

Female barn owls (*Tyto alba*) advertise good genes

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The good genes hypothesis of sexual selection postulates that ornamentation signals superior genetic quality to potential mates. Support for this hypothesis comes from studies on male ornamentation only, while it remains to be shown that female ornamentation may signal genetic quality as well. Female barn owls (*Tyto alba*) display more black spots on their plumage than males. The expression of this plumage trait has a genetic basis and it has been suggested that males prefer to mate with females displaying more black spots. Given the role of parasites in the evolution of sexually selected traits and of the immune system in parasite resistance, we hypothesize that the extent of female plumage 'spottiness' reflects immunological defence. We assessed the genetic variation in specific antibody production against a non-pathogenic antigen among cross-fostered nestlings and studied its covariation with the plumage spottiness of genetic parents. The magnitude of the antibody response was positively correlated with the plumage spottiness of the genetic mother but not of the genetic father. Our study thereby provides the first experimental support, to our knowledge, for the hypothesis that female ornamentation signals genetic quality.

Keywords: good genes; barn owls; sexual selection; ornamentation; genetic quality

1. INTRODUCTION

The evolution of sexually selected traits usually proceeds through male–male competition or female choice of the most ornamented males (Andersson 1994). This choice will allow females to select males of high genetic quality as suggested by the good genes theory of sexual selection (Andersson 1994). Although males are expected to be choosy as well (Trivers 1972; Owens & Thompson 1994; Johnstone *et al.* 1996), no trait is known to signal the genetic quality of females. To date, experimental tests of the good genes theory have been confined to species in which males are more heavily ornamented than females. These studies have shown that, by displaying such a trait, males signal viability (Norris 1993) or their ability to resist parasites (Møller 1990), and that the same trait expressed in a reduced form in females has apparently no signalling function (Hill 1993; Cuervo *et al.* 1996). This may suggest that female expression of a male trait in a reduced state is a genetic by-product (Lande 1980). In this scenario, only males are selected to develop the trait in an extravagant state. Males transmit the underlying genes of the trait into both daughters and sons, but sisters and brothers will express them differently due to the effect of sex-specific genes (Lande 1980). Because the idea that females may signal their quality to potential mates via an ornament comes from non-experimental studies (Møller 1993; Singh 1993; Møller *et al.* 1995; Potti & Merino 1996; Amundsen *et al.* 1997) and from experiments conducted in species in which females are less ornamented than males (Hill 1993; Møller 1993; Cuervo *et al.* 1996; Potti & Merino 1996; Amundsen *et al.* 1997), it

remains unclear whether female attributes serve to signal genetic quality. Consideration of model organisms in which the female is the more ornamented sex would facilitate testing this purpose.

The barn owl is distributed worldwide and females generally display more and larger black spots on the plumage of their ventral body side than males in both adults and nestlings (Roulin 1999a). The expression of plumage 'spottiness' is under genetic control and appears to be neither environmentally mediated nor condition dependent (Roulin *et al.* 1998). In Switzerland, successive females of the same males were similarly spotted. Mates of father and sons also displayed plumage spottiness to the same extent and mating was assortative with respect to this trait (Roulin 1999b). These observations suggest that male mate choice occurs and that a preference for heavily spotted females may be transmitted from father to sons. Thus, the barn owl does not match the general pattern observed in birds in which females are the choosy sex. This makes the barn owl a suitable model organism for investigating whether female traits may reflect genetic quality. Given the role of parasites in the evolution of sexually selected traits (Hamilton & Zuk 1982), we hypothesize that the extent of female plumage spottiness covaries with the level of antibody responses. Consideration of immunological mechanisms is justified since parasite resistance relies in part on the level of specific antibodies (Brossard & Girardin 1979; Gross *et al.* 1980).

We tested this hypothesis in a wild population of barn owls using a cross-fostering design, which is a useful tool for separating genetic from environmental effects on the development of a phenotypic trait by nestling birds. Offspring were randomly assigned to foster nests and their immune system challenged with sheep red blood cells (SRBCs), a non-pathogenic antigen which mimics invasion by a novel pathogen. We then correlated the

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magnitude of the specific antibody response towards SRBCs by cross-fostered nestlings to the plumage characteristics of the genetic mother and father.

2. MATERIAL AND METHODS

(a) General method

The study was conducted in 1998 in western Switzerland (46° 49' N, 06° 56' E) in an area covering 190 km². We checked nest-boxes regularly to record the breeding parameters and capture adults. Females were differentiated from males by the presence of a brood patch. At the third week of incubation, all females were weighed to the nearest gram and their tarsus length measured to the nearest millimetre. A body condition index was calculated as the residuals of the regression of body mass on tarsus length. One of the authors (A.R.) assessed the surface area of the black spots on the plumages of parents and nestlings. The number of spots was counted within a 60 mm × 40 mm frame placed on the breast, belly, flanks and underside of the wing and the diameter of three to 20 spots measured with a caliper to the nearest 0.1 mm. The proportion of the plumage surface covered by spots was calculated with the formula $100 \times \pi \times \text{number of spots} \times (\text{mean spot diameter}/2)^2 / 2400$. We averaged the values of the two flanks and the same procedure was applied to the two wings. The values found for the four body regions were then averaged. This last value was square-root transformed to normalize the data distributions and referred to as 'plumage spottiness'. The repeatability of this method is high (92%) (Roulin 1999b). To determine the sex of the nestlings a 20 µl blood sample was taken from the brachial vein at 30 days of age. DNA analyses were performed following Roulin *et al.* (1999).

(b) Cross-fostering

Barn owl parents do not discriminate between their own and unrelated nestlings (Roulin *et al.* 1999) and, thus, cross-fostering experiments are appropriate for assessing whether the antibody responsiveness of nestlings raised in foster nests towards SRBCs is related to the plumage spottiness of the genetic parents. Between pairs of nests, half of the zero- to five-day-old nestlings were exchanged without altering the brood size. Two to three hatchlings from nest A were brought to nest B and vice versa. Nests A and B are referred to as a 'pair of cross-fostered' nests. We marked the nestlings with non-toxic paint in order to record their identity before they were ringed. We determined the age of the nestlings by measuring their wing lengths (Schönfeld & Girbig 1975).

Our experimental procedure ensured that the analyses of the relationship between nestling immunocompetence and female plumage spottiness were unbiased by brood size, hatching date, size and age when the nestlings were challenged with SRBCs. Indeed, no significant correlation was found between female plumage spottiness and brood size where half of her cross-fostered offspring were raised (Spearman correlation, $r_s = 0.04$, $n = 38$ and $p = 0.81$), the mean place of these offspring in the within-brood age hierarchy ($r_s = -0.20$, $n = 38$ and $p = 0.23$), their mean age at the time of SRBC injection (Pearson correlation, $r = -0.03$, $n = 38$ and $p = 0.88$) and their hatching date ($r = -0.02$, $n = 38$ and $p = 0.91$). Differently spotted females also produced offspring which did not differ in their mean condition index which was given by the residuals from the regression of body mass on wing length at the time of injection ($r = 0.09$, $n = 38$ and $p = 0.58$). Finally, there was no resemblance in plumage spottiness between genetic and foster mothers

($r = -0.03$, $n = 38$ and $p = 0.88$) and between female and male mates ($r = -0.11$, $n = 36$ and $p = 0.51$).

(c) Measurement of antibody response towards SRBCs

The immune system of nestling birds takes several weeks to mature (Apanius 1998). We therefore injected the nestlings with SRBCs at the latest possible age, i.e. when the oldest nestling of each brood was 40 days, which is two weeks before the first flight. Thus, all nest-mates were injected with SRBCs on the same day and, since nestlings hatch every two to three days, age at injection differed. The nestlings were injected subcutaneously in the neck with 0.1 ml of a suspension of SRBCs (10% v/v in phosphate-buffered saline (PBS), with 10 mM phosphate, pH 7.4). We then took five 100 µl blood samples of each nestling from the brachial vein on day 0 (i.e. before immunization) and days 3, 8, 13 and 18 after immunization. The blood samples were centrifuged to remove the serum. We froze the serum until later analysis. We assessed antibody titres using an indirect haemagglutination assay. The samples were randomized in 96-well, round-bottomed, microtitre plates. Four microlitres of serum were diluted in 16 µl PBS and then 10 µl was serially diluted twofold with PBS (dilutions of 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640). After 30 min of incubation at 37°C and 30 min at 4°C, the plates were washed twice with PBS followed by resuspension in 100 µl of PBS. Fifty microlitres were then transferred to a new plate and 50 µl of 300-fold diluted rabbit anti-barn owl antibodies were added to these wells. The plates were incubated for 2 h at 37°C. The agglutination titres were expressed as ($\log_2 + 1$) of the reciprocal of the highest dilution showing agglutination. The rabbit anti-barn owl antibodies were prepared by immunizing a rabbit three times with 150 µg of ammonium sulphate- (40%) precipitated barn owl serum. The injections were given three weeks apart. The first injection was prepared in Freund's complete adjuvant and the following two in Freund's incomplete adjuvant. The serum of the rabbit was collected 19 days after the last injection.

(d) Statistics

The data were analysed with the JMP statistical package (Sall & Lehman 1996). The statistical tests were two-tailed and p -values ≤ 0.05 were considered as significant. Because the nestlings were not all immunized at the same age, we controlled for this factor in the statistical analyses. The heritability (h^2) of the plumage spottiness was estimated from twice the slope of the regression of the mean plumage trait of offspring raised in a foster nest on the plumage trait of each genetic parent in turn (Falconer 1989).

3. RESULTS

(a) Variation in antibody response towards SRBCs

Most of the nestlings mounted a specific antibody response towards the SRBCs (170 out of 175 nestlings). The amounts of specific antibodies progressively increased from prior to immunization (day 0) to 13 days later and then dropped slightly on day 18 (figure 1). Female and male nestlings produced a similar quantity of antibodies (mean antibody levels of same-sex nest-mates at days 0, 3, 8, 13 and 18 after immunization as repeated-measure ANOVA with sex as factor, $F_{1,173} = 0.29$ and $p = 0.59$). Therefore, we did not control for the gender of the nestlings in subsequent analyses.

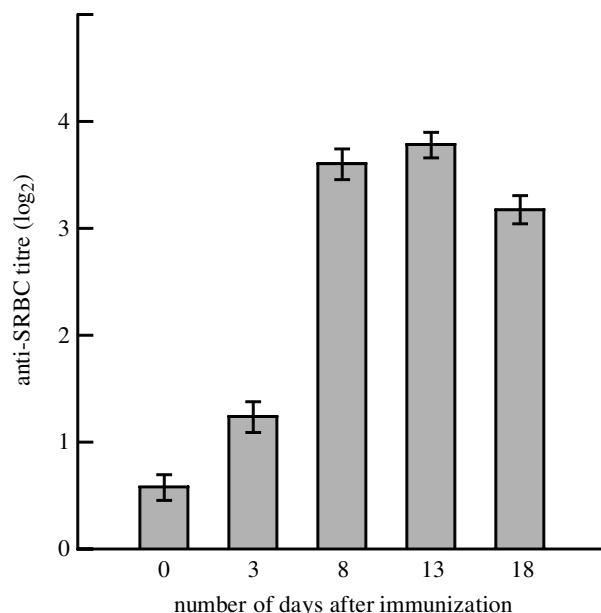


Figure 1. Time-course of the specific antibody response towards SRBCs. The sample size is 175 nestlings. When applying paired *t*-tests the mean level of antibodies differed significantly between two successive measurements except between days 8 and 13 after immunization.

(b) Covariation between plumage spottiness and antibody response

The hypothesis that the female plumage spottiness signals the antibody responsiveness of offspring towards an artificial antigenic challenge assumes that both the expression of plumage spottiness and the amounts of specific antibodies produced by nestlings are heritable. These two assumptions were verified. First, the mean plumage spottiness of offspring raised in foster nests was correlated with the plumage spottiness of their genetic parents (mother $h^2 = 0.66 \pm 0.28$, $F_{1,36} = 5.79$ and $p = 0.02$ and father $h^2 = 0.98 \pm 0.26$, $F_{1,34} = 14.22$ and $p < 0.001$). Second, siblings raised in different nests mounted a similar antibody response to the SRBCs (see the nested ANOVA analysis shown in table 1). We did not detect an effect of the nest of origin on the time-course of the immunological response (origin \times time interaction from the same previous nested ANOVA, Wilk's λ , $F_{76,451} = 1.04$ and $p = 0.40$). Therefore, we considered only the mean peak response at days 8 and 13 post-immunization (figure 1) when investigating the origin-related covariation between the magnitude of the antibody response towards the SRBCs by cross-fostered offspring and the plumage spottiness of parents.

We statistically removed the variance in antibody response due to the pair of cross-foster nests, the rearing environment and the age of the nestlings at the time of immunization from the nested ANOVA (see table 1). The residuals obtained reflect the origin-related effects on mounting an immunological response towards SRBCs. The mean residual antibody response of siblings raised in foster nests was positively correlated to the plumage spottiness of their genetic mother ($r = 0.36$, $n = 38$ and $p = 0.028$), but not to that of their genetic father ($r = -0.15$, $n = 36$ and $p = 0.39$). Thus, more heavily spotted females had offspring which produced a higher

Table 1. Mixed-model nested ANOVA on the level of anti-SRBC antibodies

(In this model, the term pair of cross-foster nests was the main effect, while the nests of rearing and nests of origin were nested in the main effect as indicated by the parentheses and the age of the nestlings at the time of injection was the covariate. For every individual the five measurements of antibody levels were used in the model as repeated measures.)

source	d.f.	<i>F</i> -ratio	<i>p</i> -value
pairs of nests	18 117	2.53	0.0020
nest of rearing (pair of nests)	19 117	1.19	0.0060
nest of origin (pair of nests)	19 117	2.43	0.0020
age at the time of injection	1 117	11.99	0.0007

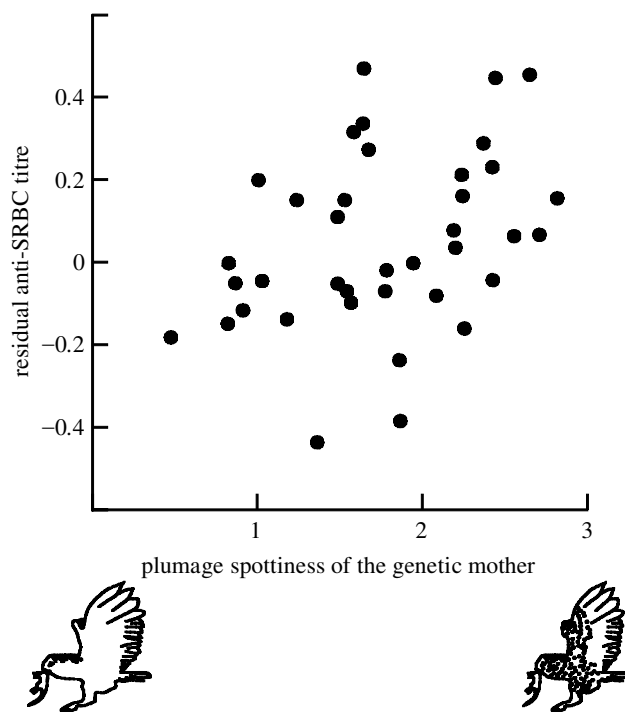


Figure 2. Relationship between the mean residual levels of anti-SRBC antibodies produced by offspring raised in foster nests and the plumage spottiness of their genetic mother. The residuals were obtained after controlling for the pair of cross-foster nests, the rearing environment and the age of the nestlings at the time of immunization.

quantity of specific antibodies against SRBCs (figure 2). We also assessed whether within nests more spotted nestlings produced more antibodies against the SRBCs. We statistically removed the variance due to the pair of cross-foster nests, the plumage spottiness of the genetic mother and the age of the nestlings at the time of injection from the nested ANOVA. Within nests more spotted nestlings produced non-significantly higher amounts of anti-SRBC antibodies (ANOVA, nestling spottiness $F_{1,37} = 2.78$ and $p = 0.10$). Since the female body condition measured during incubation was not significantly correlated to their plumage spottiness ($r = 0.19$, $n = 38$ and $p = 0.25$), maternal effects may not have inflated the relationship between the immunocompetence of the offspring and plumage characteristics of the genetic mother.

4. DISCUSSION

(a) *Genetics of parasite resistance*

Theoretical models of the evolution of parasite virulence and of host–parasite coevolution generally assume that variation in parasite resistance has a genetic basis but few field studies exist to support this assumption (Sorci *et al.* 1997). Cross-fostering experiments in the barn swallow (*Hirundo rustica*) have shown that the intensity of ectoparasite infection of nestlings is partly determined by their origin, suggesting a heritable basis for parasite resistance (Møller 1990). However, the mechanism of parasite resistance remains unclear. The immune system may play an important role because the capacity to resist endo- (Gross *et al.* 1980) and ectoparasites (Brossard & Girardin 1979) is often immunologically mediated. Recent field studies using a cross-fostering design in the barn swallow (Saino *et al.* 1997) and the great tit (*Parus major*) (Brinkhof *et al.* 1999) found that the inflammatory response of nestlings to an injection of phytohaemagglutinin was partly explained by their nest-related origin. This suggests that genetic variance in cell-mediated immunity is maintained in avian populations. In the present study we focused on humoral immunity, i.e. the production of specific antibodies. Antibody responses play an important role in conferring parasite resistance (Brossard & Girardin 1979). For instance, chickens selected for high antibody responsiveness towards SRBCs were better able to resist Newcastle disease and various bacteria including *Escherichia coli* and *Staphylococcus aureus* (Gross *et al.* 1980). Thus, SRBCs can be used to partition the variation in parasite resistance into environmental and genetic components. Genetic variance for antibody production directed against SRBCs has already been demonstrated using selection experiments with domestic fowls (e.g. Gross *et al.* 1980) but, to the best of our knowledge, not in a free-living organism. Our finding that sibling barn owls raised in different nests mounted a similar antibody response against SRBCs therefore provides, to the authors' knowledge, the first experimental support for an origin-related basis in antibody responsiveness towards a specific antigen in a wild animal population.

(b) *Signal of female quality*

In the barn owl, females are more spotted than males (Roulin 1999a,b) and the observation that more heavily spotted females produced offspring which mounted a higher antibody response towards SRBCs strongly suggests that variation in a female attribute reflects variation in the genetic quality of their offspring. It also confirms the results of an earlier study which concluded that additive genetic variance for plumage spottiness is maintained (Roulin *et al.* 1998). The absence of a significant correlation between the antibody responsiveness of their cross-fostered offspring and the plumage spottiness of the genetic father is difficult to discuss without knowledge of the frequency of extra-pair paternity. In contrast, the finding that female plumage spottiness covaried with antibody responsiveness towards SRBCs is not surprising for three reasons. First, a previous study documented that males may prefer to mate with heavily spotted females and an experiment showed that female plumage spottiness is a stimulus for males (Roulin 1999b).

Therefore, male barn owls may assess and choose heavily spotted females in order to produce more immunocompetent offspring. Second, an observational study showed that the nests of heavily spotted females were less infested by the blood-sucking fly *Carnus hemapterus* and that those flies were also less fecund (Roulin *et al.* 2000). Third, an experiment also demonstrated that flies had reduced fecundity when feeding on cross-fostered nestlings whose genetic mother was heavily spotted (Roulin *et al.* 2000). Therefore, female plumage spottiness may not only be a heritable signal of immunocompetence, as measured by SRBC antibody responsiveness in the present study, but also a heritable signal of parasite resistance. Since we cannot entirely exclude the possibility that heavily spotted females produced high-quality eggs which improved their antibody response against SRBCs, complementary studies are required in order to assess the exact role of potential maternal effects. Such effects may nevertheless be weak since the female body condition was not correlated with their plumage spottiness. In this context, the barn owl appears to be particularly promising for future studies on signals of parasite resistance displayed by females.

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