THE ROYAL SOCIETY

Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent

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The immunocompetence handicap hypothesis proposes that the immunosuppressive effect of testosterone enforces honesty of sexual signalling via a physiological trade-off between signal intensity and immunocompetence. However, evidence that testosterone is immunosuppressive is scant, particularly in birds. I studied the correlation between immunocompetence and testosterone in superb fairy-wrens (*Malurus cyaneus*), a species with intense intersexual selection. Males are seasonally dichromatic and testosterone increases during the moult from dull brown eclipse plumage into bright nuptial plumage. I determined the primary antibody response to immunization with sheep red blood cells (SRBCs) in (i) control and testosterone-implanted males in captivity, and (ii) a cross-section of free-living males with basal and elevated testosterone (in eclipse plumage, moulting and in nuptial plumage). Experimental treatment with testosterone decreased the likelihood of an antibody response to SRBCs in captive birds. In contrast, free-living males which had acquired the nuptial plumage and had naturally elevated testosterone were more likely to respond to SRBCs than males in eclipse plumage with basal testosterone levels. The association between higher immunocompetence and higher immunosuppressive testosterone could arise if both are positively correlated with male phenotypic quality. In addition, the association could result if males compensate for potential immunosuppression by enhancing their humoral immune responses, particularly since high testosterone is linked to other demanding activities such as moulting and courtship displays.

Keywords: sexual selection; immunocompetence; life-history trade-off; honest signalling; sexual dichromatism

1. INTRODUCTION

The maintenance of variability in ornaments under intense directional sexual selection is one of the most contentious issues in sexual selection theory (Andersson & Iwasa 1996). In a seminal paper, Hamilton & Zuk (1982) suggested that the answer might lie in the never-ending arms race between pathogens and their hosts, resulting in continuous evolution of optimal host-resistance types. Superior resistance results in lower pathogen load and higher condition, which is reflected in more elaborate secondary sexual ornaments. Such a condition-dependent development of sexual characters should ensure honesty in sexual signalling (Johnstone 1995).

Folstad & Karter (1992) proposed that a correlation between the level of signalling and male condition could be maintained by a trade-off between ornament size and immunocompetence which is regulated by testosterone. They postulated that testosterone acts as a double-edged sword by stimulating the development of secondary sexual characters on the one hand and suppressing the immune system on the other. The concomitant immunosuppression—an unavoidable cost of testosterone-dependent sexual signals—would ensure honesty in signalling. Thus, the three requirements of the hypothesis are that (i) females prefer more exaggerated sexual signals, which (ii) require more testosterone, which (iii) in turn is immunosuppressive. It has been shown that females prefer males with more exaggerated sexual displays in many studies of sexual selection (Andersson & Iwasa 1996). Likewise, many sexual characters and behaviours are testosterone dependent (Folstad & Karter 1992; Andersson & Iwasa 1996), although this is not always true for bright male plumage (Owens & Short 1995). However, evidence for an obligate immunosuppressive effect of testosterone is incomplete, particularly for birds.

A variety of immunosuppressive effects of testosterone on different components of the immune system have been described for mammals (Grossman 1984). In birds, many studies describing the relationship between testosterone and immunocompetence have relied on correlations which do not provide conclusive evidence of immunosuppression by testosterone. To circumvent this, some researchers have employed experimental manipulations of testosterone levels. Two studies found effects of elevated testosterone on indirect indicators of immune responsiveness: a decrease in lymphocyte numbers (Zuk *et al*. 1995), and an increase in parasite load and a transient decrease in immunoglobulin levels (Saino *et al*. 1995). A third study found that antibody responses tended to be suppressed during testosterone treatment, but this trend was not significant (Ros *et al*. 1997). However, in a detailed study of testosterone and antibody responsiveness in red-winged blackbirds (*Agelaius phoeniceus*), there was no indication of testosterone-induced immunosuppression (Hasselquist *et al*. 1999). Thus, evidence for an obligate immunosuppressive effect of testosterone remains equivocal.

I investigated the correlation between testosterone and immunocompetence in superb fairy-wrens (*Malurus cyaneus*). In this species female choice is intense and testosterone plays a pivotal role in the preferred character: early acquisition of the nuptial plumage (Peters *et al*. 2000).

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Males are seasonally dichromatic and they can moult into their bright nuptial plumage at any time from autumn to early summer (Mulder & Magrath 1994). Their testosterone levels increase during the moult and once males complete their nuptial plumage testosterone remains high, irrespective of the time of year (Peters *et al*. 2000). I compared immunocompetence in (i) control and testosterone-implanted males in captivity, and (ii) free-living males in eclipse plumage, in moult and in nuptial plumage, with basal and elevated testosterone. Immunocompetence was assessed as the humoral immune response after immunization with sheep red blood cells (SRBCs), a standard immunological assay (Bacon 1992) which has been used in a number of species of birds (Lochmiller *et al*. 1993; Saino & Møller 1996; Deerenberg *et al*. 1997; Birkhead *et al*. 1998).

Superb fairy-wrens are particularly suitable subjects for studying the relationship between testosterone and immunocompetence, in both an experimental and natural context. First, fairy-wrens adapt quickly to captivity. Second, in some males testosterone becomes elevated long before females initiate breeding, which eliminates the confounding effects of mating effort on immunocompetence (Deerenberg *et al*. 1997; Nordling *et al*. 1998). Finally, because testosterone increases during the moult and the timing of the moult is so variable, males with high and low testosterone can be compared simultaneously. This excludes the potentially substantial effects of seasonal changes in immunocompetence (Nelson & Demas 1996; Bentley *et al*. 1998).

2. METHODS

(a) *Study species*

Superb fairy-wrens are cooperatively breeding, territorial, long-lived, small passerines. The male plumage alternates between a female-like brown eclipse plumage and a bright blue/black nuptial plumage. The timing of the pre-nuptial moult is highly variable: males can start moulting any time between March and November (Dunn & Cockburn 1999). As soon as males have developed their bright plumage, they start to advertise their status by visiting neighbouring females to perform courtship displays (Mulder 1997). Completion of the nuptial plumage before mid-winter is essential if males are to obtain extra-pair paternity (Dunn & Cockburn 1999), which comprises 76% of all fertilizations in this species (Mulder *et al*. 1994). The timing of the moult is age dependent, with males moulting progressively earlier until they are five years old, and condition dependent, with males moulting later in harsh winters (Mulder & Magrath 1994). There is substantial variation within age groups and individual males show high consistency in their relative moult date between years (Dunn & Cockburn 1999).

(b) *Experimental design*

(i) *Testosterone treatment in captive males*

I compared antibody production after immunization with SRBCs in unimplanted, control-implanted and testosterone-implanted males in eclipse plumage. The birds were taken from a partially colour-banded population in an open woodland reserve on the lower slopes of Mount Ainslie, Canberra. Males were captured zero to two days before implantations and classified into two age categories (one year and two or more years) on the basis of their plumage characteristics (Peters *et al*. 2000). They were temporarily housed in individual cages placed in an aviary (20°C and a 12 L : 12 D cycle) and provided *ad libitum* with live mealworms, hard boiled egg and cheese blended with commercial insectivore mix (Wombaroo[1]) and fresh water.

The three experimental treatments were carried out in five consecutive groups of males or 'batches' between 28 April and 30 June 1998: (i) no implant (batches 1, 3 and 5, $n = 17$), (ii) control implant (batches 3 and 5, $n = 17$), and (iii) testosterone implant (batches 2 and 4, $n = 20$). The treatment batches did not vary significantly in age ($\chi^2 = 0.4$, d.f. = 2, $n = 54$ and $p = 0.8$). The testosterone-implanted males were not combined with the other treatments because control males housed in the same aviary might elevate their testosterone levels in response to their testosterone-treated neighbours (K. E. Wynne-Edwards, personal communication).

Two days prior to immunization (day -2), the birds in the implant treatments were fitted with 5–6 mm empty or testosterone-filled subcutaneous silicone implants which cause a rapid (less than two days), sustained (more than one month) elevation of testosterone (see Peters *et al*. 2000). The birds were immunized with SRBCs on day 0 and a small blood sample was taken as a pre-immunization control for heterologous antibodies and for testosterone analysis. Post-immunization blood samples were taken on day 3 (batch 1), day 6 (all batches), day 9 (all batches) and day 12 (batches 1 and 2). Body mass was measured to the nearest 0.1 g at each blood sampling. During the first two to three days of captivity the birds experienced some mass loss, but in the course of the experiment all gained body mass. There was no mortality or morbidity and all males were released at the site of capture after completion of the experiment.

(ii) *Free-living males*

Antibody production after immunization with SRBCs was compared in a cross-section of free-living males captured between 10 August and 11 September 1998. At this time of year, some males are in nuptial plumage and some are moulting, while others are still in eclipse plumage. Males were captured in and around the Australian National Botanic Gardens, Canberra, a reserve of natural sclerophyll forest and irrigated plantations of Australian native flora. A colour-banded population of superb fairy-wrens has been studied here since 1988 and most birds are of known age. I captured birds in mist-nets between 06.30 and 11.30. All birds were weighed to the nearest 0.1 g. The length of the right tarsus and head–bill length were measured to the nearest 0.1 mm. The percentage of visible colourful feathers was recorded and males were classified into three categories: (i) in eclipse plumage, showing no colourful feathers ($n = 6$), (ii) moulting, producing many colourful feathers ($n = 8$), and (iii) in nuptial plumage, with completed colourful plumage ($n = 8$). At first capture a blood sample was taken as a pre-immunization control for heterologous antibodies and for testosterone analysis and the birds were immunized with SRBCs. The second blood sample, for analysis of antibody titres, was taken at recapture between eight and ten days post-immunization. This is when antibody titres peaked (figure 1) and the day of recapture did not significantly affect the probability of an immune response in the multiple regression models ($p > 0.3$).

(c) *SRBC assay for immune responsiveness*

On day 0, the birds were injected intraperitoneally with 50 ml of fresh 2% SRBCs in phosphate-buffered saline (PBS). Pre- and post-immunization blood samples were collected in heparinized capillary tubes and immediately put on ice. After centrifugation

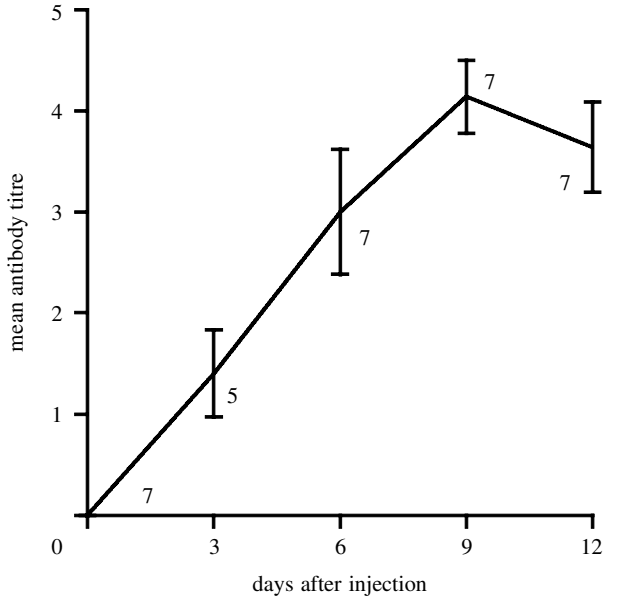

Figure 1. Time-course of the primary antibody response after intraperitoneal immunization with SRBCs on day 0. The response typically peaked on day 9: there was a very strong correlation between the titres on days 6 and 9 ($F_{2,15} = 96.7$ and $p < 0.0001$) and, apart from one exception, the titres on day 9 were higher than or equal to the titres on days 6 or 12. Samples were taken from seven males; two individuals were not sampled three days post-immunization.

for 3 min at 12 000 rpm, the haematocrit was measured and the plasma separated from the packed cells and stored at $-70\,^{\circ}$C. Antibody concentrations in the plasma sample were determined in a haemagglutination titration assay (Hudson & Hay 1976). A 20 µl sample was serially diluted in 20 µl PBS in a 96-well plate (dilution series $2–2^{12}$). The diluted samples were incubated with 20 µl of 1% SRBCs for 1 h at room temperature and the titres were expressed as the highest well number at which agglutination occurred. None of the pre-immunization samples showed signs of haemagglutination. Photographs of all plates were taken for consistency in scoring between different assays. In the comparison of immune responsiveness in free-living males, a sample of rat antiserum was included in each assay as a standard (titre 3.8 ± 0.1, $n = 5$) (antiserum provided by Dr P. McCullagh). All sheep blood was collected from the same ewe, courtesy of the Developmental Physiology Group, John Curtin School of Medical Research, Canberra.

(d) *Testosterone analysis*

Within two months of plasma collection, testosterone concentrations were measured in a commercial ^{125}I radioimmunoassay (Pantex T-direct no. 135; Pantex, Santa Monica, CA, USA) (for details see Peters *et al.* 2000). The plasma samples were analysed in duplicates of 10–20 µl and values below the assay detection limit (0.10 ng ml^{-1}) were classified as 'no detectable testosterone present'. Not all captured males yielded sufficient plasma for duplicate testosterone analysis, so the sample sizes for testosterone are lower than those for the other variables.

(e) *Statistical analysis*

(i) *Testosterone treatment in captive males*

Because the data were collected in five different batches, 'batch' was included as a factor in all analyses. The probability of mounting an antibody response to SRBCs was analysed in logistic (binomial) models, with 'response yes or no' as the dependent variable. Continuous response variables (antibody titre, body mass, haematocrit and male age) were analysed using residual maximum-likelihood (REML) models with treatment as a fixed effect and batch as a random effect. The data were analysed using Genstat^{TM} 5 release 3 (Genstat 5 Committee 1993).

(ii) *Free-living males*

The effects of testosterone level, plumage state, age, mass, haematocrit and condition on the probability of responding to SRBCs were analysed using multiple logistic regression models. Individual condition was estimated as the residual of a regression of body mass at first capture on the first principal component of tarsus and head length. Because there were strong correlations between individual condition and mass ($p < 0.001$) and testosterone, plumage category and age (all $p < 0.01$) (see also table 1), respectively, I initially used the first and second components of a principal component analysis in the regression model. The first axis, accounting for 51% of the variation, showed significant correlations with testosterone ($r = 0.61$ and $p < 0.01$), plumage category ($r = 0.56$ and $p < 0.05$) and age ($r = 0.49$ and $p < 0.05$). The second principal component, explaining 40% of the variation, was significantly correlated with body mass ($r = 0.66$ and $p < 0.01$) and condition ($r = 0.63$ and $p < 0.01$). Non-significant terms ($p > 0.05$) were progressively eliminated from the model and all eliminated variables were reintroduced to the final model to confirm the lack of contribution. The sample size did not allow robust modelling of two-factor interactions. The data for free-living males were analysed using JMP[1] 3.0.2 (SAS Institute, Inc. 1994).

The antibody titres and testosterone values were ln transformed to normalize the residuals. Back-transformed means ± s.e.s or standard errors of difference (s.e.d.s) as predicted by the models are presented in the figures and text.

3. RESULTS

(a) *Testosterone treatment in captive males*

The antibody titres on day 9 post-immunization were used as a measure of responsiveness, as this is when the response peaked (figure 1). The males were much less likely to mount an immune response when treated with testosterone ($\chi^2 = 12.5$, d.f. = 2, $n = 53$ and $p < 0.005$) (figure 2). Control-implanted males tended to be less responsive than unimplanted males ($t = -1.8$ and $p = 0.08$), while testosterone-implanted males were almost unresponsive ($t = -3.19$ and $p = 0.001$); of the latter only three out of 20 produced antibodies, far fewer than both the unimplanted (12 out of 17) or control-implanted (seven out of 16) males. The level of the response did not differ significantly between treatment groups (unimplanted = 3.5, control = 2.8 and testosterone = 1.8, s.e.d. = 1.2) (REML, $\chi^2 = 2.6$, d.f. = 2, $n = 22$ and $p = 0.3$).

The birds' testosterone levels were significantly elevated by the testosterone treatment. The average testosterone level in treated males was 8.6 ± 1.7 ng ml^{-1} ($n = 14$), which is somewhat above the natural maximum observed in this species (3.7 ng ml^{-1}), compared to 0.15 ± 0.05 ng ml^{-1} ($n = 5$) in the control-implanted males, the level of free-living males in autumn (A. Peters, unpublished data). Testosterone is an anabolic steroid (Mooradian *et al.* 1987) and treatment resulted in a rapid mass gain: their body mass at implantation (day −2) was nearly identical

Table 1. *The probability of responding to immunization with SRBCs, testosterone level, age and body mass (± s.e.) in free-living males in eclipse plumage, in moult and in nuptial plumage*

moult category	antibody response: yes	antibody response: no	testosterone (ng ml^{-1})	age (years)	body mass (g)
eclipse plumage	0	6	0.11 ± 0.2	1.3 ± 0.2	9.9 ± 0.2
moulting plumage	2	6	0.36 ± 0.2	2.4 ± 0.4	10.8 ± 0.2
nuptial plumage	5	3	0.84 ± 0.2	5.3 ± 0.7	9.7 ± 0.2
	$\chi^2 = 7.9$, d.f. = 2	$p = 0.02$	$F_{2,15} = 18.3$ $p < 0.001$	$F_{2,18} = 16.5$ $p < 0.001$	$F_{2,19} = 9.1$ $p = 0.002$

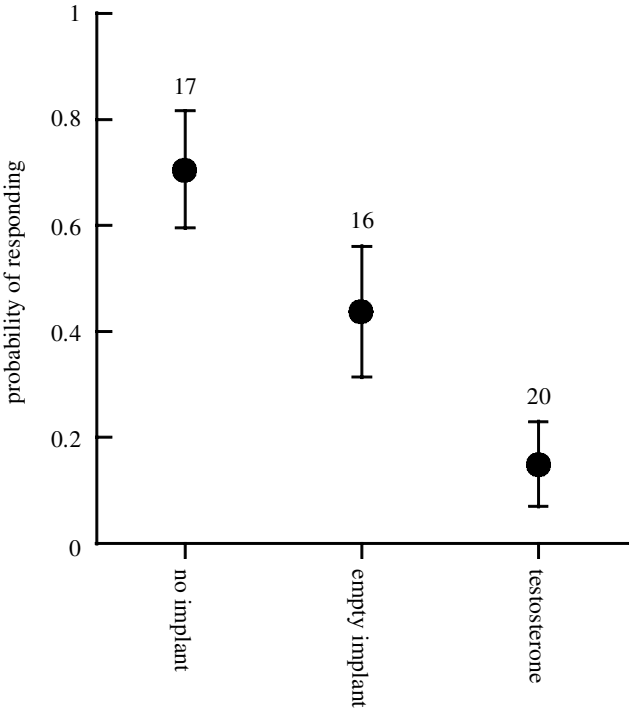

Figure 2. The probability of mounting an antibody response after intraperitoneal immunization with SRBCs in the unimplanted, control-implanted and testosterone-implanted captive males. The means ± s.e.s for the three treatments are predicted by a REML mixed model; the numbers indicate the total sample sizes.

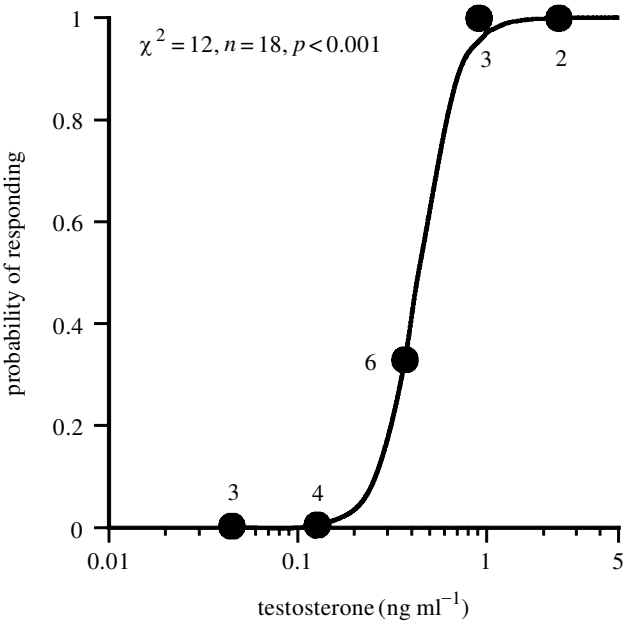

Figure 3. The relationship between circulating testosterone levels and the probability of mounting an antibody response after intraperitoneal immunization with SRBCs in free-living males. The curve represents the logistic regression of the responsiveness on the testosterone levels. The points depict the observed probabilities in males with testosterone levels of $\leqslant 0.1$, 0.1–0.3, 0.3–0.5, 0.5–0.8 and $\geqslant 0.8$ ng ml^{-1}, respectively; the numbers refer to the sample sizes for each level.

(9.4 ± 0.1 g) ($F_{1,35} = 0.05$ and $p = 0.8$), but at the time of immunization the testosterone-treated males were 5% heavier than the control-implanted males (9.9 ± 0.1 versus 9.3 ± 0.1 g) ($F_{1,35} = 15$ and $p < 0.001$). Otherwise, the three treatment groups appeared in comparable condition during the immune challenge. On average the males increased 0.4 ± 0.2 g in body mass over the course of the immune response (days 0–9) and this was not significantly different between the three treatments ($\chi^2 = 0.8$, d.f. = 2, $n = 46$ and $p = 0.7$). Moreover, the haematocrit at immunization, ($\chi^2 = 1.4$, d.f. = 2, $n = 51$ and $p = 0.5$) as well as after nine days of treatment ($\chi^2 = 2.4$, d.f. = 2, $n = 46$ and $p = 0.3$), was not significantly different between the three treatments.

(b) *Free-living males*

The probability of an immune response was correlated with plumage state: males in eclipse plumage never produced antibodies and males were most likely to respond if they had acquired nuptial plumage (table 1). Testosterone was highest in males in nuptial plumage. Moulting males had a higher body condition index ($F_{2,19} = 4.7$ and $p = 0.02$) because they were on average 1.0 g heavier (table 1). There was little variability in the magnitude of the antibody response in the free-living males which responded to immunization with SRBCs (all titres 2–3/4 and average 2.6 ± 0.2).

The birds' testosterone level appeared the key predictor of immune responsiveness: the higher the testosterone the higher the probability that the male produced antibodies to SRBCs (figure 3) ($\chi^2 = 12.7$, $n = 19$, d.f. = 1 and $p < 0.001$). Using the first and second principal components as explanatory variables in a logistic regression, it appeared that there was a highly significant effect of the principal component which summarizes testosterone, age and plumage state ($\chi^2 = 8.7$, d.f. = 1 and $p = 0.003$).

Further analysis indicated that, of these, the testosterone level had the highest explanatory power. All models which did not contain testosterone were significantly improved by the addition of testosterone, whereas both moult category ($\chi^2 = 0.1$, d.f. = 2 and $p = 0.9$) and male age ($\chi^2 = 0.4$, d.f. = 1 and $p = 0.5$) failed to improve any model which included testosterone.

Male condition had no effect on the probability of a response to SRBCs. Neither the second principal component, which reflects body condition and mass, nor either mass or body condition considered alone made a significant contribution to the regression models (all $\chi^2 < 0.6$, d.f. = 1 and $p > 0.4$), nor did haematocrit ($\chi^2 = 0.2$, d.f. = 1 and $p = 0.2$).

4. DISCUSSION

The immunocompetence handicap hypothesis proposes that testosterone-induced immunosuppression is an unavoidable link between the level of sexual signalling and immunocompetence. All three requirements of this hypothesis have now been demonstrated in superb fairy-wrens. First, females prefer males which acquire the bright nuptial plumage before mid-winter (Mulder & Magrath 1994; Dunn & Cockburn 1999). Second, an early moult requires elevated testosterone for prolonged periods (Peters *et al*. 2000). Here I show that testosterone is immunosuppressive.

(a) *Immunosuppression by testosterone*

This study provides experimental evidence of lowered immunocompetence in testosterone-treated superb fairy-wrens. Testosterone-implanted males were far less likely to mount an antibody response after immunization with SRBCs than control-implanted or unimplanted males (figure 2). There was no indication that the reduced responsiveness of testosterone-implanted males was a pathological artefact. The implants resulted in high, but physiological, testosterone levels and general body condition appeared unaffected, as indicated by the stable haematocrit and the increase in body mass during the immunological challenge.

The probability of an immune response tended to be lower in the control-implanted group than in the unimplanted males (figure 2). Although this is not quite statistically significant, this result is intriguing as it hints at an immunosuppressive effect of the implanting procedure *per se* which, to the author's knowledge, has not been reported. Immunosuppression due to handling stress is an unlikely explanation as handling is limited and similar to blood sampling (± 10 min), but perhaps an immune response associated with the insertion of a foreign body compromised the birds' responsiveness to other immunological challenges. Although this potential traumatic effect of the implanting procedure will need to be considered in future experiments, it was certainly much smaller than the effect of testosterone. After all, the probability of an immune response was three times lower in the testosterone-implanted males than in the control-implanted males.

Despite the common expectation of an immunosuppressive effect of testosterone (Folstad & Karter 1992), direct experimental evidence of this sort is limited. The two other studies which have tested the effects of testosterone treatment on antibody production found few indications of immunosuppression. Testosterone-implanted juvenile black-headed gulls (*Larus ridibundus*) showed 31% lower primary responses to SRBC immunization but, due to low statistical power, this result was not significant (Ros *et al*. 1997). Moreover, testosterone treatment did not suppress secondary antibody responses to a protein antigen in paired comparisons of captive male red-winged blackbirds (Hasselquist *et al*. 1999). This experimental design was very powerful and the negative result was surprising given that testosterone was elevated to 20–30 times natural peak levels (Hasselquist *et al*. 1999), ten times higher than in this study.

The contrasting results of these studies might reflect a lack of generality of immunosuppression by testosterone in birds. However, they may also reflect differences in the methods between studies. First, the fairy-wrens were captured just prior to the experiment, while the gulls and the blackbirds had been in captivity for many months. The extended stay in the benign conditions of captivity could have improved their immune status, making detecting immunosuppressive effects less likely. Second, whereas Ros *et al*. (1997) and this study measured the primary immune response, in a paired comparison Hasselquist *et al*. (1999) determined the secondary antibody response after repeated immunizations.

The secondary ('memory') immune response is qualitatively different from the primary response, which could be reflected in a difference in sensitivity to testosterone (Grossman 1984). A trade-off between the primary response and testosterone has been demonstrated by artificial selection experiments: in chickens selected for high or low primary responses to SRBCs, males had lower or higher testosterone, respectively (Verhulst *et al*. 1999). Moreover, there are grounds to expect that the primary response could be more sensitive to testosterone. It has been postulated that high testicular testosterone suppresses autoimmune responses to haploid, antigenic sperm (Hillgarth *et al*. 1997). If preventing recognition of sperm antigens is an adaptive immunosuppressive role of testosterone, then primary antigen recognition should be the principal target for immunosuppression. Finally, in humans, where it is well documented that women have more vigorous immunity than men, primary responses are most sexually dimorphic (Olsen & Kovacs 1996). This indicates that it is important to consider potential differences between the effects of testosterone on the primary or secondary immune response.

(b) *Why a positive correlation in free-living males?*

Both moulting males and males in nuptial plumage engage in costly activities at low winter temperatures. Moulting is one of the most energy-consuming processes in small birds and the energetic costs of moulting increase dramatically at low temperatures (Payne 1972). Likewise, males in nuptial plumage advertise their status by vigorously displaying to neighbouring females (Dunn & Cockburn 1999), undoubtedly incurring energetic costs, particularly in winter. In addition, the acquisition and maintenance of the nuptial plumage involves a substantial and sustained increase in testosterone (Peters *et al*. 2000). Thus, male superb fairy-wrens which were moulting or in

nuptial plumage were expected to be immunocompromised. However, the exact opposite is true: males in eclipse plumage showed no response to the immunological challenge, whereas some moulting males and most males in nuptial plumage did produce antibodies (table 1).

Even more surprising was the finding that the testosterone levels in immune-responsive males were higher (table 1). Indeed, it appeared that testosterone was positively correlated with immunocompetence and elevated testosterone was the best predictor of the probability of an immune response (figure 3). This is the inverse result to the captive experiment where elevated testosterone reduced the probability of responding. While this at first may seem counter-intuitive, two processes may explain such a positive correlation between immunosuppressive testosterone and immune responsiveness.

First, both immunocompetence and testosterone levels might correlate with male quality and male condition. The phenomenon of a positive correlation between two costly traits which are both related to individual condition has been discussed extensively in life-history theory (e.g. Grafen 1988). It is this correlation which necessitates experimental manipulation to demonstrate life-history trade-offs conclusively. Fairy-wren males which acquire their nuptial plumage earlier tend to be of better quality (Mulder & Magrath 1994). If individual testosterone levels and immunocompetence are both positively correlated with male quality, then males which moult earlier should have higher testosterone and stronger immune responses. This study found no evidence of a correlation between individual body condition and the likelihood of mounting an immune response. However, this could reflect the limitations of estimating condition using body mass (see, for example, Gonzalez *et al*. 1999), particularly for a bird as small as the superb fairy-wren where the diurnal variation in mass can be 10–20% (A. Cockburn, unpublished data). A more meaningful indicator of male condition, for example protein nutritional status (Lochmiller *et al*. 1993), could reveal a positive correlation of both testosterone and immunocompetence with male condition.

In addition, a positive correlation between immunocompetence and testosterone could arise through 'compensation', whereby males have enhanced immune responses in order to counter the impact of immunosuppressive testosterone. As mentioned above, moulting males and males in nuptial plumage perform many potentially immunocompromising activities. General immune functions can be boosted as compensation for immunocompromising events. For example, short days in some species are immune enhancing to overcome the stressors associated with winter (Nelson & Demas 1996; Bentley *et al*. 1998). This is compatible with the view that hyperactivation of immunity may be costly (Westneat & Birkhead 1998) and should only be expressed at maximum levels when necessary. The immune system can respond to acute stress by redistributing immune cells and resources, which led Braude *et al*. (1999) to propose that enhancement of humoral immunity should occur in response to the stress of high testosterone. Thus, male fairy-wrens which engage in stressful and demanding activities with elevated testosterone may have enhanced immune responsiveness as a compensatory measure to avoid immunosuppression. The additional expenditure in immunity will then be an unavoidable cost of nuptial plumage which only males of high quality can manage for prolonged periods.

Immunocompensation might also explain a puzzling result of this study: the considerable difference between the responsiveness of males in eclipse plumage in the captive and free-living males. The majority (70%) of the unimplanted males in the aviary mounted an immune response (figure 2), whereas in the field no males in eclipse plumage responded (table 1). This could of course arise from a seasonal change in general immune responsiveness (Nelson & Demas 1996; Bentley *et al*. 1998) or it could be that the benign temperature regime and *ad libitum* high-protein food improved the males' condition and their immunocompetence (Lochmiller *et al*. 1993). Conversely, the stress of adapting to captivity could have incited compensatory measures, resulting in boosted immunocompetence in the captive birds. A comparison of annual patterns of immunocompetence in free-living and captive males could shed more light on this fundamental issue.

5. CONCLUSION

In superb fairy-wrens the sexually selected signal of male quality is the timing of their pre-nuptial moult (Mulder & Magrath 1994). Males which acquire their nuptial plumage before mid-winter are strongly preferred in female choice (Dunn & Cockburn 1999). This led Dunn & Cockburn (1999) to propose that an early moult is a signal of endurance which only males of high quality and in good condition can produce. The present study provides strong evidence in support of this hypothesis. Males which acquire their nuptial plumage early are exposed to immunosuppressive testosterone over the winter months. Consequently, only males of high quality which are highly immunocompetent—or able to enhance their immune system for prolonged periods—can endure the extended periods of high testosterone necessary to produce this signal.

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