correlation between dominance and either breast stripe size or throat patch size. Also within sexes there was no phenotypic correlation between dominance and size of the breast stripe or size of the throat patch (Fig 3.).

DISCUSSION

Juvenile male great tits were dominant over juvenile females. In a nested ANOVA common rearing environment (including parental care of the foster parents) during early development had significant effect on dominance rank in juvenile great tits, but there was no origin related variation. The only single condition measure that was phenotypically correlated with dominance was the cell mediated immunity before fledging. This suggests that rearing related variation in dominance may be attributable to health. Fledging weight affects later survival (Alatalo et al. 1990, Tinbergen & Boerlijst 1990), territory quality in which a bird subsequently reproduces (Verhulst et al. 1997) and reproductive output of females (Haywood & Perrins 1992). Fledging weight is usually strongly environmentally determined although heritable component also exists (Merilä 1996, Merilä et al. 1999) suggesting that maternal effects through the effects of clutch size and parental food provisioning mainly determine the fledging weight. However, persistent maternal effects and their fitness consequences (linkage between early conditions and survival and reproduction) are rarely reported. In addition mechanisms explaining the influence of early maternal effects on survival and reproductive success are not clear. It has been suggested that fledging weight may be positively related to competitive ability (Garnett 1981). In a study on great tits there was stronger selection on fledging mass when the population density was high suggesting that competition affects mass-dependend recruitment (Both et al. 1999). Our results also support the view that early conditions may affect competitive ability and non genetic inheritance of dominance may be important mechanism generating between individual differences in survival and reproductive success and hence fitness.

There was no significant heritable variation in dominance. Interestingly cell mediated immunity that was phenotypically correlated with dominance had also no significant heritable variation in our study but large environmental variation (unpublished data). Instead fledging body condition that was heritable (unpublished data) was not strongly associated with dominance. Perhaps cell mediated immunity is a better measure of condition and health than body condition and hence better predictor of offspring performance later in life. Both general immunity and dominance may be traits close to fitness and hence subjects to directional selection which may have reduced genetic variation in both traits. Our results contradict high heritability of dominance reported in the red grouse (Moss et al. 1982; 1985). However, in the red grouse benefits of high dominance vary according to population density that fluctuates. Dominant types are selected for during the decline of the population cycle but subordinate types are selected during increase and at the peak density (Moss et al. 1984). Hence population cycles provide mechanism of how genetic variation in dominance is maintained in the red grouse. High heritabilities of dominance or dominance related traits reported in mammals come also from studies on species that have population cycles (Dewsbury 1990, Horne & Ylönen 1998, Oksanen et al. 1999).

We found significant origin related variation in the size of the black throat patch whereas black breast stripe showed no heritable variation. Sexual selection by female preferences for male genetic quality and/or male genetic attractiveness both require that secondary sexual characters are heritable (Andersson 1994). Our results confirm this assumption although a role of breast stripe size in female choice in this species is not clear. Common rearing environment or fledging weight had no effect on the size of either the throat patch or the breast stripe. However, body condition during moult explained major part of variation in both the size of the throat patch and the breast stripe. This result suggests that producing melanin based plumage character may be costly or individuals in poor condition may moult less conspicuous plumage characters to avoid conflicts between conspecifics. Although breast stripe size had no effect on dominance within sex it may affect the dominance relations between sexes or different age classes. Latter explanation seems to be more likely since melanin based ornaments have been regarded cheap to produce (Hill & Brawner 1998, Gonzales et al. 1999). In addition if these plumage characters were strongly condition dependent one should expect also significant effect of rearing environment, which was not found in our study.

Breast stripe size has been suggested to determine the outcome of contests between individuals with no prior experience of each other (Lemel & Wallin 1993). In our study breast stripe size or size of the throat patch did not influence dominance rank in either sex. In our study in each trial some birds originated from the same aviary and hence knew each other. Thus, our experimental design did not allow controlling for the prior knowledge of the dominance status of the opponent. However, in most trials many of the opponents were strange to each other and if there would be a strong influence of breast stripe size that would probably show in our data.

Alternatively the lack or relationship between dominance and patch of status within sexes in our study may be a result of flexibility of the expression of the patch. Many birds have coverable patches which they display only during interactions with conspecifics (Hansen & Rohwer 1986). Although the repeatability of three measurements of the breast stripe were high, we measured the latent expression of the stripe i.e. the bird was not displaying the badge. Great tits display the breast stripe during aggressive interactions and the size of the breast stripe may not be the same when measured during interactions. Social status or fighting ability is not a fixed trait. Instead, it is strongly affected by social environment, motivation and prior residency and perhaps condition or health as our results suggest and to honestly reflect status a badge should also show flexibility in its expression. In tree lizard dorsal coloration is a signal of status and males may change their coloration over a matter of minutes according to the social environment and their current status (Zucker 1994). Also in great tits breast stripe size measured during display from videotape correlated positively with aggressiveness measures, but breast stripe size measured in hand did not (unpublished data). Lemel & Wallin (1993) found that experimentally increased patch size determined the outcome of conflicts when the contestants were not familiar to each other. Without manipulation motivation decided the winner. It is possible that in the eyes of the opponents displaying and hence motivation was manipulated, not the size of the stripe itself. Perhaps the act of displaying is more important than the size of the patch itself and the patch may function as an enforcer for the more important behavioural display. Finally, the function of the breast stripe may be simply to signal intent or motivation, not quality or general fighting ability. Birds do not have facial impressions that could signal emotions or intent.

Flexible plumage characters might have this function. However, large variation in size of the breast stripe suggests that also the size of the stripe itself has a function. Our results, however, suggest that it may not be signalling general dominance status within sex or age classes.

To conclude dominance rank was environmentally determined during early development. Non genetic inheritance of dominance provides one mechanism for observed relation between fledging condition and later survival and subsequent breeding success. Breast stripe or throat patch size did not affect the dominance rank when sex was controlled for despite the large sample size. This suggests that breast stripe size may not function as a signal of status within age or sex classes. Throat patch size was heritable whereas breast stripe size was not. The function of melanin based coloration in the sexual selection in this species is unclear, but nevertheless our result suggest that if there is a genetic correlation between fitness and melanin based coloration, females may use male throat patch size as a cue of heritable quality. Both breast stripe and throat patch size were related to the body condition during moult. We interpret this result as a social cost of the character (juveniles in poor condition may moult less conspicuous plumage characters to avoid conflicts between older conspecifics) rather than a cost of producing the trait.

Acknowledgements

We thank Helinä Nisu, Matti Halonen, Panu Halme, Antti Hannuniemi and Annukka Näyhä for assistance in the field. We also thank staff at Konnevesi Research station for technical help during the experiment. This study was funded by the Academy of Finland under the Finnish Centre of Excellence Programme.

REFERENCES

Acrese, P. 1989. Intrasexual competition, mating system and natal dispersal in song sparrows. Anim. Behav. 38, 958-979.

Acrese, P. & Smith, J.N.M. 1985. Phenotypic correlates and ecological consequences of dominance in song sparrows. J. Anim. Ecol. 54, 817-830.

Alatalo, R.V., Gustafsson, L. & Lundberg, A. 1990. Phenotypic selection on heritable size traits: environmental variance and genetic response. Am. Nat. 135, 464-471.

Andersson, M. 1994. Sexual selection, Princeton, New Jersey: Princeton University press.

Both, C., Visser, M.E. and Verboven, N. 1999. Density-dependent recruitment rates in great tits: the importance of being heavier. Proc. R. Soc. Lond. B 266, 465-469.

Collias, N.E. 1943. Statistical analysis of factors which make for success in initial encounters between hens. Am. Nat. 72, 519-538.

De Laet, J.V. 1985. Dominance and aggression in juvenile great tits, Parus major major L. in relation to dispersal. In: Behavioral Ecology - Ecological consequences of adaptive behaviour (eds. Sibley, RM & Smith RH), pp 375-380.

Dewsbury, D.A. 1990. Fathers and sons: genetic factors and social dominance in deer mice, *Peromyscus maniculatus* Anim. Behav. 39, 284-289.

Drews, C. 1993. The concept and definition of dominance in animal behaviour. Behaviour 125, 3-4.

Ekman, J. & Askenmo, C.E.H. 1984 Social rank and habitat use in willow tit groups. Anim. Behav. 32, 508-514.

Engh, A.L., Esch, K., Smale, L. & Holekamp, K.E. 2000. Mechanisms of maternal rank 'inheritance' in the spotted hyaena, *Crocuta crocuta* Anim. Behav. 60, 323-332.

Garnett, M.C. 1981. Body size, its heritability and influence on juvenile survival among great tits *Parus major*. Ibis, 123, 31-41.

Gonzales, G., Sorci, G., Moller, A.P., Ninni, P., Haussy, C. & de Lope, F. 1999. Immunocompetence and condition-dependent sexual advertisement in male house sparrow (Passer domesticus). J. Anim. Ecol. 68, 1225-1234.

Goto, N., Kodama, H., Okada, K. & Fujomoto, Y. 1978. Suppression of phytohaemagglutinine skin response in thymectomized chickens. Poultry Sci. 57, 246-250.

Griffith, S.C., Owens, I.P.F. & Burke, T. 1999. Environmental determination of a sexually selected trait. Nature 400, 358-360.

Hansen, A.J. & Rohwer, S. 1986. Coverable badges and resource defence in birds. Anim. Behav. 34, 69-76.

Haywood, S. & Perrins, C.M. 1992. Is clutch size in birds affected by environmental conditions during growth? . Proc. R. Soc. Lond. B 249, 195-197.

Hill, G.E. & Brawner, W.B. 1998. Melanin-based plumage colouration in the house finch is unaffected by coccidial infection. Proc. R. Soc. Lond. B 265, 1105-1109.

Horne, T.J. & Ylönen, H. 1998. Heritabilities of dominance-related traits in male bank voles (Clethrionomys glareolus) Evolution 52, 894-899.

Järvi, T. & Bakken, M. 1984. The function of the variation in the breast stripe of the great (*Parus major*). Anim. Behav. 32, 590-596.

Järvi, T., WalsØ, Ø. & Bakken, M. 1987. Status signalling by Parus major: an experiment in deception. Ethology, 76, 334-342.

Lemel, J. 1993. Evolutionaty and Ecological Perspectives of Status Signalling in the Great Tit (*Parus major L.*) Thesis, Göteborgs Univ., Sweden.

Lemel, J. & Wallin, K. 1993. Status signalling, motivational condition and dominance: an experimental study in the great tit, Parus major L. Anim. Behav. 45, 549-558.

Maynard Smith, J. & Harper, D.G.C. 1988. The evolution of aggression: can selection generate variability? Phil. Trans. R. Soc. Ser. 319, 557-570.

McCorkle, F., Olah, I. & Glick, B. 1980. The morphology of the phytohemagglutinin.induced cell response in the chicken's wattle. Poultry Sci. 59, 616-623.

Merilä, J. 1996. Genetic variation in offspring condition: an experiment. Funct. Ecol. 10, 365-474.

Merilä, J., Przybylo, R. & Sheldon, B.C. 1999. Genetic variation and natural selection on blue tit body condition in different environments. Genet. Res. 73, 165-176.

Moss, R., Watson, A., Rothery, P. & Glennie, W. 1982. Inheritance of dominance and aggressiveness in captive red grouse Lagopus lagopus scoticus. Aggressive Behav. 8, 1-18.

Moss, R., Watson, A., Rothery, P. 1984. Inherent changes in the body size, viability and behaviour of a fluctuating red grouse (Lagopus lagopus scoticus) population. J. Anim. Ecol. 53, 171-189.

Moss, R., Rothery, P. & Trenholm, I.B. 1985. The inheritance of social dominance rank in red grouse (Lagopus lagopus scoticus). Aggressive Behav. 11, 253-259.

Nol, E., Cheng, K. & Nichols, C. 1996. Heritability and phenotypic correlations of behaviour and dominance rank of Japanese quail. Anim. Behav. 52, 813-820.

Norris, K. 1993. Heritable variation in a plumage indicator of viability in male great tits (*Parus major*). Nature 362, 537-539.

Oksanen, T.A., Alatalo, R.V., Horne, T.J., Koskela, E., Mappes, J. & Mappes, T. 1999. Maternal effort and male quality in the bank vole, *Clethrionomys glareolus*. Proc. R. Soc. Lond. B 266, 1495-1499.

Otter, K., Ramsay, S.M. & Ratcliffe, L. 1999. Enhanced reproductive success of female black-capped chickadees mated to high-ranking males. Auk 116, 345-354.

Piper, W. H., and R. H. Wiley. 1990. The relationship between social dominance, subcutaneous fat, and annual survival in wintering white-throated sparrows (*Zonotrichia albicollis*). Behav. Ecol. Sociobiol. 26, 201-208.

Price, D.K. 1996. Sexual selection, selection load and quantitative genetics of zebra finch bill colour. Proc. R. Soc. Lond. B 263, 217-221.

Qvarnström, A. & Forsgren, E. 1998. Should females prefer dominant males? Trends Ecol. Evol. 13, 498-501.

Richner, H., Schneiter, P. & Stirnimann, H. 1989. Life-history consequences of growth rate depression: an experimental study on carrion crows (Corvus *corone corone L.*) Funct. Ecol. 3, 617-624.

Rohwer, S. 1975. The social significance of avian winter plumage variability. Evolution 29, 593-610.

Roeskaft, E., Moksnes, A., Stokke, B.G., Bicik, V. & Moskat, C. 1983. The relationship between the fledging weights of captive rooks and position in the dominance hierarchy one year later. Fauna Norvegica, Ser. C., 6, 78-80.

Sandell, M. & Smith, H.G. 1991. Dominance, prior occupancy and winter residency in the great tit (Parus major). Behav. Ecol. Sociobiol. 29, 147-152.

Tinbergen, J.M. & Boerlijst, M.C. 1990. Nestling weight and survival in individual great tits *Parus major.* J. Anim. Ecol. 59, 1113-1127.

Veiga, J.P. & Puerta, M. 1996. Nutritional constraints determine the expression of a sexual trait in the house sparrow. Proc. R. Soc. Lond. B 263, 229-234.

Velando, A. 2000. The importance of hatching date for dominance in young shags. Anim. Behav. 60, 181-185.

Verhulst, S., Perrins, C.M. & Riddington, R. 1997. Natal dispersal of great tits in a patchy environment. Ecology 78, 864-872.

Westman, B. 1990. Environmental effect on dominance in young Great tits Parus major: a cross-fostering experiment Ornis Scand. 21, 46-51.

Wilson, J.D. 1992. A re-assessment of the significance of status signalling in populations of wild great tits, Parus major Anim. Behav. 43, 999-1009.

Zucker, N. 1994. Social influence on the use of a modifiable status signal. Anim. Behav. 48, 1317-1324.

TABLE 1 Component matrix of 1st principal component from a PCA, used as a dominance index.

dominance variables	**dominance factor scores**
feeding order	- 0.703
number of interactions	0.783
proportion of interactions won	0.818
variance explained	59.18 %

TABLE 2 Effects of common rearing environment and nest of origin on dominance.

	df	F	P
origin(pair)	14	0.565	0.882
foster(pair)	14	2.354	0.011
pair	13	0.598	0.806

TABLE 3 Effects of common rearing environment and nest of origin on the size of the breast stripe and size of the throat patch.

	df	F	P
Size of the breast stripe			
origin(pair)	14	1.441	0.161
foster(pair)	14	1.595	0.105
pair	13	0.895	0.578
Size of the throat patch			
origin(pair)	14	2.322	0.012
foster(pair)	14	0.955	0.508
pair	13	1.055	0.467

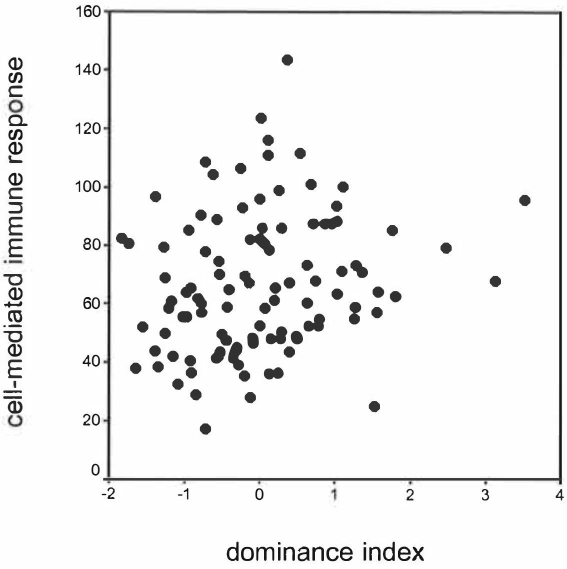

FIGURE 1 Relation between dominance index (standardized according to sex) and cell mediate immune response at fledging ($r_s = 0.232$, n = 106, p = 0.017).

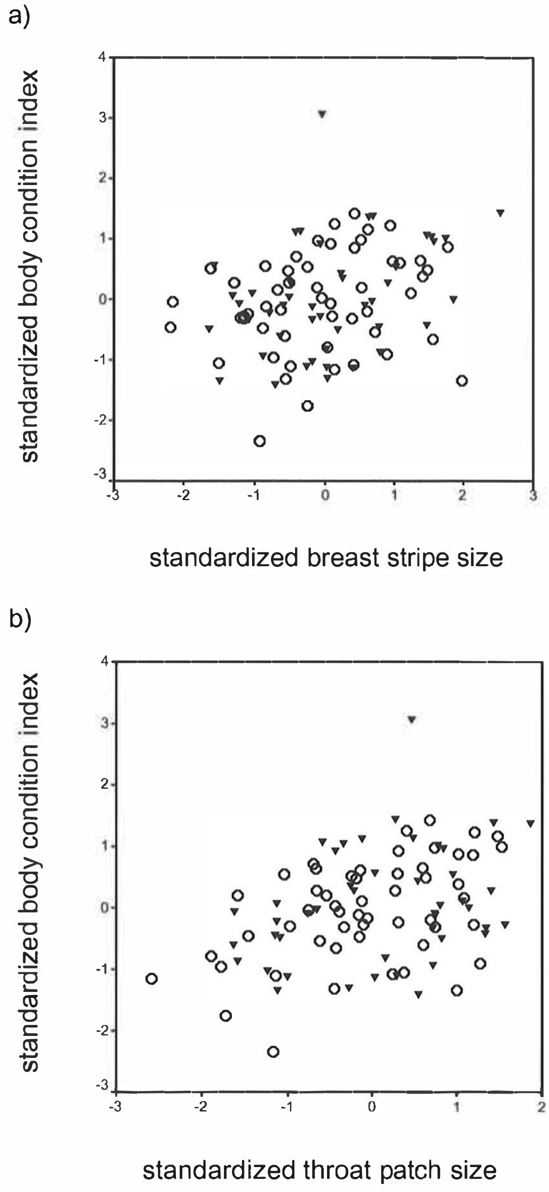

FIGURE 2 Melanin based coloration in relation to body condition index during moult (o males; ▼ females). (a) Relation between breast stripe size (standardized for each sex) and body condition ($r_s = 0.316$, $p = 0.002$, $n = 98$). (b) Relation between standardized throat patch size and body condition ($r_s = 0.358$, $p < 0.001$, $n = 98$).

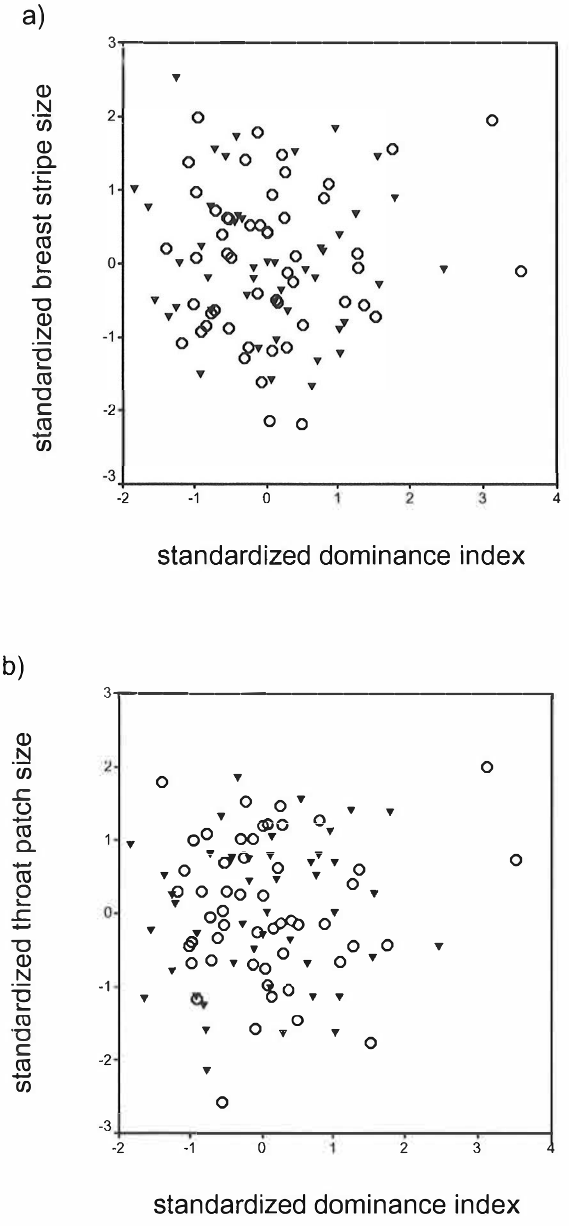

FIGURE 3 Relation between melanin based coloration and dominance (o males; ▼ females). (a) Relation between standardized breast stripe size and dominance index (r_s = -0.048, n.s., n = 98), (b) Relation between standardized throat patch size and dominance index (r_s = 0.050, n.s., n = 98)

V

PLUMAGE CHARACTERS, DOMINANCE AND WINTER SURVIVAL IN THE GREAT TIT

By

Janne Kilpimaa, Rauno V. Alatalo, Johanna Mappes & Heli Siitari

Manuscript

Plumage characters, dominance and winter survival in the great tit

Janne Kilpimaa, Rauno V. Alatalo, Johanna Mappes & Heli Siitari

Department of Biological and Environmental Science, University of Jyväskylä,
P.O. Box 35, FIN-40014 University of Jyväskylä, Finland

ABSTRACT

We investigated the role of both carotenoid based and melanin based (breast stripe size) plumage coloration on dominance and winter survival in juvenile male great tits. We also studied the flexibility of the expression of the breast stripe by measuring breast stripe size also during aggressive interactions from video captures. In our dominance experiment high level aggression determined the dominance rank with no significant impact of less intense display or the breast stripe size. Within individual birds the breast stripe expression declined with the time during the experiment suggesting that breast stripe expression is flexible. There was a positive relation between aggressiveness and breast stripe area measured from video captures during dominance trials. Breast stripe size measured in hand did not correlate with aggressiveness. This suggests that flexible breast stripe expression is used as a part of threat display to signal aggressive motivation. Males with large breast stripe size had lower fat scores at capture. This suggests that also the size of the breast stripe itself may affect dominance in certain conditions, in particular when the value of the contested resource is low. Plumage brightness of yellow breast feathers correlated positively with both dominance and aggressiveness, but saturation of yellow did not. Breast stripe size was negatively correlated with survival and we also found indirect evidence that aggressiveness and dominance may be related to survival through the effect of increased vulnerability to predation. Our results suggest that flexible breast stripe expression reflects aggressive motivation and perhaps also general dominance in some social situations. Large breast stripe is also costly in terms of reduced winter survival. Plumage brightness of yellow breast feathers correlated with dominance, but whether it is a true signal of dominance remains to be investigated.

INTRODUCTION

If males exhibit ornamental plumage outside the breeding season it is possible that ornamentation has also functions other than the sexual display. Status signalling hypothesis suggests that intraspecific plumage variability in some birds has evolved to reduce contest intensity outside the breeding season and plumage variation would signal general dominance and fighting ability (Rohwer 1975, 1982). However, it is not clear how fixed plumage characters would signal dominance status that is strongly affected by motivation, prior residency and social environment. Maynard-Smith & Harper (1988) suggested that low-cost patches of status are used only when the value of the resource is low relative to the cost of fighting. Behavioural threat display has also been suggested to be an honest signal of dominance (Parker 1974), because strong threats carry a cost of eliciting counter attacks from certain opponents (Maynard Smith 1979). However, in great tits (*Parus major*) it is thought that threat display is a graded signal of motivational state and hence flexible, not fixed signal of general status (Lange & Leimar 2003). The same role for plumage patches has rarely been investigated, probably because many plumage patches have been regarded rather fixed after moult. If plumage patches are also flexible the level of expression of the patch would signal intent or motivation in the similar way as the behavioural threat display. In the red-winged black bird (*Agelaius phoenicurus*) red epaulette patches are coverable and males use them as signals of territory ownership (Hansen & Rohwer 1986). The coverable patches enable males to adjust the size of the patch according to the status (floater/territory owner) that may change during the breeding season. In the collared flycatcher (*Ficedula albicollis*) white forehead patch that is used in male-male contests is partly coverable, but the function of the plasticity of the forehead patch expression is not known (Griffith & Sheldon 2001).

The great tit is a sexually dimorphic passerine the black breast stripe being the most conspicuous male character. It has been suggested that the black breast stripe may work in sexual selection and females may gain genetic benefits in choosing males with wide breast stripe (Norris 1993). On the other hand black breast stripe of the great tit has been often cited as a classical example of a badge of status (Järvi & Bakken 1984, Järvi et al 1987, Maynard-Smith & Harper 1988, but see Wilson 1992). Lemel & Wallin (1993) suggested that breast stripe has an impact only in conflicts between individuals of no prior experience of each other.

The black coloration of the breast stripe in great tits is melanin based like many other patches of status in birds. There is evidence that melanin based ornaments are cheap to produce (Hill & Brawner 1998, Gonzales 1999, but see Veiga and Puerta 1996). Because of the low production costs, honesty of melanin based badges is probably maintained through social costs, i.e. individuals with large patch carry a cost of increased aggression by conspecifics (Maynard-Smith & Harper 1988). Carotenoid based plumage ornamentation is regarded more costly, because carotenoids can not be synthesized by a bird and only the most efficient foragers may express the most extreme carotenoid ornamentation. Carotenoids are also immunostimulants and antioxidants used for important physiological functions like immune function (Olson & Owens 1998). Hence carotenoid colouration may reveal nutritional or immunological stress. Dual function of carotenoids as both antioxidants and colour pigments may provide a physiological mechanism behind the hypothesized trade off between ornamentation and health (Von Schantz et al. 1999). Accordingly there is lots of empirical evidence that carotenoid based plumage variation is sensitive to stress and parasitism contrary to melanin based coloration (Hill & Montgomerie 1994, Hill 1996). Because of the costliness of carotenoid based plumage colouration it has been regarded as a good candidate for honest signal in the context of sexual selection. Thus, it has often been assumed that carotenoid based colours would work in status signalling or intrasexual competition only during the breeding season when the value of contested resources (territories or females) is high. There is evidence that carotenoid colours signal dominance in male-male contests over territories in red-collared widowbirds (*Euplectes ardens*) (Pryke et al. 2001). Instead the few studies on the relationship between carotenoid variation and dominance outside the breeding season have found no evidence that carotenoid colour would signal dominance (Wolfenbarger 1999, McGraw & Hill 2000a).

The aim of this study was to investigate whether melanin based breast stripe and carotenoid plumage characters in juvenile male great tits are related to dominance and winter survival. We were also studying the flexibility of breast stripe size by measuring the breast stripe size also during aggressive interactions from video.

METHODS

Trapping and housing

The study was carried out at Konnevesi Research Station (62 ° 37′ N, 26 ° 20′ E), Central Finland during winter 1999-2000. Juvenile male great tits were trapped in mist nets during early winter (November-December) at peanut feeders in three different locations (situated a few kilometres away from each other). On capture birds were ringed both with aluminium numbered rings and up to three plastic colour rings giving an unique combination for each bird. The birds were aged and sexed on the basis of plumage (Svensson 1992). Fat in the tracheal pit was recorded on the 5 point scale (Gosler 1996). The birds were weighed to 0.1 g on a 50-g Pesola spring balance. In the laboratory males were housed solitarily in 1m x 1m x 1m cages in visual isolation from other males. During the course of the study the birds received daily replenished ad libitum food (seeds and tallow) and fresh water. After the dominance experiment all males were released at the same place where they were captured.

Dominance experiment

At each dominance trial 5 males were introduced to an aviary where they were allowed to habituate for 2 hours. During the 2 hours birds were not given food to motivate them to contest for food resources. The experiment began when the food resource was revealed without disturbing the birds by pulling the cover of the food resource from behind the one way mirror. Dominance was determined as an ability to access for food: the individual that was the first to reach a sum of one minute of feeding was ranked number 1 and the last to feed was ranked number 5. The 30 minutes experiment was videotaped and aggressive threats were calculated for each bird. Because overall aggression score was very closely correlated with the proportion of interactions won only the frequency of aggressive behaviour was used as a measure of aggression. Aggressive behaviour was classified in three types of categories: low level aggression (flattenig the crest, displaying the breast stripe), medium level aggression (spreading the wings, head held upwards, stepping towards the opponent) and high level aggression (including chase, supplanting and physical fight). As a measure of general aggressiveness a sum of all the aggressive threats weighed with the intensity of aggression was used. The distribution of aggression scores was skewed and hence aggression score was log-transformed for the statistical analysis. Mean intensity of aggression was calculated by dividing aggressiveness score with the total number of aggressive threats.

Measuring the breast stripe size

We measured the maximum breast stripe width and width of the breast stripe at the top of the keel. As a measure of breast stripe size measured in hand we used a first principal component from a PCA analysis of the covariance matrix for the two measures of breast stripe width.

We also measured the breast stripe size during the dominance experiments from the video captures. For each bird at least two video captures were obtained during each trial. Breast stripe size was obtained using image analysis program (Image Pro Plus) and the known length of the perch with food was used as a scale to get absolute area in square centimetres. The ventral stripe area was measured from the throat patch to a distance of 2 centimetres. Repeatability of the breast stripe area obtained from video captures was high (repeatability estimate for breast stripe size measured from video 0.60, df = 82, $p < 0.001$).

Carotenoid colours

We measured plumage reflectance of yellow breast feathers from feather samples (at least 10 feathers from the same region of the breast). The sample was illuminated from the proximal end of the feather sample by a Zeiss CLX xenon lamp at 45° to the surface of the feather sample, and the reflected light was collected using a Zeiss MCS 500 spectroradiometer at 135° (90° to illumination). Reflectance was calculated relative to a Spectralon™ 99% reflectance standard for the wavelength range 300 to 700 nm. Every sample was measured four times, changing the measuring spot between every recording. Mean of these four recordings were used for the analyses. For every individual, we calculated the average reflectance (brightness; $R_{300\text{-}700nm}$, Andersson et al. 1998) and saturation ($R_{700}\text{-}R_{450nm}/R_{700nm}$, Johnsen et al. 2003). The measure of saturation should reflect the influence of carotenoids, because carotenoids absorb most strongly at 450 nm.

Survival

Survival after release was recorded by observation of individually colour ringed birds during winter at capture locations and nearby feeders. Study birds were most often discovered at the same place where they were captured and dispersal between the three capture locations was rare. Only males that were released before or during December and seen after release in January were included in survival analysis to control for the effect of dispersal in late autumn. Survival was recorded in a four point scale (0 = the bird was not seen after January, 1 = not seen after February, 2 = not seen after March, 3 = seen in April).

RESULTS

Flexibility of breast stripe expression

Although the repeatability of breast stripe area measured from video captures was high, within individual bird breast stripe expression varied between the three consequent video captures (Repeated measures analysis: F(2,50) = 3.831, *P* = 0.025). Pair wise comparison shows that the breast size expression was highest in the first capture (in the beginning of the experiment) and declined in the subsequent captures: breast stripe size in the first video capture was higher than in the second capture (breast stripe size in the first capture 2.84, SD = 0.39, N = 82; breast stripe size in the second capture 2.74, SD = 0.39, N = 82, paired t-test: t(1,81) = 2.347, *P* = 0.021) and the third capture (breast stripe size in the first capture 2.84, SD=0.39, N = 51; breast stripe size in the third capture 2.70, SD = 0.37, N = 51, paired t-test: t(1,50) = 2.588, *P* = 0.013). Breast stripe size in the second capture was also higher than in the third capture (breast stripe size in the second capture 2.79, SD = 0.41, N = 51; breast stripe size in the third capture 2.70, SD = 0.37, N = 51, paired t-test: t (1,50) = 2.438, *P* = 0.018).

There was no significant relation between breast stripe width measured in hand with breast stripe area measured from video in the first video capture when the breast size expression was the largest ($r_s = 0.134$, $P = 0.218$, N = 86) whereas there was a significant relation against the second ($r_s = 0.263$, $P = 0.019$, N = 79) and the third video capture ($r_s = 0.478$, $P = 0.001$, N = 49). Hence, the breast stripe size measured in hand may reflect the latent expression of the breast stripe size which may not be strongly correlated with breast stripe size during aggressive interactions.

Threat display and dominance

General aggressiveness was related to dominance (Figure 1.; $r_s = -0.389$, $P < 0.001$, N = 138). However, this was mainly because of the despotic behaviour of the most dominant individual. There was no difference in overall aggressiveness between the four lowest ranked individuals. The mean intensity of aggression was related to feeding order so that birds that showed proportionally much high level aggression were more likely to feed first (Fig. 2, $r_s = -0.269$, $P = 0.018$, N = 77). This suggests that low level display alone is not enough to achieve high status and it must be backed up with more intense aggressiveness.

Dominance and plumage characteristics

There was a slight but significant relation between the brightness of the yellow breast feathers and dominance rank as measured in access for food (Fig. 3; $r_s = -0.177$, $P = 0.040$, N = 136). Plumage brightness was also negatively related to the time of capture ($r_s = -0.269$, $P = 0.004$, N = 113), suggesting that bright yellow birds feed earlier than others also in the wild or they are captured more easily. There was no relation between dominance and saturation of the yellowness of the breast feathers ($r_s = 0.098$, $P = 0.256$, N = 137).

There was no relation between dominance and width of the breast stripe measured in hand ($r_s = 0.049$, $P = 0.491$, N = 137) or breast stripe area measured from the video during dominance experience ($r_s = -0.027$, $P = 0.812$, N = 82). However, both measures of breast stripe size correlated negatively with fat score measured during capture corrected for capture time (relation between breast stripe size measured from video and residual fat score: $r_s = -0.277$, $P = 0.037$, N = 57; relation between breast stripe size measured in hand and residual fat score: $r_s = -0.226$, $P = 0.018$, N =110) . This suggests that breast stripe size may play some role in dominance in the wild.

Aggressiveness and plumage characteristics

Brightness of yellow breast feathers correlated positively with aggression score (Fig. 4., $r_s = 0.189$, $P = 0.028$, N = 136). Saturation of the yellow feathers was not related to aggressiveness ($r_s = 0.064$, $P = 0.457$, N = 137).

There was a positive relation between aggressiveness score and breast stripe area measured from video during the dominance trials (fig. 5; $r_s = 0.307$, $P = 0.005$, N = 82). Breast stripe width measured in hand, however was not related to aggressiveness (fig. 6.; r_s =-0.082, P = 0.346, N = 135). Residuals from regression of breast stripe area measured from video against breast stripe width measured in hand were also positively correlated with overall aggression score (r_s = 0.358, P = 0.01, N = 79), supporting the view that breast stripe size is flexible in its expression and great tits enlarge their breast stripe during aggressive interactions.

Dominance, plumage characteristics and survival

There was no linear relationship between dominance and survival. However, both dominance and aggression score were related to the capture time. The birds that were first to feed in dominance experiment were captured by us earlier in the morning than others (r_s = 0.212, P = 0.024, N = 114). Also the most aggressive birds were captured earlier (r_s =-0.220, P =0.018, N =114. This suggests that birds high in the hierarchy feed earlier or are less cautious and hence captured more easily. Capture time was also related to survival. Birds that were captured early were more likely to die after release (r_s = 0.268, P = 0.039, N = 60). These results suggest that there is a risk of being dominant or alternatively dominance correlates with some behavioural character like boldness that is related to survival.

Both breast stripe size measures, width of the breast stripe measured in hand and breast stripe area measured from the video during dominance experiment were negatively correlated with survival: relation between width of the breast stripe measured in hand and survival (Fig 7; r_s = -0.404, P = 0.001, N = 70), relation between size of the breast stripe measured from video and survival (r_s = -0.386, P = 0.011, N = 43). Brightness or saturation of the yellow breast feathers were not related to survival (relation between brightness and survival: r_s = 0.027, P = 0.827, N = 70; relation between saturation and survival: r_s = 0.069, P = 0.566, N = 71).

DISCUSSION

Breast stripe size and dominance

Breast stripe expression was flexible during the dominance experiment and individual breast stripe expression was largest in the beginning of the experiment. Breast stripe width measured in hand correlated with the breast stripe size measured from video only in the second and third captures after the decline in the breast stripe size. Hence breast stripe of the great tit is partly coverable and great tits may adjust the expression of the signal according to their motivation or intent. Indeed, mean breast stripe size measured during interactions from video correlated with aggressiveness but breast stripe measured in hand did not. Neither, latent expression of the breast stripe measured in hand or breast stripe size measured from video correlated with dominance measured as access to food resource. This suggests that flexible breast stripe size expression is related to aggressive motivation or intent, not general dominance or fighting ability. Also the threat display in great tits has been suggested to signal aggressive motivation rather than general dominance (Lange & Leimar 2003). In winter great tits display and fight frequently although they live in stable flocks where individuals should know the fighting ability of opponents (Lange & Leimar 2003). Close relation of breast stripe width expression and aggressiveness suggests that flexible expression of breast stripe width functions as a signal of aggressive intention in a similar way as behavioural display.

In our study males with large breast stripe size had lower fat reserves at capture compared to males with small breast stripe. In great tits dominant birds usually carry less fat, because higher status is correlated with the predictability of access to food resources (Gosler 1996). This suggests that males with large breast stripe may be more dominant in certain conditions. Perhaps the size of the breast stripe is an asymmetry that has an impact in outcome of the aggressive interactions, but behavioural threat display and fighting are more important when there are differences in motivation between individuals or the contested resource is too valuable. In our dominance experiment low level aggression including breast stripe display was not enough to gain access to food resources. Instead high level aggressiveness like attacks was needed. Hence strong impact of breast stripe size on general dominance was not expected. In our experiment two hours starvation may have resulted in the food resource becoming too valuable for contestants to decide the access to food by low cost breast stripe display only. There might also be differences in motivation between individuals. If males with large breast stripe are dominant in most social situations, they may not be willing to take risks in a novel environment, because they are more confident of their access to food resources in the future.

To sum up, breast stripe size is flexible in its expression and it is used as a part of threat display in aggressive interactions. However, in our study high intensity aggression decided the dominance and low level display and size of the breast size had no impact. Lower fat resources of males with large breast stripe suggest that in some social situations also the size of the breast stripe itself may have effects on dominance.

Carotenoid colouration and dominance

Brightness of the yellow breast feathers was the only plumage character that was related to dominance. Bright coloured birds were first to feed during dominance experiment. Plumage brightness was also correlated with aggressiveness. Only experimental manipulation of plumage brightness would reveal whether plumage brightness is a true signal or whether it is only a correlate of general dominance. Perhaps dominance and prior access to food resources results in plumage in better condition rather than vice versa. Chroma, saturation and hue are the commonest colour components used in studies on carotenoid signalling whereas plumage brightness is seldom used. However, there is some evidence that plumage brightness might be correlated with parasite load or quality of immune function. In siskins (*Carduelis spinus*) ectoparasite load affects plumage brightness (Figuerola et al. 2003) and in green finches (*Carduelis chloris*) plumage brightness is positively related to strength of the humoral immune response (Saks et al. 2003). Some aspects of plumage colour change according to the age of the feathers probably due to wear (Figuerola et al. 2003). It is possible that bright birds in our study were born earlier or raised in better conditions than subdominants. Hatching date (Velando 2000) and nutritional conditions (Richner et al. 1989) have been suggested to affect dominance in birds. We did not find any relation between the saturation of yellow feathers and dominance. In red-collared widowbirds collar hue signals fighting ability during breeding season (Pryke et al. 2001) whereas in house finch plumage hue did not predict dominance outside breeding season (McGraw & Hill 2000a). In northern cardinals (*Cardinalis cardinalis*) redness of the plumage correlates with dominance, but experimental manipulation of redness did not affect dominance suggesting that redness is not a signal of dominance in this species (Wolfenbarger 1999).

Plumage characters and survival

Breast stripe size was negatively related to survival. Although we did not find any direct relation between dominance and breast stripe size in juvenile males, it is possible that breast stripe size may signal dominance between different age and sex classes or also within sex/age classes in certain social situations. Hence, juvenile males with large breast stripe may pay the cost of increased aggression by adult males, which may affect their survival. Alternatively males with large breast stripe may be more conspicuous prey for predators. In our study year there were exceptionally many pygmy owls at the feeding places. Pygmy owls (*Glaucidium passerinum*) were seen hunting at each of the three study areas and many successful kills were observed. We are confident that pygmy owl was the main cause of death of our study birds. However, we don't know if males with large breast stripe were taken more often. Better survival of males with narrow breast stripe also suggests that breast stripe size is a poor predictor of condition or quality. This is in accordance with earlier studies suggesting that production costs of melanin based ornamentation are low and hence melanin based coloration may not signal general condition. Neither, saturation or brightness of yellow feathers did predict winter survival in our study. Carotenoid based plumage characters have been suggested to predict survival in the great tits (Horak et al. 2001) and in the house finch (*Carpodacus mexicanus*) (Mcgraw & Hill 2000b).

Dominance and survival

There was no linear relationship between dominance and survival. However, the most dominant and aggressive birds were captured earlier in the morning and the capture time was positively related to survival. Early capture time may mean simply that the bird comes to the feeder early or alternatively that such bird is easier to catch i.e. it is bolder or less cautious. If the first interpretation is true, the most dominant birds may be more vulnerable to predators than subdominants, because the main predator pygmy owl hunts during early hours. If the second interpretation is true, dominance may also be related to increased vulnerability to predation. It has been suggested that under predator presence subdominant great tits decrease the amount of aggression but there is no change in dominant birds (Lange & Leimar 2001). Winter feeding and subsequent high predation risk of pygmy owls at feeders is relatively new phenomenon in an evolutionary sense. Hence it is possible that under these circumstances individual behaviour may always not be adaptive and dominant birds may pay larger cost of high status than in more natural situations.

Acknowledgements

We thank Helinä Nisu and Matti Halonen for assistance in the field work. We also thank staff at Konnevesi Research station for technical help during the experiment. The experiment was carried out under permission of Animal Care Committee of the University of Jyväskylä (permission number 20 / 15.11. 1999). This study was funded by the Academy of Finland under the Finnish Centre of Excellence Programme.

REFERENCES

Andersson, S., Örnborg, J. & Andersson, M. 1998. Ultraviolet sexual dimorphism and assortative mating in blue tits. Proc. R. Soc. Lond. B 265, 445-450.

Figuerola, J., Domenech, J.C. & Senar J.C. 2003. Plumage colour is related to ectosymbiont load during moult in the serin, *Serinus serinus*: an experimental study. Anim. Behav. 65, 551-557.

Gonzales, G., Sorci, G., Moller, A.P., Ninni, P., Haussy, C. & de Lope, F. 1999 Immunocompetence and condition-dependent sexual advertisement in male house sparrow *(Passer domesticus)*. J. Anim. Ecol. 68, 1225-1234.

Gosler, A.G. 1996. Environmental and social determinants of winter fat storage in the great tit *Parus major*. J. Anim. Ecol. 65, 11-17.

Griffith, S.C. & Sheldon, B.C. 2001. Phenotypic plasticity in the expression of sexually selected traits: neglected components of variation Anim. Behav. 61, 987-993.

Maynard Smith, J. & Harper, D.G.C. 1988. The evolution of aggression: can selection generate variability? Phil. Trans. R. Soc. Ser. 319, 557-570.

Hansen, A.J. & Rohwer, S. 1986. Coverable badges and defense resource defence in birds. Anim. Behav. 34, 69-76.

Hill, G.E. 1996. Redness as a measure of the production cost of ornamental coloration. Ethol. Ecol. Evol. 8, 157-175.

Hill, G.E. & Brawner, W.B. 1998 Melanin-based plumage colouration in the house finch is unaffected by coccidial infection. Proc. R. Soc. Lond. B 265, 1105-1109.

Hill, G.E. & Montgomerie, R. 1994. Plumage colour signals nutritional condition in the house finch. Proc. R. Soc. Lond. B 258, 47-52.

Horak, P, Ots, I., Vellau, H., Spottiswoode, C. & Moller, A.P. 2001. Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits Oecologia 126, 166-173.

Johnsen, A. Delhey, K., Andersson, S. & Kempenaers, B. 2003. Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects. Proc. R. Soc. Lond. B 270, 1263-1270.

Järvi, T. & Bakken, M. 1984. The function of the variation in the breast stripe of the great (*Parus major*). Anim. Behav. 32, 590-596.

Järvi, T., WalsØ, Ø. & Bakken, M. 1987. Status signalling by Parus major: an experiment in deception. Ethology 76, 334-342.

Lange, H. & Leimar, O. 2001. The influence of predation risk on threat display in great tits. Behav. Ecol. 12, 375-380.

Lange, H. & Leimar, O. 2003. The function of threat display in wintering great tits. Anim. Behav. 65, 573-584.

Lemel, J. & Wallin, K. 1993. Status signalling, motivational condition and dominance: an experimental study in the great tit, Parus major L. Anim. Behav. 45, 549-558.

Maynard-Smith, J. 1979. Game theory and the evolution of behaviour. Proc. R. Soc. Lond. B 205, 475-488.

Maynard Smith, J. & Harper, D.G.C. 1988. The evolution of aggression: can selection generate variability? Phil. Trans. R. Soc. Ser. 319, 557-570.

Mcgraw, K.J. & Hill, G.E. 2000a. Carotenoid-based ornamentation and status signalling in the house finch. Behav. Ecol. 11, 520-527.

Mcgraw, K.J. & Hill, G.E. 2000b. Plumage brightness and breeding-season dominance in the house finch: A negatively correlated handicap? Condor 2, 456-461.

Norris, K. 1993. Heritable variation in a plumage indicator of viability in male great tits Parus major Nature 362, 537-539.

Olson, V.A. & Owens, I.P.F. 1998. Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol. Evol. 13, 510-514.

Parker, G.A. 1974. Assessment and the evolution of fighting behaviour. J. Theor. Biol. 47, 223-243.

Pryke, S.R., Lawes, M.J. & Andersson, S. 2001. Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the colour signal of both the territory owner and model intruder. Anim. Behav. 62, 695-704.

Richner, H., Schneiter, P. & Stirnimann, H. 1989. Life-history consequences of growth rate depression: an experimental study on carrion crows (Corvus *corone corone L.*) Funct. Ecol. 3, 617-624.

Rohwer, S. 1975. The social significance of avian winter plumage variability. Evolution 29, 593-610.

Rohwer, S.A. 1982. The evolution of reliable and unreliable badges of fighting ability. Am. Zool. 22, 531-546.

Saks, L., Ots, I. &Horak, P. 2003. Carotenoid-based plumage coloration of male greenfinches reflect health and immunocompetence. Oecologia 134, 301-307.

Svensson, L. 1992. Identification guide to European passerines. 4th edn. - Private publ. Stockholm.

Veiga, J.P. & Puerta, M. 1996. Nutritional constraints determine the expression of a sexual trait in the house sparrow. Proc. R. Soc. Lond. B 263, 229-234.

Velando, A. 2000. The importance of hatching date for dominance in young shags. Anim. Behav. 60, 181-185.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999. Good-genes, oxidative stress and condition-dependent sexual signals. Proc. R. Soc. London Series B 266, 1-12.

Wilson, J.D. 1992. A re-assessment of the significance of status signalling in populations of wild great tits, *Parus major* Anim. Behav. 43, 999-1009.

Wolfenbarger, L.L. 1999. Is red coloration of male northern cardinals beneficial during the nonbreeding season? : a test of status signalling. Condor 101, 655-663.

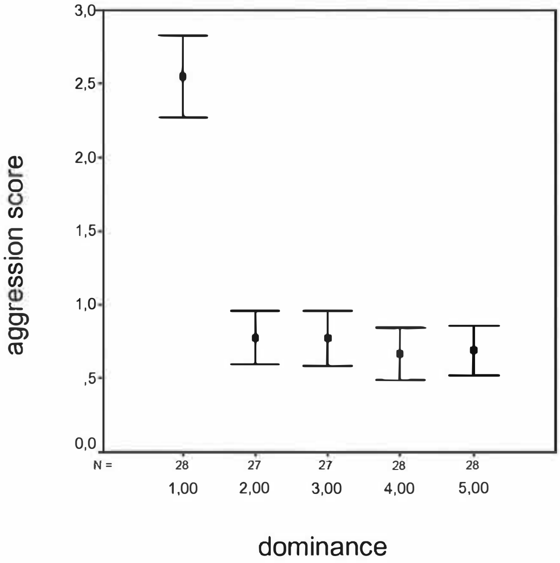

FIGURE 1 Relation between the aggression score and dominance as measured in access for food (Bars represent standard error of mean).

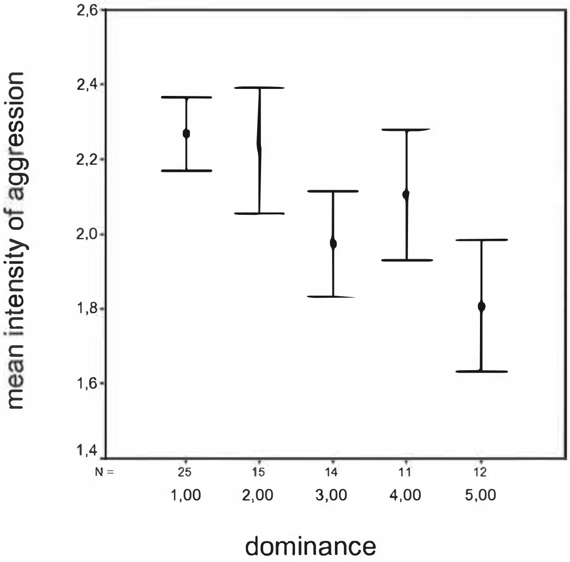

FIGURE 2 Relation between the intensity of aggression and dominance as measured in access for food (Bars represent standard error of mean).

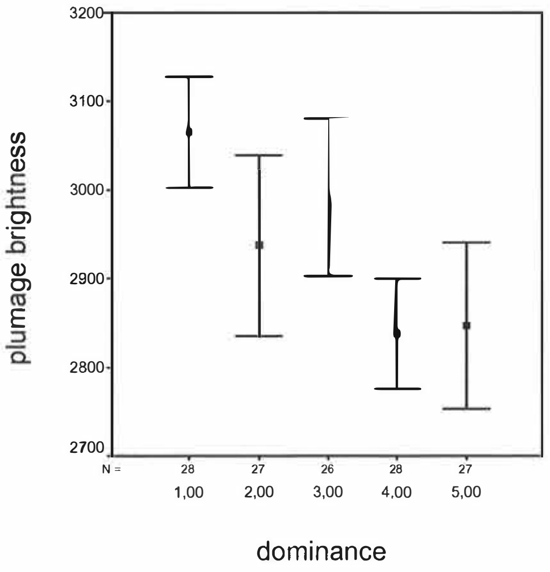

FIGURE 3 Relation between the brightness of the yellow breast feathers and dominance rank as measured in access for food (Bars represent standard error of mean).

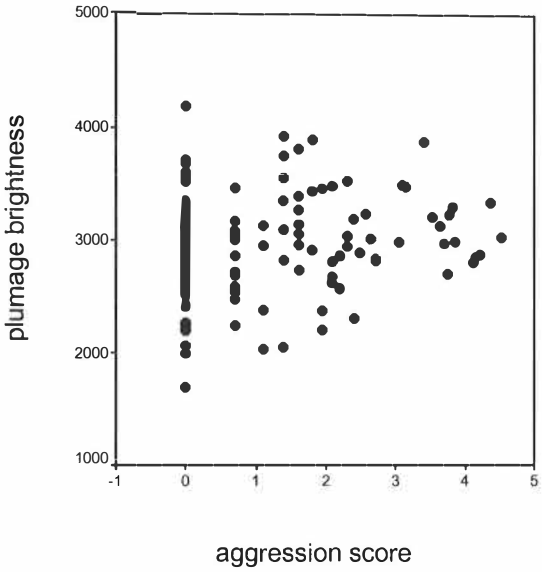

FIGURE 4 Relation between the aggression score and the brightness of the yellow breast feathers.

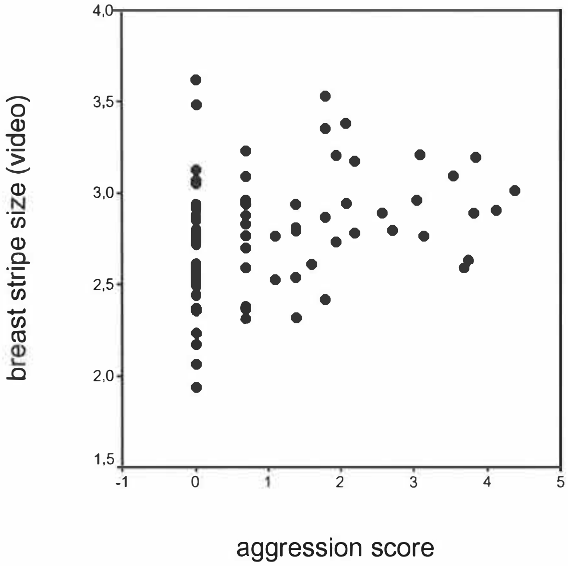

FIGURE 5 Relation between the aggression score and the breast stripe area measured from video during the dominance trials.

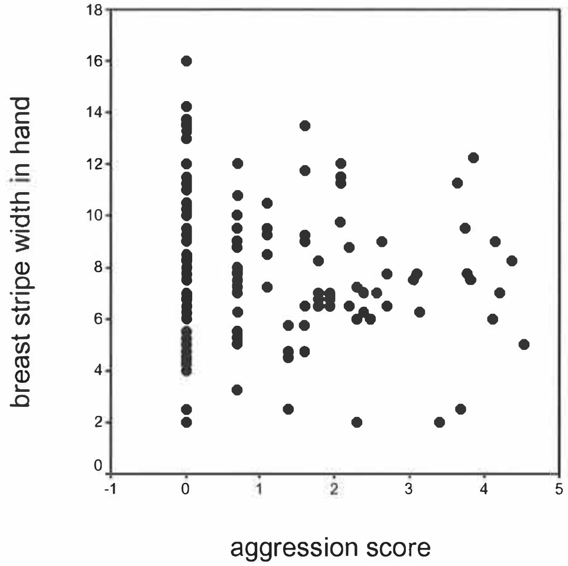

FIGURE 6 Relation between the breast stripe width measured in hand and the aggression score.

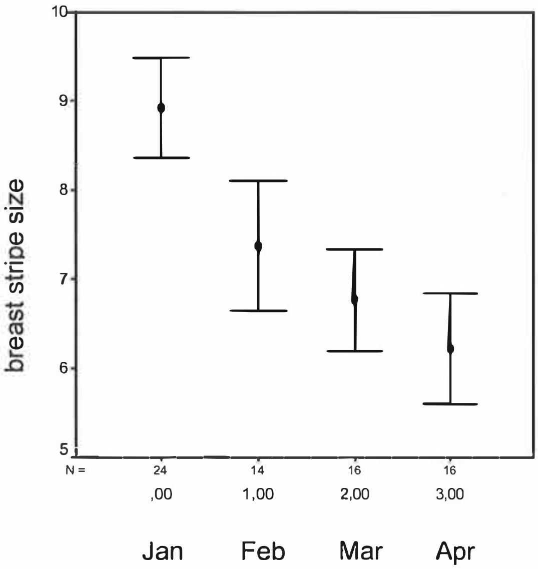

FIGURE 7 Relation between the breast stripe width measured in hand and survival (Bars represent standard error of mean).