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E-Article

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

Oliver P. Love,[*] Katrina G. Salvante,[†] James Dale,[‡] and Tony D. Williams[§]

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

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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 response when laying while receiving a high-quality (HQ) diet; however, laying while receiving a low-quality (LQ) diet significantly reduced both immune responses and reproductive outputs in a manner consistent with a facultative (resource-driven) effect of reproduction on immunity. Moreover, a reduced immune response in females who were raising offspring while receiving an HQ diet suggests a residual effect of the energetic costs of reproduction. Conversely, adult males displayed no variation in immune responses across stages, with high repeatability from the nonbreeding stage to the egg-laying stage, regardless of diet quality (HQ diet, $r = 0.51$; LQ diet, $r = 0.42$). Females displayed high repeatability when laying while receiving the HQ diet ($r = 0.53$); however, repeatability disappeared when individuals received the LQ diet. High-response females receiving the HQ diet had greater immune flexibility than did low-response females who were laying while receiving the LQ diet. Data are consistent with immunity being a highly plastic trait that is sex-specifically modulated in a context-dependent manner and suggest that immunity at one stage may provide limited information about immunity at future stages.

Keywords: cell-mediated immunity, life-history theory, sex-specific differences, individual variation, repeatability, zebra finch (*Taeniopygia guttata*).

Interpreting variation in immunocompetence is now thought to be a critical component in understanding the evolution of life histories (e.g., Zera and Harshman 2001; Viney et al. 2005; French et al. 2007*b*), sexual selection (Westneat and Birkhead 1998; McGraw and Ardia 2003), and population dynamics (Lochmiller 1996). In general, immune systems are not always expected to respond maximally (Zuk and Stoehr 2002; Viney et al. 2005), and hosts should exhibit considerable variability in defense strategies (Wakelin and Apanius 1997; Lee 2006). Moreover, short-term immunological adjustments should be managed optimally with respect to long-term effects on an animal's survival and lifetime reproductive success (Viney et al. 2005; Bertrand et al. 2006). Variation across stages must also encompass potential within-stage trade-offs between immune responses and other life-history stages, such as growth (Soler et al. 2003; Chin et al. 2005; Dubiec et al. 2006), reproduction (Bonneaud et al. 2003; Ardia 2005; Hanssen et al. 2005; French et al. 2007*a*, 2007*b*), and molt (Sanz et al. 2004; Martin 2005; Martin et al. 2006*a*). Therefore, life-history theory predicts that optimal immune responses should be plastic and context specific, where the maintenance and response of the system is managed across a wide range of environmental conditions and life-history stages (Ricklefs and Wikelski 2002; Viney et al. 2005). However, because most work to date has focused on comparing life-history measures while controlling for a single life-history stage (a productive way to begin understanding trade-offs between the immune system and other systems), we currently have an underappreciation of how the immune system is managed across the life span of an individual (Sandland and Minchella 2003; Martin et al. 2006*a*).

Immune responses are thought to be "costly" in the

* Corresponding author; e-mail: olovea@sfu.ca.

† Present address: Department of Biology, University of North Carolina, Chapel Hill, North Carolina, 27599; e-mail: ksalvante@unc.edu.

‡ Present address: Max Planck Institute for Ornithology, P.O. Box 1564, D-82305 Starnberg (Seewiesen), Germany; e-mail: jamesdale68@yahoo.com.

§ E-mail: tdwillia@sfu.ca.

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short term, on the basis of evidence of either increased energetic demands during immunological challenges (Demas et al. 1997; Ots et al. 2001; Martin et al. 2003; however, see Svensson et al. 1998) or apparent trade-offs between the immune system and life-history traits within specific life-history stages (see references above). Furthermore, many of these trade-offs can be context specific, where a significant driving force may be resource availability (Chin et al. 2005; French et al. 2007*a*, 2007*b*; Houston et al. 2007). Although there is still debate over whether individuals facultatively respond to variation in resources within stages or whether it is the stage per se that causes a change in immunity (see French et al. 2007*a*, 2007*b*), individuals cannot ignore the potential future costs of mounting an immune response in the present because immunological responses in a current life-history stage can affect both future reproduction (Hanssen et al. 2005) and survival (Moret and Schmid-Hempel 2000; Ardia et al. 2003; Hanssen et al. 2004; Jacot et al. 2004). Unfortunately, without knowledge of how animals manage their immune systems across different life-history stages, it is difficult to interpret how future performance (i.e., of the immune system, the reproductive system, etc.) will be impacted. Indeed, few studies have attempted to examine immune performance across life-history stages (however, see Martin et al. 2006*a*). Rather, many ecological immunological studies in vertebrates inherently assume that measuring components of the immune system at a static life-history stage will (1) correlate with immune function at future stages and, more important, (2) provide meaningful predictive information on both current condition and future survival and/or fitness. The current state of knowledge in vertebrates contrasts strongly with studies in invertebrates, where research encompassing numerous life-history stages has been performed to understand how immunological modulations at one life-history stage affect the immune response at future stages (Kraiijeveld and Godfray 1997; Moret and Schmid-Hempel 2000; Jacot et al. 2004, 2005*a*, 2005*b*; Rantala and Roff 2005) and survival (Moret and Schmid-Hempel 2000; Jacot et al. 2004).

To investigate sex-specific plasticity in the vertebrate immune system across life-history stages and in response to changes in resource quality, we examined a commonly employed measure of inflammatory immune responsiveness in ecological studies of vertebrates, the phytohemagglutinin (PHA) skin test (Norris and Evans 2000; Martin et al. 2006*c*; Salvante 2006), using a within-individual, repeated-measures approach. Inflammation in response to injection of PHA is driven by dynamic cellular processes involving multiple leukocyte populations, wherein most leukocytes (with the exception of heterophils and lymphocytes) increase over time following the injection (Martin et al. 2006*c*). We chose to examine the inflammatory immune response because it has been used extensively by ecologists to investigate numerous life-history trade-offs in vertebrates (Martin et al. 2006*c*; Salvante 2006) and because it should allow for unbiased repeated measures (i.e., little or no effects of priming of the immune response; see "Methods" for a detailed explanation), unlike measures of humoral immunity that indicate "memory effects." Although recent work has suggested that the inflammatory response may involve a complex dynamic of cell types (Martin et al. 2006*c*), our goal was to simply examine large-scale changes in this commonly used technique as a general measure of cell-mediated immunity within a life-history context. We used a captive vertebrate model organism, the zebra finch (*Taeniopygia guttata*), which has been used extensively to examine immune-related life-history trade-offs in birds (McGraw and Ardia 2003; Bertrand et al. 2006; Dubiec et al. 2006). However, we observed males and females concurrently through the same life-history stages under the same environmental conditions, a technique that has rarely been used in studies of immune responses (Zuk and Stoehr 2002; however, see Moreno et al. 2001). First, we examined ontogenetic variation in birds at three different life-history stages associated with varied energetic demands: nestlings at fledging, juveniles at the pre–basic molt stage, and sexually mature adults. Second, among adult birds we examined variation within and across the nonbreeding, egg-laying, and chick-rearing stages; to investigate how resource availability affects potential trade-offs between the immune and reproductive systems, we also manipulated diet quality during the egg-laying stage. Finally, to examine plasticity in the vertebrate immune system, we measured the between-stage repeatability of immune responses to test whether the inflammatory responses at future stages could be predicted from the response at a current stage, because the necessity for plasticity in the immune system may divorce relationships between individual across-stage relationships. We hypothesized that, if the immune system comprises plastic and context-specific physiological responses, then (1) inflammatory response should vary depending on both life-history stage and resource availability, with stages and resource conditions that are particularly demanding having lowered responses; (2) repeatability between stages should generally be low when decreases in resource availability force a trade-off between the immune system and other systems and higher when these trade-offs are experimentally relaxed; and (3) there should be an interaction between sex and life-history stage/resource availability on immune response because the sexes have different resource demands at specific life-history stages. Within the framework that males and females will face different costs and therefore have different evolutionary

"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 daily maintenance; (*b*) laying females should exhibit lower responses than their breeding mates because of the effects on immunity of higher resource reallocation toward egg production versus sperm production; and (*c*) this difference should be exacerbated in females who are laying while receiving a low-quality (LQ) diet. Individuals raising young should exhibit lower responses than they would during the nonbreeding stage, given that parental workload significantly rises during the chick-rearing stage (Drent and Daan 1980; Hasselquist et al. 2001; Love et al. 2004; Ardia 2005), potentially diverting energy away from being able to immunologically respond.

3. We predicted that the inflammatory responses of individuals should exhibit some degree of plasticity across life-history stages given differential resource allocation to the immune system within and potentially between particular life-history stages. As such, we predicted significant interindividual variation in responses within stages; although individuals may display similar general patterns in relation to changing resources within specific stages, we predict low within-bird repeatability of responses across stages.

Importantly, the predicted differences between the sexes allow us to test whether potential trade-offs between the immune system and other systems at various stages are obligatory or facultative (i.e., resource driven; French et al. 2007*a*, 2007*b*). For example, obligatory trade-offs would occur if either one sex at a given stage or all reproductively active individuals always display lower immune responses, whereas facultative trade-offs would be observed only when individuals have limited access to resources (French et al. 2007*b*).

Methods

Study Species and Dietary Manipulation

Zebra finches are size monomorphic but sexually dichromatic as adults (Zann 1996). In captivity, female zebra finches lay four to six eggs per clutch, with one egg laid per day. They incubate the eggs for 10–11 days and fledge nestlings 20–23 days after hatching. Both parents attend the nestlings during the chick-rearing stage (Zann 1996). At fledging, males and females exhibit monomorphic gray plumage with dark black beaks; at approximately 8 weeks after hatching, juveniles begin to molt into adult plumage (Zann 1996). In this study, all zebra finches were housed in an indoor room at the Simon Fraser University Animal Care Facility (Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada) under controlled environmental conditions (temperature, 19–23°C; humidity, 35%–55%; constant light schedule of 14L : 10D). Experiments and animal husbandry were performed under a Simon Fraser University Animal Care Committee permit (no. 558B) and followed the guidelines of the Canadian Committee on Animal Care (1984). All birds received mixed seed (Panicum and white millet, 50 : 50, approximately 12.0% protein, 4.7% lipid; Jameson's Pet Food, Vancouver) as a maintenance diet, in addition to water, grit, and cuttlefish bone (calcium) ad lib. and a multivitamin supplement that was added to the drinking water once a week.

Breeding pairs ($n = 24$) were housed individually in cages (61 cm × 46 cm × 41 cm) equipped with an external nest box (15 cm × 15 cm × 15 cm). Adult females were randomly paired with an experienced adult male who had at least 28 days between any previous breeding attempts. As part of our regular breeding protocol, pairs were provided with an egg-food supplement ("high quality" [HQ] diet: 6 g of a mixture of hard-boiled egg, cornmeal, and bread crumbs [3 : 1 : 1 w/w, 30.2% protein and 13.0% lipid by dry mass]) daily between pairing and clutch completion in addition to the ad lib. seed diet. The LQ diet consisted of the ad lib. maintenance seed diet only. These diets are intended to best mimic diets in the wild, where nonbreeding birds have access to seed-only diets whereas breeding birds have access to sprouted (protein-rich) seeds (Zann 1996). As such, nestlings and chick-

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	>120	Seed and egg food
Egg laying (HQ diet)	>120	Seed and egg food
Egg laying (LQ diet)	>120	Seed
Nonbreeding	>120	Seed

Note: HQ = high quality; LQ = low quality.

rearing birds also had access to the HQ diet as a part of the standard breeding procedure.

Experimental Design

Two groups of birds (juveniles and adults) were used in the overall experiment. Individuals within each group were observed through the life-history stages outlined in table 1. Although the only manner in which to measure within-individual responses in juveniles is sequentially across developmental stages, we followed our experimental design for adult birds rather than use a possible fully factorial design because the former was the most logistically feasible given our particular set of questions (e.g., adult birds produced offspring that were observed as a part of this study). Inflammatory responses were measured in juveniles ($n = 77$) and in adults ($n = 48$) when nestlings were 17 or 18 days of age (defined as the fledging stage for nestlings and the chick-rearing stage for adults; table 1). Following fledging, juveniles were housed in communal cages until molt began (60–80 days of age; table 1), at which time birds could be sexed on the basis of sexually dichromatic plumage, and inflammatory responses were again measured. Following inflammatory response measurement, birds were placed in same-sex communal cages. Finally, at 120 days of age, inflammatory responses were again measured in the juveniles, who were now considered to be sexually mature (nonbreeding) adults (table 1). Following the chick-rearing period, adult birds were rested for 45 days, at which time they were paired once again with the same mate; inflammatory responses were measured at the first-egg stage of laying while individuals were receiving the HQ diet (table 1). After clutches were complete, birds were unpaired and allowed to rest for 90 days. Following this, birds were paired again (usually with the same mate; 22/24 pairs), and inflammatory responses were again measured during the egg-laying stage while birds now received the LQ diet (table 1). Finally, following another rest period of 90 days, inflammatory responses were measured in birds now considered to be nonbreeding adults (table 1). When not paired for breeding, adult birds were housed in same-sex cages but were not visually or acoustically isolated from birds of the opposite sex. In total, 24 pairs of adults were

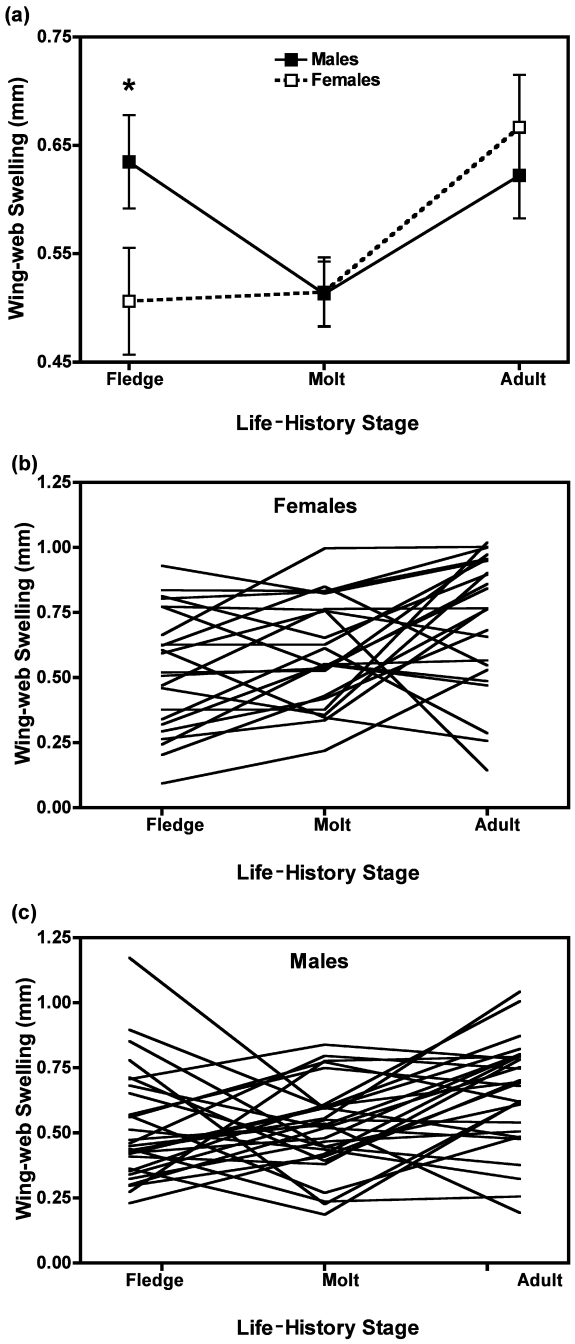

Figure 1: Intersexual variation in the inflammatory immune response across three life-history stages in juvenile zebra finches: (*a*) LSM ± SEM (asterisk represents $P < .05$ for within-stage analysis) and individual variation in (*b*) females and (*c*) males.

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 μg PHA (PHA-p, Sigma: L-9132) in 30 μL of sterile phosphate buffered saline (PBS) using a monoject insulin syringe with an integrated 27-gauge needle. The same volume of sterile PBS that was used in the PHA injection was injected, using a second needle, into the left wing web. Patagium thickness was measured three times at each wing web to 0.01 mm using a gauge micrometer (Dyer, model 304–196) immediately before and 24 h after injection. The same person (O. P. Love, who had examined more than 1,000 individual birds before this study; Chin et al. 2005; Love et al. 2005; Rowland et al. 2007; Love and Williams 2008) performed all bird measurements in this study. To ensure similarity of measurement technique across individuals, the specific nature of skin twisting was used as the cue as to when the micrometer reading should be taken. The difference between the pre- and postinjection responses was calculated for each wing independently, and then the change in thickness of the PBS-injected wing was subtracted from the change in thickness of the PHA-injected wing. The similarity of both preinjection ($r = 0.986$, $P < .0001$) and postinjection ($r = 0.974$, $P < .0001$) within-bird, within-stage measurements was high, and we used mean values of these three measurements for a within-stage response value. Juveniles and adults were weighed ($\pm$0.01g) and body size was measured (beak to tarsus; $\pm$0.01mm) immediately following injections. Only one previous study examining the within-individual repeatability of the immune response to PHA in birds had been conducted before we performed our study, and the authors report no evidence that exposure to the antigen at one stage had a priming effect on the response at a future stage (Granbom et al. 2004). If priming of the cell-mediated immune response had a potential confounding effect in this study, we would have predicted a significant relationship between the number of exposure events and the immune response. However, there was no evidence in our data to suggest consistent within-individual patterns of this response with time over multiple exposure episodes (see fig. 1*b*, 1*c*; fig. 2*b*, 2*c*), and, as such, we are confident that repeated exposure to the PHA antigen did not cause confounding or "priming" effects.

Statistical Analysis

Mixed-model repeated-measures ANCOVA was used to examine changes in immune response across stages for both juveniles and adults, using immune response as the

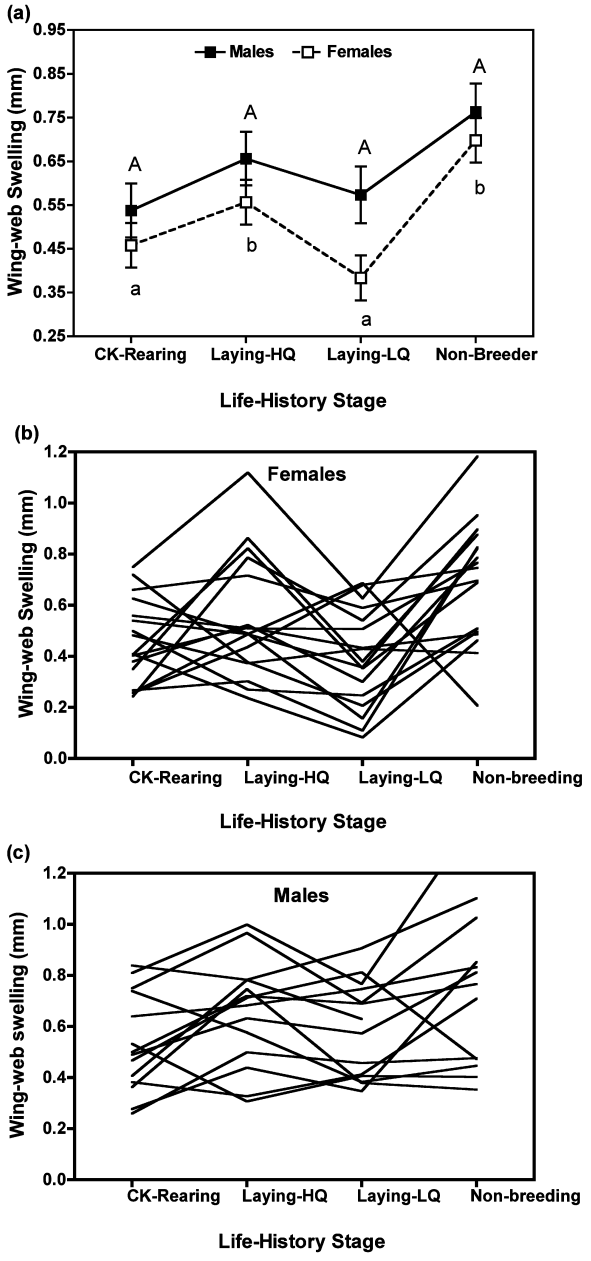

Figure 2: Intersexual variation in the inflammatory immune response across three life-history stages in adult zebra finches: (*a*) LSM $\pm$ SEM (different letters represent statistically significant differences between stages within a given sex, as calculated using the sequential Bonferroni post hoc 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).

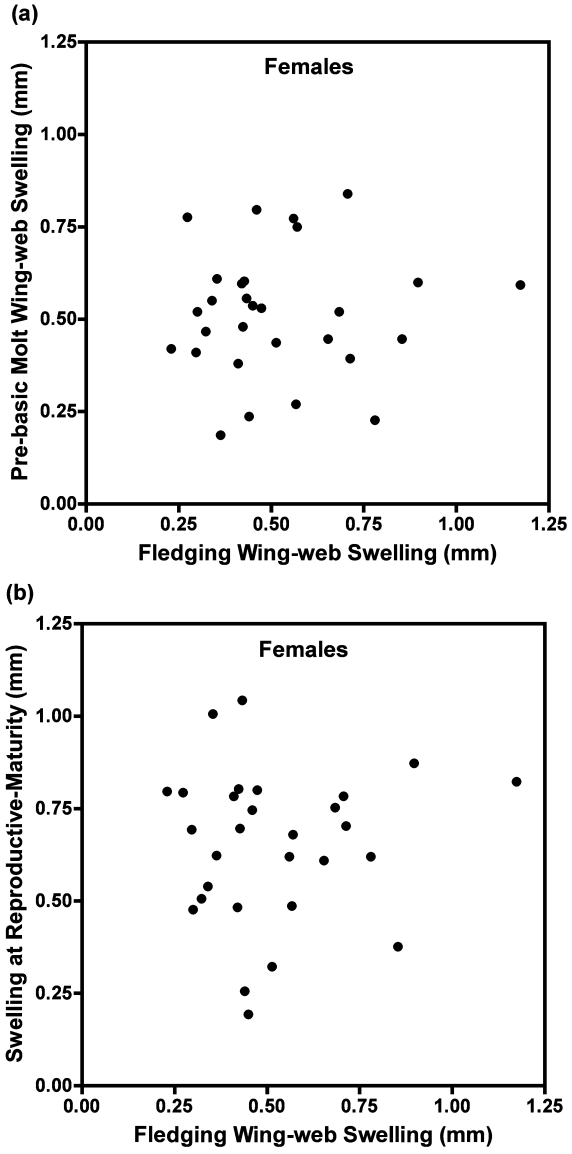

Figure 3: Interstage repeatability in the inflammatory immune response in juvenile female zebra finches (*a*) from the fledging to the molt stages and (*b*) from the fledging to the reproductively mature stages.

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: $F = 2.51$, df $= 28, 86$, $P = .01$; fig. 1*b*; male ANOVA: $F = 1.89$, df $= 47, 142$, $P = .004$; fig. 1*c*). Males displayed significantly higher inflammatory responses than females did at fledging (ANOVA: $F = 6.26$, df $= 1, 75$, $P = .016$), whereas both sexes displayed similar inflammatory responses at the pre–basic molt and reproductively mature stages (ANOVA: $F = 3.28$, df $= 1, 52$, $P = .08$, and $F = 1.11$, 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: $F = 2.38$, df $= 2, 97$, $P <$

.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 $P = .05$, respectively); however, no differences were observed between individuals in the fledging and pre–basic molt stages for females (Bonferroni post hoc analysis: $P = .88$). In contrast, inflammatory responses in males were similar at the fledging and reproductively mature stages (Bonferroni post hoc analysis: $P = .85$) and were even higher at both these stages compared with responses during the pre–basic molt stage (Bonferroni post hoc analysis: $P = .008$ and $P = .008$, respectively).

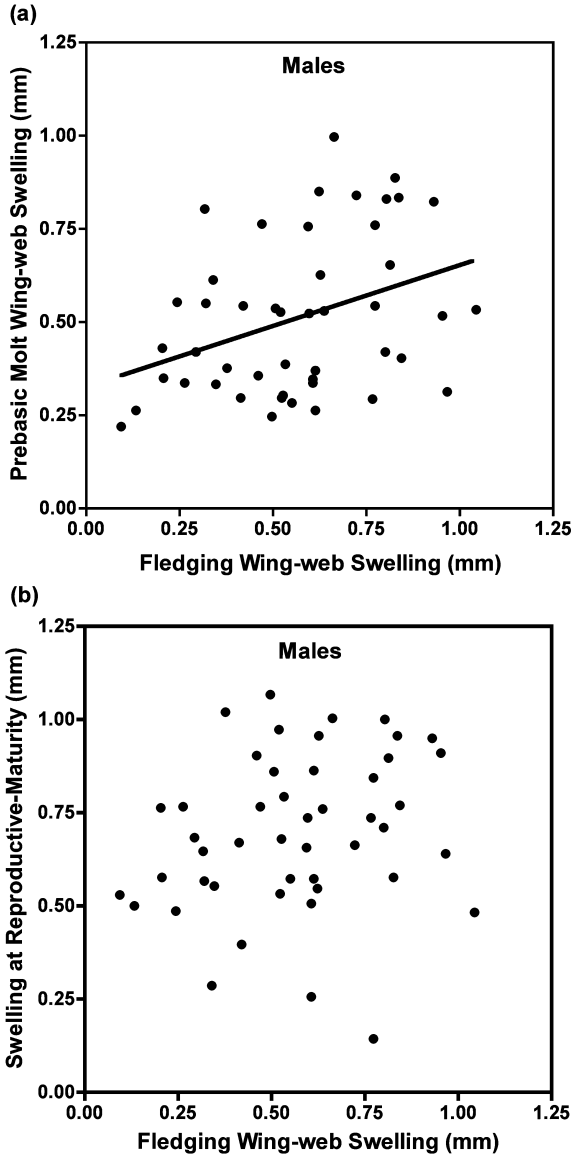

Figure 4: Interstage repeatability in the inflammatory immune response in juvenile male zebra finches (*a*) from the fledging to the molt stages and (*b*) from the fledging to the reproductively mature stages.

Adults: Individual and Life-History Stage Variation in Inflammatory Immune Responses

As in juveniles, individual variation across stages in adult inflammatory responses was high for both sexes (female ANOVA: $F = 2.33$, df $= 24, 85$, $P = .004$; fig. 2*b*; male ANOVA: $F = 2.23$, df $= 23, 83$, $P = .007$; fig. 2*c*). Across stages, males displayed significantly higher inflammatory responses than females did (repeated-measures ANCOVA: $F = 7.39$, df $= 1, 29$, $P = .011$; fig. 2*a*). More important, however, adult female responses varied significantly across life-history stages, whereas male responses did not (repeated-measures ANCOVA, stage × sex effect: $F = 7.21$, df $= 3, 31$, $P < .01$; fig. 2*a*). However, although female inflammatory responses that occured during the egg-laying stage while individuals received the HQ diet did not differ significantly from those measured during the nonbreeding stage (Bonferroni post hoc comparison, $P = .01$; fig. 2*a*), inflammatory responses that occured during the laying stage while individuals were receiving the LQ diet and those that occured during chick rearing were significantly lower than those that occurred during the nonbreeding stage (Bonferroni post hoc comparison: $P = .0004$ and $P = .0014$, respectively; fig. 2*a*). Furthermore, inflammatory responses in females who were laying while receiving the LQ diet and those that occurred during chick rearing were significantly lower than those that occurred while individuals were laying while receiving the HQ diet (Bonferroni post hoc comparison: $P = .005$ and $P = .003$, respectively; fig. 2*a*). In contrast, overall variation in adult male immune responses was much lower compared with that in females, and, unlike that with females, no pair-wise comparisons were statistically significant between stages for males (Bonferroni post hoc comparisons: all $P > .01$; fig. 2*a*). Finally, females displayed similar positive relationships between clutch size (reproductive effort) and the inflammatory response, regardless of diet quality (linear regression analysis: HQ diet, $r^2 = 0.27$, $P = .01$, slope $= 0.026$; LQ diet, $r^2 = 0.22$, $P = .03$, slope $= 0.022$). We did not observe a similar relationship for egg size for either diet (linear regression analysis: HQ diet, $P = .20$; LQ diet, $P = .11$).

Repeatability of the Inflammatory Immune Response and Body Mass

In juvenile females, immune responses were not repeatable between the fledging and the pre–basic molt stages ($F = 1.14$, df $= 28, 57$, $P = .36$; fig. 3*a*) or between the

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 ($F = 0.43$, df $= 9, 57$, $P = .90$) or the reproductively mature stage ($F = 0.16$, df $= 9, 57$, $P = .99$). In males, body mass was also not repeatable either between the fledging and the pre–basic molt stages ($F = 0.31$, df $= 26, 95$, $P = .99$) or between the fledging and the reproductively mature stages ($F = 0.17$, df $= 26, 95$, $P = .99$).

Inflammatory responses in nonbreeding adult birds significantly predicted their responses during breeding under high-quality resource conditions. In adults of both sexes, when comparing inflammatory responses between the nonbreeding and the egg-laying stages (while receiving the HQ diet), repeatability was 50.6% in females ($F = 3.05$, df $= 16, 33$, $P = .014$; fig. 5*a*) and 53.5% in males ($F = 3.29$, df $= 12, 25$, $P = .02$; fig. 5*b*). However, when laying while receiving the LQ diet, although the responses at the nonbreeding and egg-laying stages were repeatable for males (42.1%; $F = 2.45$, df $= 12, 25$, $P = .06$; fig. 5*b*), they were not so for females ($F = 0.59$, df $= 16, 33$, $P = .85$; fig. 5*a*). Comparing body masses at the nonbreeding and egg-laying stages for individuals receiving the HQ diet resulted in high repeatability in both males (78.6%) and females (72.4%; $F = 8.36$, df $= 12, 25$, $P = .0003$ for males; $F = 6.24$, df $= 16, 33$, $P = .0003$ for females). Moreover, unlike inflammatory responses, body mass was also strongly repeatable for both sexes when comparing the nonbreeding stage to the egg-laying stage for individuals receiving the LQ diet (54.9%, $F = 3.44$, df $= 12, 25$, $P = .018$ for males; 68.6%, $F = 5.37$, df $= 16, 33$, $P = .0006$ for females). Finally, intraindividual variation in plasticity in the laying inflammatory immune response across diets was dependent on the initial inflammatory response that occurred while the individual was receiving the HQ diet. The slope of the relationship between the inflammatory response when individuals were receiving the HQ diet and that when individuals were receiving the LQ diet was 0.040—significantly lower than a slope of 1 ($t = -3.0897$, df $= 19$, $P = .006$; fig. 6). This indicates that individuals who experienced high inflammatory responses while receiving the HQ diet displayed significantly greater flexibility than did individuals who experienced low inflammatory responses while receiving the HQ diet when each group had to lay while receiving the LQ diet.

Discussion

Although many ecological studies have focused on the activity and responsiveness of the vertebrate immune system within specific life-history stages, little attention has been paid to understanding the proximate and ultimate

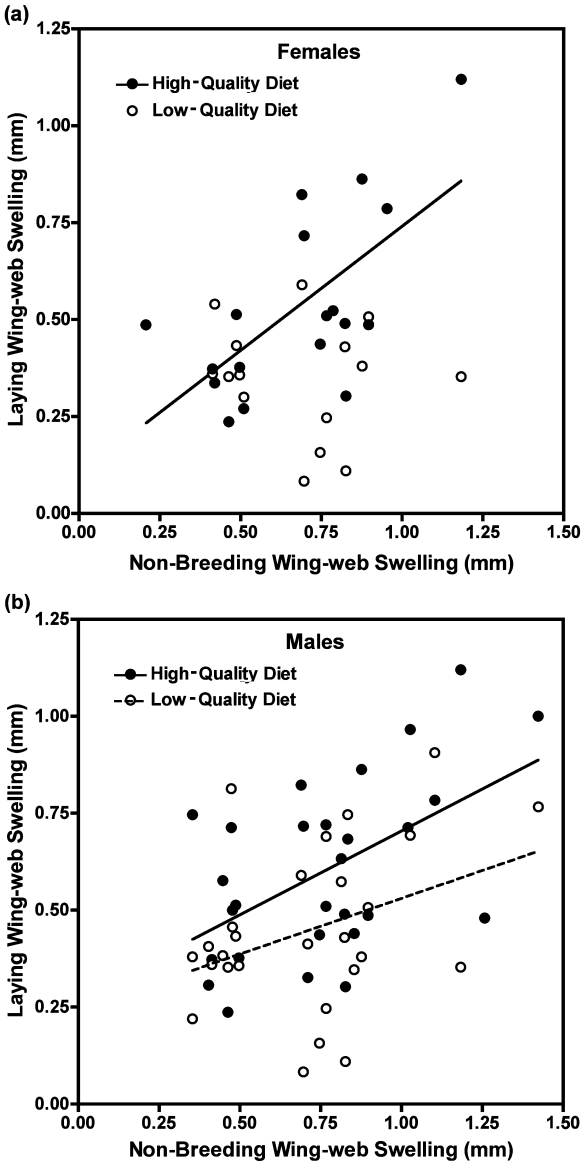

Figure 5: Interstage repeatability in the inflammatory immune response between the nonbreeding and the egg-laying stages in adult (*a*) female and (*b*) male zebra finches in relation to dietary treatment during laying (while receiving either a high-quality diet or a low-quality diet).

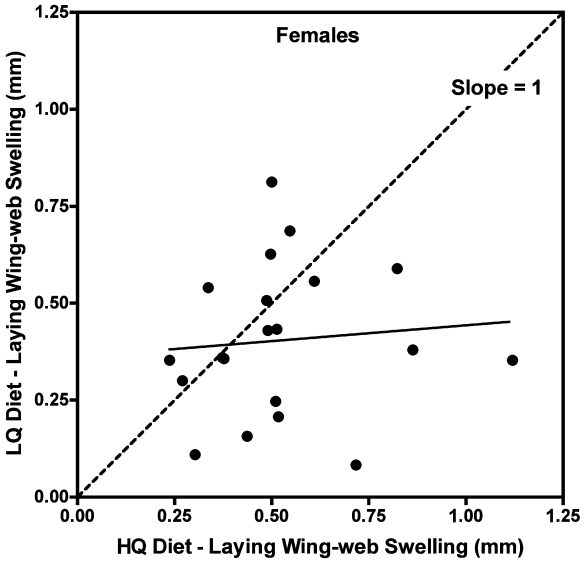

Figure 6: Intraindividual variation in plasticity in the inflammatory immune response of females laying while receiving a high-quality (HQ) diet and then a low-quality (LQ) diet. Slope is significantly <1 (*dashed line*), indicating that individuals who had high inflammatory responses while receiving the HQ diet displayed significantly greater immune flexibility than did individuals when laying while receiving the LQ diet and who had low responses while receiving the HQ diet.

reasons for how and why the immune system might display variation across life-history stages within individuals (Møller et al. 1998; Martin et al. 2006*a*). Understanding this "life span" scale variation is important for understanding the role of the immune system in shaping the evolution of life histories (Viney et al. 2005). In fact, recent work in invertebrates has underscored that recognizing life-history stage variation may be key in correctly interpreting optimal immune responses and trade-offs with other physiological systems within different life-history stages (Moret and Schmid-Hempel 2000; Jacot et al. 2004, 2005*a*, 2005*b*; Rantala and Roff 2005). The results of our study first indicate that the commonly used measure of inflammatory response in vertebrates varies significantly through ontogeny and into adulthood, with variation and potential trade-offs between responses and reproduction being sex specific and dependent on resource availability. Second, within-individual variation in inflammatory responses was not repeatable between early development and key future life-history stages. However, adults who had access to abundant resources during reproduction displayed high repeatability in inflammatory responsiveness between the nonreproductive and egg-laying stages. Nevertheless, when faced with limited resources, reproductive females appeared to facultatively reallocate resources/energy between reproduction and inflammatory responsiveness, which resulted in the disappearance of within-individual repeatability across stages. Taken together, our results indicate that the immune system can display significant and seemingly facultative sex-specific plasticity in response to the demands of various life-history stages and variable resource quality in vertebrates. Below we discuss our results within the framework of the predictions we developed above.

Ontogenetic Changes in the Inflammatory Response

Two common hypotheses often used to examine sex-specific differences in immune responsiveness across a number of taxa are sexual size dimorphism (SSD; Møller et al. 1998; Moore and Wilson 2002; Rolff et al. 2005) and sex differences in potentially resource-costly male displays (Hill 1999; Møller et al. 1999; Ryder and Siva-Jothy 2000; McGraw and Ardia 2003; Jacot et al. 2004; Kilpimaa et al. 2004; Rantala et al. 2007). Zebra finches provide an ideal model to differentiate between these two hypotheses because they are not size dimorphic and yet they exhibit temporal variation in the development of dimorphic visual displays (i.e., no differences at the fledging stage, significant differences during the pre–basic molt stage). However, contrary to our prediction of similar responses across the sexes at fledging, we observed higher inflammatory responses in males at the fledging stage. While some studies support the role of SSD in sex-specific trade-offs in resource allocation to immune function in species with larger males (Müller et al. 2003; Tschirren et al. 2003; Chin et al. 2005; Dubiec et al. 2006), research in a species exhibiting reversed SSD indicate that males still exhibit lower inflammatory responses than females do (Fargallo et al. 2002). Moreover, it is generally accepted that male mammals exhibit lower immune responses regardless of size and ornamentation (Nelson and Demas 1996; Moore and Wilson 2002; Nelson 2002; Zuk and Stoehr 2002; Krasnov et al. 2005), although results in insects appear to be mixed (Vainio et al. 2004; Rantala et al. 2007). Whether it is important for vertebrate females to redirect energy away from the immune system toward postnatal growth to maximize reproductive output as adults is largely unknown. However, if true, this suggests that females may redirect resources away from the functioning of the immune system during development to increase fitness, meaning that reduced nestling inflammatory responses are beneficial in the long run.

The second proposed hypothesis to explain lower inflammatory responses in males—namely, developing secondary sexual traits such as ornamental plumage or skin coloration—is based on the potentially high resource cost of developing these displays or obtaining limited resources

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, $P = .0014$; 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 highlight yet another physiological mechanism (namely, the inflammatory response) by which mothers balance egg production and other physiological processes under conditions of limited resources.

We found no evidence for variation in adult male inflammatory responses across life-history stages, regardless of resource quality during reproduction. This result in males may be explained by their putatively lower relative energetic contribution (namely, sperm) during the egg-laying stage, compared with females, and may also indicate that it is the accumulated energetic costs of laying and incubation that result in immune deficits in females during the chick-rearing stage because males and females in this species are thought to work equally hard at chick rearing (Zann 1996). However, although the energetic costs of chick rearing are predicted to be significant (Drent and

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 inflammatory immune response should be a plastic trait allowing individuals to express responses in a context-dependent manner given the energetic costs of mounting an immune response (see Houston et al. 2007). The immune system would therefore be expected to have a high degree of environmental determination, allowing individuals to adjust responses to "optimize" overall energy investment. Interindividual variation in inflammatory responses was marked both (1) among individuals within specific life-history stages and (2) within individuals across stages. Of particular interest is that many individuals do not display the "average" among-stage pattern as represented by the mean of the group, something that emphasizes the potential for individually optimal immune management strategies to exist. The importance of allowing the reader to view and interpret the unmasked variation is the primary reason why we have provided both the clearly individually variable patterns across life-history stages and the mean values for groups. In fact, studying individual variation in physiological traits has the potential to increase our ability to understand how and why physiological mechanisms influence fitness (Williams 2008).

In juveniles, responses measured at the end of postnatal development did not predict responses at key future life-history stages, namely, the pre–basic molt and reproductively mature stages. The lack of repeatability in inflammatory responses likely reflects the different within-stage influences that environmental quality has in shaping the response of the immune system across these stages (Klasing 2002; Sandland and Minchella 2003; Chin et al. 2005) as well as the many non-resource-related sources potentially influencing immunes responses between stages such as exposure to pathogens (Christe et al. 1998) and sexually selected differences (Møller et al. 1999; Kilpimaa et al. 2004). Measurement of the inflammatory response in fledgling birds is particularly prevalent in many ecological studies (e.g., Soler et al. 2003; Tschirren et al. 2003; Chin et al. 2005; Dubiec et al. 2006). Practically speaking, our results suggest that measuring the inflammatory immune response at only the fledging stage provides limited information about how the immune system of these individuals will respond during future life-history stages and may even be misleading as to how the current response will impact future reproduction and fitness.

In adults, both sexes displayed significant repeatability in both inflammatory responses and body mass between the nonbreeding and the egg-laying stages when neither sex was challenged by resource limitation (i.e., when laying while receiving the HQ diet). However, repeatability in adults who are breeding under HQ conditions is not necessarily evidence of a strong genetic component to the immune response measured here but instead might reflect the effect of common environment (i.e., responses are expressed at a given value when resources are plentiful). This point is emphasized by the lack of repeatability across these two key life-history stages for females laying eggs while receiving limited resources (LQ diet), whereas males displayed high repeatability in their inflammatory responses when faced with the same diet constraints. This indicates that it is likely the energetic costs of egg production under limited conditions, rather than the reproductive stage per se that forces females to reallocate resources away from the immune system. Interestingly, regardless of the quality of resources available, both sexes displayed high repeatability of body mass, indicating that across-stage physiological adjustments can be made without interfering with body condition. Moreover, inflammatory responses were not repeatable in females laying on the LQ diet, whereas body mass was, which suggests that repeatability in responses is strongly influenced by resource availability in egg-laying females. Furthermore, the amount of flexibility available to individual females in their ability to respond immunologically when forced to lay while receiving the LQ diet is related to their ability to respond when laying while receiving the HQ diet; that is, females with high inflammatory responses when laying while receiving the HQ diet displayed a greater flexibility in being able to reduce responses when laying while receiving the LQ diet compared with individuals who had low inflammatory responses while receiving the HQ diet (i.e., fig. 6). These results may also indicate a critical lower limit for the inflammatory immune response below which individuals may be significantly at risk of infection.

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 in resources, and stage-specific demands such as reproduction. As has been recently appreciated, it is furthermore difficult to predict how the various components will interact within each other in relation to variation in these demands across stages (Martin et al. 2006*d*). In this light, constitutive innate immunity (which has a mixture of humoral and cellular components; Tieleman et al. 2005) may be one of the best immune responses to examine in the context of a life-history stage in future studies. Our purpose in this study is to highlight for ecological immunologists the idea that individuals may manage immune responses differentially across their life span to maximize lifetime fitness. Practically, our results suggest that examining immunity within a single life-history stage (i.e., the nestling or reproduction stage) or without knowledge of the variation in environmental quality ignores how plastic the immune system can be and, therefore, potentially provides misleading information about how individuals are managing their immune systems across their entire life spans. Second, because individuals may be capable of redistribution among various arms of the immune system (Martin et al. 2006*d*), future studies measuring multiple types of responses or using a more holistic measure of immunity in individuals will be better at understanding management of the immune system. Finally, it is important to appreciate that species with differing life-history strategies (Tella et al. 2002; Martin et al. 2006*d*) and even subspecies differing in breeding schedules and resource availability (Martin 2005; Martin et al. 2006*b*) may differ in their immunological responses to antigens. As such, it is more relevant than ever for immunoecologists to appreciate the complexity of the life history of their study species before interpreting how immunological differences between individuals influence fitness.

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