ANIMAL BEHAVIOUR, 2003, **65**, 1125–1131
doi:10.1006/anbe.2003.2142

# Bill colour and immunocompetence in the European blackbird

**B. FAIVRE*, M. PRÉAULT*, F. SALVADORI†, M. THÉRY‡, M. GAILLARD* & FRANK CÉZILLY***
*Equipe Ecologie Evolutive (UMR CNRS 5561), Université de Bourgogne
†Laboratoire d'Immunologie comparée (UMR CNRS 5548), Université de Bourgogne
‡Laboratoire d'Ecologie Générale, Equipe Evolution des Systèmes Sociaux (UMR CNRS 8571 ECOTROP),
Muséum National d'Histoire Naturelle

*(Received 11 January 2002; initial acceptance 9 April 2002;
final acceptance 15 October 2002; MS. number: 7199)*

The level of expression of secondary sexual characters has been suggested to signal male ability to resist parasitic infestations. To test this idea, several studies have examined the link between sexual signals and immunocompetence in birds. However, most of them have used only a single aspect of immune response to evaluate immunocompetence. We investigated the relation between bill colour and immunocompetence in captive male European blackbirds, *Turdus merula*, during the breeding season by assessing both cell-mediated and humoral components of the immune system. The blackbird is a sexually dimorphic species with bill colour varying from yellow to orange in males. Humoral immunity was assessed by measuring both primary and secondary responses to sheep red blood cell inoculation. Cell-mediated immunity was estimated with a delayed cutaneous hypersensitivity response to an injection of a mitogen (phytohaemagglutinin). No relation was found between male bill colour and the primary humoral response. However, males with orange bills showed a lower secondary humoral response but a higher cell-mediated immune response than males with yellow bills. Thus, the relation between immunocompetence and a secondary sexual trait may differ markedly depending on which component of the immune system is under consideration. We discuss our results in relation to mechanisms involved in sexual selection.

Over the last 20 years, increasing interest has been devoted to the role of parasites and diseases in sexual selection (Hamilton & Zuk 1982; Loye & Zuk 1991; Zuk 1996). More recently, several studies have examined the possible trade-off between investment in secondary sexual characters and immunocompetence, particularly in male birds (Saino et al. 1997a, b; Zuk & Johnsen 1998; Gonzalez et al. 1999a, b; Verhulst et al. 1999). The results of these studies are extremely variable and sometimes confusing. Some have found that highly ornamented males have reduced immunocompetence. For example, lymphocyte concentration was negatively correlated with comb size in male red junglefowl, *Gallus gallus* (Zuk et al. 1995). Conversely, other studies found a positive relation between measures of immunocompetence and the expression of sexual signals. For instance, Gonzalez et al. (1999a) observed that cell-mediated immune response increased with badge size in male house sparrows, *Passer domesticus*, after the breeding season.

This apparent discrepancy between studies may result from several factors. Various methods have been used to assess immunocompetence including measuring immunologically active cell numbers (Ots & Hõrak 1996; Zuk & Johnsen 1998) and the relative size of immunological organs such as the spleen or bursa of Fabricius (Møller et al. 1996). The reliability of these techniques when used as assays of immunocompetence has, however, been questioned (Siva-Jothy 1995; Ots et al. 1998; Zuk & Johnsen 1998; Owens & Wilson 1999; Norris & Evans 2000). Consequently, behavioural ecologists have more recently used challenge techniques, in which the immune system is exposed to a novel antigen, and the subsequent immune response is then quantified (Deerenberg et al. 1997; Saino et al. 1997a, b; Zuk & Johnsen 1998; Gonzalez et al. 1999a, b). Nevertheless, most ecological studies have used a single technique that quantifies only one component (humoral or cell mediated) of the immune system. If a trade-off exists between investment in different immune components, studies that consider only a single immune system

*Correspondence: B. Faivre, Equipe Ecologie Evolutive (UMR CNRS 5561), Université de Bourgogne, 6 Blvd Gabriel, 21000 Dijon, France (email: bfaivre@u-bourgogne.fr). M. Théry is at Laboratoire d'Ecologie Générale, Equipe Evolution des Systèmes Sociaux (UMR CNRS 8571 ECOTROP), Muséum National d'Histoire Naturelle, 4 Avenue du Petit Château 91800 Brunoy, France.*

0003–3472/03/$30.00/0

component could produce biased results (Norris & Evans 2000).

Different morphological characters have also been examined in relation to immunocompetence measures. For instance, some studies considered plumage characteristics such as the length of tail feathers (Møller et al. 1996) or coloration (Gonzalez et al. 1999a, b), and others investigated variation in comb size in relation to immune response (Zuk & Johnsen 1998; Verhulst et al. 1999). However, endocrinal control and patterns of development and temporal variation differ for such traits (Owens & Short 1995).

Third, seasonal variability in the relation between the sexual signal and immunocompetence remains poorly explored and is rarely discussed. However, Gonzalez et al. (1999a) observed that male house sparrows with a large black bib presented a T-cell reactivity that was lower during the breeding season, but higher outside the breeding season, when these males were dominant over access to trophic resources. Similarly, Zuk & Johnsen (1998) have reported a negative correlation between comb size of male red junglefowl and T-cell reactivity during the breeding season and a positive one before the breeding season. Thus, showy males may pay only temporary costs of immunosuppression.

To elucidate the importance of trade-offs between life history traits and immunocompetence, studies that link sexual signals with more than one immune component are required. As emphasized by Norris & Evans (2000), no ecological study has assessed both cell-mediated and humoral components of the immune system within a single experiment on a wild population.

We investigated the relation between bill coloration and two measures of immune response in male European blackbirds, *Turdus merula*. This socially monogamous species (Snow 1958; Desrochers & Magrath 1996) shows a marked sexual dimorphism. The plumage is entirely black in males, and brownish and relatively cryptic in females. In addition, the bill and eye ring in males are bright yellow to orange, and some variation in the intensity of bill colour between individuals can be observed within natural populations. Females have brown to dark brown bills, with small dull yellow areas in some individuals (Cramp 1988). In addition, early breeding females in good body condition mate with males with more orange bills (Faivre et al. 2001).

Blackbirds are a suitable model for testing the relation between sexual signal and immune function because (1) bill colour is based on carotenoid pigments (Fox & Vevers 1960; F. Balleville-Nabet, personal communication) which are involved in the elaboration of immune defences (Lozano 1994, 2001; Olson & Owens 1998; Owens & Wilson 1999) and (2) bill colour appears to be related to some measures of immune capacity. Hatchwell et al. (2001) observed a strong tendency for bill colour to be paler in male blackbirds with high blood parasite loads, suggesting that bill colour might be a reliable index of male ability to resist infection. In addition, Møller et al. (2000) reported that male blackbirds with brighter bills have smaller spleens. In this study, we examined how the bill colour of breeding male blackbirds correlates with two components of immune function: the T-cell-mediated immune response and the humoral immune response.

## METHODS

### General Methods

We caught male blackbirds with mist nets in Dijon, eastern France, and its surroundings, from the end of May to mid-July during the second part of the 1999 breeding season. Birds were kept in individual indoor cages (0.60 × 0.40 m and 0.40 m hight) under an artificial photoperiod that matched the natural light cycle (16–17 h of light/day), at a controlled temperature of 20°C. The birds could not see each other, but could hear each other. Water and food were provided ad libitum throughout the trial. Bill colour was assessed (by BF and MP) when birds were caged, before injection, by comparison with a coloration index derived by the Yolk Colour Fan Roche (Neuilly-sur-Seine, France) ranging from index 1 (pale yellow) to index 15 (orange). The selected colours were characterized by tristimulus values of the CIE-1931-standard colorimetric system. In a previous study, this coloration index was compared with an objective measure of bill coloration using a quantum spectroradiometer (Endler 1990; Endler & Théry 1996), and was consistent with the measurements obtained with the spectroradiometer (Faivre et al. 2001), justifying the use of the colour index in the field.

### Ethical Note

We caught 40 males during the breeding season. Each individual was kept in a cage for 4 weeks and was returned to its place of capture after the experiment. Birds were fed with complete feeding stuff for birds (Orlux, Roeselare, Belgium; Provenfig—Deltazur, Tours, France), and especially for mynahs and thrushes. Earthworms and apple were provided daily. Individuals were carefully restrained by hand in a specific room during bill colour measurement and injections. One person held the bird and another injected the SRBC or PHA (see below). The procedures lasted up to 10 min and we verified that the birds resumed ‘normal’ behaviour (preening, feeding, etc.) a few minutes afterwards. The use of anaesthetics was not advised by the regional veterinary agency for these manipulations because anaesthesia (1) decreases blood pressure which complicates blood sampling and (2) is not necessary before injections of PHA and SRBC. We verified that birds had maintained their body weight, and that the injections did not cause visible wounds or skin irritation, before releasing them. Males that were known to be rearing nestlings at the time of capture were released immediately. Several injected males have been recaptured at the capture site, even 1–2 years after release, suggesting that the injections and blood sampling had no adverse effect on survival or fidelity to the breeding site.

The study was done under licences to catch and keep birds (Autorisation de capture d’oiseaux Museum Paris, Convention ONCFS) and to conduct physiological experiments on vertebrates (from the Ministère de l'Agriculture et de la Pêche).

## Humoral Immune Response

To assay the primary humoral response, we used intraperitoneal injections of 400 μl of a phosphate-buffered saline solution (PBS, Sigma diagnostics, L'Isle D'Abeau Chesne, France) containing $5 \times 10^5$ sheep red blood cells (SRBC)/μl of solution (2% SRBC, Biomérieux, Lyon, France) to immunize 31 males 1 week after capture (Saino & Møller 1996; Ros et al. 1997). The amount of injected SRBC suspension was adjusted to the size of this bird species (Aitken & Parry 1974). A subsample of 15 randomly chosen birds were injected again in the same way 14 days later to assess the secondary humoral response. We investigated the time course of antibody responses by taking blood samples (200 μl) before the first immunization, as well as on days 3 (J3), 7 (J7) and 14 (J14) after the first immunization, and on days 3 (RJ3) and 7 (RJ7) after the second immunization (Fig. 1). Blood samples were taken from the brachial vein into heparinized capillary tubes. After centrifugation (4000 rpm, 15 min, 4°C), plasma was stored at −20°C to await analysis.

We estimated individual antibody responses with the haemagglutination method (Abbas et al. 1994). The plasma was heated to 56°C for 30 min to prevent the lysis of red blood cells by complement. It was then serially diluted in PBS in U-shaped microtitre plates (dilution: 1, 1/2, 1/4, 1/8, 1/16 . . .). An equal volume of SRBC in NaCl (0.15 M) (SRBC 1%) was added to these dilutions. After agitation, the plates were covered by parafilm and incubated at 37°C for 60 min; agglutination was visually determined. Titres were expressed as the reciprocal of the highest dilution of serum showing positive haemagglutination. Agglutination tests were blind with respect to bill colour.

## T-cell-mediated Immune Response

We estimated cell-mediated immunity with a delayed cutaneous hypersensitivity response (Lochmiller et al. 1993; Zuk & Johnsen 1998; Gonzalez et al. 1999a, b). Injection of a mitogen, the phytohaemagglutinin (PHA), causes T-lymphocytes to proliferate and react to foreign antigens by producing a local swelling. For 31 caged males, we marked a point on the wing web (patagium) of both wings with permanent nontoxic ink. The thickness of each patagium was measured to the nearest 0.01 mm with a pressure-sensitive spessimeter (Teclock, SM-112). One of the wing webs was then injected intradermally with 125 μl of phytohaemagglutinin (Sigma diagnostics) suspended in phosphate-buffered saline solution (PBS) (10 mg/ml), and the other wing web was injected with 125 μl of PBS. After 24 h, we measured the web thickness at both wing points. The cellular immune response was calculated as the difference in patagium thickness 24 h after the injection with PHA compared to the PBS control.

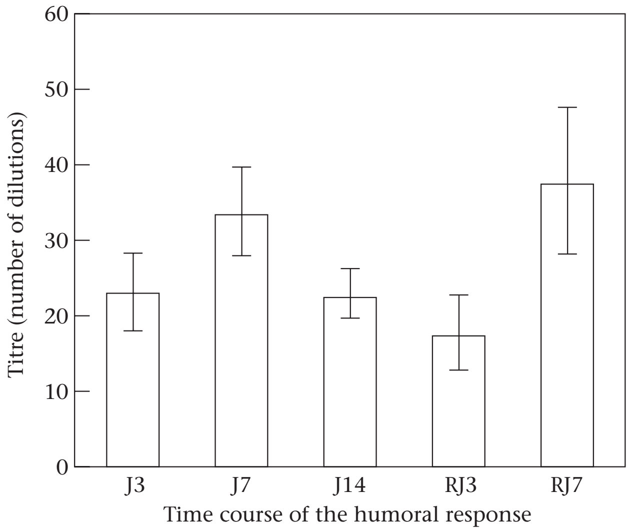

**Figure 1.** Time course of the primary humoral response ($N$=31) 3 (J3), 7 (J7) and 14 (J14) days after a first injection of sheep red blood cells, and of the secondary humoral response ($N$=12) 3 (RJ3) and 7 (RJ7) days after a second injection. Means are given±SE.

For all measurements, the wing web was measured twice to assess measurement error. To detect any relation between the two immune response measurements, the 31 males were allocated to one of three groups: seven were injected once with SRBC 2 weeks before the PHA injection (SRBC1+PHA); 15 received two injections of SRBC, 3 weeks and 1 week before the PHA injection (SRBC2+PHA); and nine were treated only with PHA (PHA). The 22 individuals injected with SRBC before PHA injection were included in samples used to estimate humoral responses.

For each bird, immunological challenge began 1 week after its capture.

We used two-tailed statistical tests for comparisons and correlations.

# RESULTS

## Humoral Immune Response

### *Primary antibody response*

Antibody production was highest 7 days after the first SRBC injection (Friedman test: $\chi^2_2$=10.065, $P$=0.006; Fig. 1). For each male, the primary humoral response was measured as the difference between the titre observed 7 days after the injection and the titre obtained before immunization. No significant correlation was observed between the primary humoral response and bill colour index (Spearman rank correlation: $r_S$=0.116, $N$=31, $P$=0.527).

### *Secondary antibody response*

Among the 15 males that received the booster, three were removed from the analysis because we did not obtain sufficient blood on day 7. Antibody production

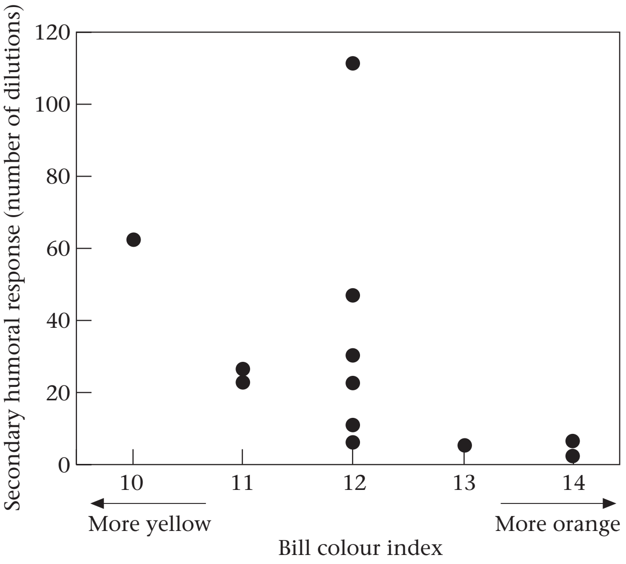

**Figure 2.** Relation between bill colour index and the secondary humoral response of captive male blackbirds to a second injection of sheep red blood cells.

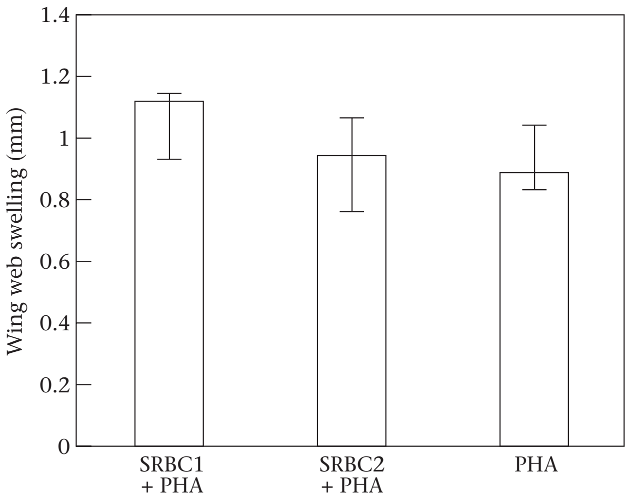

**Figure 3.** Median values for the wing web swelling of captive male blackbirds in response to injection of phytohaemagglutinin (PHA). Vertical lines show the interquartile range. Among the 31 males injected with PHA, seven had been previously injected once with SRBC (SRBC1+PHA), 15 had received two injections with SRBC (SRBC2+PHA) and nine were injected only with PHA (PHA).

was greatest 7 days after the booster (Wilcoxon test: $T$=3.00, $N$=12, $P$=0.02; Fig. 1). For each male, the secondary humoral response corresponded to the titre observed 7 days after the booster (RJ7) minus the titre obtained before immunization (J0). The secondary humoral response was negatively and significantly correlated with colour index during the breeding season (Spearman rank correlation: $r_S = -0.669$, $N$=12, $P$=0.026; Fig. 2). Because one point (a bird with a colour score of 12) appeared to be an outlier, we did the analysis without this point and the correlation was still significant ($r_S = -0.765$, $N$=11, $P$=0.015). Males with more orange bills showed a lower response to injection of SRBC than males with more yellow bills. There was no significant correlation between the primary and the secondary humoral response ($r_S = -0.058$, $N$=12, $P$=0.847).

## T-cell-mediated Immune Response

Measurement errors of each wing web thickness were lower than asymmetry between right and left wing web patagium thickness (mixed-model two-way ANOVA with repeated measures: $F_{30,62}$=41.76, $P$<0.0001). Bill colour index did not differ significantly between the three groups of treated males (seven SRBC1+PHA, 15 SRBC2+PHA and nine PHA; Kruskal–Wallis test: $H_2$=1.916, $P$=0.383). The measured wing web swellings also did not differ significantly between these three groups ($H_2$=2.567, $P$=0.277; Fig. 3). Therefore, the three groups were pooled for subsequent analyses. In addition, the cell-mediated response was not correlated with either the primary humoral response in the SRBC1+PHA group (Spearman rank correlation: $r_S = -0.143$, $N$=7, $P$=0.571), or the secondary humoral response in the SRBC2+PHA group ($r_S$=0.095, $N$=12, $P$=0.813). There was a positive and significant relation between wing web swelling and colour index ($r_S$= 0.442, $N$=31, $P$=0.015), males with more orange bills showing a greater response to PHA injection than males with more yellow bills (Fig. 4).

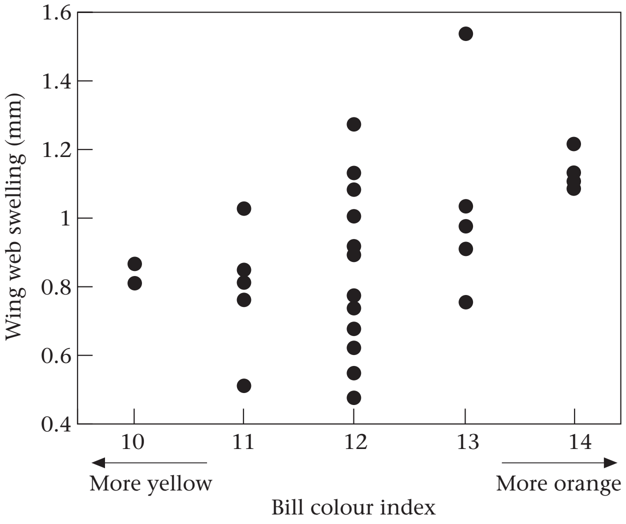

**Figure 4.** Relation between bill colour index and wing web swelling in captive male blackbirds. The thickness of the swelling in response to injection of phytohaemagglutinin into the wing web is a measure of cell-mediated immunity.

## Power Analysis

We performed a power analysis for the correlation between primary humoral response and bill colour index with $N$=31 (size of the analysed sample) and values of $r_S$ corresponding to those obtained (1) between bill colour index and the secondary humoral response ($r_S = -0.669$) and (2) between bill colour index and the cell-mediated response ($r_S$=0.442). Power for the correlation coefficients was, respectively, 0.90 and 0.65.

## DISCUSSION

The secondary humoral response was negatively correlated with the bill colour index of male blackbirds during the breeding season. This negative association between immune response and the development of a secondary sexual character supports the findings of Verhulst et al. (1999) who showed that comb size was negatively associated with immune response in male domestic chickens, *Gallus gallus domesticus*. Assuming that bill colour is testosterone dependent (Rothschild 1975) and that testosterone reduces immunity (Duffy et al. 2000), our results could be interpreted in the framework of the immunocompetence handicap hypothesis (Folstad & Karter 1992). However, a negative relation between immune system and sexual signal could also be the result of resource reallocation by males during the breeding season from immune function to the production of an intersexual signal (Gonzalez et al. 1999a).

In contrast to the humoral response, the T-cell-mediated immune response was higher in males with more orange bills. Therefore, bill colour may reflect a male's ability to develop a cell-mediated immune response. This result is similar to that obtained by Zuk & Johnsen (1998), who reported a positive correlation between comb size and cell-mediated immunocompetence during the breeding season in red junglefowl. However, such results contrast with those obtained by Gonzalez et al. (1999a) who found a negative correlation between male badge size and wing web swelling in the house sparrow during the breeding season, suggesting that males with more developed traits are less immunocompetent. Our results suggest that bill colour might be an honest signal of male quality in terms of cellular immunocompetence: given the cost of immune function (Råberg et al. 1998), only high-quality males would be able to cope with this cost and could bear extravagant traits (Hamilton & Zuk 1982).

Finally, a previous treatment with SRBC had no effect on the response to the PHA injection. Unfortunately, a restriction in the permitted number of captured birds prevented us from testing the reciprocal association (PHA followed by SRBC). However, our results suggest that the two components of the immune system may work independently of each other to some degree in birds. This suggestion is supported by the differences observed between the activation mechanisms of both immune responses (Roitt 1984; Abbas et al. 1994) and the selective influence of certain drugs that are known to suppress particular immune system components (Norris & Evans 2000).

Overall, the relations between bill colour and immunocompetence appear to be conflicting. Males with more orange bills were less immunocompetent when the humoral immune system was tested, and more immunocompetent when the cell-mediated immune system was tested. Only one study has previously reported a similar pattern. Zuk & Johnsen (1998) observed that, during the breeding season, male red junglefowl with large combs had lower levels of lymphocytes (an indirect estimate of humoral immunity) and a greater cell-mediated immunity, but before the breeding season, both the proportion of lymphocytes and cell-mediated immunity were positively correlated with comb size. Although we have no information on male blackbird immunocompetence in relation to bill colour outside the breeding season, our results, together with those of Zuk & Johnsen (1998), suggest that males could pay a cost for elaborate secondary sexual characters by decreasing investment in only one component of the immune system. They also support Norris & Evans's (2000) hypothesis that investment in one component of the immune system could compromise an individual's ability to invest in other components.

In the context of a trade-off in investment between different components of the immune system, the cell-mediated component may be strategically more important for male blackbirds during the breeding season, because competition and conflicts between males are frequent at that time and add to the stress associated with courtship interactions (Braude et al. 1999). Aggressive encounters between male blackbirds occur usually during territory defence but also during pair formation or competition for food, and, occasionally, injuries may lead to the death of one of the fighting males (Snow 1958). Consequently, cell-mediated immunity may often be mobilized to heal injuries and subsequent infections. High-quality males may be able to pay the cost of showing a highly developed sexual signal while maintaining the more strategic component of the immune system in the breeding season, that is cell-mediated immunity. Alternatively, if high levels of testosterone induce increased levels of aggression in male blackbirds, as observed in other songbirds (Harding 1989), selection may occur for the reallocation of resources towards the cellular component of the immune system in males with high testosterone levels, independent of their intrinsic quality, thus explaining the observed correlations between bill colour and immunocompetence.

Although further investigations are required to understand how resource allocation between the various components of the immune system is regulated, our results show that a simple trade-off may exist not between a secondary sexual character and immune function, but between the two components of immune function themselves. This highlights the importance of measuring more than one aspect of the immune system in studies of immuncompetence (Norris & Evans 2000).

Bill colour in male blackbirds is a carotenoid-dependent trait (Rothschild 1975; F. Belleville-Nabet, personnal communication), which may act as an honest signal of immunocompetence (Møller et al. 2000; Hatchwell et al. 2001; this study). Particular attention has been devoted to carotenoid-based coloration in studies of sexual selection because carotenoid pigments have a positive action on both the immune system and free-radical scavenging (Bendich 1989; Chew 1993; Lozano 1994, 2001; Møller et al. 2000). Because birds cannot synthesize carotenoids directly, they must be obtained from food and hence are supposedly a limited resource (Goodwin 1984; Hill 1992). Recent work suggests that males may face a trade-off between allocating carotenoids to coloration and immunological defences (Møller et al. 1996; Hill 1999; Saino et al. 1999). Carotenoid-based pigmentation would thus be a condition-dependent trait showing the ability of an individual to secure high-quality resources (Brush & Power 1976; Hill 1992; Gray 1996) and safeguard its health and immunocompetence (Hudon 1994; Zuk & Johnsen 1998; Møller et al. 2000). Recently, carotenoid-based plumage coloration has been shown to reflect parasite infection in two passerine species (McGraw & Hill 2000; Hõrak et al. 2001).

The precise mechanism of a possible trade-off in allocation of carotenoids between coloration and immunological defence remains uncertain (Hill 1999; Lozano 2001). More specifically, little is known about the interplay between the role of carotenoids as stimulants, and the role of testosterone as suppressant, of the immune system. Although there is some evidence that the production of sexual traits is stimulated by a rise in circulating testosterone levels in some bird species (Eens et al. 2000; Peters 2000; Stoehr & Hill 2000), little is known of the relation between bill colour and circulating levels of testosterone in male blackbirds. In addition, the immunosuppressive effect of testosterone has been questioned (e.g. Hasselquist et al. 1999). The ability of testosterone-dependent traits to reflect male quality may be difficult to assess because of numerous interactions with other hormones such as corticosterone (Evans et al. 2000), such that the effect of testosterone on secondary sexual characters may be modulated by the general condition of individuals (Birkhead et al. 1998; Duckworth et al. 2001). Further studies should assess the relative importance of carotenoids and testosterone in the relation between bill colour and immunocompetence in blackbirds.

## Acknowledgments

Paul Alibert, Philipp Heeb, Stewart Plaistow, Alexandre Roulin and Matt Wood provided helpful comments on the manuscript. Véronique Laurens provided valuable advice on immunological techniques. Catherine Hamelin and Jean Claude Branellec (ROCHE) kindly provided Yolk Colour Fan. This study was supported by the Contrat de plan Etat-Région Bourgogne, the Programme National: Dynamique de la Biodiversité et Environnement—Populations fragmentées, extinctions, sélection d'habitat et Biologie de la Conservation, the Institut Français de la Biodiversité and the GDR CNRS 2155 Ecologie Comportementale. This work would not have been possible without technical assistance from Yann Doisneau and Jean Secondi.

## References

**Abbas, K. A., Lichtman, A. H. & Pober, J. S.** 1994. *Cellular and Molecular Immunology*. 2nd edn. Philadelphia: W. B. Saunders.

**Aitken, I. D. & Parry, S. H.** 1974. The comparative serological response of the chicken, pheasant and quail to soluble and particulate antigen. *Immunology*, **24**, 623–629.

**Bendich, A.** 1989. Carotenoids and the immune response. *Journal of Nutrition*, **119**, 112–115.

**Birkhead, T. R., Fletcher, F. & Pellatt, E. J.** 1998. Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. *Behavioral Ecology and Sociobiology*, **44**, 179–191.

**Braude, S., Tang-Martinez, Z. & Taylor, G. T.** 1999. Stress, testosterone, and the immunoredistribution hypothesis. *Behavioral Ecology*, **10**, 346–350.

**Brush, A. H. & Power, M.** 1976. House finch pigmentation: carotenoid metabolism and the effect of diet. *Auk*, **93**, 725–739.

**Chew, B. P.** 1993. Role of carotenoids in the immune response. *Journal of Dairy Science*, **76**, 2804–2811.

**Cramp, S.** 1988. *Handbook of the Birds of Europe, the Middle East and North Africa. Vol. 5*. Oxford: Oxford University Press.

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

**Desrochers, A. & Magrath, R. D.** 1996. Divorce in the European blackbird: seeking greener pastures? In: *Partnerships in Birds* (Ed. by J. M. Black), pp. 177–191. Oxford: Oxford University Press.

**Duckworth, R. A., Mendonça, M. T. & Hill, G. E.** 2001. A condition dependent link between testosterone and disease resistance in the house finch. *Proceedings of the Royal Society of London, Series B*, **268**, 2467–2472.

**Duffy, D. L., Bentley, G. E., Drazen, D. L. & Ball, G. F.** 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. *Behavioral Ecolology*, **11**, 654–662.

**Eens, M., Van Duyse, E., Bergham, L. & Pinxten, R.** 2000. Shield characteristics are testosterone-dependent in both male and female moorhens. *Hormones and Behavior*, **37**, 126–134.

**Endler, J. A.** 1990. On the measurement and classification of color in studies of animal color patterns. *Biological Journal of the Linnean Society of London*, **41**, 315–352.

**Endler, J. A. & Théry, M.** 1996. Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest dwelling birds. *American Naturalist*, **148**, 421–452.

**Evans, M. R., Goldsmith, A. R. & Norris, S. R. A.** 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behavioral Ecology and Sociobiology*, **47**, 156–163.

**Faivre, B., Préault, M., Théry, M., Secondi, J., Patris, B. & Cézilly, F.** 2001. Pairing pattern, morphological characters and individual quality in an urban population of blackbirds *Turdus merula*. *Animal Behaviour*, **61**, 969–974.

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

**Fox, D. L. & Vevers, G.** 1960. *The Nature of Animal Colours*. London: Sedgewick & Jackson.

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

**Gonzalez, G., Sorci, G., Møller, A. P., Ninni, P., Haussy, C. & De Lope, F.** 1999b. Immunocompetence and condition-dependent advertisement in male house sparrows (*Passer domesticus*). *Journal of Animal Ecology*, **68**, 1225–1234.

**Goodwin, T. W.** 1984. *The Biochemistry of Carotenoids. Vol 2. Animals*. 2nd edn. New York: Chapman & Hall.

**Gray, D. A.** 1996. Carotenoids and sexual dichromatism in North-American passerine birds. *American Naturalist*, **148,** 453–480.

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

**Harding, C. F.** 1989. Interactions of androgens and estrogens in the modulation of social behavior in male songbirds. In: *Ethoexperimental Approaches to the Study of Behavior* (Ed. by R. J. Blanchard, P. F. Brain, D. C. Blanchard & S. Parmigiani), pp. 558–579. Dordrecht: Kluwer Academic.

**Hasselquist, D., Marsh, J. A., Sherman, P. W. & Wingfield, J. C.** 1999. Is avian humoral immunocompetence suppressed by testosterone? *Behavioral Ecology and Sociobiology*, **45,** 167–175.

**Hatchwell, B. J., Wood, M. J., Anwar, M., Chamberlain, D. E. & Perrins, C. M.** 2001. The haematozoan parasites of common blackbirds *Turdus merula*: associations with host condition. *Ibis*, **143,** 420–426.

**Hill, G. E.** 1992. Proximate basis of variation in carotenoid pigmentation in male house finches. *Auk*, **109,** 1–12.

**Hill, G. E.** 1999. Is there an immunological cost to carotenoid-based ornamental coloration? *American Naturalist*, **154,** 589–595.

**Hõrak, P., Ots, I., Vellau, H., Spottiswoode, C. & Møller, A. P.** 2001. Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. *Oecologia*, **126,** 166–173.

**Hudon, J.** 1994. Showiness, carotenoids, and captivity: a comment on Hill 1992. *Auk*, **111,** 218–221.

**Lochmiller, R. L., Vestrey, M. R. & Boren, J. C.** 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk*, **110,** 503–510.

**Loye, J. E. & Zuk, M.** 1991. *Bird–Parasite Interactions: Ecology, Evolution and Behaviour*. Oxford: Oxford University Press.

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

**Lozano, G. A.** 2001. Carotenoids, immunity, and sexual selection: comparing apples and oranges? *American Naturalist*, **158,** 200–203.

**McGraw, K. J. & Hill, G. E.** 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proceedings of the Royal Society of London, Series B*, **267,** 1525–1531.

**Møller, A. P., Kimball, R. T. & Erritzøe, J.** 1996. Sexual ornamentation, condition, and immune defence in the house sparrow *Passer domesticus*. *Behavioral Ecology and Sociobiology*, **39,** 317–322.

**Møller, A. P., Biard, C., Blount, J. D., Houston, D. C., Ninni, P., Saino, N. & Surai, P. F.** 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Poultry and Avian Biology Reviews*, **11,** 137–159.

**Norris, K. & Evans, M. R.** 2000. Ecological immunology: life-history trade-offs and immune defense in birds. *Behavioral Ecology*, **11,** 19–26.

**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.

**Ots, I. & Hõrak, P.** 1996. Great tits *Parus major* trade health for reproduction. *Proceedings of the Royal Society of London, Series B*, **263,** 1443–1447.

**Ots, I., Murumägi, A. & Hõrak, P.** 1998. Haematological health states indices of reproducing great tits: methodology and sources of natural variation. *Functional Ecology*, **12,** 700–707.

**Owens, I. P. F. & Short, R. V.** 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends in Ecology and Evolution*, **10,** 44–47.

**Owens, I. P. F. & Wilson, K.** 1999. Immunocompetence: a neglected life history trait or conspicuous red herring? *Trends in Ecology and Evolution*, **14,** 170–172.

**Peters, A.** 2000. Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. *Proceedings of the Royal Society of London, Series B*, **267,** 883–889.

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

**Roitt, I. M.** 1984. *Essential Immunology*. 5th edn. London: Blackwell Scientific.

**Ros, A. F. H., Groothuis, T. G. G. & Apanius, V.** 1997. The relation among gonadal steroids, immunocompetence, body mass and behavior in young black-headed gulls (*Larus ridibundus*). *American Naturalist*, **150,** 201–219.

**Rothschild, M.** 1975. Remarks on carotenoids in the evolution of signals. In: *Coevolution of Animals and Plants* (Ed. by L. E. Gilbert & P. H. Raven), pp. 20–51. Austin: University of Texas Press.

**Saino, N. & Møller, A. P.** 1996. Sexual ornamentation and imunocompetence in the barn swallow. *Behavioral Ecology*, **7,** 227–232.

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

**Saino, N., Galeotti, P., Sacchi, R. & Møller, A. P.** 1997b. Song and immunological condition in male barn swallows (*Hirundo rustica*). *Behavioral Ecology*, **8,** 364–371.

**Saino, N., Stradi, R., Ninni, P. & Møller, A. P.** 1999. Carotenoid plasma concentration, immune profile and plumage ornamentation of male barn swallows (*Hirundo rustica*). *American Naturalist*, **154,** 441–448.

**Siva-Jothy, M. T.** 1995. 'Immunocompetence': conspicuous by its absence. *Trends in Ecology and Evolution*, **10,** 205–206.

**Snow, D. W.** 1958. *A Study of Blackbirds*. London: Allen & Unwin.

**Stoehr, A. M. & Hill, G. E.** 2001. The effects of elevated testosterone on plumage hue in male house finches. *Journal of Avian Biology*, **32,** 153–158.

**Verhulst, S., Dieleman, S. J. & Parmentier, H. K.** 1999. A trade off between immunocompetence and sexual ornamentation in domestic fowl. *Proceedings of the National Academy of Sciences, U.S.A.*, **96,** 4478–4481.

**Zuk, M.** 1996. Disease, endocrine-immune interactions, and sexual selection. *Ecology*, **77,** 1037–1042.

**Zuk, M. & Johnsen, T. S.** 1998. Seasonal changes in the relationship between ornamentation and immune response in red jungle fowl. *Proceedings of the Royal Society of London, Series B*, **265,** 1631–1635.

**Zuk, M., Johnsen, T. S. & MacLarty, T.** 1995. Endocrine-immune interactions, ornaments and mate choice in red jungle fowl. *Proceedings of the Royal Society of London, Series B*, **260,** 205–210.