

Pheasant sexual ornaments reflect nutritional conditions during early growth

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Differences in growth conditions during early life have been suggested to cause long-lasting effects on morphology and quality of adult birds. We experimentally investigated the effect of early growth conditions on the expression of sexual ornaments later in life in male ring-necked pheasants (*Phasianus colchicus*). We also investigated the effects on immune function, as it could be a functional link between early nutrition and ornament expression. We manipulated the dietary protein intake during the first eight weeks post hatching. Males receiving fodder with 27% protein during the first three weeks of life grew larger and more colourful wattles when sexually mature than males receiving a low-protein diet (20.5% protein). Spur length was unaffected by diet treatment. Manipulation of food protein levels during weeks 4–8 after hatching had no effect on the development of ornaments. The different protein treatments had no long-term effect on either humoral or cell-mediated immune responses. There was, however, a positive relationship between spur length and cell-mediated immune responsiveness. Our study shows that expression of a sexual ornament in adult pheasants reflects nutritional conditions early in life. Because the expression of secondary sexual ornaments is affected by conditions during early growth, by selecting more ornamented males, females would choose mates that are superior at handling early nutritional stress. If the susceptibility to early nutritional stress also has a hereditary basis, females may benefit by obtaining ‘good genes’.

Keywords: early nutrition; immunocompetence; male ornaments; enzyme-linked immunosorbent assay; delayed-type hypersensitivity; *Phasianus colchicus*

1. INTRODUCTION

Some ‘good genes’ models of sexual selection propose that females assess male genetic quality through the expression of secondary sexual characters (Andersson 1994). These models assume that there is a link between genetic quality and expression of ornaments, such that genetic quality modifies environmental effects on condition, with males in a higher condition developing larger ornaments (David *et al.* 2000). Genetic quality may, for instance, be expressed as the ability to find or process food (David *et al.* 2000), to fight diseases or parasites (Hamilton & Zuk 1982; Westneat & Birkhead 1998) or to deal with oxidative stress (von Schantz *et al.* 1999).

The early stages of somatic growth and development constitute a critical period, with high energetic demands. A poor ability to handle nutritional stress during this period may result in retarded growth and smaller size, or even death (e.g. Boag 1987; Richner 1989; Dahlgren 1990; De Kogel & Prijs 1996; Lindström 1999). It has recently been suggested that there may be links between early development and later expression of ornaments. Nowicki *et al.* (1998) argued that critical phases exist during early development in songbirds, when nutritional stress may affect the development of song-related brain nuclei. As a consequence, the song-repertoire size of adult songbirds may reflect nutritional condition during early development. This hypothesis is supported by a recent study on great

reed warblers, *Acrocephalus arundinaceus* (Nowicki *et al.* 2000), a species in which song-repertoire size is a sexual ornament (Hasselquist *et al.* 1996). We suggest that early nutrition could also affect other kinds of sexual ornaments in a similar way. Large ornaments may signal that males have received good paternal care early in life (altricial species), or that the parents brought newly hatched young to food-rich areas (precocial species). However, large male sexual ornaments may also signal a high ability to handle nutritional stress early in life (altricial and precocial species). By selecting a male that was able to better handle early nutritional stress, females may obtain direct benefits or, assuming that there is genetic variation in the ability to cope with nutritional stress early in life, ‘good genes’.

The expression of ornaments may be dependent on the bearer’s ability to defend itself against pathogens (Folstad & Karter 1992; von Schantz *et al.* 1996; Westneat & Birkhead 1998). For example, red pigmentation has been shown to relate to infestation of parasites (Milinski & Bakker 1990; Brawner *et al.* 2000). According to this theory, the bearer of a high-quality ornament signals a high resistance to parasites and diseases. Food quality during the first weeks post hatching is likely to be important for the development of the immune system, because during this period there is a rapid expansion of leucocyte populations and seeding of lymphoid organs (Klasing 1998). A deficiency (or an extreme excess) of nutrients during this critical period may affect immunity later in life (Klasing 1998). Thus, we suggest that immune function is a potential functional link between early nutrition and a later ability to develop ornaments.

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The ring-necked pheasant is a highly sexually dimorphic species, with males having an array of presumably sexually selected traits like body size, elongated tail feathers, spurs, ear tufts and wattles. Female pheasants are smaller and have a dull, cryptic plumage. The social mating system in pheasants is harem polygyny, and males use sexual ornaments when attracting females (e.g. von Schantz *et al.* 1989; Mateos & Carranza 1995; Mateos 1998). The exact function of the different ornaments in intra- and intersexual contexts is controversial. Males with long spurs have been shown to be more attractive to females (von Schantz *et al.* 1989; Göransson *et al.* 1990; Grahn & von Schantz 1994; but see also Hillgarth (1990)). Also, characteristics of the wattle, ear tufts and tail have been found to be important for female choice in this species (Mateos & Carranza 1995). Male pheasants exhibit conspicuous red wattles that are enlarged during sexual displays and females prefer males with brighter wattles (Hillgarth & Wingfield 1997). The redness of combs and wattles in other birds has been shown to be attractive to females (Zuk *et al.* 1990; Rintamäki *et al.* 2000). The red colour of the pheasant's wattle is caused by storage of the carotenoid pigment astaxanthin (Lucas & Stettenheim 1972). Carotenoid pigmentation can be used as a signal of individual condition in birds (e.g. Hill & Montgomerie 1994; Olson & Owens 1998). These pigments cannot be synthesized by vertebrates and must therefore be ingested (e.g. Brush 1978). Theory predicts that only individuals in good condition can afford a full expression of such ornaments and carotenoid-based pigmentation is inhibited by infestation of parasites, especially coccidia (reviewed by Hill (1999)). Thus, carotenoid pigmentation of sexual ornaments may function as a reliable signal of individual condition.

This paper investigates whether ornaments (spur length and wattle colour and size) reflect nutritional stress during early development by raising male pheasants under different food regimens. Moreover, we test whether nutritional effects on immune function can be a mechanism that links the development of sexual ornaments to early nutrition by quantifying both humoral and cell-mediated immune responsiveness.

2. METHODS

(a) *Experimental set-up*

In an experiment on caged pheasants, chicks were provided with either low- or high-quality fodder during the first eight weeks of life. When the birds were 20 and 40 weeks old, we investigated the long-term effects of the protein treatments on male ornaments and immune responsiveness.

Nutrition was manipulated by varying protein content in the food during early ontogeny. Chicks received two different kinds of food: either a commercial turkey starter containing 27% protein (high-protein treatment: Kalkon start, Skånska Lantmännen, Malmö, Sweden) or a commercial chicken starter containing 20.5% protein (low-protein treatment: Fenix start, Skånska lantmännen). The chicken starter contained a little more energy than the turkey starter (12.3 MJ kg⁻¹ and 11.8 MJ kg⁻¹, respectively), but was otherwise similar in nutritive value. We chose to use the chicken starter as our low-protein-treatment fodder, because mortality among newly hatched pheasant chicks increases when they are given food with

less than 20% protein (Woodard *et al.* 1977), and we used the turkey starter as our high-protein-treatment fodder because we wanted to use a fodder that met the known protein requirements of ring-necked pheasant chicks (Woodard *et al.* 1977; Warner *et al.* 1982).

To distinguish the importance of food quality during different phases of early ontogeny, the experiment was designed as a 2 × 2 factorial, with randomly selected chicks obtaining high- and low-protein-content fodder during three weeks 0–3 (early treatment) and weeks 4–8 (late treatment). During weeks 9–40, all chicks were fed the same fodder containing 16% protein (Allfoder.veg; Skånska Lantmännen, Malmö, Sweden).

(b) *Housing of the pheasants*

On the day of hatching, the pheasants were divided into two groups of equal size by random match (weight) and kept indoors in two adjacent 3.5 m × 3.5 m rooms. Because of a lack of sexual dimorphism at hatching, pheasants could not be sexed initially. Therefore, initially the high-protein treatment consisted of 25 males and the low-protein treatment consisted of 29 males. Only a thin wooden wall separated the rooms, which had identical light and temperature conditions. After three weeks, we randomly selected every second chick in each treatment for the high-protein treatment and the rest for the low-protein treatment during weeks 4–8. During this period, the high-protein treatment consisted of 23 males and the low-protein treatment of 26 males. The pheasants were finally sexed at the ages of 4–7 weeks. When the birds were eight weeks old, they were transferred to an outdoor aviary, which was divided into two similar sections. The size of each aviary section was 40 m² and each contained a house in which the birds could avoid any inclement weather. To avoid competition between chicks, both water and food were available *ad libitum*, provided at several feeders in each compartment, throughout the entire study (from hatching until 40 weeks of age).

(c) *Measurements of morphology, ornament size and colour*

Pheasants were obtained from a local breeder at Höör, southern Sweden. The study animals came from eggs of wild-caught pheasants from a semi-natural population. All eggs were weighed on the day they were laid and then individually hatched in a standard incubator in early June 1998.

On the day of hatching (day 0), all chicks were weighed (to the nearest 0.1 g) when they had dried, on a dynamic digital balance (Mettler Toledo AB, Stockholm, Sweden). When 20 and 40 weeks old, the thickness of their tarsi and length of their spurs were measured with digital callipers to the nearest 0.01 mm. Spur length was measured from the tip to the distal edge of the tarsus. From this measurement we then subtracted the thickness of the tarsus to obtain a measure of the actual length of the spur. We also measured the size and colour of their wattles. The wattles were photographed with a digital camera (Fuji DX-7; Fuji Photo Film Co. Ltd, Tokyo, Japan). Included on each photo was a ruler to facilitate calibration. The size (height) of the wattles was measured to the nearest 0.1 mm using a graphical software program (ADOBE PHOTOSHOP 5.5 (Adobe Systems Inc. 1998)). In some cases the head of the bird was not placed exactly horizontal and therefore could not be measured accurately; in such cases no measurements were taken. The area and the height of the wattle are highly correlated in male pheasants (Briganti *et al.* 1999; T. Ohlsson, personal

observation). Thus, we have used the height (vertical axis) of the wattle as the measure of size.

The colour of the wattles was measured using a colorimeter (Colortron II, Light Source Inc., San Rafael, CA, USA; Light Source Inc. 1994). At 20 weeks of age three measurements were taken on each wattle, in total six measurements on each pheasant. At 40 weeks of age only two measurements of each wattle were made. For each pheasant we excluded the two extreme measurements and then used the Colortron software to calculate mean values of hue, saturation and brightness for the remaining measurements. The overall colour score for each pheasant was then calculated as the first principal component scores of the mean values of hue, saturation and brightness (Hill 1998). We always measured wattle colour at the same area, just above the eye.

(d) Measurements of immune responses

As a measure of humoral immune responsiveness we measured the antibody response to non-pathogenic antigens, human diphtheria–tetanus vaccine. All birds were immunized with 140 μ l diphtheria–tetanus vaccine (SBL, Stockholm, Sweden) intramuscularly in the pectoral muscle when they were about 20 weeks old. For practical reasons all measurements of immune functions were measured on the same occasion for all birds. Thus, 20 weeks is an average age, since the birds hatched over a two-week period in early June.

The diphtheria–tetanus vaccine contains two different protein antigens, diphtheria and tetanus toxoid; hence we obtained two measures of immune responsiveness. Blood samples (*ca.* 300 μ l, taken from the wing vein) were collected in tubes with heparine and stored on ice until centrifugation (3000 r.p.m. for 7 min) later the same day, after which plasma was extracted and stored at -20°C until later analysis.

Antibody titres were analysed using an enzyme-linked immunosorbent assay (ELISA), (see Svensson *et al.* 1998; Hasselquist *et al.* 1999, 2001). High-binding 96-well plates (Costar; Merck EuroLab Ltd, Stockholm, Sweden) were coated overnight with either diphtheria toxoid or tetanus toxoid (3 $\mu\text{g ml}^{-1}$ in 0.15 M carbonate buffer, pH 9.6). After three washings with phosphate-buffered saline (PBS)/Tween 20 (0.01 M PBS with 0.05% Tween 20), wells were blocked with 3% milk powder in PBS/Tween 20 for 2 h at room temperature. After two washings, dilutions of plasma (1 : 1000 for primary responses and 1 : 3000 for secondary responses, in PBS/Tween 20 with 1% milk powder) were added in duplicates. The plates were then incubated overnight at 4°C . The following day, plates were washed three times and thereafter incubated for 1 h at 37°C with a peroxidase-conjugated rabbit-anti-chicken IgG antibody diluted 1 : 10 000 (Cat. A 9792; Sigma-Aldrich, Sweden AB, Stockholm, Sweden). Finally, plates were washed twice and a colour reaction was achieved by the addition of ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) and H_2O_2 in citrate buffer (200 μ l of 0.2 mM ABTS (Cat. A1888, Sigma) and 80 μ l of 30% H_2O_2 (diluted 1 : 50 in double-distilled water), in 20 ml citrate buffer (pH 4.0)). The kinetics of colour reactions was measured by reading plates every 30 s for 14 min using a V_{max} MAXline microplate reader (Molecular Devices, Sunnyvale, CA, USA). Antibody titres are obtained as the slope of the substrate conversion over time in the unit 10^{-3} Optical Density per minute (mOD min^{-1}).

To compare antibody (Ab) titres of samples run on different plates, we ran a serially diluted standard (pooled serum from pheasants with very high antibody titres to diphtheria–tetanus)

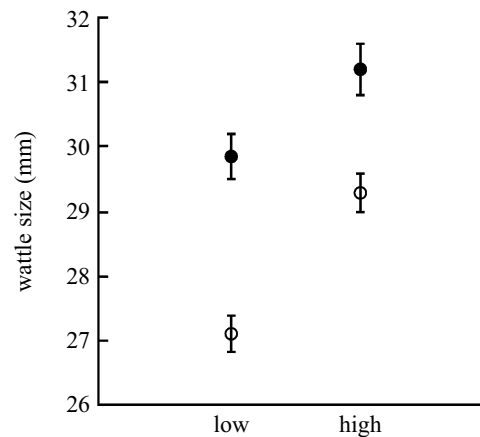


Figure 1. Male pheasant wattle size (mean \pm s.e.) at 20 (open circles) and 40 (filled circles) weeks of age in relation to feeding high- or low-protein fodder during the first three weeks following hatching.

on all plates. This made it possible to recalculate OD values of individual samples to a common 'Ab-titre unit'.

As a second measure of immunocompetence, the delayed-type hypersensitivity (DTH) test was used with phytohaemagglutinin (PHA) as antigen to measure *in vivo* T-cell-mediated immune responsiveness (e.g. Cheng & Lamont 1988). We injected 1 mg of PHA (Cat. L-8754, Sigma), dissolved in 0.1 ml of sterile PBS intradermally in the wing web (patagium). Following the recommendations of Smits *et al.* (1999), PBS was not injected and the swelling measured in the other wing as a control. We measured the thickness of the patagium in the right wing to the nearest 0.01 mm with a micrometer immediately before and 24 h after injection. Measurements were taken as follows: one person held the bird down and feathers were plucked or turned aside from the swelled area, and the micrometer was tightened just until the skin began twisting. The mean of three measurements was used. All measurements were taken blind to experimental treatment by the same person (L.R.). In all analyses, we calculated statistics with the difference in thickness caused by the PHA injection (after – before).

Repeated measures of wattle colour, wattle size and spur length were analysed using restricted maximum-likelihood estimation (REML) of mixed models (Littell *et al.* 1996). When selecting variance–covariance structures we followed the recommendations for repeated measures analyses by Littell *et al.* (1996). For wattle size and spur length, unstructured variance–covariance structures were used; for wattle colour, a compound-symmetrical variance–covariance structure was used.

Statistics were performed using SYSTAT (Wilkinson 1987) and SAS (Littell *et al.* 1996). We always tested for interactions, but excluded them unless significant. All probability distributions are two-tailed.

3. RESULTS

(a) Early protein intake and ornament quality

At the age of 20 weeks, males that received the high protein treatment during the first three weeks of life (early treatment) had wattles that were *ca.* 2 mm larger than males in the low-protein treatment (29.3 ± 1.4 and 27.1 ± 0.93 , respectively \pm s.d., ANOVA with treatments as factors, the effect of early treatment: $F_{1,43} = 26.7$, $p < 0.0001$; the effect of late treatment: $F_{1,43} = 0.39$, $p = 0.54$; figure 1). The difference in wattle size between

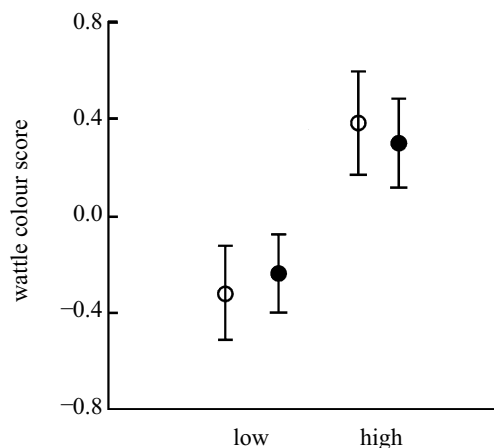


Figure 2. Male pheasant wattle colour score (mean \pm s.e.) at 20 (open circles) and 40 (filled circles) weeks of age, in relation to feeding high- or low-protein fodder during the first 3 weeks following hatching. Colour scores were calculated as the first score from a PCA including the hue, saturation and brightness values.

males on high- and low-protein treatments was still significant when males were 40 weeks old, because the average wattle size in the high-protein-food group was 31.2 ± 1.3 mm, compared with 29.8 ± 1.2 mm in the low-protein group (ANOVA as above, the effect of early treatment: $F_{1,22} = 6.81$, $p = 0.016$; the effect of late treatment: $F_{1,22} = 0.02$, $p = 0.90$; figure 1). This difference was highly significant when analysed on the full dataset using the repeated measures mixed model (early and late treatment as factors, the effect of early treatment: $F_{1,35.2} = 24.87$, $p < 0.0001$). The late treatment, weeks 4–8, had no effect on wattle size ($F_{1,37.1} = 0.46$, $p = 0.50$). Because treatment affects the size of pheasants (Ohlsson & Smith 2001), the effect of treatment on wattle size could be caused by pheasants becoming generally larger. However, the effect of the early treatment on wattle size was significant also when including tarsus length as a covariate (early treatment as factor and tarsus length as covariate, the effect of early treatment $F_{1,40.2} = 18.53$, $p = 0.0001$).

When analysing the effect of treatments on wattle colour, we used the scores from the principal component analysis (PCA) of the values of hue, saturation and brightness. The effect of early treatment (weeks 0–3) was significant both at 20 and at 40 weeks of age (ANOVA with treatments as factors, at 20 weeks: $F_{1,41} = 6.01$, $p = 0.018$; at 40 weeks: $F_{1,36} = 5.07$, $p = 0.030$; figure 2), whereas there was no effect of the late treatment (weeks 4–8) (20 weeks: $F_{1,41} = 0.19$, $p = 0.66$; 40 weeks: $F_{1,36} = 0.13$, $p = 0.72$). We also analysed the full dataset using REML estimation of mixed models for repeated measures, which showed that pheasants that received the high-quality fodder during the early treatment had ‘redder’ wattles when adult (the effect of early treatment: $F_{1,37.3} = 7.53$, $p = 0.0093$; the effect of late treatment: $F_{1,37.1} = 0.08$, $p = 0.77$). The effect of treatment on wattle colour was significant also when including tarsus length as a covariate (early treatment as factor and tarsus length as covariate, the effect of early treatment: $F_{1,37.2} = 4.18$, $p = 0.048$). The wattle colour score (PCA with hue, saturation and brightness) was highly correlated with the value of satu-

ration ($r = 0.87$) and hue ($r = -0.76$), and less so with the value of brightness ($r = 0.27$). For both saturation and hue a higher principal component meant more intense red.

There was no significant difference in spur length between food treatments. Neither the early nor the late treatments had any effect either at 20 or 40 weeks of age (20 weeks of age, high: 6.62 ± 1.2 mm; low: 6.47 ± 1.2 mm; ANOVA with treatments as factors, the effect of early treatment: $F_{1,45} = 0.17$, $p = 0.68$; the effect of late treatment: $F_{1,45} = 0.16$, $p = 0.69$; 40 weeks of age, high: 8.24 ± 1.4 mm; low: 8.56 ± 1.5 mm; ANOVA as above, the effect of early treatment: $F_{1,37} = 0.48$, $p = 0.49$; the effect of late treatment: $F_{1,37} = 0.62$, $p = 0.44$). The results were the same when we analysed the complete dataset using the repeated-measures mixed model with treatments as factors (the effect of early treatment: $F_{1,44.3} = 1.88$, $p = 0.18$; the effect of late treatment: $F_{1,44.2} = 0.03$, $p = 0.86$).

(b) *Early protein intake and immune function*

Immune responses were measured when the birds were 20 weeks old. Hence, in all analyses of immune function, the week 20 values of ornament sizes have been used. The responses to the diphtheria and tetanus injections were congruent. Therefore, we used PCA to create a single score of the primary and secondary antibody responses to the two antigens. This score has been used in all analyses of humoral immunity.

There was no trend regarding early protein intake and any of the measured immune responses. Neither cell-mediated (to PHA injection) nor humoral (antibody responses to diphtheria–tetanus) immune responses were affected by the experimental treatments (ANOVA with the effect on cell-mediated immunity as the dependent variable and early and late treatments as factors, the effect of early treatment: $F_{1,43} = 0.18$, $p = 0.67$; the effect of late treatment: $F_{1,43} = 0.02$, $p = 0.88$; ANOVA as above with the effect on humoral immunity as the dependent variable, the effect of early treatment: $F_{2,46} = 0.92$, $p = 0.34$; the effect of late treatment: $F_{2,46} = 0.16$, $p = 0.69$).

(c) *Ornament size/quality and immune functions*

Long-spurred males responded with a significantly larger wing-web swelling than males with shorter spurs (Pearson correlation: $r = 0.46$, d.f. = 43, $p = 0.0045$; figure 3). There was no correlation between the magnitude of the wing-web swelling and either wattle size (Pearson correlation: $r = 0.22$, d.f. = 40, $p = 0.15$) or wattle-colour saturation ($r = 0.17$, d.f. = 40, $p = 0.29$).

There was no correlation between antibody responsiveness and any measure of male ornaments (Pearson correlation: wattle size, $r = 0.14$, d.f. = 43, $p = 0.35$; wattle colour score, $r = 0.05$, d.f. = 41, $p = 0.74$; spur length, $r = 0.04$, d.f. = 45, $p = 0.81$).

4. DISCUSSION

Our experiment showed that food quality during early ontogeny had long-lasting effects on ornament expression. The wattles grew larger and the colour of the wattles became redder at the age of sexual maturity among birds receiving a high-quality food (higher protein content) during the first three weeks post hatching. By contrast, spur

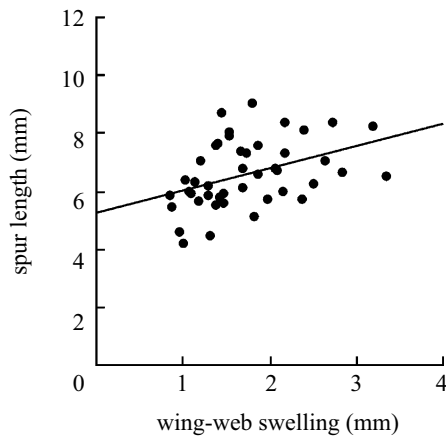


Figure 3. Male pheasant cell-mediated immunity measured as wing-web swelling, in response to an injection with PHA, in relation to spur length.

length, which has been shown to be a sexually selected trait associated with viability (von Schantz *et al.* 1989), was not affected by food quality early in life. Furthermore, neither humoral nor cell-mediated immune responsiveness was affected by food quality during early ontogeny. Even though our food-quality treatment was small (27% versus 20.5% protein), we found long-lasting effects on wattle ornament. Hence, even quite small differences in nutritional conditions during early development can have important effects on trait expression later in life. It is probable that a difference as large as this (and most probably much larger) exists under natural conditions between broods that feed in high-quality versus low-quality territories or between individuals in any given environment when, for example, age, experience, dominance or health affect food intake.

Because pheasants were housed in groups, this study includes an element of pseudoreplication. However, after the initial three-week treatment period, pheasants undergoing the two experimental treatments were randomly mixed. Thus, the differences between the experimental groups in this study are caused by factors differentiating the two groups during the first three weeks, i.e. effects of environmental influence on early ontogeny on the later development of sexual ornaments. Because of the effort made to make conditions for the two experimental groups physically similar (the only obvious difference was the diet provided to the two groups), we believe that the most plausible explanation is that the nutritional treatment caused the differences in ornament expression.

There is growing evidence of critical phases during early development when exposure to nutritional stress might have detrimental effects on future expression of ornaments. For example, Nowicki *et al.* (1998) discuss critical phases during brain development when sensitivity to nutritional stress is high and may lead to poorer song capacity in adult songbirds. In the present study, we found an effect on the expression of an ornament by a short and modest manipulation of food quality during early ontogeny. These data imply that the first three weeks post hatching constitute a critical period, when differences in food quality can have important effects on the expression of ornaments later in life. Some other studies, manipulating nutritional conditions during early ontogeny, have

shown long-lasting effects on various sexually selected traits. For example, Gustafsson *et al.* (1995) manipulated brood size in collared flycatchers (*Ficedula albicollis*) and found that male forehead patch size the following year was negatively related to the size of the brood in which they were raised. De Kogel & Puijs (1996) manipulated brood size in zebra finches (*Taeniopygia guttata*) and found that birds reared in small broods were not only larger, but also had redder beaks as adults. David *et al.* (2000) showed that male eyespan in stalk-eyed flies (*Cyrtodiopsis dalmanni*) was affected by food quality during development. Some fly genotypes developed large eyespan as adults in spite of low food quality during development, indicating genetic variation in stress patience. These studies suggest that females choosing a male with exaggerated ornaments may obtain a mate with a capability to withstand nutritional stress during critical periods of development. By this, females may either gain direct benefits or, if the susceptibility to handle early nutritional stress has a hereditary basis, 'good genes'.

Only the manipulation during the first three weeks had an effect, whereas there was no effect of the same manipulation during weeks 4–8. This may be because the early growth period is a more critical period. However, the effect of the two experiments might not be independent. Zuk & Johnsen (2000) demonstrated that social dominance affected ornament expression in red jungle fowl. If social dominance in pheasant chicks is affected by their size, the early treatment might cause dominance hierarchies that are not reversed by the later treatment, because of a social inertia of dominance (cf. Piper 1997).

We found that the red pigmentation of the males' wattles was more saturated in the high-protein group. Nutritional conditions, either as measured as a factor of quality or quantity, have been reported to influence the expression of carotenoid pigmentation (Hill 2000). In addition, red pigmentation has been suggested to be particularly costly to produce (Hill 1996). Thus, the brightness of carotenoid coloration may act as a reliable signal of quality (e.g. Hill & Montgomerie 1994; De Kogel & Puijs 1996; Hill 2000). In accordance with this, in several species and taxa, it has been shown that females prefer to mate with males that have redder ornaments, e.g. plumage (house finch, *Carpodacus mexicanus* (Hill 1990), combs (jungle fowl, *Gallus gallus* (Zuk *et al.* 1990)), bills (zebra finch (Burley & Coopersmith 1987)) or red skin coloration (sticklebacks, *Gasterosteus aculeatus* (Milinski & Bakker 1990)). The importance of wattle colour variation for female choice in pheasants remains to be investigated.

We found no effect of the nutritional treatment on the growth of the spurs. A possible explanation for this might be that spur length, as opposed to wattle size, had not reached its maximum at the time when the measurements ended. Alternatively, spurs are affected by both growth and wear so that any effect of early nutritional condition is masked. In this context it is interesting to note that spur length had no effect on female choice and did not reflect viability in 1-year-old pheasants (Grahn & von Schantz 1994), in contrast to the pattern found in males more than 1 year old (von Schantz *et al.* 1989, 1994). In species with multiple ornaments, different ornaments may reflect different aspects of quality (Møller & Pomiankowski 1993; Wedekind 1994). Females may therefore use the accumu-

lated information they receive from different ornaments when choosing a partner. In the pheasant, it is possible that wattle size and colour may reflect factors affected by conditions during early development, whereas spur length may reflect factors affecting male condition later in life.

In our study, nutritional levels during early ontogeny did not affect the quality of either humoral or cell-mediated immunity at the age of 20 weeks. This was surprising, given the well-documented effect of nutrition on immune function (Sapolsky 1992; Klasing 1998). A possible explanation for the lack of effect of the experiment on immunity may be that the tests of immune responsiveness only reveal the immediate nutritional status, and that effects of protein conditions early in life had no effect on immunity 12–20 weeks later. Another explanation is that our rather modest manipulation of protein levels was not severe enough to affect the development of the immune system. Klasing (1998) states that the nutritional costs of maintaining an immune system are very low compared with the costs of, for example, growth. However, northern bobwhite (*Colinus virginianus*) chicks receiving a low level of protein in the diet during weeks 4–10 post hatching had a compromised cell-mediated immune response compared with chicks receiving higher levels of protein (Lochmiller *et al.* 1993). In that study, however, the low-protein-treatment group received as little as 8% protein, whereas in our study the low-treatment group received 20.5% protein. Vitamins and minerals may be more important for the immune system than deficiencies in fat, carbohydrates or proteins. For example, vitamin A deficiency during embryonic growth results in impaired immunity and decreased resistance to several diseases in chickens (Sklan *et al.* 1994).

The capacity of the immune system may be a functional link between early nutrition and the ability to develop large ornaments later in life. According to this hypothesis, both secondary sexual ornaments and immune function should be sensitive to early nutrition, and there should be a positive correlation between ornamentation and immune function. The only support for this from our study was the correlation between DTH response and spur length, but neither was affected by early nutrition. On the contrary wattle colour and size, which were affected by early nutrition, were not related to immune function. The link between early nutrition and expression of these ornaments thus demands further study.

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REFERENCES

- Adobe Systems Inc. 1998 *ADOBE PHOTOSHOP 5.5*. San José, CA: Adobe Systems Inc.
- Andersson, M. 1994 *Sexual selection*. Princeton University Press.
- Boag, P. T. 1987 Effects of nestling diet on growth and adult size of zebra finches (*Poephila guttata*). *Auk* **104**, 155–166.
- Brawnner III, W. R., Hill, G. E. & Sundermann, C. A. 2000 Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *Auk* **117**, 952–963.
- Briganti, F., Papeschi, A., Mugnai, T. & Dessì-Fulgheri, F. 1999 Effect of testosterone on male traits and behaviour in juvenile pheasants. *Ethol. Ecol. Evol.* **11**, 171–178.
- Brush, A. H. 1978 Avian pigmentation. In *Chemical zoology*, vol. 10. *Aves* (ed. A. H. Brush), pp. 141–164. New York: Academic Press.
- Burley, N. & Coopersmith, C. B. 1987 Bill color preferences of zebra finches. *Ethology* **76**, 133–151.
- Cheng, S. & Lamont, S. J. 1988 Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poultry Sci.* **67**, 989–995.
- Dahlgren, J. 1990 The significance of arthropods in the Grey Partridge diet. In *Proc. Perdix V Symp. Mankato, Minnesota, USA* (ed. E. Church, R. E. Warner & S. J. Brady), pp. 201–214. Pratt, KS: Kansas Department of Wildlife and Parks.
- David, P., Bjorksten, T., Fowler, K. & Pomiankowski, A. 2000 Condition-dependent signalling of genetic variation in stalk-eyed flies. *Nature* **406**, 186–188.
- De Kogel, C. H. & Pijrs, H. J. 1996 Effects of brood size manipulations on sexual attractiveness of offspring in the Zebra Finch. *Anim. Behav.* **51**, 699–708.
- Folstad, I. & Karter, A. J. 1992 Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* **139**, 603–622.
- Göransson, G., von Schantz, T., Fröberg, I., Helgée, A. & Wittzell, H. 1990 Male characteristics, viability and harem size in the pheasant, *Phasianus colchicus*. *Anim. Behav.* **40**, 89–104.
- Grahn, M. & Von Schantz, T. 1994 Fashion and age in pheasants: age differences in mate choice. *Proc. R. Soc. Lond. B* **255**, 237–241.
- Gustafsson, L., Qvarnström, A. & Sheldon, B. C. 1995 Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. *Nature* **375**, 311–313.
- Hamilton, W. D. & Zuk, M. 1982 Heritable true fitness and bright birds: a role for parasites. *Science* **218**, 384–387.
- Hasselquist, D., Bensch, S. & Von Schantz, T. 1996 Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature* **381**, 229–232.
- Hasselquist, D., Marsh, J. A., Sherman, P. W. & Wingfield, J. C. 1999 Is avian immunocompetence suppressed by testosterone? *Behav. Ecol. Sociobiol.* **45**, 167–175.
- Hasselquist, D., Wasson, M. F. & Winkler, D. W. 2001 Humoral immunocompetence correlates with date of egg-laying and reflects work load in female tree swallows. *Behav. Ecol.* **12**, 93–97.
- Hill, G. E. 1990 Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Anim. Behav.* **40**, 563–572.
- Hill, G. E. 1996 Redness as a measure of the production cost of ornamental coloration. *Ethol. Ecol. Evol.* **8**, 157–175.
- Hill, G. E. 1998 An easy, inexpensive means to quantify plumage coloration. *J. Field Ornithol.* **69**, 353–363.
- Hill, G. E. 1999 Is there an immunological cost to carotenoid-based ornamental coloration? *Am. Nat.* **154**, 589–595.
- Hill, G. E. 2000 Energetic constraints on expression of carotenoid-based plumage coloration. *J. Avian Biol.* **31**, 559–566.
- Hill, G. E. & Montgomerie, R. 1994 Plumage colour signals nutritional condition in the house finch. *Proc. R. Soc. Lond. B* **258**, 47–52.
- Hillgarth, N. 1990 Pheasant spurs out of fashion. *Nature* **345**, 119–120.
- Hillgarth, N. & Wingfield, J. C. 1997 Parasite-mediated sexual selection: endocrine aspects. In *Host-parasite evolution*.

- General principles and avian models (ed. D. H. Clayton & J. Moore), pp. 78–104. Oxford University Press.
- Klasing, K. C. 1998 Nutritional modulation of resistance to infectious diseases. *Poultry Sci.* **77**, 1119–1125.
- Light Source Inc. 1994 *Colortron user manual*. San Rafael, CA: Light Source Inc.
- Lindström, J. 1999 Early development and fitness in birds and mammals. *Trends Ecol. Evol.* **14**, 343–348.
- Littell, R. C., Milliken, G. A., Stroup, W. W. & Wolfinger, R. D. 1996 *SAS system for mixed models*. Cary, NC: SAS Institute.
- Lochmiller, R. L., Vestey, M. R. & Boren, J. C. 1993 Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk* **110**, 503–510.
- Lucas, A. M. & Stettenheim, P. R. 1972 *Avian anatomy: integument*. Part II. Washington DC: US Government Printing Office.
- Mateos, C. 1998 Sexual selection in the ring-necked pheasant: a review. *Ethol. Ecol. Evol.* **10**, 313–332.
- Mateos, C. & Carranza, J. 1995 Female choice for morphological features of male ring-necked pheasants. *Anim. Behav.* **49**, 737–748.
- Milinski, M. & Bakker, T. C. M. 1990 Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**, 330–333.
- Møller, A. P. & Pomiankowski, A. 1993 Why have birds got multiple sexual ornaments? *Behav. Ecol. Sociobiol.* **32**, 167–176.
- Nowicki, S., Peters, S. & Podos, J. 1998 Song learning, early nutrition and sexual selection in songbirds. *Am. Zool.* **38**, 179–190.
- Nowicki, S., Hasselquist, D., Bensch, S. & Peters, S. 2000 Nestling growth and song repertoire size in great reed warblers: evidence for song learning as an indicator mechanism in mate choice. *Proc. R. Soc. Lond. B* **267**, 2419–2424. (DOI 10.1098/rspb2000.1300.)
- Ohlsson, T. & Smith, H. G. 2001 Early nutrition causes persistent effects on pheasant morphology. *Phys. Biochem. Zool.* **74**, 212–218.
- Olson, V. A. & Owens, I. P. F. 1998 Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**, 510–514.
- Piper, W. H. 1997 Social dominance in birds: early findings and new horizons. In *Current ornithology*, vol. 14 (ed. V. Nolan Jr, E. D. Ketterson & C. F. Thompson), pp. 125–187. Plenum Press: New York.
- Richner, H. 1989 Habitat-specific growth and fitness in carrion crows (*Corvus corone corone*). *J. Anim. Ecol.* **58**, 427–440.
- Rintamäki, P. T., Höglund, J., Karvonen, E., Alatalo, R. V., Björklund, N., Lundberg, A., Rätti, O. & Vouti, J. 2000 Combs and sexual selection in black grouse (*Tetrao tetrix*). *Behav. Ecol.* **11**, 465–471.
- Sapolsky, R. M. 1992 Neuroendocrinology of the stress response. In *Behavioural endocrinology* (ed. J. B. Becker, S. M. Breedlove & D. Crews), pp. 287–324. Cambridge, MA: MIT Press.
- Sklan, D., Melamed, D. & Friedman, A. 1994 The effect of varying levels of dietary vitamin A on immune response in the chick. *Poultry Sci.* **73**, 843–847.
- Smits, J. E., Bortolotti, G. R. & Tella, J. L. 1999 Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol.* **13**, 567–572.
- Svensson, E., Råberg, L., Koch, C. & Hasselquist, D. 1998 Energetic stress, immunosuppression and the costs of an antibody response. *Funct. Ecol.* **12**, 912–919.
- von Schantz, T., Göransson, G., Andersson, G., Fröberg, I., Grahn, M., Helgée, A. & Wittzell, H. 1989 Female choice selects for a viability-based trait in pheasants. *Nature* **337**, 166–169.
- von Schantz, T., Grahn, M. & Göransson, G. 1994 Intersexual selection and reproductive success in the pheasant *Phasianus colchicus*. *Am. Nat.* **144**, 510–527.
- von Schantz, T., Wittzell, H., Göransson, G., Grahn, M. & Persson, K. 1996 MHC genotype and male ornamentation: genetic evidence for the Hamilton–Zuk model. *Proc. R. Soc. Lond. B* **263**, 265–271.
- von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999 Good genes, oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond. B* **266**, 1–12. (DOI 10.1098/rspb1999.0597.)
- Warner, R. E., Darda, D. M. & Baker, D. H. 1982 Effects of dietary protein level and environmental temperature stress on growth of young ring-necked pheasants. *Poultry Sci.* **61**, 673–676.
- Wedekind, C. 1994 Mate choice and maternal selection for specific parasite resistances before, during and after fertilizations. *Phil. Trans. R. Soc. Lond. B* **346**, 303–311.
- Westneat, D. F. & Birkhead, T. R. 1998 Alternative hypothesis linking the immune system and mate choice for good genes. *Proc. R. Soc. Lond. B* **265**, 1065–1073.
- Wilkinson, L. 1987 *SYSTAT. The system for statistics*. Evanston, IL: Systat Inc.
- Woodard, A. E., Vohra, P. & Snyder, R. L. 1977 Effect of protein levels in the diet on the growth of pheasants. *Poultry Sci.* **56**, 1492–1500.
- Zuk, M. & Johnsen, T. 2000 Social environment and immunity in male red jungle fowl. *Behav. Ecol.* **11**, 146–153.
- Zuk, M., Thornhill, R., Ligon, J. D., Johnson, K., Austad, S., Ligon, S. H., Thornhill, N. W. & Costin, C. 1990 The role of male ornaments and courtship behavior in female mate choice of red jungle fowl. *Am. Nat.* **136**, 459–473.

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