A label of health: a previous immune challenge is reflected in the expression of a female plumage trait

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Studies of ornaments as indicators of individual quality have hitherto focused on males, while studies of female ornaments have been almost absent. However, females within the same species may show large variation in both outer appearance and individual quality. We experimentally examined the effect of an immune challenge on the size of white fringes in the wing feathers produced one to two months after the challenge in female common eiders (*Somateria mollissima*), a long-lived sea duck. Immune-challenged females produced feathers with smaller white fringes, showing that a previous immune challenge may affect the expression of a plumage trait. We also report an unexpected difference in the expression of this white plumage trait within the immune-challenged group, related to the specific immune response against one of the injected antigens.

Keywords: immune challenge; diphtheria; tetanus; female ornament

1. INTRODUCTION

Individual variation in physical characteristics (e.g. colour intensity, size of ornaments) may be used by congeners to judge the quality, condition or status of the individual, which may be important when selecting partners or evaluating competitors (e.g. [Espmark et al. 2000](#)). In order for an ornament to be available for use as such a 'quality label', it is assumed that the individual variation in quality is linked to the individual variation in the expression of the ornamental character. The ornament may ensure honesty if it is costly to produce or carry (reviewed in [Andersson 1994](#)). Alternatively, the ornament may be an amplifier, i.e. it is a character that reveals individual quality without itself being costly to produce or carry (e.g. [Hasson 1989](#); [Fitzpatrick 1998](#)). White feather tips may, for instance, reveal overall feather quality, as the absence of melanin in white parts of the feather weakens it ([Burt 1986](#)). Thus, if the overall quality

of a feather is low, then the white tip will wear off faster revealing the lower feather quality ([Fitzpatrick 1998](#); [Kose & Møller 1999](#)). Experimental studies on wild animals linking aspects of individual quality to ornament variation are still scarce, but for captive birds two recent studies on males in zebra finches and blackbirds have shown that manipulating immune function leads to rapid changes in bill colour ([Blount et al. 2003](#); [Faivre et al. 2003](#)). In a previous observational study, we found that the size of the white fringes in the wing feathers of female common eiders correlated with previously experienced reproductive costs and immune function ([Hanssen et al. 2006](#)). These wing feathers are produced one to two months after breeding has ended. Female common eiders are cryptic brown with two white wing bars consisting of depigmented feather fringes (ranging in size from 0 to 6 mm; [figure 1](#); [Carney 1992](#)). Female eiders incubate their eggs and care for the young alone or in cooperation with other females ([Hanssen et al. 2004](#); [Hanssen 2006](#)). Reproductive costs are large in common eiders. Facultative anorexia during incubation and egg laying leads to a mass loss of approximately 40% ([Parker & Holm 1990](#)), and many females subsequently abandon their nest and ducklings ([Bustnes & Erikstad 1991](#)). Moreover, female eiders with experimentally enlarged incubation costs suffer reduced immune function and future reproductive success ([Hanssen et al. 2005](#)). In the present study, we experimentally manipulated incubation costs and immune function in breeding common eider females. In this way, we aimed at examining the potential roles of reproductive and immune costs in shaping the expression of a female plumage character.

2. MATERIAL AND METHODS

The study was conducted in a common eider breeding colony (approx. 400 pairs) on Grindøya, near Tromsø, northern Norway (69°49' N, 18°15' E) during the breeding seasons (May–July) in 2000–2002. Eider females were caught on their nest 5 days after clutch completion. They were randomly assigned to a combination of two treatments: (i) large (six eggs) or small (three eggs) clutch size (modulating incubation costs, natural range three to six eggs) and (ii) injection of diphtheria–tetanus vaccine or sterile saline (modulating immune system activation costs) in a 2×2 crossed factorial design. Sample sizes in the four combinations of treatments were as follows: small clutch/no immune challenge (S/C, $n=35$); small clutch/immune challenge (S/I, $n=26$); large clutch/no immune challenge (L/C, $n=27$); and large clutch/immune challenge (L/I, $n=34$). Because eiders moult their feathers during August–September, wing feathers were collected 1 year after the experiment from the females returning to breed. The experimental treatments did not affect the probability of being captured at the nest the following year (return rates: 51.4% returned (S/C), 46.1% returned (S/I), 44.4% returned (L/C) and 50% returned (L/I); experimental clutch size: $\chi^2_{1,121}=0.03$, $p=0.86$; immune challenge: $\chi^2_{1,121}=0.00$, $p=0.99$; experimental clutch×immune challenge: $\chi^2_{1,121}=0.35$, $p=0.55$). We were able to collect feathers from 52.5% of the returning birds; thus, the final sample sizes for the analysis of the white wing bars were $n=6$ (S/C), 7 (S/I), 10 (L/C) and 7 (L/I). No individual was included in both years. Statistical analyses were performed using the R v. 2.6.1 software package (<http://r-project.org>).

(a) Plumage

We collected the middle feather of the secondary remiges and greater secondary coverts from the left wing ([figure 1](#)). The size of the white fringe from the tip to the end of the white area on each feather was measured to the nearest 0.01 mm with a digital calliper. A digital spectrometer (Avantes) was used to measure the 'whiteness' of the light area on each feather. The spectrometer registered the following three aspects of the 'colour' of the white in the

Figure 1. Inner part of wings from females with (a) more and (b) less whiteness in their secondary coverts (cover wing feathers) and secondary remiges (flight feathers).

feathers: (i) the amount of light reflected (lightness); (ii) the hue (i.e. the wavelength of the colour), and (iii) the chroma (i.e. the density of pigmentation). In order to simplify the analyses, we entered all the variables in a principal component analysis. The first principal component (PC1) explained 49% of the variation, whereas PC2 explained 24%. Higher values of PC1 reflect more whiteness in the feather. The factor loadings for PC1 were negative for the chroma in both the inner and the outer feathers (-0.34 and -0.31 , respectively), and positive for the rest of the variables (0.44 and 0.11 for the hue in the inner and outer feathers, respectively; 0.25 and 0.37 for the lightness in the inner and outer feathers, respectively; and 0.46 and 0.37 for the inner and outer size of the white fringe, respectively). We therefore use PC1 as a measure of whiteness in eider feathers.

(b) Immune responses

In the immune-challenged group ($n=14$), we measured the specific antibody responses against the antigens diphtheria and tetanus toxoids, respectively (for a detailed description of ELISA method in eiders, see Hanssen *et al.* 2004). The females were injected with $150\ \mu\text{l}$ diphtheria–tetanus vaccine in the pectoral muscle (SBL Vaccin AB, Stockholm; diphtheria toxoid 38 Lf (flocculation entities) and tetanus toxoid 7.5 Lf mixed with the adjuvant aluminium phosphate $5\ \text{mg ml}^{-1}$). Out of the 14 inoculated individuals, six produced a detectable amount of antibodies against diphtheria toxoid and four against tetanus toxoid. We therefore classified individuals according to their response against the two antigens.

3. RESULTS

The whiteness of the wing bars was not significantly affected by increased incubation cost (low cost: mean whiteness $= 0.21 \pm 0.55$, $n=13$; large cost: mean whiteness $= -0.16 \pm 0.50$, $n=17$; see below for statistics). However, the immune challenge treatment induced a decrease in the whiteness of the wing bars (figures 1 and 2a; immune challenge: $F_{1,27}=4.41$, $p=0.045$; experimental incubation cost: $F_{1,27}=0.61$, $p=0.44$). The interaction term ($F_{1,26}=0.0002$, $p=0.99$) was removed from the final model. Moreover, within the group of females that were exposed to an immune challenge, we measured whether the females produced a specific antibody response against the two injected antigens (diphtheria and tetanus toxoids). The females that responded against diphtheria toxoid had the same amount of whiteness in the wing bars as control birds, whereas the antigen-challenged females that did not mount a specific antibody response against diphtheria toxoid developed significantly less white wing bars ($F_{1,12}=7.66$, $p=0.017$; figure 2b). There was no difference in the wing bar whiteness in the females responding or not against the second antigen, the tetanus toxoid ($F_{1,12}=0.27$, $p=0.61$). Year was included in all the initial models but was subsequently removed (all $p>0.23$).

Figure 2. (a) The effect of an experimental immune challenge (injection with diphtheria–tetanus vaccine versus sham-injected controls) on the expression of the white wing bars (PC1) in female common eiders. (b) Same as (a), but here the immune-challenged group is split into two, depending upon whether they responded against the immune challenge by production of specific antibodies against diphtheria toxoid (triangle) or not (circle).

4. DISCUSSION

The present study found that an immune challenge led to smaller white fringes in wing feathers in female common eiders, while experimentally increased mass loss (through clutch size manipulation) did not affect this trait. Investment of energy and resources into incubation and immune function are processes that may be traded off against each other (Sheldon & Verhulst 1996). Previous studies confirm that increased reproductive investment can lead to reduced immune function (e.g. Deerenberg *et al.* 1997; Hanssen *et al.* 2005); conversely, immune challenges lead to reduced feeding rates, increased mass loss or increased metabolic rates (e.g. Råberg *et al.* 2000; Ots *et al.* 2001; Hanssen 2006). In the present experiment, where we simultaneously manipulated mass loss and immune investment, it seems that immune function is the more important factor affecting the development of white wing plumage as only the immune challenge affected plumage whiteness.

We found that females that did not produce antibodies against diphtheria toxoid produced less conspicuous white wing bars than females that responded against the antigen. One explanation may be that mounting an acquired antibody response led to a downregulation of the possibly more costly innate responses, e.g. inflammation (Fearon & Locksley 1996; Medzhitov & Janeway 2000; Råberg *et al.* 2002). Thus,

the females that did not produce specific antibodies may have suffered increased costs from their innate immune defence, which may have led to the reduced expression of white wing bands. Alternatively, females able to mount the response against diphtheria toxoid may also be of better overall quality, when compared with the rest of the challenged group, and may thus be able to maintain white feather tips throughout the winter.

Ornamental display of quality in females is not expected to evolve by sexual selection, unless males become a limited resource for which females may compete (Emlen & Oring 1977). However, we suggest that the cost of male investment in mate guarding and courtship in some socially monogamous species may be underestimated. The information contained in the conspicuousness of the white wing bars of females may be important for males as they perform courtship and mate guarding for several months before breeding and male reproductive success is solely dependent on the fecundity of their female partner. Hence, information about the quality of females should be of importance for male fitness in this socially monogamous species. Also, females often cooperate during brood rearing (e.g. Bustnes & Erikstad 1991), and studies have indicated that females evaluate each others' status during this period (e.g. Ost et al. 2003), which may mean that ornaments related to quality are also important in female–female interactions. These possible adaptive roles of this female quality-revealing plumage character await further study.

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