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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.
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 wattles, 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 comb 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 & Montgomery 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 (Woodard et al. 1977), and 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 x 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 Lantmännens, 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 x 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 PHOTO SHOP 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 measurement).
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 µl diphtheria–tetanus vaccine (SBL, Stockholm, Sweden) intramuscularly in the pectoral muscle when they were about 20 weeks old. For practical reasons all measures 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 reponsiveness. Blood samples (ca. 300 µl, taken from the vein wing) 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 µg ml⁻¹ in 0.15 M carbonate buffer, pH 9.6). After three washings with phosphate-buffered saline (PBS)/Twente 20 (0.01 M PBS with 0.05% Tween 20), wells were blocked with 3% milk powder in PBS/Twente 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/Twente 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 (G) 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 H₂O₂ in citrate buffer (200 µl of 0.2 mM ABTS (Cat. A1888, Sigma) and 80 µl of 30% H₂O₂ (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 Vmax 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) 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) 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 ± s.d.), ANOVA with treatments as factors, the effect of early treatment: F1,43 = 26.7, p < 0.0001; the effect of late treatment: F1,43 = 0.39, p = 0.54; figure 1). The difference in wattle size between
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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
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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 & Prijs (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 (Cyrtocephus 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 an exaggerated ornament 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 & Montgomery 1994; De Kogel & Prijs 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 (Moller & 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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