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ARTICLE

Condition-dependent sex difference in nestling House Wren (*Troglodytes aedon*) response to phytohaemagglutinin injection

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Abstract: Adult male and female birds typically respond differently to immunological challenges, but whether this difference is present in altricial nestlings is not well-documented. Furthermore, the timing of the development of different axes of the immune system might vary in nestlings and also be affected by differences in condition and health state. We tested for sex-related differences in the immune response of nestling House Wrens (*Troglodytes aedon* Vieillot, 1809) to the injection of phytohaemagglutinin (PHA) and in the bacteria-killing capacity of their plasma. Based on prior work, we predicted that if there were sex-related differences in immune responsiveness, they would most likely occur when the sexes differed in some measure of condition or health state. Female nestlings had a stronger response to the injection of PHA than males in the one of three breeding seasons in which the condition of nestling females exceeded that of males, suggesting that the response to PHA injection is condition-dependent rather than sex-dependent. The sexes, however, did not differ in bactericidal activity, suggesting that different axes of the immune system may not be affected to the same degree by differences in sex or in condition.

Key words: House Wren, *Troglodytes aedon*, sex- and condition-dependent immune response, PHA test, plasma bactericidal assay.

Résumé : Si les oiseaux adultes mâles et femelles réagissent généralement différemment à des stimuli immunologiques, cette différence n'est pas bien documentée chez les oisillons nidicoles. En outre, le moment du développement de différents axes du système immunitaire pourrait varier chez les oisillons et être influencé par des différences sur le plan de l'embonpoint et de l'état de santé. Nous avons vérifié la présence de différences associées au sexe en ce qui concerne la réaction immunitaire d'oisillons de troglodyte familier (*Troglodytes aedon* Vieillot, 1809) à l'injection de phytohémagglutinine (PHA) et la capacité bactéricide de leur plasma. À la lumière de travaux antérieurs, nous avions prédit que d'éventuelles différences immunitaires associées au sexe seraient plus susceptibles de se produire en cas de différences entre les sexes sur le plan de l'embonpoint ou de l'état de santé. Les oisillons femelles présentaient une réaction plus forte à l'injection de PHA que les mâles pour celle des trois périodes de reproduction durant laquelle l'embonpoint des oisillons femelles était supérieur à celui des mâles, ce qui indiquerait que la réaction à l'injection de PHA dépend de l'embonpoint plutôt que du sexe. L'activité bactéricide ne variait pas selon le sexe, ce qui porte à croire que différents axes du système immunitaire pourraient ne pas être influencés dans la même mesure par des différences associées au sexe ou à l'embonpoint. [Traduit par la Rédaction]

Mots-clés : troglodyte familier, *Troglodytes aedon*, réaction immunitaire dépendant du sexe et de l'embonpoint, essai à la PHA, épreuve du pouvoir bactéricide du plasma.

Introduction

Sexual dimorphism in immunoresponsiveness is widespread among invertebrate and vertebrate taxa (e.g., McKean and Nunney 2005; Love et al. 2008; Nunn et al. 2009; Gershman et al. 2010; Aisenberg and Peretti 2011; Steiger et al. 2011). In adult vertebrates, males typically respond to immunological challenges less robustly than females (Møller et al. 1998; Klein 2000; Hasselquist 2007; Nunn et al. 2009; Pap et al. 2010; but see Tieleman et al. 2010), but when this difference in immune function manifests itself is less well-documented, particularly in birds. In altricial bird species, young hatch in an extremely immature state (Ricklefs 1983) and then occupy a common nest, rich in both micro- and macro-parasites, for an extended period of time (Tschirren and Richner 2006). Thus, we might expect any sex-related differences in immune function to develop after leaving the nest. Alternatively, the immune system of male and female nestlings might develop differently in the common nest environment if selection favors either allocating different levels of resources to developing structures and systems, such as the immune system, in the two sexes (Dubiec et al. 2006; van der Most et al. 2011) or differential priming in ovo of the nestling immune system (e.g., Grindstaff et al. 2006). Under some circumstances, as for example when males as adults must compete intensely for access to resources or mates, selection may favor male nestlings that maximize their size, stamina, and strength at the expense of the development of their immunity, while selection may favor females that do the opposite (Bowers et al. 2012).

Complicating matters is that different axes of the immune system might develop at different rates (e.g., Smits and Williams 1999; Palacios et al. 2009), with the adaptive axis perhaps lagging developmentally behind components of the innate branch. In addition, maternal effects could obscure any developmental differences between the sexes. Mothers may, for example, prime the adaptive humoral response of their offspring by incorporating antibodies (Grindstaff et al. 2006; Hasselquist and Nilsson 2009) or

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components of the constitutive innate defense, such as lysozyme, into eggs (Saino et al. 2007). By varying egg size and incubation temperatures, females may also influence other constitutive components of nestling immune defense (Arriero et al. 2013).

Given the myriad of factors potentially affecting immune responsiveness, it is not surprising that some studies have detected sexual dimorphism in the response of altricial nestlings to the injection of phytohemagglutinin (PHA) (Fargallo et al. 2002; Tschirren et al. 2003; Chin et al. 2005; Bize et al. 2005; Dubiec et al. 2006; Bowers et al. 2012), whereas others have not (Saino et al. 2002; Dubiec and Cichoń 2005; Wilk et al. 2007; Arriero 2009). Such decidedly mixed results may reflect differences in the nature and extent of natural immune challenges between species and even populations. However, conflicting results could also stem from differences in the physical condition or health state of nestlings involved in different studies. Dubiec et al. (2006) found that male nestling Blue Tits (*Cyanistes caeruleus* (L., 1758)) have a more robust cutaneous immune response to PHA injection than female nestlings under normal feeding conditions, but not when food is limited. Similarly, in Eurasian Kestrels (*Falco tinnunculus* L., 1758), a species in which males are the smaller sex, male nestlings show a weaker immune response than females, but only when food is limited (Fargallo et al. 2002). Other studies have also reported an effect, often sex-dependent, of nestling mass, condition, parasite load, or health state on the cutaneous immune response (Tschirren et al. 2003; Westneat et al. 2004; Chin et al. 2005; Palacios et al. 2009).

In contrast to our knowledge of the factors affecting the response to injection of PHA, comparatively little is known about the effect of the environment or sex on the bactericidal capacity of blood, a measure of the response by the innate axis of the immune system to the presence of potential pathogens. However, effects of body condition and inbreeding have been documented in nestlings (Townsend et al. 2010). Clearly, additional studies are needed to determine whether sex-related differences in immune responsiveness occur in altricial nestlings, and such studies should control for effects of body condition and general health on immune responses.

We tested for sex-related differences in immune response of nestlings in an altricial passerine, the House Wren (*Troglodytes aedon* Vieillot, 1809). In addition to examining the cutaneous immune response to the injection of PHA, we also tested for a difference in the sexes in plasma bactericidal activity. Based on results of previous research on the study population (Forsman et al. 2008, 2010) and of those described above, we predicted that if sex-related differences were found in immune response, they would most likely occur in situations in which the sexes differed in body condition index or health state.

Materials and methods

Study species and study site

The House Wren is a small (≈11 g), migratory, insectivorous, secondary-cavity-nesting songbird that is sexually monomorphic in size and plumage. At hatching, House Wrens weigh about 1.0 g and after a 10-day period of sigmoidal growth reach the approximate adult mass of 10 g (Johnson 1998).

House Wrens readily accept nest boxes in which to build their nests, because suitable, high-quality cavities are often in short supply in their natural habitat. Males compete for this valuable resource, and their mating success is directly related to their ability to secure exclusive access to one or more nest sites (Johnson and Kermott 1991; Eckerle and Thompson 2006). The intensity of competition among males for access to females is further enhanced by opportunities for attracting multiple social mates to their territories and in engaging in extra-pair matings off-territory (summarized in Johnson 1998). On the study site, individual House Wrens are typically double-brooded, with the eggs of the first brood laid in May and those of the second brood in early July. Modal clutch size is seven eggs in the first brood and six eggs in the second. For additional information on the House Wren's breeding biology see Johnson (1998).

We conducted this study during the 2004, 2005, and 2006 breeding seasons on the Mackinaw study site (40°40′N, 88°53′W) in north-central Illinois, USA, in a second-growth, deciduous forest bordering the Mackinaw River. This site contains nest boxes of uniform construction (see Lambrechts et al. 2010) that are arranged in a grid pattern (5.4 nest boxes/ha). All nest boxes were mounted on 1.5 m metal poles that had been greased or under which 48.3 cm diameter aluminum baffles had been mounted to discourage nest predators. This research was carried out under Illinois State University Institutional Animal Care and Use Committee protocols 17-2003 and 15-2006.

Field procedures

We visited nest boxes at least twice weekly during the May–August breeding season to document the progress of active nests and to determine the date the first egg of each clutch was laid. We later visited boxes daily to pinpoint the date eggs began hatching (brood-day 0; hereafter "hatching date"). On brood-day 11 or 12, we banded nestlings with one US Fish and Wildlife Service numbered aluminum band, weighed them on an electronic balance (Acculab, Pocket Pro 250-B) to the nearest 0.1 g, and measured their tarsus length with dial calipers to the nearest 0.1 mm. At the same time, we collected a blood sample (≈75 μL) from the brachial vein in heparinized microcapillary tubes that were then stored on ice in coolers in the field. Blood was taken to the laboratory later the same day to be centrifuged in a Hematastat at 1610*g* for 60 s (Separation Technologies, Sanford, Florida, USA) to separate cellular and plasma components. Red blood cells were stored at 4 °C and plasma at –20 °C in microcapillary tubes until further analysis.

Condition and health-state measures

We calculated a body condition index for each nestling as the residual value from a least-squares regression of body mass on tarsus length of all nestlings. We used two measures of health state: (1) hematocrit, percent volume of whole blood occupied by packed red blood cells, determined after centrifugation as the mean of three readings; and (2) the plasma albumin/γ-globulin (A/G) ratio, determined, as described in Forsman et al. (2010), using a Helena Laboratories kit (Quick-Gel System, Beaumont, Texas, USA). Hematocrit and the plasma A/G ratio reflect different aspects of health state, with low hematocrit indicative of an anemic condition and high A/G ratios with adequate amino acid reserves and lack of a recent humoral immune response (see Ots et al. 1998). Repeatability of the hematocrit measures was $r = 0.99$ (see Forsman et al. 2010).

Immune responsiveness

In all 3 years of the study, we induced cutaneous immune activity (Martin et al. 2006) on brood-day 11 by injecting in the nestling prepatagium (wing web) 50 μL of sterile phosphate-buffered saline (PBS) containing PHA (Sigma Aldrich, St. Louis, Missouri, USA) at a concentration of 5 mg/mL (see Smits et al. 1999). PHA, a plant-derived protein, induces a measurable tissue swelling as a result of responses from both the innate and the humoral axis of the immune system. We used the change in mean wing-web thickness as a measure of cutaneous immune activity (Martin et al. 2006). We used a digital thickness gauge (Mitutoyo No. 547-500; Mitutoyo America Corp., Aurora, Illinois, USA) to measure wing-web thickness (mean of three successive readings) prior to PHA injection and again ≈24 h after injection. Repeatability of the before and after injection thickness measures were both $r = 0.95$. The mean (±SE) interval between before and after injection measures was

Table 1. Minimal adequate mixed-model ANOVAs of nestling House Wren (*Troglodytes aedon*) immunity and condition in relation to year, sex of offspring, and hatching date.

	df	*F*	*P*
Immune responses			
PHA response			
Year	2, 130	11.56	<0.0001
Sex	1, 567	3.28	0.0708
Hatching date	1, 133	1.68	0.1972
Year × sex	2, 568	2.98	0.0513
Hatching date × year	2, 131	13.09	<0.0001
Hatching date × sex	1, 567	3.05	0.0815
Bactericidal response			
Sex	1, 121	2.07	0.1532
Hatching date	1, 26.6	0.66	0.4222
Hatching date × sex	1, 121	1.84	0.1772
Condition and health-state measures			
Condition index			
Year	2, 126	1.91	0.1516
Sex	1, 576	1.72	0.1900
Year × sex	2, 574	3.47	0.0319
Hematocrit			
Year	2, 128	5.66	0.0044
Hatching date	1, 132	5.43	0.0213
Hatching date × year	2, 129	5.42	0.0055
Albumin/γ-globulin ratio			
Year	2, 130	7.20	0.0011

Note: PHA, phytohaemagglutinin.

23.4 ± 0.11 h in 2004, 24.0 ± 0.10 h in 2005, and 24.6 ± 0.09 h in 2006 (see Forsman et al. 2010).

In addition to measuring the cutaneous immune response, we determined bactericidal activity of plasma (Matson et al. 2006) in 1 year, i.e., 2006. Bactericidal activity measures constitutive innate immunity but also includes an antibody component (Forsman et al. 2010). Within 12 h of blood centrifugation, we incubated duplicate samples of 5 μL of fresh plasma with ≈200 colony-forming units (CFUs) of *Escherichia coli* (ATCC #8739) for 45 min in 100 μL sterile CO_2-independent media (Gibco-Invitrogen, Carlsbad, California, USA) enriched with 5% fetal bovine serum and 4 mmol/L L-glutamine. The plasma–bacteria mixtures were then plated on sterile tryptic soy agar plates, incubated overnight at 37 °C to allow surviving CFUs to grow into visible colonies. The mean number of colonies on duplicate sample plates and on duplicate control plates (no plasma added) was determined 24 h later, and the percentage of total bacteria killed was calculated as [1 – (duplicate sample mean number of colonies/duplicate control mean number of colonies)] × 100. For additional procedural details see Matson et al. (2006) and Forsman et al. (2010).

Sex determination

We extracted DNA from red blood cells following the procedures of Bruford et al. (1992). Sex was determined by amplifying sex-specific introns of the *CHD-1* gene. Polymerase chain reactions (PCR) were carried out with sexing primers 1237L and 1272H (Kahn et al. 1998) using a touchdown protocol as described in Johnson et al. (2002), and the products electrophoresed through 2% agarose gels and stained with ethidium bromide. DNA isolated from adult House Wrens of known sex was included in all sets of PCR runs as controls, and their PCR products were always electrophoresed with those of nestling samples. Because some nestlings did not survive to brood-day 11–12 and some DNA samples did not successfully amplify, not all nestlings in each nest were sexed.

Sample sizes and statistical analyses

In total, we obtained data from 664 nestlings from 129 broods, 19 broods in 2004, 63 broods in 2005, and 47 broods in 2006. We used SAS, version 9.1.3, statistical software (SAS Institute Inc. 2004) for all analyses. We employed mixed-model analyses of variance in PROC MIXED to examine the effect of sex on nestling immune function, the condition index, and measures of health state (hematocrit and the plasma A/G ratio). Measures of PHA response, bactericidal activity, and hematocrit were square root transformed to achieve normality. Nest was included as a random effect to account for the statistical nonindependence of nestlings within a brood. Year was included as a fixed factor (except in the case of the bactericidal assay, which was only employed in 2006) and hatching date was included as a covariate. Parameter estimates were obtained using REML and degrees of freedom (df) were estimated using the Satterthwaite approximation (Littell et al. 2006). To obtain minimal adequate models (Crawley 1993), we employed sequential backward elimination to remove nonsignificant terms ($P > 0.15$), beginning with interactions of the highest order. Therefore, the results reported in Table 1 are from the reduced models. To determine the source of significant interactions involving nestling sex, we used the slice option in PROC MIXED to compare male and female responses in each year of the study.

Fig. 1. Sex differences in phytohaemagglutinin (PHA) response of nestling House Wrens (*Troglodytes aedon*). Back-transformed least-squares means (±SE) are plotted.

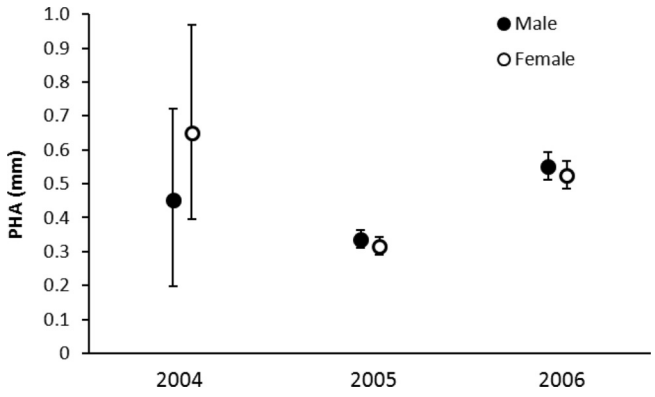
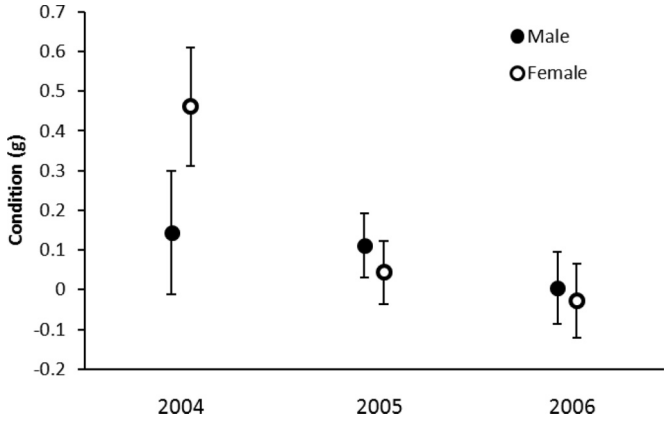


Fig. 2. Sex differences in condition of nestling House Wrens (*Troglodytes aedon*). Least-squares means (±SE) are plotted.



Results

Nestling immune responses

The PHA response differed significantly among broods (Wald $Z = 6.77$, $P < 0.01$). There was a marginally significant interaction between sex and year in their effect on the PHA response (Table 1). Females had a significantly higher PHA response than males in 2004 ($F_{[1,570]} = 5.64$, $P = 0.02$), but there was no difference between

Fig. 3. Phytohaemagglutinin (PHA) response (SQRT, square root transformed) of nestling House Wrens (*Troglodytes aedon*) as a function of their condition (i.e., residual body mass).

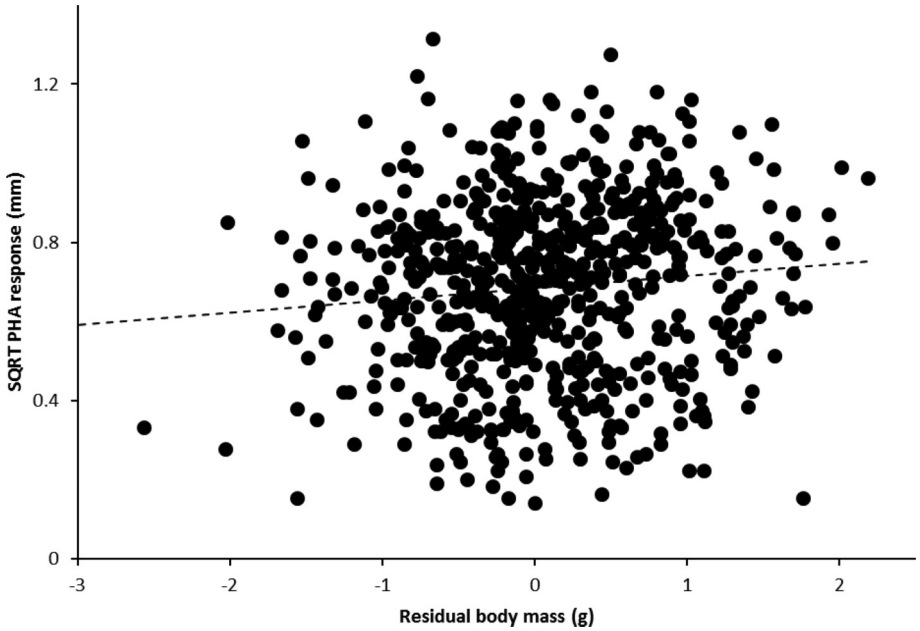


Table 2. Yearly least-squares means (±SE) of nestling House Wren (*Troglodytes aedon*) hematocrit and albumin/γ-globulin ratio (means with different letters are significantly different at $p < 0.05$).

	2004		2005		2006	
	No. of broods	Mean ± SE	No. of broods	Mean ± SE	No. of broods	Mean ± SE
Hematocrit (%)	19	55.0±5.1^{A}	63	41.1±0.6^{B}	47	41.9±0.7^{B}
Albumin/γ-globulin ratio	19	6.09±0.38^{A,B}	63	6.49±0.21^{A}	47	5.31±0.23^{B}

Note: There were no differences between the sexes in these measures.

Table 3. Minimal adequate mixed-model ANOVA of the effect of year, sex, and the condition index on the phytohaemagglutinin (PHA) response of nestling House Wrens (*Troglodytes aedon*).

	df	*F*	*P*
Year	2, 132	10.84	<0.0001
Sex	1, 562	0.04	0.8368
Condition index	1, 637	7.56	0.0061
Condition × year	2, 638	4.42	0.0125
Condition × sex	1, 558	3.32	0.0689

the sexes in 2005 ($F_{[1,561]} = 1.25$, $P = 0.26$) and 2006 ($F_{[1,573]} = 0.83$, $P = 0.36$) (Fig. 1).

Bactericidal activity differed significantly among broods (Wald $Z = 3.07$, $P < 0.01$), but was unrelated to sex or hatching date (Table 1). Percent killing of plasma from male nestlings was 56.3% (7.2–6.8) (back-transformed mean + upper SE – lower SE), whereas percent killing of plasma from female nestlings was 49.2% (6.8–6.3).

Condition index and health state

The condition index differed significantly among broods (Wald $Z = 6.41$, $P < 0.01$). Nestling condition index varied with sex, but only in 1 year (Table 1). Females had significantly higher condition indexes than males in 2004 ($F_{[1,580]} = 5.66$, $P = 0.02$), but there were no differences between the sexes in 2005 ($F_{[1,564]} = 1.12$, $P = 0.29$) and 2006 ($F_{[1,574]} \approx 0.0$, $P = 0.66$) (Fig. 2). Because the sex difference in the condition index paralleled the sex difference in the response to PHA injection in 2004, we explored the influence of condition on the PHA response in a follow-up mixed model with year, sex, and condition as fixed effects. Condition had a significant positive influence on the PHA response (parameter estimate ± SE = 0.0424 ± 0.0185, $F_{[1,637]} = 7.56$, $P = 0.0061$), but the effect of sex was no longer significant ($F_{[1,562]} = 0.04$, $P = 0.84$) (Fig. 3). There was a significant interaction between condition and year in their effect on the PHA response ($F_{[1,638]} = 4.42$, $P = 0.0125$), but the interaction between condition and sex was not quite significant ($F_{[1,558]} = 3.32$, $P = 0.0689$) (Table 3).

Hematocrit varied significantly among broods (Wald $Z = 6.49$, $P < 0.01$). Year and hatching date had a significant influence on hematocrit, and there was a significant interaction between hatching date and year (Tables 1 and 2). Hematocrit was negatively correlated with hatching date in 2004 ($N = 78$, $r = -0.48$, $P < 0.01$) and 2005 ($N = 327$, $r = -0.17$, $P < 0.01$), and positively correlated with hatching date in 2006 ($N = 259$, $r = 0.17$, $P < 0.01$). There was no significant effect of nestling sex on hematocrit.

The A/G ratio varied significantly among broods (Wald $Z = 6.49$, $P < 0.01$) and among years (Tables 1 and 2). The A/G ratio of nestlings was significantly higher in 2005 than in 2006 ($t_{[123]} = 3.79$, $P < 0.01$), but there was no significant difference in the A/G ratio in 2004 and 2005 ($t_{[137]} = -0.93$, $P = 0.35$) or in 2004 and 2006 ($t_{[135]} = 1.72$, $P = 0.09$). There was no significant effect of nestling sex on the A/G ratio.

Discussion

In 1 of the 3 years of the study, females produced a more robust response to PHA injection than males. This was also the only year in which the sexes differed in their condition indexes, with females being in better condition than males. Male and female nestlings did not differ in either measure of health state (hematocrit

and A/G ratio) in any of the 3 years. Thus, the cutaneous immune response of female nestlings was more robust than that of males only in the year that female condition exceeded that of males. This suggests that the response to PHA injection in nestlings is more likely condition-dependent than sex-dependent. Indeed, when the condition index was included in the analysis of the PHA response, the effect of sex was eliminated, whereas the cutaneous immune response positively covaried with the condition index across all nestlings (Fig. 3). Moreover, the absence of a significant interaction between sex and condition on the PHA response suggests that the effect of condition on the PHA response does not differ between the sexes. The positive relationship between cutaneous immune response and the condition index is consistent with that reported in most studies of altricial nestlings in a variety of species (e.g., Westneat et al. 2004; Moreno et al. 2005; Lobato et al. 2008; but see Sandell et al. 2009).

Experimental studies have demonstrated that covariance between the response to PHA injection and condition measures often reflects an underlying direct causal link mediated by access to critical resources. When food availability is manipulated during the nestling stage, the positive relationship between condition and PHA response arises only when food is restricted and body mass is negatively affected compared with controls (Alonso-Alvarez and Tella 2001). A similar result has been reported when clutch or brood size is manipulated, creating a situation in which aspects of nestling growth and survival are adversely affected because food resources apparently are limited (Fargallo et al. 2002; Dubiec et al. 2006). This evidence for condition-dependence of the response to PHA injection may explain why there are conflicting reports in the literature on the relationship between the sex of nestlings and the cutaneous immune response to PHA injection.

Components of the cutaneous immune response involve not only the adaptive axis of the immune system but also the innate axis (Martin et al. 2006). The underlying molecular and cellular mechanisms of sexual dimorphism in the innate immunity typically found in adults are unknown, but recent evidence from studies of mammalian models point to antigen-presenting cells (APC) as key mediators. Females generally have a more efficient acute inflammatory response linked to both a higher number of tissue-resident APC and increased activity of these cells (Rettew et al. 2009; Scotland et al. 2011). In mammals, these changes are reported to be driven by estrogen receptors present on macrophages and dendritic cells, as well as T and B cells (reviewed in Fish 2008). Given this important mechanism mediating sexual dimorphism, the PHA assay chosen here, which relies heavily upon tissue-resident APC, may be more likely to reflect early developmental sex-specific differences compared with other immune measures, such as the bactericidal assay. The response to PHA injection should be particularly robust in cases where individuals are in good body condition.

If, in fact, the stronger cutaneous immune response of female nestlings in 2004 can be attributed to their higher condition index, we can offer no obvious explanation as to why female nestlings were in better condition than males in that year. We think it unlikely that parents differentially provisioned nestlings according to the sex of their young. A more recent study of this same population has revealed that when marked differences in age and size among nestlings occur, male House Wren offspring apparently trade off investment in immune responsiveness for increased growth, whereas females sacrifice growth for increased immune responsiveness (Bowers et al. 2012). This sex difference, along with the sex difference documented in the present study, may reflect a sex-specific trade-off between growth and immunity in which male fitness is tied most closely to size, stamina, or other measures of vigor, whereas female fitness is optimized through increased allocation to immune function.

We did not find sex-based differences in the bactericidal activity of nestling plasma in the 1 year in which we measured it, which is consistent with the findings of others when comparing adult birds (Rubenstein et al. 2008, Tieleman et al. 2010; Houdek et al. 2011). However, in a subsequent study of the same House Wren population, the bactericidal activity of male, but not of female, nestlings increased significantly as the breeding season progressed (Bowers et al. 2012). We think, therefore, that it would be premature to suggest that male and female nestlings of altricial species of birds are unlikely to differ in this important component of their immune defense. We obtained data on bactericidal activity from only one breeding season, and no other studies have applied this assay to any other species to investigate sex-related differences in altricial nestlings. We think it is possible, therefore, that bactericidal activity varies with adult and nestling condition and health state, as do other measures of immune function. This caution seems further justified because of the considerable differences in bactericidal activity observed not only interspecifically (e.g., Millet et al. 2007; Girard et al. 2011; Horrocks et al. 2012) but also intraspecifically under different environmental conditions (e.g., Buehler et al. 2008, 2009; Rubenstein et al. 2008; Pap et al. 2010) and among broods in the study population (Forsman et al. 2008). In addition, comparisons among studies are complicated by whether whole blood (plasma plus cellular components) or plasma only is used for the assay (e.g., Millet et al. 2007; Morrison et al. 2009, respectively).

In summary, we found evidence of a sex-related difference in nestling immune responsiveness in only one of three breeding seasons, with females mounting a more robust cutaneous immune response than males in the only year in which females were in better condition than males. Based on these results and the lack of a difference between the sexes in condition, health state, and in the bactericidal activity of their plasma in the 1 year it was measured, we conclude that the difference in immune responsiveness that was detected is more likely a condition-related response than a sex-related response.

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