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Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*)

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Summary

1. We tested the condition-dependent hypothesis of sexual advertisement in house sparrows (*Passer domesticus*). Male house sparrows have a bib of black feathers which serves as both a badge of social status and as a cue for female choice. We manipulated environmental conditions during the premoult period of juvenile house sparrows kept in outdoor aviaries. Birds were assigned to two treatments differing in the amount of dietary proteins, which are known to affect the expression of immune response in birds. We tested whether birds in the protein-rich group had better immune responses and developed larger bibs than birds reared on a protein-poor diet. We also checked whether immune response was a predictor of survival and parasite resistance.

2. Individuals with higher cellular immune response at capture had greater probability to survive during the 3 months of the experiment, and they had a higher probability to recover from infection with *Haemoproteus* sp. (a blood parasite). Conversely, birds with high immunoglobulin concentrations at capture had a higher probability of mortality.

3. Birds on the protein-rich diet had a higher cellular immune response compared to birds in the protein-poor treatment. Humoral immune response showed the opposite pattern, being higher for birds in the protein-poor treatment. We did not find any effect of food quality on the development of the badge, assessed as the size of the trait and its colour properties.

4. In conclusion, our results support the view that immune defences are important for survival and parasite resistance in natural populations, and that they might be costly to produce. On the other hand, we did not find support for the condition-dependent hypothesis of sexual advertisement, suggesting that the badge may not be a costly trait to produce. However, badge size could reflect other aspects of condition. The kind of pigments involved in colour signals may be the key factor determining the production costs of such traits.

Key-words: immune response, immunoglobulins, parasite resistance, sexual signals, survival, T-cell response.

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Introduction

Models of sexual selection have focused on the benefits a female can acquire from mating with a male possessing particular phenotypic traits (Andersson

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1994). Recent evidence suggests that many secondary sexual characters demonstrate condition-dependent expression, and that females may obtain either direct or indirect fitness benefits by using such traits as cues for mate choice (reviews in Andersson 1994; Johnstone 1995). Cheating can be prevented if sexual signals are costly to produce and maintain. Parasites and diseases have been implicated to play an important role in sexual selection because only individuals in prime condition, and hence in superior health status, will be able to develop the most extreme expression of costly secondary sexual characters (Hamilton & Zuk 1982; review in Møller, Christe & Lux 1999). Folstad & Karter (1992) provided a physiological framework for the trade-off between parasite resistance and sexual signals. They suggested that secondary sexual traits which are hormone-dependent might be honest indicators of male quality because androgens may have immunosuppressive effects. Therefore, only males of high phenotypic and/or genetic quality could develop exaggerated sexual signals and afford to pay the cost of immunosuppression, with sexual signals reliably signalling immunocompetence (the ability to raise an efficient immune response to a novel challenge). Without evoking the immunosuppressive effects of androgens, it has been suggested that sexual advertisement and immune defences can be traded-off because both functions are costly to produce and maintain (Fig. 1) (Wedekind & Folstad 1994), an issue currently debated (see Råberg *et al.* 1998; Westneat & Birkhead 1998).

Trade-offs between the expression of secondary sexual traits and immune defences could represent the underlying mechanism resulting in the classical trade-off between reproduction and survival. To be the case, exaggerated secondary sexual traits should correlate with higher reproductive output, and immune defences with better survival prospect. Positive correlations between sexual advertisement and reproductive success have been reported for several species where females show a preference for males with exaggerated traits (see Andersson 1994 for a review). Much less evidence is available on the importance of immune defences for survival in natural populations (Saino, Bolzern & Møller 1997), and whether assays used to estimate immunocompetence actually reflect resistance to parasitic infections in free-ranging individuals (Siva-Jothy 1995).

The house sparrow (*Passer domesticus* L. 1758) is a small dimorphic passerine; adult males have a bib of black feathers on the throat and the chest, whereas females are uniformly brown. Males with larger bibs are dominant and may have better access to food resources in flocks (Barnard & Sibly 1981; Møller 1987a,b). Females appear to have a preference for large bib males in some populations (Møller 1988), but to prefer small-badged males in others (Griffith, Owens & Burke 1999).

We conducted an experiment to test the condition-dependent hypothesis of sexual advertisement in juvenile house sparrows kept in aviaries during the moulting period. Birds were assigned to two food treatments to test whether individuals living under better environmental conditions had higher immunocompetence and moulted into larger sexual signals. We also tested whether mortality and blood parasite prevalence were negatively correlated with immunocompetence.

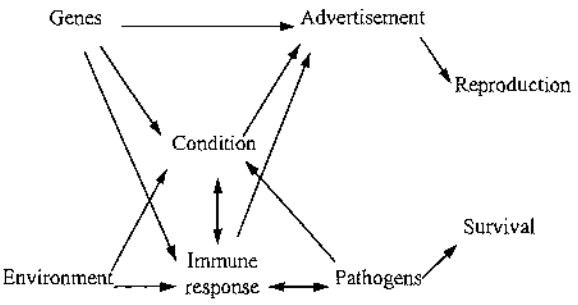

Fig. 1. Path diagram illustrating links between genes and environment with condition, immune response, pathogens and sexual advertisement. Genetic and environmental variation contribute to determine general condition, immune response and sexual advertisement. Individuals in prime condition can invest more resources in immune defence and sexual signals. Pathogens interfere with the optimal allocation of resources to immune defence and sexual signals, by diverting resources. Ultimately, resource allocation to immune defences and sexual advertisement determines fitness via the effect on survival (i.e. parasite resistance) and reproduction (i.e. mate attraction). Modified from Westneat & Birkhead (1998).

Methods

The experiment was carried out on a house sparrow population at Badajoz (Extremadura, south-west Spain). During July 1997 we captured juvenile (i.e. born in the spring) house sparrows in mist nets. As for most altricial birds, post-fledgling house sparrows still depend on their parents which continue to feed them for up to 14 days after fledging (Summers-Smith 1988). Therefore, we limited our sample to independent birds which obviously also made the sample more homogeneous with respect to age. Birds were then banded, their tarsus length measured with a digital calliper with an accuracy of 0·01 mm, and body mass on a Pesola spring balance with an accuracy of 1 g. Sparrows were subsequently randomly released in 10 adjacent outdoor aviaries located on the campus of the University of Extremadura (Badajoz). Eight aviaries were 3·5 × 1·1 × 2·5 m and contained between nine and 12 birds; two were 3·5 × 0·6 × 2·5 m and contained five birds each. Birds were randomly assigned to two

food regime treatments. Birds in the first group received a commercial mixture of seeds for canaries (Ornyzoo S. L., Spain), provided *ad libitum*. The second group received the same mixture of seeds plus a supplement in animal protein (commercial diet protein complement 'Pasta de huevo', Pet-bon). All birds had water *ad libitum* supplemented with vitamins. The experiment lasted until 15 October 1997 (12 weeks).

MEASUREMENT OF IMMUNOCOMPETENCE

An estimate of immunocompetence would require information on the following components of immune function: (1) the relative mass of lymphoid organs (spleen and thymus); (2) response to immunization with sheep red blood cells to obtain an *in vivo* measure of B- and T-cell activity; (3) response to immunization with *Brucella abortus* to obtain *in vivo* response of B-cells independent of T-cells; (4) response to phytohaemagglutinin to obtain *in vivo* response of T-cells; and (5) *in vitro* assay of nitrous oxide production by macrophages from spleen cells (e.g. National Research Council 1992). Since some of these components of immune function are based on *in vitro* methods, there will often be a conflict between practical considerations (the fitness consequences of phenotypic variation can only be estimated for individuals that are still alive following assessment of immune function) and a broad scale estimate of immunocompetence. Furthermore, ethical considerations will also often be at conflict with complete assessment of immune function. We have attempted to achieve a reasonable balance between these conflicting demands by obtaining information on two components of the immune response: T-cell mediated immunity and immunoglobulin concentration. T-cell response was assessed using the following assay: at capture we injected each bird intradermally in the centre of the right wing web (after having marked the injection site with a pen) with 0·025 mg of phytohaemagglutinin (PHA; Sigma Chemical Co., St Louis, Missouri, USA) in 0·04 mL of physiological saline solution (PBS) (Bausch & Lomb Co.). The left wing web was used as a control by injecting 0·04 mL of PBS. The thickness of each wing web was measured with a pressure-sensitive spessimeter (to the nearest 0·01 mm) at the injection site before and 24 h (± 15 min) after injection. The wing web swelling (WWS) was estimated as the change in thickness of the right wing web from the day of injection with PHA until the following day minus the change in thickness of the left wing web from the day of injection until the following day. Wing web swelling has been shown to be repeatable in another group of house sparrows (Gonzalez, Sorci & de Lope, unpublished), as well as in other species (Sorci, Soler & Møller 1997). The same experimental protocol was used to assess T-cell mediated immunity 3 months later, at the end of the experiment.

After having measured the thickness of the wing web, we collected a blood sample from the brachial vein in a heparinized capillary. The capillaries were centrifuged for 5 min at 14 000 r.p.m., the plasma was isolated and stored at -30 °C for immunoglobulin analysis. We immunized the sparrows by injecting intraperitoneally a suspension of sheep red blood cells (SRBS), containing 5×10^7 SRBS in 100 μL of PBS. Blood samples were taken 10 days after immunization and stored as described above. Immunoglobulin concentrations were assayed by densitometric analysis after electrophoretic separation of plasma proteins on agarose gels (Paragon SPE Kit, Beckman). Five microlitres of plasma were applied to agarose gels and proteins were allowed to migrate for 25 min at constant voltage (100 V). After electrophoresis, gels were air-dried and subsequently analysed using the procedure 'Densitometric analysis of 1-D gels' of the image analysis software NIH Image 1·54 (NIH Image is a public domain image analysis program which is available by anonymous FTP from zippy.nimh.nih.gov). The relative concentration of immunoglobulins (Ig) and other proteins that comigrate during electrophoresis was expressed as the ratio between the area of the densitometric profile corresponding to the immunoglobulins and the total area of the densitometric profile (Saino *et al.* 1997). The total area of the densitometric profile was used to check whether any change in immunoglobulin concentration between food regimes was due to changes in total plasma proteins between treatments. Ten individuals were ran twice to investigate the repeatability of immunoglobulin concentration. The repeatability was high and statistically significant ($P < 0·001$).

Finally, we screened sparrows for haematozoan infections. This was done by collecting a drop of blood for a blood smear at the beginning and the end of the experiment. The smear was subsequently air-dried, fixed in absolute methanol and stained with Giemsa. We screened 10 000 red blood cells in each smear and counted the number of parasitized cells. This was carried out at ×1000 magnification using an optic microscope. Prevalence and intensity were defined as the proportion of infected birds and the percentage of infected cells, respectively.

MEASUREMENT OF BADGE SIZE AND COLOUR

Badge length and width were measured with a ruler with an accuracy of 1 mm. After moult, the black feathers of the badge have white tips which partially cover the underlying feathers. Our measurements refer to the total area covered by black feathers

[total and visible area are positively correlated (Møller & Erritzøe 1992)]. The area of the badge was estimated using a regression equation derived by Møller (1987a): badge size (mm^2) = 166·7 + 0·45 × badge length (mm) × badge width (mm), and was log-transformed. Badge colour was measured with a spectrophotometer (Ocean Optics Europe) and decomposed into three colour parameters: hue, saturation and tone (Hill & Brawner 1998). Each bird was measured twice in order to estimate repeatability of the three colour parameters, and of badge size. Repeatability were high and significant for all variables (all $P < 0·001$).

STATISTICAL ANALYSES

Surviving birds were assigned a value of 1 and non-surviving a value of zero. Juvenile house sparrows are sexually monomorphic. Since dead individuals were not examined post-mortem, we were unable to investigate the effect of sex on survival. We used a stepwise multiple logistic regression to test the correlation between survival and wing web swelling, immunoglobulin concentration and body size. The food treatment was also included in the model to account for different patterns of mortality in the two food regimes. We also computed the mean wing web swelling and immunoglobulin concentrations for surviving and non-surviving birds for each aviary. We then computed the difference (surviving minus non-surviving) between the means within aviaries. Under the null hypothesis of no association between survival and immune responses, these differences should have a mean of zero. We tested this by using a paired *t*-test (Sokal & Rohlf 1981).

The effect of food treatment on the immune response and badge size was estimated using a repeated-measures nested ANOVA, to take into account the effect of replicates (i.e. aviaries). The correlation between different components of the immune response was assessed using linear regression models, based on both individuals and aviaries as independent observations. Linear and log-linear regressions were used to test the correlation between immune responses and intensity and prevalence of haematozoa.

Since hue, saturation and tone may be intercorrelated among them, we used MANOVA and MANCOVA to test whether food manipulation affected colouration.

All analyses were performed using SAS (SAS Release 6·12, SAS 1996).

Results

IMMUNOCOMPETENCE AND SURVIVAL

A total of 60 individuals of initially 96 (63%) survived until the end of the experiment. Most mortality occurred during the first 2 weeks and no birds died in the last 2 weeks of the experiment. Survival rate differed among aviaries (Fisher's exact test, $P = 0·021$) and between food treatments (high protein diet: 51·1% survival, $n = 47$; low protein diet: 73·5% survival, $n = 49$; Fisher's exact test, $P = 0·035$). This last surprising result was probably due to an initial difference in wing web swelling between the two treatments. In spite of being randomly assigned to the two food regimes, it transpired that birds on the protein-rich diet had lower initial values of WWS (mean ± SE = 0·409 ± 0·028, $n = 47$) compared to birds on the protein-poor diet (mean ± SE = 0·544 ± 0·047, $n = 48$) (one-way ANOVA with food as factor: $F_{1,93} = 5·90$, $P = 0·017$). None of the other variables was initially different between the two treatments.

The wing web swelling and immunoglobulin concentration can depend on individual condition. For this reason, we first regressed WWS and Ig on two potential correlates of body condition: body mass and tarsus length. A stepwise multiple regression showed that only body mass was a significant predictor of wing web swelling; birds with higher body mass having a larger wing web swelling than lighter birds (slope ± SE, b = 0·304 ± 0·099, $n = 95$, $P = 0·003$). The same multiple regression on Ig showed that neither body mass nor tarsus length were significantly correlated with Ig (all $P > 0·1$).

Wing web swelling was a significant predictor of survival probability. A stepwise logistic regression with survival as the dependent variable and wing web swelling, body mass and food treatment as independent variables revealed that surviving birds had on average a higher WWS than non-survivors (mean ± SE, non-survivors, 0·341 ± 0·032 (mm), $n = 35$; survivors, 0·528 ± 0·038 (mm), $n = 60$; Wald $\chi^2 = 6·281$, d.f. = 1, $n = 95$, $P = 0·012$), and that surviving birds had higher body mass than non-survivors (Wald $\chi^2 = 6·131$, d.f. = 1, $n = 95$, $P = 0·013$). Cellular immunity and body mass thus had an additive positive effect on survival probability. In a second regression model, we replaced WWS by Ig. Here again body mass was significantly positively correlated with survival probability (Wald $\chi^2 = 8·527$, d.f. = 1, $n = 92$, $P = 0·004$), whereas immunoglobulin concentration was negatively correlated with survival (mean ± SE, non-survivors, 29·68 ± 1·141 (%), $n = 33$; survivors, 22·48 ± 0·80 (%), $n = 59$; Wald $\chi^2 = 5·861$, d.f. = 1, $n = 92$, $P = 0·015$). A stepwise logistic regression with both measurements of immune response and body mass showed that the three variables were significant predictors of survival (body mass: Wald $\chi^2 = 6·389$, d.f. = 1, $P = 0·012$; immunoglobulins: Wald $\chi^2 = 5·53$, d.f. = 1, $P = 0·019$; WWS: Wald $\chi^2 = 4·09$, d.f. = 1, $P = 0·043$, $n = 92$).

As previously reported, survival rate differed among aviaries. We therefore checked the consistency of the association between immune responses and survival among aviaries. This was performed by comparing mean WWS (after correcting for body mass) and mean immunoglobulin concentration for survivors and non-survivors with a paired *t*-test. In one aviary all birds survived and therefore we could not use it to compute a difference between survivors and non-survivors. In eight of nine aviaries survivors had higher WWS than non-survivors (paired $t = 3{\cdot}17$, d.f. = 8, $P < 0{\cdot}02$), showing that individuals with higher WWS were consistently better able to survive irrespective of any environmental heterogeneity among aviaries. The difference in immunoglobulin concentration between surviving and non-surviving birds also tended to be consistent among aviaries. In seven of nine aviaries survivors had lower immunoglobulin concentrations than non-survivors, although this difference was not significant (paired $t = 2{\cdot}2$, d.f. = 8, $0{\cdot}05 < P < 0{\cdot}1$).

Birds were infected with *Haemoproteus* sp. In July, the prevalence was 32·3% ($n = 96$). *Haemoproteus* infections (prevalence and intensity) did not correlate significantly with survival probability (prevalence: Wald $\chi^2 = 2{\cdot}84$, d.f. = 1, $n = 92$, $P = 0{\cdot}092$; intensity: Wald $\chi^2 = 1{\cdot}055$, d.f. = 1, $n = 92$, $P = 0{\cdot}304$); nor were immunoglobulins, WWS and body mass correlated with *Heamoproteus* prevalence and intensity (prevalence: immunoglobulins, Wald $\chi^2 = 0{\cdot}28$, d.f. = 1, $n = 92$, $P = 0{\cdot}59$; WWS, Wald $\chi^2 = 0{\cdot}067$, d.f. = 1, $n = 92$, $P = 0{\cdot}79$; body mass, Wald $\chi^2 = 1{\cdot}65$, d.f. = 1, $n = 92$, $P = 0{\cdot}129$; intensity: immunoglobulins, $r_s = -0{\cdot}045$, $n = 92$, $P = 0{\cdot}67$; WWS, $r_s = 0{\cdot}015$, $n = 95$, $P = 0{\cdot}89$; body mass, $r_s = 0{\cdot}142$, $n = 96$, $P = 0{\cdot}17$).

A potential problem with our results is that we observed 37% mortality during the 12 weeks of the experiment. However, comparisons with data available on post-fledging survival in free-living house sparrows show similar patterns of mortality, suggesting that high mortality could be an intrinsic characteristic due to age rather than potential bias in our experimental protocol (i.e. food quality, crowding, aviaries). To test this hypothesis, we repeated the experiment in 1998, using adult male house sparrows. As in 1997, birds were caught and their cellular immunity estimated using injection with PHA (we did not estimate humoral immunity). Birds were then released into the same aviaries, fed with the same mixture of seeds and therefore experienced the same overall experimental conditions. Only 7·8% (4/51) of adult birds died. A logistic regression on survival probability with age and WWS as independent variables showed significant effects of both age (Wald $\chi^2 = 11{\cdot}28$, d.f. = 1, $n = 147$, $P = 0{\cdot}0008$) and WWS (Wald $\chi^2 = 15{\cdot}20$, d.f. = 1, $n = 147$, $P = 0{\cdot}0001$), with the interaction being non-significant (Wald $\chi^2 = 2{\cdot}69$, d.f. = 1, $n = 147$, $P = 0{\cdot}101$).

ENVIRONMENTAL VARIABILITY AND IMMUNE RESPONSE

Food treatment significantly affected both T-cell response and immunoglobulin concentration of surviving birds. At the beginning of the experiment neither WWS nor Ig differed between protein-rich and protein-poor diets (two-way ANOVA: WWS, food: $F_{1,54} = 1{\cdot}21$, $P = 0{\cdot}276$, sex: $F_{1,54} = 4{\cdot}60$, $P = 0{\cdot}036$; Ig, food: $F_{1,53} = 0{\cdot}81$, $P = 0{\cdot}372$, sex: $F_{1,53} = 1{\cdot}58$, $P = 0{\cdot}214$). However, at the end of the experiment wing web swelling was higher for birds receiving the protein-rich treatment compared to protein-poor individuals (nested ANOVA: food: $F_{1,45} = 28{\cdot}90$, $P < 0{\cdot}001$, replicate within food: $F_{8,45} = 1{\cdot}70$, $P = 0{\cdot}125$, sex: $F_{1,45} = 0{\cdot}08$, $P = 0{\cdot}924$, interaction terms: $P > 0{\cdot}5$). Conversely, Ig were significantly lower for birds belonging to the protein-rich treatment (nested ANOVA: food: $F_{1,45} = 4{\cdot}53$, $P = 0{\cdot}039$, replicate within food, $F_{8,45} = 2{\cdot}90$, $P = 0{\cdot}011$, sex: $F_{1,45} = 0{\cdot}08$, $P = 0{\cdot}927$, interaction terms: $P > 0{\cdot}5$). Birds in the protein-poor regime were unable to raise their WWS as time progressed, whereas birds in the protein-rich regime strongly increased the WWS (Fig. 2a). This resulted in a highly significant interaction term between food treatment and date (repeated-measures nested ANOVA: $F_{1,45} = 23{\cdot}57$, $P < 0{\cdot}001$). The opposite trend was observed for Ig which only increased for birds in the protein-poor treatment (Fig. 2b). However, in this case the food by date interaction was not significant (repeated-measures nested ANOVA: $F_{1,45} = 3{\cdot}52$, $P = 0{\cdot}067$).

The analysis of the total densitometric profile of plasma proteins showed that the amount of plasma proteins did not change between food treatments nor between the two sampling dates (repeated-measures nested ANOVA: date: $F_{1,45} = 0{\cdot}01$, $P = 0{\cdot}917$; food: $F_{1,45} = 0{\cdot}07$, $P = 0{\cdot}794$, food*date: $F_{1,45} = 0{\cdot}08$, $P = 0{\cdot}785$). This shows that the change in the percentage of Ig among plasma proteins was not due to a change of overall plasma proteins.

Body mass significantly increased between the two sampling dates, although the increase in body mass was independent of food regimes (repeated-measures nested ANOVA: date: $F_{1,45} = 126{\cdot}56$, $P < 0{\cdot}001$; food: $F_{1,45} = 0{\cdot}03$, $P = 0{\cdot}869$, food*date: $F_{1,45} = 0{\cdot}52$, $P = 0{\cdot}476$). It is therefore unlikely that the differences observed for WWS and Ig were the results of the confounding effect of body mass. Indeed, both WWS and Ig were still significantly correlated with food treatment after correcting for body mass (nested ANCOVA: WWS, food $F_{1,46} = 31{\cdot}33$, $P < 0{\cdot}001$; body mass: $F_{1,46} = 5{\cdot}84$,

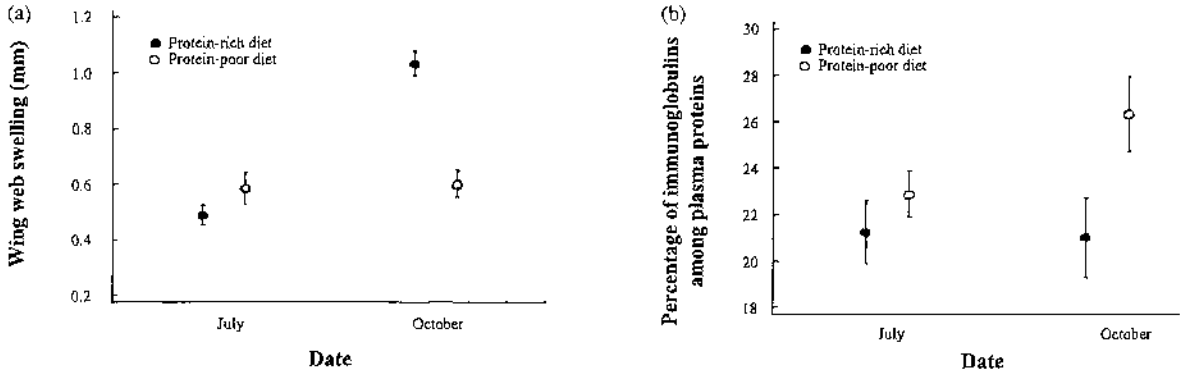

Fig. 2. Change in wing web swelling (a) and the percentage of immunoglobulins among plasma proteins (b) of juvenile house sparrows from July to October 1997 depending on protein food regime. Bars represent standard errors.

$P = 0{\cdot}019$; Ig, food: $F_{1,46} = 5{\cdot}58$, $P = 0{\cdot}023$; body mass: $F_{1,46} = 1{\cdot}95$, $P = 0{\cdot}169$).

At the end of the experiment, Ig and WWS were negatively and significantly correlated, after correcting for body mass (stepwise linear regression, slope $\pm$ SE = $-0{\cdot}083 \pm 0{\cdot}039$, $P = 0{\cdot}037$, $n = 57$). The negative correlation between Ig and WWS was also significant when considering the aviaries as independent observations (stepwise linear regression, slope $\pm$ SE = $-0{\cdot}159 \pm 0{\cdot}055$, $P = 0{\cdot}020$, $n = 10$, Fig. 3).

VARIATION IN IMMUNE RESPONSES AND PARASITIC STATUS

In July, 31·6% of the birds which were still alive in October harboured parasites, whereas the proportion of infected birds decreased to 12·3% at the end of the experiment in October. This was due to 12 birds changing their status from infected to non-infected, whereas only one individual acquired the infection during the experiment ($\chi^2_1 = 10{\cdot}82$, $P < 0{\cdot}001$). The proportion of infected birds in July was not significantly correlated with any of the predictor variables (log-linear model: WWS: $\chi^2 = 0{\cdot}57$, $P = 0{\cdot}449$; Ig: $\chi^2 = 0{\cdot}010$, $P = 0{\cdot}912$; body mass: $\chi^2 = 1{\cdot}670$, $P = 0{\cdot}196$; sex: $\chi^2 = 0{\cdot}650$, $P = 0{\cdot}421$). However, at the end of the experiment both WWS and food treatment were significantly correlated with *Haemoproteus* prevalence, birds with higher WWS and in the protein rich regime having lower probabilities of being infected (log-linear model: WWS: $\chi^2 = 6{\cdot}51$, $P = 0{\cdot}011$; food treatment: $\chi^2 = 4{\cdot}75$, $P = 0{\cdot}029$; WWS * food treatment: $\chi^2 = 0{\cdot}29$, $P = 0{\cdot}519$); all other variables (Ig, sex, body mass) not being significantly correlated with prevalence (all $P > 0{\cdot}2$). Moreover, prevalence did not vary among aviaries (Fisher's exact test, $P = 0{\cdot}452$), which allows the use of each individual as independent observations. The correlation between *Haemoproteus* parasitism and WWS was also present when based on intensity of parasitism instead of prevalence. Again intensity in July was not significantly correlated with WWS, Ig, body mass or sex (all $P > 0{\cdot}3$) whereas, in October, birds

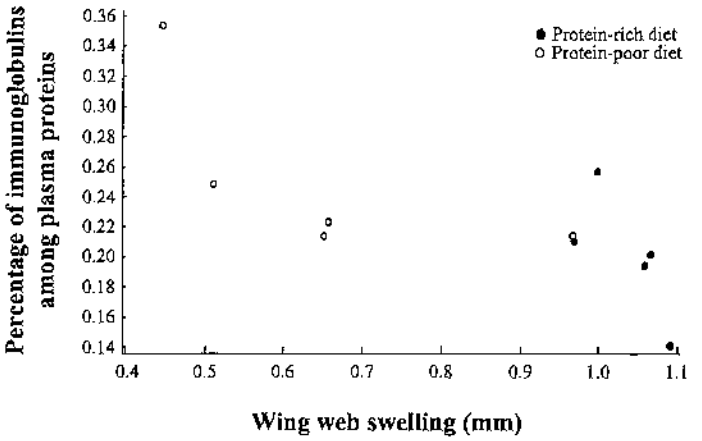

Fig. 3. Negative correlation between the percentage of immunoglobulins among plasma proteins and wing web swelling in juvenile house sparrows. Aviaries were considered here as independent observations. Full and empty dots represent protein-rich and protein-poor regimes, respectively.

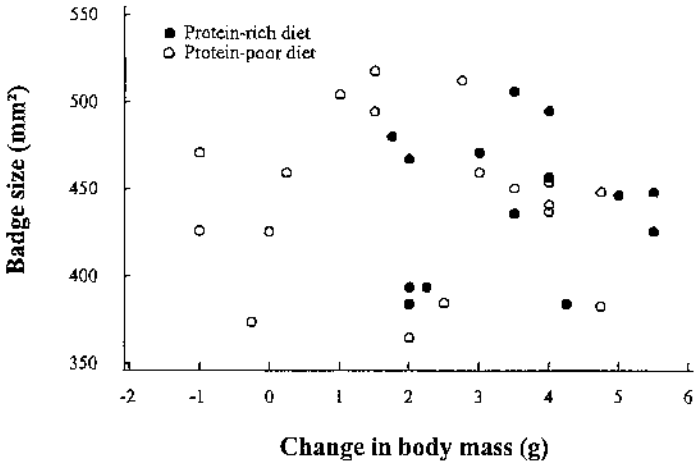

Fig. 4. Badge size (mm^2) in relation to change in body mass (g) between the two sampling dates (July and October) for birds in the protein-rich and protein-poor diet. Full and empty dots represent protein-rich and protein-poor regimes, respectively.

with the highest intensity of parasitism were those with the smallest WWS (Pearson's partial correlation coefficient, $r = -0{\cdot}344$, $P = 0{\cdot}010$, $n = 56$). Intensity was not significantly correlated with Ig (Pearson's partial correlation coefficient, $r = 0{\cdot}096$, $P = 0{\cdot}483$, $n = 56$). Intensity did not vary among aviaries (one-way ANOVA, $F_{1,8} = 1{\cdot}00$, $P = 0{\cdot}455$).

ENVIRONMENTAL VARIATION, IMMUNE RESPONSE AND SEXUAL ADVERTISEMENT

Badge size was not affected by food treatment (nested ANOVA: food: $F_{1,24} = 0{\cdot}010$, $P = 0{\cdot}913$, replicate within food: $F_{8,24} = 0{\cdot}85$, $P = 0{\cdot}573$). Badge size was not significantly correlated with variation in body mass (i.e. the difference of body mass between the two sampling dates) (Fig. 4), WWS and Ig (nested ANOVA: food: $F_{1,20} = 0{\cdot}00$, $P = 0{\cdot}966$; replicate within food: $F_{8,20} = 0{\cdot}75$, $P = 0{\cdot}646$; body mass: $F_{1,20} = 0{\cdot}00$, $P = 0{\cdot}965$; WWS: $F_{1,20} = 0{\cdot}010$, $P = 0{\cdot}909$; Ig: $F_{1,20} = 0{\cdot}55$, $P = 0{\cdot}469$), nor was it correlated with values of body mass, WWS (Fig. 5) and Ig in October (nested ANOVA: food: $F_{1,21} = 0{\cdot}04$, $P = 0{\cdot}836$; replicate within food: $F_{8,21} = 1{\cdot}11$, $P = 0{\cdot}394$; body mass: $F_{1,21} = 0{\cdot}20$, $P = 0{\cdot}661$; WWS: $F_{1,21} = 0{\cdot}990$, $P = 0{\cdot}330$; Ig: $F_{1,21} = 3{\cdot}53$, $P = 0{\cdot}074$).

Similarly, badge colour was not affected by food treatment, nor was it correlated with body mass, WWS or Ig (MANCOVA: Food: Wilks' lambda = $0{\cdot}772$, $F_{3,26} = 2{\cdot}55$, $P = 0{\cdot}078$; body mass: Wilks' lambda = $0{\cdot}889$, $F_{3,26} = 1{\cdot}084$, $P = 0{\cdot}373$; WWS: Wilks' lambda = $0{\cdot}919$, $F_{3,26} = 0{\cdot}758$, $P = 0{\cdot}528$; Ig: Wilks' lambda = $0{\cdot}865$, $F_{3,26} = 1{\cdot}354$, $P = 0{\cdot}279$). Finally, badge colour and size were not

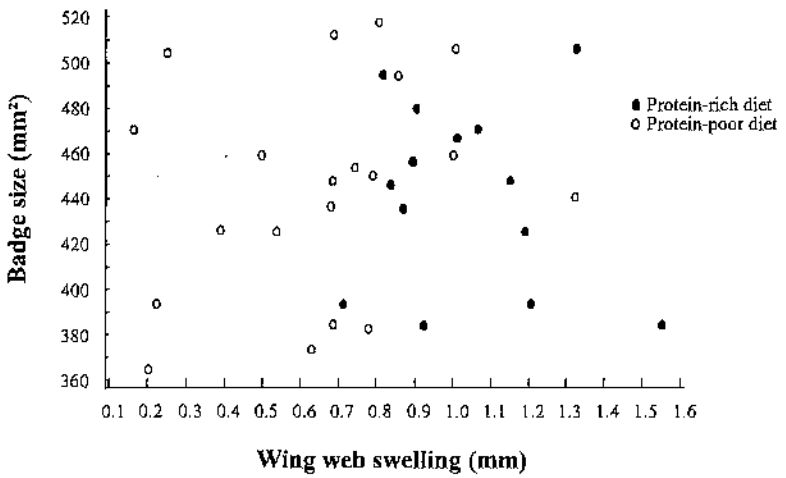

Fig. 5. Relationship between badge size (mm^2) and wing web swelling (mm) measured at the end of the experiment. Full and empty dots represent protein-rich and protein-poor regimes, respectively.

significantly correlated (MANCOVA: badge size, Wilks' lambda = 0·976, $F_{3,29}$ = 0·976, P = 0·869).

Discussion

IMMUNOCOMPETENCE, PARASITISM AND SURVIVAL

Wing web swelling, a measure of T-lymphocyte proliferation, was positively correlated with survival prospects in house sparrows. Conversely, we found a negative correlation between immunoglobulin concentration and survival probability. High levels of immunoglobulins are likely to reflect exposure to infectious agents, and therefore the negative correlation between immunoglobulins and survival might not be surprising. For instance, work on the house martin (*Delichon urbica* L. 1758) has shown that experimentally manipulated parasitism by *Oeciacus hirundinis* (Jenyns 1839) (a haematophagous hemipteran) increases immunoglobulin concentration (de Lope, Møller & de la Cruz 1998).

All organisms are exposed to parasites and pathogens which may significantly reduce host fitness (e.g. reviews in Lehmann 1993; Møller 1997). The immune system of vertebrates probably represents the most efficient mechanism to control parasitic infections, as illustrated by dramatic fitness costs of immunodeficient individuals (Mitchison 1990). In spite of the straightforward predictions concerning the correlation between the efficiency of the immune system and host fitness components, experimental evidence for such a relationship is still scarce (see Saino *et al.* 1997). Our results add evidence for the role played by immune defences in determining survival prospects in natural populations. In this study, individuals were maintained under homogeneous environmental conditions that preclude any effect of differential exposure to parasites or predators as a cause of the observed effect. A potential bias in our study could be the 'unrealistically' high juvenile mortality observed in the aviaries. However, comparisons with data available on post-fledging survival in the house sparrow show similar patterns of mortality for natural populations (Summers-Smith 1988). Most mortality observed in the aviaries occurred during the first 2 weeks after the beginning of the experiment, suggesting that some birds were more susceptible to a changing and potentially stressful environment. The relationship between cellular immune response and survival was consistent in juvenile and adult birds. Survival rate of adult house sparrows maintained under the same experimental conditions during an equivalent period of time was significantly higher than survival of juveniles, which again suggests that juvenile mortality was not due to bias in our experimental protocol.

ENVIRONMENTAL VARIABILITY AND IMMUNOCOMPETENCE

Environmental conditions are known to have a large impact on the expression of the immune response in vertebrates and on risks of parasitism. Among other factors, food quantity and quality can significantly modify the level of immunocompetence (Chandra & Newberne 1977; Lochmiller, Vestey & Boren 1993). We found that juvenile house sparrows were able to mount a better cell-mediated immune response when feeding on a protein-rich food compared to individuals experiencing a protein-poor diet. Humoral immunity, assessed as the percentage of immunoglobulins among plasma proteins after immunization with SRBC, showed the opposite pattern, tending to be lower for birds in the protein-rich treatment. Differences between components of the immune response in their ability to respond to different food treatments have already been reported for other species (e.g. Lochmiller *et al.* 1993).

Assuming that combating an immune challenge is costly for a host, and that the different components of the immune system confer protection against different pathogens, one might expect that individuals invest more into the components which proved to be more protective against present parasitic challenge. Cytotoxic T-cells are responsible for the destruction of host cells infected by viruses and other intracellular parasites, whereas antibodies (i.e. immunoglobulins) can provide a major barrier either against extracellular pathogens or extracellular stages of parasites (e.g. by restricting the spread of virus in the blood stream) (Roitt, Brostoff & Male 1996). Cell-mediated immunity could, thus, be more effective against haemosporidians which mostly have intratissue and intracellular stages. In agreement with this hypothesis, we found that the probability of recovering from *Haemoproteus* infection was positively correlated with wing web swelling, an index of T-lymphocyte proliferation, but not with immunoglobulin concentrations. Experimental manipulation of parasites with different 'niches' (intra- vs. extracellular) are needed for a better understanding of the adaptive nature of this relationship.

COST OF SEXUAL SIGNALS AND IMMUNITY

The condition-dependent hypothesis of sexual advertisement is based on the assumption that sexual signals are costly to produce (see Westneat & Birkhead 1998). Females may obtain direct and indirect benefits from mating with males with exaggerated sexual signals, if these signals indicate phenotypic and/or genetic quality (Andersson 1994). However, under the assumption of limited resources, allocation to one function can only be achieved at the expense of other resource-demanding functions.

Using the reasoning of the principle of resource allocation, Wedekind & Folstad (1994) suggested that sexual advertisements might indicate resistance to parasites because resources allocated to signals cannot be allocated to mounting immune defences. The view of resource constraints on immune functioning is currently under debate (König & Schmid-Hempel 1995; Sheldon & Verhulst 1996; Deerenberg *et al.* 1997; Råberg *et al.* 1998; Westneat & Birkhead 1998). Some empirical evidence seems to suggest that immune defences are resource-demanding, while other studies have failed to find significant impact of immune defences on basal metabolic rate (see Westneat & Birkhead 1998 for a review). Our finding of an increase of cellular immune response in birds belonging to the protein-rich group is also in agreement with the hypothesis of nutrition limiting immune defences.

Costs of immune defences are certainly difficult to assess; however, the cost of producing and maintaining sexual signals might also be difficult to estimate, and few studies have attempted to manipulate body condition and assess the effect on the development of secondary sexual traits. A further difficulty in considering secondary sexual traits as signals of condition or quality is that each trait is likely to be associated with different costs. Morphological, behavioural and colour-based traits have different physiological pathways and probably different resource demands for their expression. Moreover, in birds, secondary sexual traits involving feathers are usually produced once a year during moult (Svensson 1994). Therefore, the cost of producing feathers, if any, should reflect body condition during the period when feathers are produced (i.e. moult). Finally, pigments involved in colour signals are not all equally expensive to produce and/or acquire. Carotenoid-based colours, for example, are supposed to reflect quality because carotenoids cannot be synthesized by birds, but must be ingested with food (Kodric-Brown 1989; Hill & Montgomerie 1994). Only birds in good nutritional condition with access to large quantities of carotenoids could thus develop brighter colouration. Moreover, carotenoids might provide the mechanistic link between immunity and sexual signals because they play an important role in stimulating both B-and T-lymphocytes (see Lozano 1994). Individuals would thus face the dilemma of immobilizing carotenoids in the signals at the expense of immune defence (Olson & Owens 1998). The bib of black feathers in the house sparrow is a melanin-based trait. Contrary to carotenoids, melanin is synthesized by birds from basic dietary components (i.e. tyrosine), and it has been suggested that melanin-based colours can be cheap to produce (Hill & Brawner 1998). To our knowledge only one study has provided supportive evidence for the condition-dependent hypothesis of sexual advertisement based on melanin pigments (Veiga & Puerta 1996). Our results are in agreement with those reported by Hill & Brawner (1998), since we did not find any effect of food quality on the development of badge size and colour.

In this study we focused only on potential production costs of a sexual signal. Secondary sexual traits, however, might be more expensive to maintain than to produce, in particular traits involved in social interactions which are used throughout the year. As already stated, the badge of house sparrows is used in male–male contests as a signal of social dominance (Møller 1987a,b). Dominant males might have better access to food resources, but are also challenged by subordinates and are therefore under social control of their status. Large-badged males might also have higher testosterone titres (Evans *et al.* unpublished), and they are involved in sexual displays at higher rates than small-badged males (Møller 1990). All these activities are potentially costly and stressful, and they could determine the immunological status of individuals at a given time. In other words, we suggest that for traits which are produced once a year at low costs, the causal link between sexual signals and immunocompetence might involve costs of maintaining sexual advertisement. According to this hypothesis, sexual signals would determine immunocompetence and not the reverse. Observations carried out on male house sparrows have shown seasonal variation in the relationship between wing web swelling and badge size, the relationship being positive in autumn and winter, and negative in spring during the reproductive season (Gonzalez, Sorci & de Lope 1999).

Our finding of low production costs of sexual signal in male house sparrows raises the question of honesty of the badge as an indicator of male quality. Social control of deception might prevent cheating, suggesting that maintenance costs could be prohibitively high for low quality birds. Manipulation of badge size has provided evidence for both the view of social control of dominance status (Møller 1987b) and for survival costs of experimentally enlarged badges (Veiga 1995). We suggest that the currency of such maintenance costs may be the immune status of an individual.

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References

Andersson, M. (1994) *Sexual Selection*. Princeton University Press, Princeton.

Barnard, C.J. & Sibly, R.M. (1981) Producers and scroungers: a general model and its application to captive flocks of house sparrows. *Animal Behaviour*, **29**, 543–550.

Chandra, R.K. & Newberne, P.M. (1977) *Nutrition, Immunity and Infection*. Plenum Press, New York.

Deerenberg, C., Apanius, V., Daan, S. & Bos, N. (1997) Reproductive effort decreases antibody responsiveness. *Proceedings of the Royal Society London B*, **264**, 1021–1029.

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

Gonzalez, G., de Sorci, G. & Lope, F. (1999) Seasonal variation in the relationship between cellular immune response and badge size in male house sparrows (*Passer domesticus*). *Behavioral Ecology Sociobiology*, **46**, 117–122.

Griffith, S.C., Owens, I.P.F. & Burke, T. (1999) Female choice and annual reproductive success favour less-ornamented male house sparrows. *Proceedings of the Royal Society London B*, **266**, 765–770.

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

Hill, G.E. & Brawner,W.R. III (1998) Melanin-based plumage coloration in the house finch is unaffected by coccidial infection. *Proceedings of the Royal Society London B*, **265**, 1105–1109.

Hill, G.E. & Montgomerie, R. (1994) Plumage colour signals nutritional condition in the house finch. *Proceedings of the Royal Society London B*, **265**, 1105–1109.

Johnstone, R.A. (1995) Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biological Reviews*, **70**, 1–65.

Kodric-Brown, A. (1989) Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behavioral Ecology Sociobiology*, **17**, 199–205.

König, C. & Schmid-Hempel, P. (1995) Foraging activity and immunocompetence in workers of the bumble bee, *Bombus terrestris* L. *Proceedings of the Royal Society London B*, **260**, 225–227.

Lehmann, T. (1993) Ectoparasites: direct impact on host fitness. *Parasitology Today*, **9**, 8–13.

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.

de Lope, F., Møller, A.P. & de la Cruz, C. (1998) Parasitism, immune response and reproductive success in the house martin *Delichon urbica*. *Oecologia*, **114**, 188–193.

Lozano, A.G. (1994) Carotenoids, parasites, and sexual selection. *Oikos*, **70**, 309–311.

Mitchison, N.A. (1990) The evolution of acquired immunity to parasites. *Parasitology*, **100**, S27–S34.

Møller, A.P. (1987a) Variation in badge size in male house sparrows *Passer domesticus*: evidence for status signalling. *Animal Behaviour*, **35**, 1637–1644.

Møller, A.P. (1987b) Social control of deception among status signalling house sparrows *Passer domesticus*. *Behavioral Ecology Sociobiology*, **20**, 307–311.

Møller, A.P. (1988) Badge size in the house sparrow *Passer domesticus*: effects of intra- and intersexual selection. *Behavioral Ecology Sociobiology*, **22**, 373–378.

Møller, A.P. (1990) Sexual behaviour is related to badge size in the house sparrow *Passer domesticus*. *Behavioral Ecology Sociobiology*, **27**, 23–29.

Møller, A.P. (1997) Parasitism and the evolution of host life history. *Host–Parasite Evolution. General principles and avian models* (eds D.H. Clayton & J. Moore), pp. 105–27. Oxford University Press, Oxford.

Møller, A.P., Christe, P. & Lux, E. (1999) Parasite-mediated sexual selection: effects of parasites and host immune function. *Quarterly Review of Biology*, **74**, 3–20.

Møller, A.P. & Erritzøe, J. (1992) Acquisition of breeding coloration depends on badge size in male house sparrows *Passer domesticus*. *Behavioral Ecology Sociobiology*, **31**, 271–277.

National Research Council (1992) *Biologic Markers in Immunotoxicology*. National Academy Press, Washington.

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

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

Roitt, I., Brostoff, J. & Male, D. (1996) *Immunology*. Mosby, London.

Saino, N., Bolzern, A.M. & Møller, A.P. (1997) Immunocompetence, ornamentation, and viability of male barn swallows (*Hirundo rustica*). *Proceedings of the National Academy of Science USA*, **94**, 549–552.

SAS (1996) *SAS User's Guide: statistics*. Version 6.12 edition. SAS Institute, Cary.

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

Siva-Jothy, M. (1995) 'Immunocompetence': conspicuous by its absence. *Trends in Ecology and Evolution*, **10**, 205–206.

Sokal, R.R. & Rohlf, F.J. (1981) *Biometry*. Freeman, San Francisco.

Sorci, G., Soler, J.J. & Møller, A.P. (1997) Reduced immunocompetence of nestlings of replacement clutches in the European magpie (*Pica pica*). *Proceedings of the Royal Society London, B*, **264**, 1593–1598.

Summers-Smith, J.D. (1988) *The Sparrows*. T. & A.D. Poyser, Calton.

Svensson, L. (1994) *Identification Guide to European Passerines*. 3rd edn. L. Svensson, Stockholm.

Veiga, J.P. (1995) Honest signalling and the survival cost of badges in the house sparrow. *Evolution*, **49**, 570–572.

Veiga, J.P. & Puerta, M. (1996) Nutritional constraints determine the expression of a sexual trait in House sparrow, *Passer domesticus*. *Proceedings of the Royal Society London B*, **263**, 229–234.

Wedekind, C. & Folstad, I. (1994) Adaptive or non adaptive immunosuppresion by sex hormones? *American Naturalist*, **143**, 936–938.

Westneat, D.F. & Birkhead, T.R. (1998) Alternative hypotheses linking the immune system and mate choice for good genes. *Proceedings of the Royal Society London B*, **265**, 1065–1073.

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