

Cutaneous Immune Activity Varies with Physiological State in Female House Sparrows (*Passer domesticus*)

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ABSTRACT

Many vertebrates show seasonality in immune defenses, perhaps because of trade-offs with other physiological processes. Trade-offs between reproduction and immune function have been well studied, but how other life cycle events such as molt affect immune function remains unclear. Here, we hypothesize that one possible explanation is that accumulative dissociated processes (e.g., resource deficits generated over the long term by physiological processes) can have delayed effects on immune activity. To test this hypothesis, we compared cutaneous immune responses in groups of captive female house sparrows (*Passer domesticus*) photoperiodically induced into six different life cycle stages. We predicted that if delayed trade-offs occur, immune activity would be reduced after a mature life state was reached (e.g., postmolt) and not just compromised when other tissues were actively growing (instantaneous trade-off). We found evidence for both types of trade-offs: immune responses were weakest in sparrows that had just completed postnuptial molt, but they were also weak in birds growing reproductive tissues or feathers. Birds in mature reproductive states or light molt had strong immune responses comparable with birds in a nonbreeding/nonmolting state. Altogether, our results indicate that immune activity in female house sparrows can be influenced by both instantaneous and delayed trade-offs.

Introduction

Immune defenses are critical for survival (Norris and Evans 2000), so it is surprising that they are often weaker at some

times of the year than at others (Nelson and Demas 1996; Nelson 2004). In temperate vertebrates, immune function is generally enhanced in the nonbreeding season and reduced in the breeding season (Lochmiller et al. 1993; Bentley et al. 1998; Moore and Siopes 2000; Bilbo et al. 2002; Lozano and Lank 2003; Greenman et al. 2005; but see Hasselquist et al. 1999). One mechanistic hypothesis for this seasonal variation involves trade-offs between costly immune activity and other physiological processes (Nelson and Demas 1996; Martin et al. 2003, 2004). Limited resources coupled with the high costs of immune function and other physiological processes are thought to require some activities to be decreased if others are increased (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Norris and Evans 2000; but see Svensson et al. 1998). Much experimental evidence supports this hypothesis. For instance, increased reproductive effort (e.g., via experimentally enlarged brood size) can lead to weakened immune responses (Gustafsson et al. 1994; Deerenberg et al. 1997; Nordling et al. 1998; Moreno et al. 1999; Lifjeld et al. 2002; Ardia et al. 2003, 2005). Conversely, induction of immune activity can decrease reproductive output (Bonneaud et al. 2003; but see Williams et al. 1999), reduce growth (Prendergast et al. 2002; Fair et al. 2003), or even lead to mortality (Moret and Schmid-Hempel 2000; Hanssen et al. 2004).

Although trade-offs between immunity and reproduction have been convincingly demonstrated, interactions between immune function and other equally costly life cycle stages remain unclear. For example, passerine birds typically undergo a period of complete feather renewal immediately after the breeding season (a postnuptial molt), which incurs substantial nutritional, energetic, and thermoregulatory costs (Murphy and King 1992; Lindström et al. 1993). The few studies that have tested whether molt affects immune function have produced equivocal results; some report increases in immune activity (Silverin et al. 1999; Moreno et al. 2001), while others indicate decreases (Martin 2005) or no effects (Greenman et al. 2005). One potential explanation for the varying effects of molt on immune function involves the distinct timescales over which molt and immune responses occur; immune responses usually take days to manifest and resolve, but molt requires weeks to months for completion. Trade-offs between immune function and other physiological activities therefore may depend on the temporal aspects of the cost function of the physiological process associated with a particular life cycle stage ("delayed trade-off" hypothesis; Greenman et al. 2005). That is, impairment of immune function may manifest (1) instantaneously during pe-

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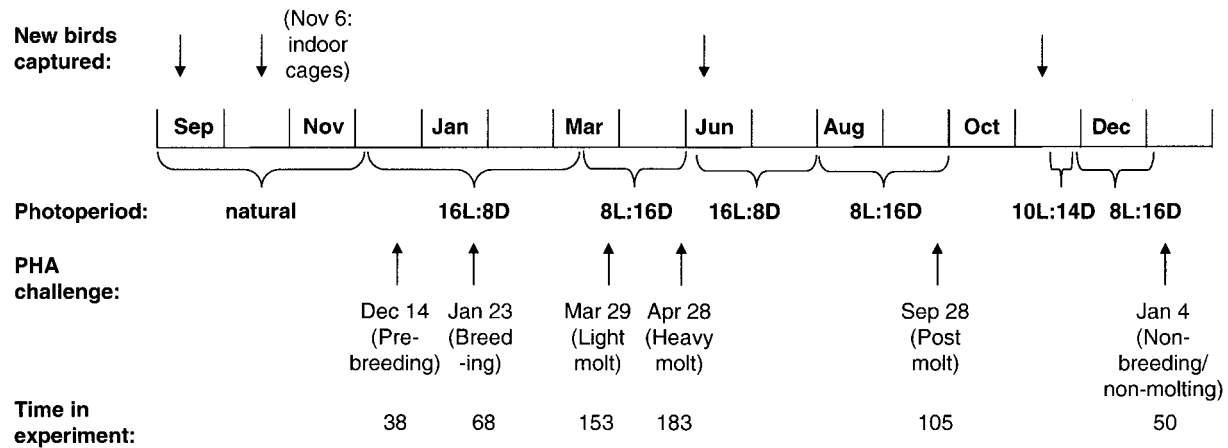


Figure 1. Timeline of experiments. Arrows pointing down on time axis indicate times when birds were captured. Arrows pointing up indicate dates of PHA injections in the different groups. Photoperiod treatments are indicated below time axis. Numbers indicate the duration (d) that each group spent in experimental conditions.

riods of tissue growth (e.g., feathers, reproductive tissue), (2) after some time when significant energy/resource deficits have been generated (e.g., once molt or reproductive maturation is complete), or (3) both.

Here we sought to determine which type of trade-off impinges on cutaneous immune activity in female house sparrows (*Passer domesticus*). Specifically, we photoperiodically induced groups of female house sparrows into different life cycle stages: prebreeding, breeding, light molt, heavy molt, postmolt, and nonbreeding/nonmolting. We expected that if instantaneous trade-offs mediate seasonal variation in immune function, immune function would be weak in animals generating reproductive tissue or feathers. Alternatively, if delayed trade-offs were important, we expected that immune activity would be weakest once birds reached a mature life cycle state but not during the generation process of tissues itself.

We conducted this experiment on female house sparrows for two reasons. First, we chose a small passerine because we expected that both instantaneous and delayed trade-offs between immune activity and other physiological processes, if they existed, would be detectable in an income-breeding species with little capacity to store resources for later investment (Drent and Daan 1980). Second, we aimed to test whether females would show a similar pattern of seasonal variation in immune activity as males of this species; in a previous study, we found that male house sparrows exhibited weak immune responses during the reproductive period and stronger immune response during the nonbreeding stage (Greenman et al. 2005). We asked whether a similar pattern would manifest in females of this species because the costs of reproduction are purportedly greater for this sex in passerines (King 1973; Ricklefs 1974; Bryant and Newton 1994). Similar to our previous study (Greenman et al. 2005), we conducted this experiment on wild-

caught, captive-housed birds. This approach allowed us to provide animals with comparable climatic environments and diets. In this way, we could reduce variation in experience among treatment groups or individuals (e.g., frequency of aggressive interactions, intensity of nestling provisioning, quality and quantity of food resources), which could have biased our results.

Material and Methods

Bird Capture and Housing

Forty-three female house sparrows were caught near Princeton, New Jersey (40°21'N, 74°40'W), in September 2001 ($n = 12$), October 2001 ($n = 12$), June 2002 ($n = 13$), and November 2003 ($n = 6$). After capture, birds were held in outdoor aviaries for a short period and then brought indoors (September and October 2001 birds), or they were captured and immediately brought inside (all others). For the duration of the experiments, birds were housed in groups of six in cages (47 cm × 75 cm × 98 cm) kept inside ventilated, noiseproof, and lightproof chambers. Birdseed (Kaytee Supreme), chick starter mash (Belle Mead Farmers Co-op, Belle Meade, NJ), and tap water were provided ad lib.; Kaytee calcium-fortified grit was also provided to the prebreeding and breeding groups (see below). White fluorescent tubes provided illumination to each chamber, yielding approximately 300 lux at cage height. Temperature was maintained at $25^\circ \pm 2^\circ\text{C}$; relative humidity mirrored ambient conditions. At the end of the experiments, all birds were released to the areas where they were originally captured. All work was approved by the Princeton University Institutional Laboratory Animal Care and Use Committee (protocol 1428).

Photoperiod Manipulation

Figure 1 provides a graphical depiction of the progression of our experiment. Birds caught in September and October 2001 ($n = 24$) were transferred to indoor cages on November 6, 2001, and randomly divided into four groups. On December 4, 2001, all four groups were photostimulated with 18 h of light (photoperiod of 18L:6D, a photoperiod under which these house sparrows would breed; Summers-Smith 1988). Ten days after photostimulation, one group of birds (prebreeding) was injected with phytohemagglutinin (PHA; see below for details); 40 d after photostimulation, a second group was injected with PHA (breeding). The remaining two groups of birds were kept on an 18L:6D photoperiod for a total of 15 wk, and then photoperiod was shortened to 8L:16D. Birds then started to molt. Ten days and 40 d after this switch to a short photoperiod, birds from each group were injected with PHA (light-molt and heavy-molt groups, respectively).

Birds caught in June 2002 were immediately photostimulated (18L:6D) for 40 d. After this period, photoperiod was shortened to 8L:16D and remained short until 7 d after all individuals completed molt (after 65 d on this light schedule). At this time, all birds were injected with PHA (postmolt). Birds caught in November 2003 were molting at time of capture. They were kept under an ambient photoperiod of 10L:14D for 10 d until all individuals had completed molt. After this period, photoperiod was shortened to 8L:16D for 40 d, and then birds were injected with PHA (nonbreeding, nonmolting). In total, birds were kept indoors in experimental conditions for 38 d (prebreeding), 68 d (breeding), 138 d (light molt), 168 d (heavy molt), 105 d (postmolt), or 55 d (nonbreeding/nonmolting).

Cutaneous Immune Activity

PHA was used to induce cutaneous immune activity in each bird. Derived from the red kidney bean (*Phaseolus vulgaris*), PHA is a mitogen that triggers proliferation of many cells, which incites local infiltration of immune cells including heterophils, macrophages, basophils, and other lymphocytes and causes edema and swelling at the injection site (Stadecker et al. 1977; Goto et al. 1978). Each injection consisted of 100 μL of a 0.1 mg mL⁻¹ solution of purified crystalline PHA-P (Sigma L9017) dissolved in pyrogen-free phosphate buffered saline (Sigma P3823) into the left wing web (Greenman et al. 2005). Just before each injection and 24, 48, 72, and 96 h postinjection, the thickness of the left wing web was measured to the nearest tenth of a milli-inch using a pressure-sensitive spessimeter (Teclock SI-510 pocket thickness gauge or Starrett 1010M thickness gauge). All data were later transformed to metric units. Three readings were taken per bird, and the average of these three values was recorded. Repeatability of these three measurements ranged from 0.96 to 0.99 (respective r values as calculated after

Lessells and Boag [1987]). PHA-induced swelling was determined by subtracting the thickness of the wing web before injections from later thickness measures. Although we used two different spessimeters in the experiment, no significant differences were observed between them (paired-sample t -test, $P = 0.76$). Wing measurements obtained by two different experimenters (P. Han and J. Kwong) yielded similar results (paired-sample t -test, $P = 0.68$, $n = 6$, for repeatedly measured birds).

Before PHA injections, body mass was determined to the nearest 0.1 g, and tarsus length was measured to the nearest 0.05 mm for each bird. Additionally, the color of the culmen and rictus of each bird was scored on a scale of 0 (uniform light yellow color) to 3 (solid black) and then summed. Wing and body molt were also scored on a scale of 0 (no molt) to 3 (heavy molt) and later summed. Finally, an index of body condition was calculated by dividing body mass by the third power of tarsus length, multiplied by 100 (Romero and Wikelski 2001).

Assessment of Reproductive Condition

Two days after the last wing web measurement, all birds from the prebreeding and breeding groups were laparotomized to determine reproductive state. For this procedure, birds were lightly anesthetized with isoflurane vapors (Enflurane, Abbott Laboratories), a small incision was made between the last and the second-to-last rib, and the diameter of the largest ovarian follicle was measured to the nearest 0.1 mm with modified calipers (Hau et al. 2004). All birds exposed to short-day photoperiods had yellow bills and were molting and were thus assumed to have regressed gonads (Donham et al. 1982). To ensure that this assumption was valid, a subsample of birds from each short-day group was laparotomized (for sample sizes, see Fig. 3b). Laparotomies were approved before the experiment by the Princeton University Institutional Animal Care and Use Committee.

Statistical Analysis

Data did not deviate from normality, and therefore parametric multivariate statistics were used. Repeated-measures ANOVA was thus used, followed by Tukey HSD post hoc tests to compare wing-web swelling responses within and among treatment groups. One-way ANOVA followed by Tukey HSD post hoc tests were used to compare PHA-induced swelling at 24 h, body condition, mass, follicle size, and beak color score among groups. To compare molt scores among groups, a Kruskal-Wallis test was used, followed by post hoc Mann-Whitney U -tests (pairwise comparisons of groups in successive life cycle stages, Bonferroni-corrected $\alpha = 0.01$). Data could be obtained from $n = 6$ individuals in each group except for postmolt and nonbreeding, nonmolting birds ($n = 5$ for both groups), and

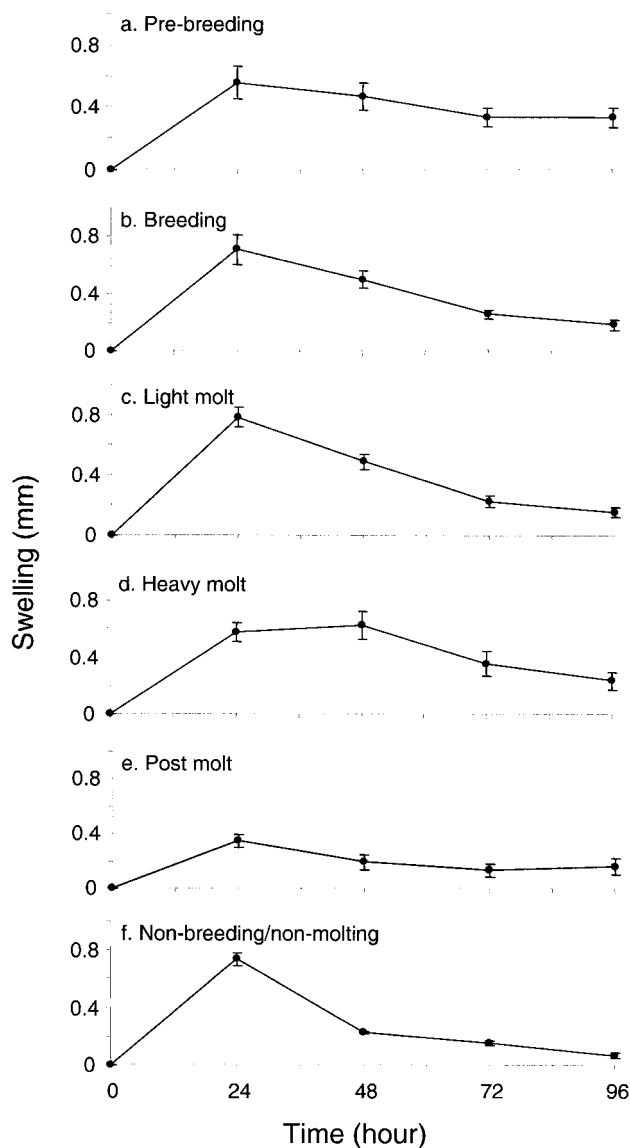


Figure 2. PHA swelling response over 96 h (means \pm 1 SE) of female house sparrows varies with photoperiodically induced life cycle stage ($n = 6$ for groups a-d; $n = 5$ for e-f).

the data were analyzed with SPSS software (ver. 10, SPSS, Chicago), setting $\alpha = 0.05$.

Results

Immune Activity

Females in all life cycle stages showed significant increases in wing web thickness after PHA injection (time: $F_{3,26} = 95.16$, $P < 0.001$; Fig. 2). However, the pattern of the PHA swelling response over 96 h differed with seasonal state (effect of life cycle stage: $F_{5,28} = 7.93$, $P < 0.05$; interaction time \times life cycle stage: $F_{15,84} = 4.22$, $P < 0.001$); the heavy-molt group in partic-

ular retained wing web swelling for a longer period of time than all other groups (Fig. 2). Swelling at 24 h, the most commonly used index of the cutaneous PHA responses in birds, also differed among the groups (Fig. 3a; $F_{5,28} = 3.46$, $P = 0.015$). Light-molt and nonbreeding, nonmolting groups had significantly higher 24-h PHA responses than the postmolt group (post hoc Tukey HSD; postmolt \times light molt: $P = 0.013$; postmolt \times nonbreeding, nonmolting: $P = 0.042$), and breeding birds tended to have higher 24-h responses than postmolt birds (postmolt \times breeding: $P = 0.055$). Prebreeding and heavy-molt groups had intermediate 24-h responses to the other groups (Fig. 3a).

Life Cycle Stage

Breeding birds had larger follicles than all other groups (Fig. 3b; $F_{2,20} = 47.1$, $P < 0.001$). Reproductive maturity in the breeding group was also confirmed by the appearance of two unfertilized eggs in birds' cages 34 and 37 d after photostimulation. Body mass of birds did not vary among groups ($F_{5,28} = 0.229$, $P = 0.947$), but body condition did (Fig. 3c; $F_{5,28} = 3.53$, $P = 0.013$), with postmolt birds having lower body condition than nonbreeding, nonmolting birds (post hoc Tukey HSD: $P = 0.003$). Feather growth also varied significantly with photoperiod treatment (Fig. 3d; $\chi^2 = 32.7$, $P < 0.001$). Light-molt birds were growing some feathers, heavy-molt birds exhibited intensive feather growth, but all other groups did not show molt. Molt scores of light-molt birds were higher than those of breeding birds, and heavy-molt birds had higher molt scores than light-molt and postmolt birds (post hoc Mann-Whitney U -tests; Fig. 3d). Bill color score also showed a seasonal pattern ($F_{5,28} = 27.63$, $P < 0.001$); breeding birds had significantly darker bills than all other groups (post hoc Tukey HSD: $P < 0.001$), and prebreeding birds had darker bills than heavy-molt birds (post hoc Tukey HSD: $P = 0.006$).

Discussion

Our results indicate that cutaneous immune activity varies with life cycle stage in female house sparrows. Cutaneous immune activity was lowest in female sparrows shortly after the conclusion of molt. Immune responses were intermediate in females just entering reproductive condition and in heavy molt. Immune responses were greatest in breeding, light-molt, and nonbreeding/nonmolting birds.

Do Trade-Offs Underlie Seasonal Variation in Immune Defenses?

Vertebrate immune defenses are seasonally labile (Nelson and Demas 1996; Nelson 2004). If trade-offs are the basis for such seasonal variation, immune activity must impart costs of a similar magnitude as reproduction and other seasonally ex-

pensive physiological activities (Sheldon and Verhulst 1996; Norris and Evans 2000). So far, several studies have supported this prerequisite. First, metabolic rates in birds increase after an immune challenge (Ots et al. 2001; but see Svensson et al. 1998), comparable in magnitude to increases detected due to other physiological activities (Martin et al. 2003). Second, nutrients such as vitamins, iron, and protein are critical for development of immune cells and synthesis of effector molecules (Lochmiller and Deerenberg 2000).

To directly demonstrate that seasonal variability in immune activity is due to trade-offs with other costly processes, however, one must either (1) directly identify shifts in the usage of resources such as protein or energy between immune defense and other physiological processes or (2) compare the level of immune defense in animals in different, costly life cycle states under controlled conditions. Here, we used the second approach and regarded the nonbreeding/nonmolting group as a "baseline" group because we expected that nonbreeding/nonmolting birds would be in the least expensive physiological condition. This decision was based on two observations. First, in wild female house sparrows, body mass and fat depots are typically highest in nonbreeding birds after the completion of molt (Hegner and Wingfield 1986). Second, only this group of birds was not actively undergoing other observable physiological processes, namely, growing gonads or feathers, but they had recently completed the same activities.

Reproduction

Reproductive condition is assumed to be energetically expensive due to growth and maintenance of reproductive tissues, expression of sexual behaviors, production of gametes, and care of offspring (King 1973; Ricklefs 1974; Bryant and Newton 1994). However, in this experiment, immune activity was only slightly compromised in females that had just begun to generate their reproductive tracts (prebreeding birds; Fig. 3), whereas females with developed reproductive systems (breeding birds) tended to have immune responses comparable to light-molt and nonmolting/nonbreeding birds. This decrease in immune function in prebreeding birds complements previous findings that gonadal recrudescence in female white-crowned sparrows (*Zonotrichia leucophrys*) costs about 8.34 kcal d^{-1} (King 1973). The lack of immune suppression in reproductively mature (breeding) birds was unexpected, however, because females typically have high resource requirements during this period. In small passerines, egg production and laying can increase basal metabolic rate (BMR) by 45% (Ricklefs 1974).

One explanation of these results may involve the modest reproductive output of birds in this study compared with wild birds. Even though all the females in our study were developing follicles and were therefore in breeding condition (Fig. 3b), only two females actually laid eggs. Further, our captive females were limited in the expression of many behaviors associated

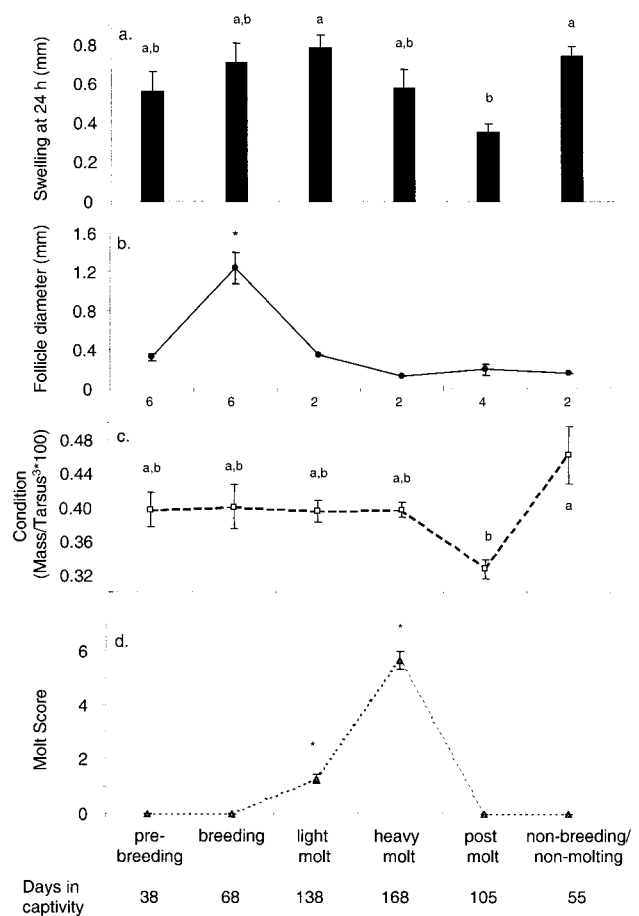


Figure 3. *a*, Swelling response to PHA at 24 h postinjection; *b*, follicle diameter; *c*, body condition; and *d*, molt score of female house sparrows in photoperiodically induced life cycle stages. All data are means \pm 1 SE, sample size for each group = 6, except for postmolt and nonbreeding/nonmolting birds, where sample size = 5. Letters on graphs indicate significant differences between groups by Tukey HSD post hoc tests. In *b*, asterisk indicates significant difference in labeled group versus all others by Tukey HSD test, and numbers below X-axis give number of birds for which follicle sizes were determined. Asterisks in *d* indicate differences between successive groups (post hoc pairwise Mann-Whitney *U*-tests).

with reproduction, including courtship, copulation, nesting, incubation, and nestling provisioning. These activities might contribute substantially to the costs of the breeding state (Monaghan and Nager 1997). Last, trade-offs between immune activity and other physiological processes in general may be less dramatic in captivity than they would be in the wild (Peters 2000). Ad lib. food, the benign thermal environment, and the absence of predators and disease-causing agents could have diminished evidence for immunosuppression in captivity. The manifestation of trade-offs under lab conditions (when the only constraint animals face is resource processing time or limitations of essential amino acids) suggests that trade-offs in the wild must be dramatic.

Molt

Immune responses of female house sparrows at the onset of molt (light molt) were indistinguishable from those of non-molting/nonbreeding birds. However, birds that had just completed molt (postmolt) showed the lowest immune responses of all experimental groups, and birds in heavy molt showed immune function that was intermediate between these groups. Taken together, these results indicate that initiating and sustaining molt (instantaneous trade-offs) do not compromise cutaneous immune activity dramatically, at least not when PHA challenges were made here (but see Martin 2005). Completing a full molt apparently does generate a nutrient or energy deficit, however, which eventually suppresses immune activity.

These results are consistent with studies that indicate trade-offs between immune function and molt. For instance, circulating leukocyte densities were decreased in molting domestic chickens (*Gallus gallus*; Holt 1992; Alodan and Mashaly 1999). In house sparrows, Martin (2005) found (1) a negative relationship between the number of feathers that birds were actively molting and their PHA response and (2) slower feather regrowth in birds over 3 wk after a PHA injection. Such impairments of immune activity during molt may be due to competition for energetic and/or nutritional resources. In house sparrows, the total cost of feather production ranges from 416 to 835 kJ g⁻¹ feathers (Lindström et al. 1993), and the energetic cost of peak molt is 58% daily resting metabolic rate, exceeding that expended on reproduction per diem (34% BMR; Murphy and King 1992). Regrowth of the integument and formation of feather sheaths and stratum corneum during molt in similar-sized species are also very nutrient demanding (Murphy and King 1992; Lindström et al. 1993). Tissues involved in molt can equal 10% of body mass and 25% total body protein contents (mainly keratin; Murphy 1996). Cysteine, the main component of keratins, is a particularly important resource that may be involved in trade-offs, as it can only be derived metabolically from methionine, an essential amino acid (e.g., one that cannot be generated endogenously; Murphy 1996).

Our finding of suppression of immune function after the completion of molt could indicate delayed trade-offs. Delayed trade-offs between molt and immune function might help explain the lack of molt effects on immune function in other studies. As in this experiment on females, captive male house sparrows in early molt stages did not show immunosuppression (Greenman et al. 2005). Alternatively, we might not have found immune suppression during some molt stages because we were assessing one aspect of immune function, cell-mediated immunity. Molt could have led to immune suppression in other arms of the immune system, but this possibility remains unaddressed.

Some aspects of molt-immune interactions remain enigmatic. For example, it has been reported that immune function may be enhanced during molt. Specifically, molting male pied

flycatchers (*Ficedula hypoleuca*) exhibited a positive relationship between their molt score and their PHA response (Moreno et al. 2001). Indeed, one might expect that cutaneous immune activity might be actively upregulated during parts of molt to guard against the presumably greater risk of infection during feather growth. In support, lightly molting sparrows in this study showed the highest swelling responses of all other groups. Clearly, more work on immune activity during molt in captive and wild species is needed to resolve this paradox.

What Factors Mediate Immunological Trade-Offs?

Many factors may mediate seasonal variability in immune activity. One possibility includes variability in body reserves of animals across the year. Generally, body condition is low after demanding physiological activities such as breeding (Hegner and Wingfield 1986). Further, animals in poor body condition tend to exhibit weak responses to PHA (Alonzo-Alvarez and Tella 2001; Martin et al. 2004; but see Greenman et al. 2005). In our study, postmolt birds had the lowest body condition and exhibited the weakest swelling responses, suggesting that even in captivity, birds generate some resource deficits because of costly physiological processes.

A second possibility involves the hormonal profile of birds at different times of year. Part of the differences between this study and our previous work on male birds of this species (Martin et al. 2004; Greenman et al. 2005) may be due to the divergent natural hormone profiles of the two sexes. In male vertebrates, testosterone (T) is typically elevated during the breeding season, but in females it is typically present at low levels year-round (Nelson 2005). Although the significance of T in the modulation of wild animal immune defenses remains unresolved (Hasselquist et al. 1999; Duffy et al. 2000; Evans et al. 2000; Poiani et al. 2000; Casto et al. 2001; Duckworth et al. 2001; Buchanan et al. 2003; Owen-Ashley et al. 2004; Berger et al. 2005; Greenman et al. 2005), immune activity can be affected by this and other androgens (Grossman 1984; Nelson and Demas 1996). Even though female vertebrates generally have lower circulating T levels than males, they experience fluctuations in other steroid hormones, such as estradiol, which may affect immune function during certain life cycle stages (Nelson and Demas 1996). Levels of estradiol across the breeding season in wild house sparrows do in fact mirror the levels of immune activity of females in our experiment (Hegner and Wingfield 1986).

A final influence on the results of our experiments includes methodological limitations, such as the type of immune defense we measured and whatever stress animals might have experienced during and before experiments. We may have missed some seasonal variation in immune activity because of our choice of immune defense for study; other immune defenses may have been affected by reproductive state, but they were not measured (Norris and Evans 2000; Schmid-Hempel and

Ebert 2003; Adamo 2004). Moreover, the functional underpinnings of the immune response we did measure warrants further study. Although it is commonly assumed that more PHA-induced swelling is "better," some studies suggest that this assumption may be invalid (Martin et al., forthcoming). Besides these limitations, the different durations of captivity that groups experienced (an unavoidable consequence of our experimental design) may also have differentially exposed birds to stress and affected the duration or intensity of immune responses (Sapolsky et al. 2000; Martin et al. 2005). We do not feel that this factor confounds our study, however, because time in captivity was not directly related to PHA-induced swellings (Fig. 3). Still, this assumption warrants testing; although many studies have examined the physiology of wild birds in captivity (Bentley et al. 1998; Casto et al. 2001; Owen-Ashley et al. 2004; Greenman et al. 2005), rarely have they made efforts to determine whether and how captivity itself affects the results they have obtained (but see Martin et al. 2004, 2005).

Here, we have shown that immune activity in female house sparrows varies with physiological state, even when birds are held under benign environmental conditions. The slight decrease in immune activity we found in birds during early breeding and heavy molt and the strong suppression of immune function after molt suggest that both instantaneous and delayed physiological trade-offs are important for mediating seasonal variation in immune activity. Further, the lack of immunosuppression in female birds in breeding condition suggests that behavioral costs must contribute substantially to immune suppression during reproduction in wild birds. Females may not have shown impairments of their cutaneous immune responses because they did not display as vigorous aggressive and competitive interactions as males (Summers-Smith 1988). In a previous study, we found that captive male house sparrows in a breeding stage had suppressed cutaneous immune responses, compared with nonbreeding/nonmolting males (Greenman et al. 2005). Because the energetic costs of growing and maintaining active testes are fairly low, we attributed this immune suppression to the behavioral costs of reproduction that these socially housed males experienced in captivity (increased aggressive interactions). Further studies should test this hypothesis, help identify the specific components of each life cycle stage that impart costs, and determine whether steroid hormones mediate trade-offs.

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