
Reproductive State, but Not Testosterone, Reduces Immune Function in Male House Sparrows (*Passer domesticus*)

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ABSTRACT

The immune system requires energetic and nutritional resources to optimally defend organisms against pathogens and parasites. Because resources are typically limited, immune function may require a trade-off with other physiologically demanding activities. Here, we examined whether photoperiodically induced seasonal states (breeding, molting, or nonbreeding) affected the cutaneous immune response of captive male house sparrows (*Passer domesticus*). To assess immune function in these birds, we injected the mitogen phytohemagglutinin (PHA) into the patagium and measured the resulting wing web swelling. Molting and nonbreeding birds had similar immune responses to PHA injection. However, males in a breeding state showed lower immune responses than both molting and nonbreeding birds even though they did not actually breed. We tested whether this decrease in the PHA swelling response in birds in a breeding state was due to elevated plasma concentrations of testosterone (*T*) by administering *T* to birds in a nonbreeding state. Contrary to some evidence in the literature, *T* did not suppress the response to PHA in house sparrows. Our data show that passerine birds show seasonal modulation in immune function, even in benign environmental conditions. However, even though *T* is often cited as a strong immunosuppressant, it is not fully responsible for this seasonal modulation.

Introduction

The immune system provides organisms with a defense against parasites and pathogens, thus increasing host longevity and reproductive output (Clayton and Moore 1997). However, these benefits do not come without costs, as some aspects of immune activity require energetic and nutritional investment (Lochmiller and Deerenberg 2000; Norris and Evans 2000). For example, the metabolic cost of an immune response to the mitogen phytohemagglutinin (PHA) in small passerine birds can be of the same magnitude as egg production or molt (reviewed by Martin et al. 2003). Because resources are typically limited, the cost of immunocompetence may become important when competing physiological systems need resources. This may lead to trade-offs between immune function and other energetically demanding activities (Sheldon and Verhulst 1996; Westneat and Birkhead 1998). For example, a PHA-induced response in white-footed mice (*Peromyscus leucopus*) leads to a reduction in testis mass (Derting and Compton 2003). During certain stages of an organism's life, such as reproduction or molt, resources may have to be shunted away from immune function, resulting in seasonal variability in immune function.

Seasonal variability in immune function has been examined in considerable detail in mammals (Nelson and Demas 1996; Nelson et al. 2002) and to a limited extent in some lower vertebrates (e.g., Muñoz and De la Fuente 2001). In birds, few seasonal comparisons of immunity have been conducted (e.g., Bentley et al. 1998; Hasselquist et al. 1999; Moore and Siopes 2000; Lozano and Lank 2003; Martin et al. 2004). However, most studies so far have contrasted the reproductive with the nonreproductive state without including molt (but see Silverin et al. 1999).

Reproduction is indeed an expensive life-history state, in particular for female birds that face demanding activities like egg production, incubation, and maternal care (King 1973; Ricklefs 1974; Drent and Daan 1980; Carey 1996; Monaghan and Nager 1997). When engaging in these reproductive processes, female birds show suppressed immune function (e.g., Deerenberg et al. 1997; Nordling et al. 1998; Moreno et al. 1999; Bonneaud et al. 2003; but see Ilmonen et al. 2002; Liffield et al. 2002). However, even in males, reproductive costs may be great enough to warrant trade-offs with immune function. During the breeding season, male birds typically display costly activities like courtship (e.g., song; Oberweger and Goller 2001; Thomas 2002) and intraspecific aggression (Hogstad 1987; Bryant and Newton 1994). Males also develop and maintain sec-

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ondary sexual characteristics (reviewed in Andersson 1994) and often provide substantial parental care (Ketterson and Nolan 1994). Exaggerated secondary sexual characters in males are presumed to entail energetic and nutritional costs (Hamilton and Zuk 1982; Andersson and Andersson 1994; Gustafsson et al. 1995), and increased brood size has been shown to impair immune function in male birds that provide substantial parental care (Deerenberg et al. 1997).

Molt immediately follows reproduction in most passerine birds (Jenni 1994). The costs of molt include keratin synthesis and the maintenance of feather-producing tissues (Lindström et al. 1993; Murphy 1996), which, together with increased thermoregulatory costs, can lead to an overall increase in metabolic rate (Dolnik 1965; Klaassen 1995). Increased thermoregulatory costs during molt may result from decreased ambient temperatures and loss of insulation. The few studies that examined interactions between molt and immune function produced conflicting results. Some studies report an upregulation of immune tissue and function during the molting season (Silverin et al. 1999; Moreno et al. 2001), while others found immunosuppression (Kogut et al. 1999; Kuenzel 2003; L. B. Martin II, unpublished data). However, some of these latter studies induced molt via food deprivation, which in itself may suppress immune function (Holt 1992; Alodan and Mashaly 1998; Lochmiller and Deerenberg 2000).

The physiological processes that underlie seasonal allocation of energy resources and hence immunosuppression are still unclear, but they may be mediated by changes in concentrations of circulating hormones (Folstad and Karter 1992; Marsh 1992; Wedekind and Folstad 1994; Zuk 1996; Ricklefs and Wikelski 2002). In male vertebrates, the hormone testosterone increases in the circulation during the breeding season (reviewed in Nelson 2000) at the same time immune function is compromised. Testosterone promotes the development of many male secondary sexual characteristics (Emerson 2000), and Folstad and Karter (1992) suggested that it might simultaneously suppress immune function. According to their theory, exaggerated sexual ornaments are an honest signal of male quality because they can only be expressed if a male can cope with the simultaneous elaboration of a sexual trait and the concomitant immunosuppression.

Indeed, testosterone has traditionally been considered immunosuppressive (reviewed by Grossman 1984; Alexander and Stimson 1988; Folstad and Karter 1992), but recent *in vivo* studies on birds have revealed a more complex picture. Testosterone administration does suppress immune function and increase ectoparasite loads in some bird species (Saino et al. 1995; Duffy et al. 2000; Eens et al. 2000; Poiani et al. 2000; Casto et al. 2001; Duckworth et al. 2001), but in others, it had no effect (Hasselquist et al. 1999), or its effects were condition dependent. For example, in black-headed gull chicks (*Larus ridibundus*), testosterone treatment increased immune function during early development, but it had no effect on immune

activity in older chicks (Ros et al. 1997). In male greenfinches (*Carduelis chloris*), testosterone decreased the severity of a viral infection during the early stages of viremia, but it increased the severity of infection later (Lindström et al. 2001).

To increase our understanding of trade-offs between costly life-history states and immune activity, we examined the immune response of captive male house sparrows (*Passer domesticus*) in photoperiodically induced states of reproduction, molt, and overwintering (i.e., nonbreeding, nonmolting). We further tested whether the reproductive hormone testosterone was involved in mediating an expected trade-off between immune function and reproduction during the breeding season by administering it to birds and measuring immune activity during the nonbreeding season.

Material and Methods

Experiment 1: Seasonal Variation in Immune Function

Bird Capture and Maintenance. In June 2002, we caught 25 male house sparrows using mist nets on farms near Princeton, New Jersey, and immediately housed them in 47 × 75 × 98-cm cages in groups of six to nine birds. Cages were situated inside noise- and lightproof chambers, and no-waste seeds (Kaytee Wild Finch mix), chick starter mash, and water were provided *ad lib*. Vitamins (Avitron) were added every second week to the drinking water, and cages were cleaned every 3 d. Temperature was kept at ~20°C.

Photoperiodic Manipulation. Initially, we exposed birds to a long-day (LD) photoperiod that mimicked the ambient day length in New Jersey during the month in which they were captured. Birds concluded breeding condition at the end of August, as evidenced by the development of light-colored beaks and molt (house sparrows have black beaks during the breeding season due to increased plasma concentrations of testosterone; Donham et al. 1982). On August 28, 2002, we changed photoperiod to short days (SD) of 8 h of light and 16 h of darkness per day. After house sparrows stopped molting in mid-December, we changed the photoperiod again on December 20, this time to long days (18L : 6D), to reinduce the breeding state (as evidenced by the development of black beaks).

Immune Activity. PHA injection into the patagium (wing web) of birds induces infiltration of heterophils and mononuclear cells by 1 h after injection (McCorkle et al. 1980) followed by an accumulation of blood-borne macrophages and basophils that peak around 24 h after injection and decline by 48 h after injection (Goto et al. 1978; McCorkle et al. 1980). Swelling after 48 h is due to edema, recirculated lymphocyte cells (Goto et al. 1978), and endothelial cells that underwent mitosis in the presence of PHA (McCorkle et al. 1980).

We administered PHA to three different groups of males (*a*) at the onset of molt (on September 10, 2001; *n* = 6), (*b*) 12

wk later during the nonbreeding state after molt had terminated (on December 14, 2001; $n = 7$), and (c) 7 wk after photostimulation during the breeding state (on February 4, 2002; $n = 9$; as determined by black beaks). Birds in all groups were naive to PHA at the time of each injection. For each injection, we used purified PHA-P (Sigma L 9017) dissolved in phosphate-buffered saline to a 0.1 mg/mL concentration. Before injection, the patagium of each bird was cleaned with alcohol, and then 100 μ L of the PHA solution was injected subdermally into the left wing web with a 26-gauge needle attached to a tuberculin syringe. The thickness of the patagium was measured to the nearest tenth of a milli-inch using a pressure-sensitive spessimeter (Teclock pocket thickness gauge, model SI-510) immediately before the injection and at 24, 48, 72, and 96 h postinjection. Timing of the wing web swelling measurements was accurate ± 1 h. Each patagium was measured between three and five times, and average thickness was calculated for each individual.

Morphometrics. Before injection, we weighed birds to the nearest 0.1 g using a Pesola spring scale. Body molt was scored using a scale of 0 (none) to 3 (heaviest), and wing molt was described as either 1 (present) or 0 (absent). Beak color was assessed separately in upper and lower beak on a scale of 0 (lightest) to 3 (darkest), and scores were then added for each individual.

Experiment 2: Testosterone Administration to Short-Day Birds

On January 2 and 3, 2004, we captured 12 male house sparrows from a residential neighborhood in Princeton, New Jersey. Groups of six birds were housed in two 47 \times 75 \times 98-cm cages. All birds were kept on short days (8L : 16D); care was identical to the procedure described above. On January 8, we implanted six of these birds with one 10-mm piece of silastic tubing (Dow Corning; 1.47 mm i.d., 1.96 mm o.d.) packed with crystalline testosterone (*T*; Sigma T-1500). We implanted the remaining six birds with empty implants as a control group. For implantation, we made a small incision in the skin of each bird on its left flank under a light isoflurane anesthesia and then inserted one implant under the skin on the back between the wings. The incision was sealed with a medical-grade adhesive (Vetbond 1469). Just before implantation, all birds were weighed and beak colors were assessed as before.

Nine days after implantation, all birds were injected in the left wing web with 100 μ L PHA-P (Sigma L9017) diluted to a concentration of 0.1 mg/mL with saline. The same morphometric measurements and assessments were taken as in experiment 1. This time, birds were also weighed at 24, 48, 72, and 96 h postinjection. At 96 h after injection, blood samples (\sim 250 μ L) were taken from the right wing of all birds. We took all blood samples within <3 min from opening the chambers in which the birds were housed, to avoid a stress-induced increase

in plasma corticosterone concentrations (Wingfield et al. 1982). We then centrifuged blood samples at 500 g for 4 min, removed the plasma, and froze it at -20°C . Plasma concentrations of total androgens (*T* and dihydrotestosterone) and corticosterone were each determined in a single direct radioimmunoassay (e.g., Hau et al. 2000; Tarlow et al. 2003). Recoveries for *T* were $63.42\% \pm 1.96\%$ (mean ± 1 SE), lower detection limit was at 0.1 ng/mL, and upper detection limit was at 13.73 ng/mL. Samples below the lower detection limit of the assay were set at the detection limit as a conservative estimate for statistical analysis. Recoveries for corticosterone were $62.5\% \pm 1.4\%$ (mean ± 1 SE), and lower detection limit was at 2 ng/mL. All methods used in this study were approved by the Princeton University Institutional Animal Care and Use Committee (protocol no. 1492).

Analysis

We processed data using SPSS, version 10 (SPSS, Chicago). We calculated the wing web swelling response to PHA by subtracting the thickness of the wing web of a bird before injection from the thickness after injection. When appropriate, groups were compared using repeated-measures ANOVA (rmANOVA) or one-way ANOVA. In experiment 1, post hoc Tukey HSD tests were performed to compare wing web swelling in each group separately for each time point. Hormone concentrations between groups were compared using nonparametric statistics. For the correlation analysis between PHA response and molt, scores for wing and body molt were summed. All tests were two-tailed, and significance was accepted at $\alpha = 0.05$.

Results

Experiment 1: Seasonal Variation in Immune Function

All three groups showed significant increases in wing web thickness after PHA injection (rmANOVA; effect of time, $F_{3,17} = 14.47$, $P < 0.001$; Fig. 1). However, the time course of PHA response differed with seasonal state of the birds (effect of season, $F_{2,19} = 5.36$, $P = 0.014$; interaction time \times season, $F_{6,34} = 1.24$, $P > 0.3$), with the birds in breeding condition having a significantly lower PHA response than molting birds; the nonbreeding birds had an intermediate response (post hoc Tukey's HSD tests; see Fig. 1).

Molting and nonmolting SD birds had significantly lighter beaks than LD birds (beak scores: molting birds, 1.17 ± 0.4 ; nonmolting birds, 1.28 ± 0.25 ; LD birds, 5.94 ± 0.06 ; one-way ANOVA, $F_{2,19} = 187.6$, $P < 0.001$, followed by Tukey's HSD test). Each bird in the molt group indeed showed molt, but no birds in any of the other groups showed molt. There was no correlation between the degree of molt and 24-h swellings in the molting group (Spearman rank test, $P > 0.2$). Seasonal groups did not differ in body mass (molting birds, $27.53 \pm$

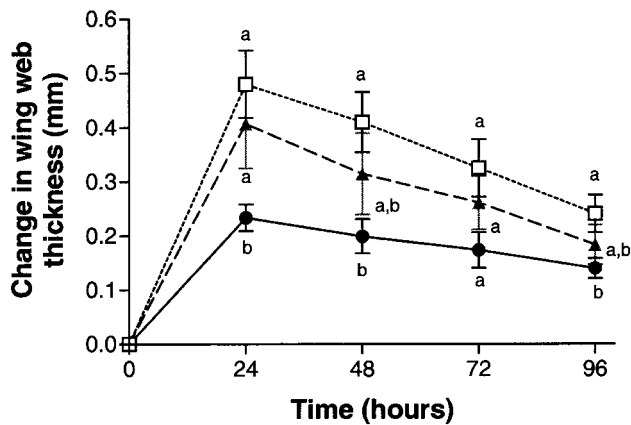


Figure 1. Time course of wing web swelling response to PHA injection in male house sparrows in breeding, molting, and nonbreeding condition (experiment 1). *Open squares*, short-day molting birds ($n = 6$); *gray triangles*, short-day nonbreeding, nonmolting birds ($n = 7$); *solid circles*, long-day breeding birds ($n = 9$). Data are means \pm 1 SE. Post hoc Tukey HSD tests were used to compare groups separately for each time point (letters denote differences between groups).

0.53 g; nonmolting birds, 25.98 ± 0.63 g; LD birds, 25.83 ± 0.59 g; one-way ANOVA, $F_{2,19} = 2.175$, $P > 0.1$).

Experiment 2: Testosterone Administration to SD Birds

The *T*-implanted birds had significantly higher plasma androgen concentrations than control-implanted birds (mean \pm 1 SE; *T*-implanted birds, 12.58 ± 0.79 ng/mL; control birds, 0.13 ± 0.024 ng/mL; Mann-Whitney $Z = -2.9$, $P < 0.001$). Plasma corticosterone concentrations were undetectable in all birds, except for one individual in the *T*-implanted group that had 3.34 ng/mL. Wing web swelling significantly increased after PHA injection in both *T* and control birds (rmANOVA; effect of time, $F_{4,40} = 20.3$, $P < 0.001$), but this increase was not different between *T* and control-treated birds (effect of treatment, $F_{1,10} = 0.975$, $P > 0.3$; interaction hormone treatment \times time, $F_{4,40} = 0.85$, $P = 0.5$; Fig. 2).

The PHA-induced wing web swelling did not differ between the nonbreeding birds from experiment 1 and the nonbreeding controls from experiment 2 (effect of experiment number, $F_{1,11} = 1.49$, $P > 0.2$; interaction of experiment number \times time, $F_{4,44} = 1.26$, $P = 0.3$), suggesting our results are repeatable using house sparrows kept in captivity for different lengths of time (the birds from experiment 1 were kept for \sim 6 mo before injection, whereas the controls from experiment 2 were kept for just over 2 wk before injection).

From time of implantation to PHA injection, birds in *T*- and control-implanted groups lost mass (control group at implantation, 26.7 ± 0.78 g; at PHA injection, 25.32 ± 0.6 g; *T*-implant group at implantation, 26.67 ± 0.87 g; at PHA injection, 25.98 ± 0.55 g; rmANOVA, effect of time, $F_{1,10} =$

14.70, $P = 0.003$), but there was no difference in mass loss between the two groups (effect of hormone treatment, $F_{1,10} = 0.11$, $P > 0.7$; interaction time \times hormone treatment, $F_{1,10} = 1.69$, $P > 0.2$). Birds did not change body mass following PHA injection (rmANOVA; effect of time, $F_{1,10} = 3.028$, $P = 0.11$), and there were no differences in mass between treatment groups (effect of hormone treatment, $F_{1,10} = 4.21$, $P > 0.5$; interaction time \times hormone treatment, $F_{1,10} = 0.013$, $P > 0.9$).

Discussion

Seasonal Variation in Immune Activity

Our results show that photoperiodic induction of the breeding state suppresses immune activity in male house sparrows. These data complement results on photoperiodically manipulated European starlings (*Sturnus vulgaris*), in which males in a breeding state had reduced in vitro cell-mediated immune function relative to photorefractory males (Bentley et al. 1998). Similarly, the vast majority of small mammals studied so far show reduced immune activity under long days (which induces the breeding state) as compared with short days (reviewed in Nelson and Demas 1996; see Demas and Nelson 2003 for an exception in a tropical rodent). In contrast, female Japanese quail (*Coturnix japonica*) had similar cellular and humoral immune responses under both short and long days (Moore and Siopes 2000).

The resource allocation hypothesis states that trade-offs exist between costly seasonal events such as reproduction and the immune system (Sheldon and Verhulst 1996; Westneat and Birkhead 1998). In this study, we did not directly measure energetic trade-offs between life-history states and immune function, but our results are compatible with the resource al-

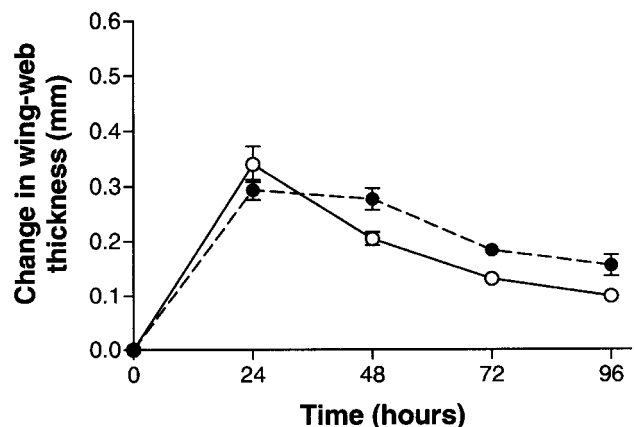


Figure 2. Time course of the PHA response in short-day nonbreeding male house sparrows implanted with testosterone (*solid circles*, $n = 6$) or an empty implant (*open circles*, $n = 6$). Data are means \pm 1 SE. Scale of Y-axis has been matched to that of Figure 1 to facilitate comparison. PHA-induced wing web swelling did not differ between nonmolting, nonbreeding birds in experiment 1, and control birds in experiment 2 (see "Results").

location hypothesis. Immunosuppression during the breeding season has typically been demonstrated in birds that actually engaged in breeding activities in the wild (e.g., Nordling et al. 1998; Silverin et al. 1999; Moreno et al. 2001; Lozano and Lank 2003; Martin et al. 2004; but see Hasselquist et al. 1999). It is therefore interesting that we observed a trade-off between reproduction and immunity even in captivity, when males were prevented from engaging in most of the costly activities associated with reproduction such as courtship, copulation, and parental care. The costs that birds in a breeding state in our experiment may have incurred include gonad growth and increased intraspecific aggression. Metabolic costs of testis growth and maintenance are notoriously hard to determine, but they have been estimated to not exceed 2% of basal metabolic rate in birds (King 1973; Ricklefs 1974). Aggressive behavior and dominance also raise the energy metabolism of birds, increasing resting metabolic rate by about 3% (Bryant and Newton 1994) and daytime oxygen consumption rate by about 10%–20% (Hogstad 1987). Taken together, these costs could be sufficient to cause immunosuppression of birds in a breeding state even in captive conditions. Further, reproductive processes and immune function could be competing for limited nutrients rather than overall energy (Klasing et al. 1987).

The observed immunosuppression during the breeding season in our birds is also compatible with three alternative (though not mutually exclusive) hypotheses. First, immunosuppression during the breeding season might be adaptive to prevent the production of antisperm antibodies (Hillgarth et al. 1996). In vertebrates, sperm achieve haploidy long after the ontogenesis of the immune system, which renders them antigenic and vulnerable to autoimmune responses. Second, during periods of heavy workload such as during the breeding season, immunosuppression could be vital to reduce the risk of autoimmune responses from hyperactivation of the immune system (Råberg et al. 1998; Westneat and Birkhead 1998). Third, reduced immune function in one part of the immune system might in fact be due to a redistribution of immune cells to another compartment of the immune system rather than a general immunosuppression (Braude et al. 1999; Dhabhar 2000).

Molt did not affect the PHA response of male house sparrows kept on short days, yet this result can still be reconciled with the resource allocation hypothesis. First, the cost of molt in this species may not be significant enough to warrant a trade-off with immune function. Although the cost of molt varies between species, it has been documented as a substantial energetic expense in other small passerines such as European stonechats (*Saxicola torquata*; Klaassen 1995), common redpolls (*Carduelis flammea*), and blue-throats (*Luscinia svecia*; Lindström et al. 1993). However, in other bird species molt can be less expensive (e.g., in the larger-bodied nonpasserine kestrel *Falco tinnunculus*; Dietz et al. 1992). Second, molt might be costly, but our captive birds provided with ad lib. food could

have compensated for it. Third, molt may have had no effect on PHA responses in our experiment due to a delay between investing resources in feather production and immunosuppression. We injected our birds with PHA as soon as molt appeared, but molt continued over a 10-wk period. In order to test this “delayed trade-off” hypothesis, separate groups of male house sparrows could be challenged at different intervals during the molting season. An increase in immunosuppression toward the end of the molting season would suggest that the two processes trade-off asynchronously. Indeed, L. B. Martin II (unpublished data) found a suppressed response to PHA injection in both male and female house sparrows at the peak of molt.

Testosterone and the PHA Response

Exogenously administered testosterone did not suppress immune function in SD nonmolting male house sparrows. Testosterone implantation resulted in approximately twice the androgen concentrations that are found in wild male house sparrows during the breeding season (Hegner and Wingfield 1990), but such levels are still probably within the physiological range for this species. Our results demonstrate that the PHA-induced wing web swelling is not reduced even when *T* is present at high levels in the circulation. There are two possible explanations for this finding: either testosterone does not mediate immunosuppression in male house sparrows, or it might do so only during the breeding season. The idea that vertebrates may be able to seasonally modulate the sensitivity of immune tissue to testosterone (e.g., by varying the number of androgen receptors in immune tissue; Olsen and Kovacs 1996; Tanriverdi et al. 2003) is attractive, although to our knowledge it has yet to be experimentally tested. There is already evidence that testosterone has a seasonally dependent effect on the neural circuit that produces song in European starlings. Photorefractoriness makes the song nuclei of starlings less sensitive to the stimulatory effects of testosterone (Bernard and Ball 1997).

If the action of testosterone on the immune system is dependent on time of year, some of the conflicting evidence about the immunosuppressive qualities of testosterone in the literature might be reconciled. Exogenously administered testosterone indeed suppressed the immune response in some bird species during the breeding season (Evans et al. 2000; Peters 2000; Casto et al. 2001; Buchanan et al. 2003). However, at a similar time of year in other species, the effects of testosterone on immune function were absent or complex (Hasselquist et al. 1999; Lindström et al. 2001). Immunosuppression by exogenous testosterone during the nonbreeding state has also been reported (Duffy et al. 2000). Thus, interspecific variation in the responsiveness to exogenous testosterone, as well as methodological differences between studies such as the concentration of testosterone administered and the duration of testosterone treatment, might be additional important factors. Finally, di-

vergent findings regarding the immunosuppressive effects of testosterone might also depend on which components of the immune system are being assessed. For example, a recent study on male house sparrows in which testosterone was manipulated during the breeding and postbreeding season failed to demonstrate immunosuppressive effects on the PHA-induced wing web swelling, but it did indicate a suppressive effect of *T* on the humoral response to injections of sheep red blood cells (Buchanan et al. 2003; the effect perhaps being mediated by differences in corticosterone levels; see also section below).

Testosterone implantation may also affect other circulating hormones and thus indirectly alter immune function. For instance, elevated corticosterone levels have been noted in some studies where exogenous testosterone impaired immune function (Duffy et al. 2000; Evans et al. 2000; Casto et al. 2001; Buchanan et al. 2003). Because corticosterone is a known immunomodulator (McEwen et al. 1997; Sapolsky et al. 2000), it may be responsible for the immunosuppressive effects often attributed to testosterone. In this study, however, plasma corticosterone concentrations were below detection limit in all but one bird, and *T* administration thus did not increase circulating corticosterone concentrations (similar findings were obtained by Hegner and Wingfield 1987).

We did not observe seasonal changes in body mass of our experimental birds, suggesting that body condition had no effect on immune response. Also, PHA injection did not result in mass loss of birds in experiment 2. This suggests that the PHA-induced immune response is not severe enough to significantly affect body condition. Mass loss during immune up-regulation might arise from reduced food intake (Klasing et al. 1987), increased protein breakdown in the muscle tissue to supply amino acids for the synthesis of immune cells (Klasing and Austic 1994), or host damage caused by a pathogen.

Conclusions

We found seasonal variation in PHA-induced immune activity in male house sparrows kept in captivity under benign environmental conditions and prevented from engaging in many of the costs associated with an actual breeding event. Testosterone administered to nonbreeding birds did not mimic the reduction in PHA response observed in birds in a breeding state. Thus, hormones might mediate trade-offs between the immune system and life-history events but probably function in a state-dependent manner. Additional experiments under controlled conditions and on animals in defined seasonal states are needed to unravel the physiological mechanisms underlying seasonal immunomodulation. It also remains to be determined whether the observed immunosuppression during the breeding season is the result of an acute trade-off with other body functions or whether it has been evolutionarily fixed and is thus expressed even though costs are not actually fully borne (Schmid-Hempel 2003).

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