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E-Article

Sex-Specific Variability in the Immune System across Life-History Stages

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abstract: Organisms theoretically manage their immune systems optimally across their life spans to maximize fitness. However, we lack information on (1) how the immune system is managed across life-history stages, (2) whether the sexes manage immunity differentially, and (3) whether immunity is repeatable within an individual. We present a within-individual, repeated-measures experiment examining life-history stage variation in the inflammatory immune response in the zebra finch (*Taeniopygia guttata*). In juveniles, age-dependent variation in immune response differed in a sex- and context-specific manner, resulting in no repeatability across stages. In adults, females displayed little stage-dependent variation in immune

“goals” at each of the life-history stages, we generated three main predictions.

1. During postnatal development and pre–basic molt, resource-mediated, sex-specific differences in the responsiveness of the immune system are predicted in species exhibiting sexual size dimorphism (Møller et al. 1998; Moore and Wilson 2002; Rolff et al. 2005; Dubiec et al. 2006) or sexually dimorphic ornaments/displays (Hill 1999; Møller et al. 1999; Ryder and Siva-Jothy 2000; McGraw and Ardia 2003; Jacot et al. 2004; Kilpimaa et al. 2004). Because of different stage-specific resource demands among sexes, we therefore predicted that there would be (*a*) no sex difference in inflammatory responses in fledging zebra finches because the sexes are similar in size and have monomorphic plumage but that (*b*) sex differences in inflammatory responses would occur at molt, when males develop sexually dimorphic plumage.

2. Immune responses are generally thought to be resource dependent (Dubiec et al. 2006; French et al. 2007*a*, 2007*b*; Houston et al. 2007); as such, we predicted that the inflammatory response would decrease during life-history stages when resource competition with other physiological systems increases (e.g., French et al. 2007*b*). Specifically, (*a*) in adults, responses during nonbreeding should be the highest of all stages and similar between sexes, given that birds should have relatively low energetic costs other than those associated with daibutweiologiriolog-640lowwhse should gxhibiti-385.1(sow)r geleponses thea

Table 1: Life-history stages used for measurement of the inflammatory immune response in zebra finches

Stage	Age (days)	Diet
Juvenile:		
Fledging	17–18	Seed and egg food
Pre–basic molt	60–80	Seed
Reproductively mature	120	Seed
Adult:		
Chick rearing	1120	Seed and egg food
Egg laying (HQ diet)	1120	Seed and egg food
Egg laying (LQ diet)Egm(n)795.70 7.9699 185.4999 595.7516 Tm()Tj/F4 1 Tf8()Tj/F4 1f8()T6(yi)5.6(n)5.6(g)-350((H)5.5Tegg food		

measured across three life-history stages and two resource levels, and a total of 77 nestlings from these 24 nests were observed across three life-history stages.

Measurement of the Innate Cutaneous Inflammatory Response

We used a PHA injection assay to evaluate, in vivo, an innate cutaneous inflammatory immune response; this technique is widely used in ecological immunology studies (Tella et al. 2002; Martin et al. 2006*c*; Salvante 2006). We intradermally injected the right wing web (patagia) of each bird with 30 g PHA (PHA-p, Sigma: L-9132) in 30 L of sterile phosphate buffered saline (PBS) using a monoject

Figure 2: Intersexual variation in the inflammatory immune response across three life-history stages in adult zebra finches: (*a*) LSM □ SEM (different letters represent statistically significant differences between stages within a given sex, as calculated using the sequential Bonferroni post hoc method; see "Methods") and individual variation in adult (*b*) females and (*c*) males. Laying birds were examined while they were receiving a high-quality (HQ) and a low-quality (LQ) diet.

repeated measure (repeated across stages), sex as a fixed factor, body mass at each stage as a covariate (when related to responses), and adult pair number as a random factor to control for differences between nests owing to parental environmental/genetic quality. Post hoc comparisons were performed using the adjusted Bonferroni post hoc procedure, with the *P* value corrected for the number of pairwise comparisons made depending on the type of analysis used (Rice 1989; corrected $P = .017$ for the three possible stage comparisons among juveniles and $P = .0083$ for the six possible stage comparisons among adults). To examine whether inflammatory responses and body mass are predictive from one life-history stage to the next under varying resource conditions, we calculated the repeatability for these two traits for both juveniles and adults according to Lessells and Boag (1987). As defined by Lessells and Boag (1987, p. 116), "repeatability is a measure used in quantitative genetics to describe the proportion of variance in a character that occurs among rather than within individuals"; therefore, our repeatability index reflects the proportion of variation in either the inflammatory response or the body mass among, rather than within, individuals. We included calculations of the repeatability of body mass in this study as a baseline trait that is often correlated with inflammatory responses and is expected to reveal some plasticity across stages, but perhaps less than the immune system is. To perform repeatability calculations in juveniles we included individual identity in the model, nested within brood, to avoid pseudoreplication when using multiple nestlings from a given nest (C. Lessells, personal communication). It should be noted that sample sizes reported for adults decreased across the course of the experiment (1) because of natural mortality in this short-lived species and (2) because some adults did not breed while receiving the LQ diet. As such, repeatability calculations involving the LQ diet are limited by the number of females remaining in the experiment that would breed under LQ diet conditions. Where relevant, nonsignificant interactions were backward eliminated; results are presented for reduced models. Repeated-measures analysis was performed using PROC MIXED in SAS (ver. 9); all other analyses were conducted using JMP (ver. 6.0).

Results

Ontogenetic Variation in Inflammatory Immune Responses

Individual variation in immune responses across the three life-history stages examined in juvenile birds was high for both sexes (female ANOVA: $F = 2.51$, $\mathrm{df} = 28, 86$, $P = .01$; fig. 1*b*; male ANOVA: $F = 1.89$, $\mathrm{df} = 47, 142$, $P = .004$; fig. 1*c*). Males displayed significantly higher inflammatory responses than females did at fledging (ANOVA: $F = 6.26$, $\mathrm{df} = 1, 75$, $P = .016$), whereas both sexes displayed similar inflammatory responses at the pre–basic molt and reproductively mature stages (ANOVA: $F = 3.28$, $\mathrm{df} = 1, 52$, $P = .08$, and $F = 1.11$, $\mathrm{df} = 1, 52$, $P = .30$, respectively). Despite large individual variations within stages, juvenile zebra finches exhibited consistent intersexual differences in the pattern of changes in the response from fledging through the pre–basic molt stage to the reproductively mature stage (repeated-measures ANCOVA, stage × sex effect: $F = 2.38$, $\mathrm{df} = 2, 97$, $P <$

Figure 3: Interstage repeatability in the inflammatory immune response in juvenile female zebra finches (*a*) from the fledging to the molt stages and (*b*) from the fledging to the reproductively mature stages.

.05; fig. 1*a*). In females, individuals at the reproductively mature stage displayed stronger inflammatory responses than they had at both the fledging and the pre–basic molt stages (Bonferroni post hoc analysis: $P = .008$ and

Figure 4: Interstage repeatability in the inflammatory immune response in juvenile male zebra finches (*a*) from the fledging to the molt stages and (*b*) from the fledging to the reproductively mature stages.

fledging and the reproductively mature stages ($F = 0.97$, df = 28, 57, $P = .53$; fig. 3*b*). In juvenile males, although immune responses were somewhat repeatable between the fledging and the pre–basic molt stages (34.13%, $F = 2.04$, df = 47, 94, $P = .008$; fig. 4*a*), they were not repeatable between the fledging and the reproductively mature stages ($F = 1.27$, df = 47, 94, $P = .21$; fig. 4*b*). In general, therefore, fledging-stage immune responses did not predict variation in immune responses at subsequent stages. Similarly, female fledging-stage body mass did not predict body mass at either the pre–basic molt stage (, ,) or the reproductively ma-

Figure 5: Interstage repeatability in the inflammatory immune response between the nonbreeding and the egg-laying stages in adult (*a*) female and (*b*) male zebra finches in relation to dietary treatment during laying (while receiving either a high-quality diet or a low-quality diet).

used for displays (Hill 1999; Møller et al. 1999; McGraw and Ardia 2003; Kilpimaa et al. 2004). As such, we predicted that males should experience the lowest inflammatory responses at the pre–basic molt stage; however, the sexes were similar in their inflammatory responses. Nevertheless, inflammatory responses in males did decrease from the fledging stage to the molt stage, which may indicate that males may prepare in advance for the increased energetic demand of molting, similar to that which has been observed to occur in the seasonal preparation of the immune system for the stress of winter conditions in small mammals (Demas and Nelson 1998). Mechanistically, it is possible that the pleiotropic effects of hormones involved in the molt process (such as thyroid hormone; Payne 1972) may contribute to this decrease in inflammatory response given the hormone's role in B cell development (reviewed in Dorshkind and Horseman 2000). At reproductive maturity, the sexes displayed similar inflammatory responses. In general, our analysis of the variation in cutaneous inflammatory responses in juvenile zebra finches reveal that the sexes experience distinct immune management strategies during development and similar responses when they have reached adulthood.

Variation in the Inflammatory Response of Adults

The immune system is often regarded as a resource-driven physiological trait (Klasing 2002), and recent experimental manipulations indicate that the trade-off between reproduction and the immune system in females may be facultative (resource driven; French et al. 2007*a*, 2007*b*). On the basis of the idea of a direct trade-off between these two systems under limited-resource conditions, we predicted that inflammatory responses of adult females would decrease from the nonbreeding stage to the egg-laying stage as females attempt to maintain reproductive output, because the immune system may compete with egg production for a potentially limited pool of resources. In support of recent experimental work in lizards (French et al. 2007*a*, 2007*b*), we found that, although inflammatory responses in reproductive (egg-laying) females with access to an HQ diet were not different from the inflammatory responses experienced when the birds were in the nonbreeding stage, individuals in the egg-laying stage who received an LQ diet experienced a significant decrease in their inflammatory responses. However, even when mothers were able to lay and raise nestlings with access to an HQ diet, the inflammatory response while raising offspring was significantly lower than the one measured when individuals were classified as nonbreeders. Importantly, despite a reduction in the inflammatory responses experienced by laying individuals that were receiving the LQ diet, mothers do not appear to directly trade off immune function for reproduction (contrary to what we predicted for this short-lived species; Zann 1996; as proposed by Birkhead et al. [1999]).

We observed that females displayed the same positive relationship (and identical slopes) between clutch size and the inflammatory response while receiving both diets (see "Results"). Moreover, females experienced both reduced inflammatory responses and reduced reproductive effort (clutch size and egg size) when laying while receiving the LQ diet compared with those receiving the HQ diet (egg size: paired *t*-test, P ! .0001; clutch size paired *t*-test, P p .0014; O. P. Love, K. G. Salvante, J. Dale, and T. D. Williams, unpublished data). Whether females have flexibility in both systems (i.e., they are able to modulate both reproductive effort and inflammatory responses simultaneously without facing a direct trade-off for either) or whether decreased resources cause a fixed decrease in both systems is unknown and requires more investigation. However, given that female zebra finches with access to an HQ (protein-rich) diet displayed no reduction in inflammatory responses during the egg-laying stage, our results also suggest that modulation during reproduction is a facultative (resource-driven) response rather than an obligatory side effect of going through reproduction per se, as reported recently in a manipulative study in lizards (French et al. 2007*a*, 2007*b*).

Although ecologists have traditionally considered egg laying to be highly energetically costly in terms of both the nutrients placed in eggs (Perrins 1996; Monaghan and Nager 1997) and the overall metabolic demand associated with egg production (Nilsson and Råberg 2001; Vézina and Williams 2002), recent work has revealed that this metabolic increase may be small in relation to increases found at other life-history stages (K. G. Salvante, F. Vézina, and T. D. Williams, unpublished data). In fact, females can employ many behavioral and energetic reallocation tactics to maintain daily energy expenditure (Vézina and Williams 2003; Vézina et al. 2006), and our results high-

Daan 1980; Hasselquist et al. 2001; Love et al. 2004; Ardia et al. 2003), we understand that males who were raising offspring did so under captive conditions and when they had access to the HQ diet. Nonetheless, taken together, our measurements of the inflammatory response in adults during reproduction suggest a sex-specific modulation of the immune system in response to the energetically demanding phases of both egg laying and chick rearing that is enhanced by the quality of available resources. Furthermore, females may modulate the immune system within stages to manage trade-offs between current and future reproduction and survival to maximize fitness.

Individual Variation and Repeatability of Inflammatory Responses

Physiological ecologists are beginning to appreciate that how the immune system responds during a given stage should be dependent on both the immediate and the future costs of the current response (Viney et al. 2005; Bertrand et al. 2006). As such, we hypothesized that the inflam-

Conclusions and Future Considerations

The value in the experimental design of this study lies in our ability to examine (1) whether the sexes differ in their management of the immune system across stages, (2) whether the immune system exhibits plasticity across stages and in response to resource variation, and (3) whether inflammatory response or the state of the immune system at a given stage predicts how the system will respond at future life-history stages. Nevertheless, it is important to note that, within the inflammatory response itself, there exists significant temporal variation in the various cell types that are recruited during the response (Martin et al. 2006*c*). Moreover, the organismal immune response is highly complex and is differentiated into humoral, cell-mediated, and innate components (Tieleman et al. 2005; Salvante 2006). As such, despite at least a decade of research by ecological immunologists, we still have a relatively poor understanding of how each of these three systems should both respond and work together in shaping life-history evolution (Zera and Harshman 2001; Tella et al. 2002; Zuk and Stoehr 2002; Viney et al. 2005). The juvenile and adult data presented here are consistent with the inflammatory immune response being a highly plastic and sex-specific trait reacting in a context-dependent manner. Although not possible with this present data set, relating intraindividual variation in inflammatory responses (and potentially any physiological trait) to variation in fitness is now possible via a reaction norm approach—something ecophysiologists are beginning to appreciate (Williams 2008). It is difficult to make extensions to the innate and humoral responses, but it is reasonable to suggest that both responses would be expected to reveal a similar degree of plasticity as we have demonstrated here for the cellular response given that one would predict that individuals will be exposed to the existence of seasonal and stage-specific pathogens, variation

Demas, G. E., and R. J. Nelson. 1998. Photoperiod, ambient temperature and food availability interact to affect reproductive and immune function in adult male deer mice (*Peromyscus maniculatus*