Juvenile Nutritional Stress Affects Growth Rate, Adult Organ Mass, and Innate Immune Function in Zebra Finches (*Taeniopygia guttata*)

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ABSTRACT

Developmental conditions may influence many aspects of adult phenotype, including growth and immune function. Whether poor developmental environments impair both growth and immune function or induce a trade-off between the two processes is inconclusive, and the impact of the timing of stress in determining this relationship has so far been overlooked. We tested the hypothesis that the long-term effects of nutritional stress on growth, body composition, and immune function in zebra finches (*Taeniopygia guttata*) are different depending on whether stress is experienced during an early or a juvenile phase (i.e., before or after nutritional independence, respectively). We raised birds on high (H) or low (L) food conditions until posthatch day (PHD) 35 and switched treatments for half of the birds in each of the H and L groups from PHD 36 to 61. We found that unfavorable juvenile conditions (PHD 36–61) increased somatic growth rates and liver mass, body fat, and some aspects of immune function. We also observed a positive relationship between growth and immune function, as individuals that grew faster as juveniles also had better innate immune responses as adults. There was no effect of treatment on basal metabolic rate. These findings demonstrate the importance of juvenile developmental conditions in shaping multiple aspects of the adult phenotype.

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Introduction

Poor developmental environments can have short- and long-term consequences on adult phenotype (Birkhead et al. 1999; Lindström 1999; Tschirren et al. 2009). When faced with limited and insufficient resources organisms may prioritize the development and maintenance of particular processes over others, thus inducing a trade-off between these processes. In the short-term, stressful developmental conditions may induce a trade-off between growth and immune function (Fair 1999; Hoi-Leitner et al. 2001; Soler et al. 2003; Brommer 2004; Chin et al. 2005; Brzęk and Konarzewski 2007). In the long term, there is some evidence that favorable early environments enhance while unfavorable early environments impair growth and adult immune function (Birkhead et al. 1999; Tella et al. 2001; Naguib et al. 2004; Stjernman et al. 2008; Butler and McGraw 2011; De Coster et al. 2011). However, Tschirren et al. (2009) found the opposite pattern, with adult birds raised in larger broods mounting a stronger T-cell-mediated immune response. Råberg et al. (2003) also reported no correlation between nutritional status of nestling blue tits and adult antibody responsiveness. Thus, further investigation is required to resolve the contradiction regarding the persistent effects of developmental conditions on growth and immune function in birds.

One factor that may affect this relationship is the developmental period at which poor environments are encountered. This factor has so far been understudied, even though it may play a critical role in determining the direction of the relationship. Both growth and immune function have prolonged developmental schedules: in more than half the bird species examined by Ricklefs (1968), somatic growth continued through the juvenile period after young fledged the nest. Development of the innate and adaptive components of the immune system also continues well past the nestling stage (Mauck et al. 2005; Palacios et al. 2009; Strambaugh et al. 2011). Conceivably, development and function of physiological processes that are likely to be most affected by early environmental conditions are those that are maturing at that time when substandard conditions are experienced. Thus, growth and development of the immune system may be differentially affected depending on whether poor environmental conditions are experienced early (e.g., nestling phase) or later in development (e.g., juvenile phase).

The developmental period at which stress is experienced is important also because organisms may be able to compensate for detriments incurred during periods of poor conditions if conditions subsequently improve. Developmentally disadvantaged organisms may accelerate growth to match the size of conspecifics in adulthood, although this "catch-up" growth may

have long-term costs (reviewed in Metcalfe and Monaghan 2001). Additionally, compensation may not occur for all detriments or be fully complete, as zebra finches in poor developmental conditions were able to match control subjects on wing length (de Kogel 1997) but not mass (de Kogel 1997; Tschirren et al. 2009) in adulthood. Thus, further research is needed to understand how the timing of developmental stressors affects growth and immune function and how this timing influences potential compensation or catch-up growth.

In this study, we tested the hypothesis that the long-term effects of developmental stress on growth, body composition, and adult immune function in zebra finches are dependent on the developmental period at which stress is experienced. Captive zebra finches were subjected to high (H) or low (L) food conditions, before or after nutritional independence (phase 1 and 2, respectively). We then measured basal metabolic rate, body fat percentage, immune function, and organ mass of the same birds as adults. Our results demonstrate that poor developmental conditions differentially affect adult phenotypes depending on the stage of development they occur but that unfavorable conditions experienced after nutritional independence (during the juvenile phase) have the strongest effect on growth, adult body condition, and immune function.

Material and Methods

Subjects and Manipulation

We randomly paired adult male and female zebra finches from our breeding colony. Each pair was housed in a 36 × 43 × 42-cm cage with access to an external nest box (20 cm × 13.5 cm × 13.5 cm) and kept on 14L : 10D photoperiod at 22°C. Pairs received grit, cuttlefish bone, seed (Living World Premium Finch Seed; 11.0% protein, 5.9% lipid) and water ad lib. and were supplemented with daily portions of egg-food (hardboiled chicken eggs, cornmeal, bread). All animal care and husbandry protocols were approved by the Animal Use Subcommittee at the University of Western Ontario (2007-089) and followed guidelines of the Canadian Council on Animal Care. We used a total of 9 pairs that produced 33 experimental offspring in this study. Nonindependence of nest-mates was controlled statistically (see below). Nests were monitored daily for nesting activity and randomly assigned to treatment conditions after the first egg hatched.

Experimental treatment began when the oldest nestling was 6 days posthatch (PHD 6) and lasted until approximately PHD 61. Only broods with 4 or 5 nestlings at the start of treatment were included in our experiment. We manipulated food accessibility (Spencer et al. 2003), where broods in the H treatment were given access to 65 g seed and 13.5 g egg-food daily, while broods in the L treatment were given access to 50 g total of seed in a mixture containing a 1 : 3 ratio of seeds and wood chips (by volume), and 6.5 g egg-food daily. This treatment forces parents in the L treatment to search longer for seeds from the time treatment starts until offspring reach PHD 35 and become nutritionally independent. Similar feeding treatments have been shown by Lemon (1993), Spencer et al. (2003), Buchanan et al (2004), and Zann and Cash (2008) to negatively affect body mass, adult song control brain regions, and song characteristics of zebra finches raised in these conditions.

Our experiment was designed to test effects of feeding treatment during two phases: an early phase (PHD 6–35, before offspring were nutritionally independent) and a juvenile phase (PHD 36–61, after offspring were nutritionally independent). We raised zebra finches on H or L food conditions during the early phase and then switched feeding treatments for half of the birds in each of the H and L groups during the juvenile phase. After the juvenile phase, all birds were given seed ad lib. and after PHD 90 were housed in same-sex groups of four to five birds.

Growth and Body Fat

To calculate growth, we weighed subjects daily using an electronic scale accurate to 0.1 g from hatch (PHD 1) to approximately PHD 65. Body fat was quantified on approximately PHD 25, 50, 59, 180, and 600 using quantitative magnetic resonance (QMR) body composition analysis, using an instrument designed for small birds (model MRI-B, Echo Medical Systems, Houston, TX). With QMR, we are able to obtain a more comprehensive measure of body condition, as we can examine both total mass and percentage of body fat in proportion to total body mass. This QMR analysis has been shown to accurately and precisely detect lean and fat mass in various bird species including zebra finches (Gerson and Guglielmo 2011; Guglielmo et al. 2011), and here we report the values accurate to 0.01 g. The QMR analyzer was calibrated with 5 or 2.1 g of canola oil before each measurement. Coefficients of variation for fat and lean mass are 3% and 0.5%, respectively, and relative accuracies are ±11% and ±1%, respectively (Guglielmo et al. 2011). Lean and fat content were adjusted (raw value × 0.94 for fat mass, raw value × 1.021 for lean mass) according to calibration equations for zebra finches (Gerson and Guglielmo 2011; Guglielmo et al. 2011).

Organ Mass

At approximately 600 PHD, birds were killed and whole organs (heart, liver, kidney, and pancreas) were extracted and weighed immediately on a digital balance to the nearest 0.001 g to determine wet mass.

Basal Metabolic Rates

We measured basal metabolic rates (BMR) when birds were approximately PHD 180, using a flow-through respirometry system similar to Gerson and Guglielmo (2011). Birds were weighed and placed into individual well-sealed respirometry chambers overnight and maintained at a constant temperature of 35°C, which is within the thermoneutral zone for zebra finches (Calder 1964). We recorded five birds per night, and the baseline was taken from a channel sampling air within the room. Incurrent air was scrubbed of CO_2 and water vapor using

soda lime and Drierite, respectively, and the chambers received a constant flow of 350 mL/min. Birds were fasted for 3 h in the chambers, and only data from the remaining 7 h of the night were used to ensure that measurements would reflect metabolic rates of birds in postabsorptive state. Excurrent air was subsampled at a rate of 150 mL/min and was passed through a Drierite column to the CO_2 (CA-2A, Sable Systems, Las Vegas, NV) and O_2 analyzers (FC-1B, Sable Systems), with CO_2 and H_2O scrubbing between the analyzers. Gas analyzers were calibrated with a certified standard containing 20.9% O_2–1.0% CO_2 balanced with N_2 (Praxair, London, Canada). Multiplexing enabled measurements of a 10-min baseline and 10-min samples from each chamber every hour. All instruments were connected to an analog-to-digital converter (UI-2, Sable Systems), which was connected to a laptop computer. Expedata software (Sable Systems) was used for both data collection and analysis. We corrected for lag time between O_2 and CO_2 measurements and used equations 10. 6 and 10.7 in Lighton (2008) to calculate VO_2 (mL/min) and VCO_2 (mL/min) based on the mean of the final 5 min of each 10-min sampling interval (Gerson and Guglielmo 2011). We also calculated the respiratory quotient (RQ) as VCO_2/VO_2 (Lighton 2008). The RQ value indicates energy production resulting from pure lipid catabolism to pure carbohydrate catabolism, with values ranging from 0.7 to 1.0, respectively.

Immune Function

Innate Constituent Immunity. The antimicrobial capability of blood from each subject was assessed with microbe-killing assays against a strain of *E. coli* (ATTC 8739, Epower Microorganisms, catalog 0483E7, MicroBiologics) and *Candida albicans* (ATTC 10231, Epower Microorganisms, catalog 0443E7, MicroBiologics). Killing of *E. coli* is dependent on complement proteins while killing of *C. albicans* is dependent on interactions between plasma factors and phagocytosis (Millet et al. 2007). We first conducted optimization assays to determine optimal microbe concentration, blood to cell media dilution, and incubation times following procedures outlined by Liebl and Martin (2009). For optimization of the *C. albicans* assay, we reconstituted the lyophilized pellet of *C. alibcans* and diluted the stock with sterile phosphate buffered saline (PBS) to obtain a working solution of 1×10^4 CFU/mL. We then incubated the *C. albicans* working solution in a 1 : 24 dilution of fresh blood to cell media for 15 min. For optimization of the *E. coli* assay, we reconstituted the lyophilized pellets of microorganisms according to the manufacturer's instructions and diluted the stock concentration with sterile PBS to obtain a working solution of 1×10^5 CFU/mL. We then incubated the *E. coli* working solution in a 1 : 6 dilution of previously frozen whole blood to cell media for 30 min (see below for details).

When subjects were approximately PHD 150, sterile blood samples were collected in less than 5 min from the brachial vein. We first cleaned the skin with cotton balls soaked with 70% ethanol, waited for the ethanol to dry, collected the blood with sterile heparinized capillary tubes, and then transferred the blood into sterile microcentrifuge tubes. A portion of the fresh blood was immediately aliquoted out into separate sterile microcentrifuge tubes containing cell media (CO2-Independent Medium, Invitrogen 18045-088; L-glutamine, Sigma-Aldrich; fetal bovine serum, Invitrogen) for the *C. albicans* assay. The remaining whole blood was stored at −80°C for the *E. coli* assay and assayed within 10 d of sample collection.

For each assay, we made three replicates of an assay positive control (APC) and two replicates of an assay blank control (ABC). APCs contained microbes but no blood (36 μL sterile PBS, 12.5 μL working solution, 250 μL tryptic soy broth). Consequently, APC values reflect unhindered bacterial growth and were used to ensure that microbe concentrations reached a particular absorbance range for spectrophotometry. Too-low APC absorbance values would have made it difficult to detect small absorbance difference between samples. On the other hand, ABCs contained no blood or microbes (48.5 μL sterile PBS and 250 μL tryptic soy broth). ABCs were used as basal values for APCs to account for any effects on absorbance that were due to the color of the soy broth. Furthermore, each subject had an individual blank control (IBC) to account for individual variation in blood coloration. IBCs contained blood but no microbes (36 μL blood, 12.5 μL sterile PBS, 250 μL tryptic soy broth). Antimicrobial activity was assessed by subtracting the absorbance of the same subjects' IBC from the absorbance of a subject's sample (i.e., absorbance of each sample was adjusted according to the corresponding IBC). All controls (APC, ABC, and IBC) were made alongside samples and incubated with samples to ensure that all tubes were exposed to the same conditions.

Microbe-killing assays for *C. albicans* commenced immediately after sample collection. For each individual bird, we had three sterile microcentrifuge tubes, and each tube contained 36 μL of a 1 : 24 dilution of blood to cell media. Two of the tubes were sample replicates and the other served as the individual blank control. We made a working solution of 1×10^5 CFU/mL, added 12.5 μL of the working solution to the samples, vortexed all tubes thoroughly, and incubated them at 30°C for 15 min. We then vortexed and added 250 μL tryptic soy broth (product 1.05459, EMD Chemicals) to all tubes. Samples were then incubated at 30°C for 24–48 h, after which the absorbance (abs) of each sample was measured using a Nanodrop spectrophotometer (Nanodrop 2000c, Thermoscientific). We used the average value of sample replicates in our calculations. Percentage of microbes killed in each sample was quantified as $(abs_{sample} - absIBC/absAPC - abs_{ABC}) \times 100$. Quantifying antimicrobial activity as the proportion of microbes killed in samples relative to APCs accounted for any differences in secondary incubation times between assays.

Similar procedures were used in the microbe-killing assays for *E. coli*. For each individual, we had three sterile microcentrifuge tubes, each containing 36 μL of a 1 : 6 dilution of previously frozen blood to cell media. We then made a working solution of 1×10^4 CFU/mL, added 12.5 μL of the working solution to all samples, vortexed, and incubated them at 37°C for 30 min. We then vortexed samples, added 250 μL tryptic soy broth, and

incubated samples at 37°C for 12 h. Absorbance readings of samples were then taken using the Nanodrop spectrophotometer and percentage of microbes killed in each sample was quantified as (abs_{sample} − absIBC/absAPC − abs_{ABC}) × 100).

Humoral Immunity. Our aim was to assess effects of treatment on subjects' ability to mount an immune response to a foreign novel antigen. We used a hemagglutination assay to assess levels of antibody-mediated agglutination after exposure to sheep red blood cells (SRBC; Deerenberg et al. 1997; McGraw and Ardia 2005). We did not distinguish between SRBC-specific and nonspecific natural antibodies because we were not interested in differences in production of specific antibodies.

We first diluted a stock of 10% suspension of SRBC (catalog 55876, MP Biomedicals) to 2% with sterile PBS. When birds were approximately PHD 360, they were injected intraperitoneally with 100 μL of 2% SRBC using an insulin syringe with a 29-gauge needle. Blood samples were again taken 6 d later to assess primary antibody response (Birkhead et al. 1998). Plasma was extracted after centrifugation at 13,000 g for 10 min, stored at −80°C, and assayed within 10 d of collection.

For the assay we followed procedures described by Matson et al. (2005). In 96-well round-bottom microtiter plates, we serially diluted 20 μL of plasma in 20 μL of PBS (1 : 1 dilution). Each plate also had a negative control (PBS only) and a positive control (chicken plasma). We then added 20 μL of 1% SRBC to all wells and incubated the plates at 37°C for 90 min. After incubation the plates were rested on a tilted stand for 20 min and then scanned with a flatbed scanner (Epson Perfection 4990 Photo) at 300 dpi with the "positive transparency" setting. An experimenter blind to treatment conditions scored a plate by comparing hemagglutination of the samples to that of the positive and negative controls on the plate. Hemagglutination scores were expressed as $\log_2$ of the highest dilution showing agglutination.

Statistics

Statistical analyses were conducted using SPSS 19.0. We used linear mixed models with restricted maximum likelihood to analyze the effect of treatment on all our dependent measures (i.e., growth, immune function, body composition, metabolic rates, organ mass). This analysis is appropriate for our data because we can control for the nonindependence of our samples (i.e., relatedness of siblings in each nest) to avoid pseudoreplication. In all of the analyses described below we account for brood identification (ID) and individual ID by entering them as random effects in the full statistical models; treating these variables as separate or nested (individuals nested in broods) random effects yielded similar results, so below we report results of models that treat random effects as separate. As these random effects did not contribute significantly to all models, some results are reported with these random effects excluded from the final model. For all analyses, stepwise deletion of nonsignificant terms was applied to obtain the most parsimonious model of the data.

Growth Rates. We calculated growth rate constants (k; following Morton et al. 1985) for the early and juvenile phase and analyzed each phase using separate linear mixed models. To analyze the effects of feeding treatments on growth during the early phase, growth rate k was entered as the dependent variable, with early feeding treatment (H or L), sex, and early feeding treatment × sex interaction as fixed effects. Brood ID was also included as a random effect in the final model to control for repeated observations within a brood. For the juvenile phase, early feeding treatment (H or L), juvenile feeding treatment (H or L), and sex and all higher-order interactions were entered as fixed effects. Brood ID and individual ID were nonsignificant and dropped from the final model. To control for parental effects we initially included parent mass (average of mother and father mass) as a fixed covariate, but it did not significantly contribute to the model and was removed. Significant main effects of age interval were analyzed using Sidak adjustment for pairwise comparisons.

Body Fat. To examine effects of feeding treatment on body fat, grams of body fat was entered as the dependent variable, with diet (H or L), age (PHD 25, 50, 59, 180, and 600), sex, and all higher-order interactions as fixed effects and total mass minus fat mass as a fixed covariate (Christians 1999). Brood ID and individual ID were not significant and were excluded in the final model. Significant main effects of age were analyzed with Sidak adjustment for pairwise comparisons.

Adult Body Mass and Organ Mass. To examine effects of feeding treatment on organ mass, separate linear mixed models were used for each organ (heart, liver, kidney, pancreas). Organ mass was entered as the dependent variable, with early condition (H or L), juvenile condition (H or L), sex, and all higher-order interactions as fixed effects, and body mass at PHD 600 as a fixed covariate. Brood ID was retained in the final model for all organs except kidney. To clarify whether any differences in organ mass could have been due to differences in general growth, we ran the model again without body mass as a covariate and with a separate linear mixed model with body mass at PHD 600 as a dependent variable and with early condition (H or L), juvenile condition (H or L), sex, and all higher-order interactions as fixed effects. Brood ID was retained as a random effect in the final model. Significant differences in organ mass without corresponding differences in body mass would suggest that organ mass specifically was affected by treatment.

Metabolic Rates. To analyze effects of feeding treatment on metabolic rates, BMR (W) was entered as the dependent variable, with early condition (H or L), juvenile condition (H or L), sex, and all higher-order interactions as fixed effects and with body mass as a fixed covariate. For RQ, the RQ value was used as the dependent variable, and all fixed effects and covariates were identical to BMR. For both BMR and RQ, brood ID and individual ID were not significant and were excluded in the final model.

Immune Function. To analyze effects of feeding treatment on innate constitutive immune function, we ran separate linear mixed models for microbicidal capacity against *C. albicans* and *E. coli*. For both analyses, percent killing was the dependent variable, with early condition (H or L), juvenile condition (H or L), sex, and all higher-order interactions as fixed effects. Brood ID and individual ID were not retained in the final model. For humoral immunity, we entered agglutination scores as the dependent variable and early conditions (H or L), juvenile conditions (H or L), sex, and all higher-order interactions as fixed effects. Brood ID and individual ID were not retained in the final model.

Relationship between Growth and Immune Function. To analyze whether growth rates during development influenced adult immune function, separate linear mixed models were used for each of the dependent variables (percent killing *E. coli*, percent killing *C. albicans*, and hemagglutination score), with early condition (H or L) and juvenile condition (H or L) as fixed effects, and with early and juvenile growth rates (k) as covariates. Brood ID and individual ID were included as a random effect in the final model. Only interactions between main effects were considered in these models.

Results

Growth Rates

Our results indicate that juvenile, but not early, feeding treatment significantly affected growth rates. Growth during the early phase was not significantly affected by feeding treatment or sex (early $F_{1,6.56} = 0.331$, $P = 0.584$, n for H = 16 and L = 17; sex ($F_{1,23.41} = 0.305$, $P = 0.586$; fig. 1*a*). On the other hand, growth during the juvenile phase was significantly affected by juvenile feeding treatment ($F_{1,28} = 7.36$, $P = 0.011$, n for H = 15 and L = 18) and the interaction of early feeding treatment × juvenile feeding treatment ($F_{1,28} = 32.28$, $P < 0.001$). Pairwise comparisons of the main effect indicated that L juvenile feeding treatment caused faster growth than H juvenile feeding treatment ($P = 0.011$). The early × juvenile feeding treatment interaction indicated that birds that experienced juvenile feeding treatments that were different from early feeding treatments grew faster during the juvenile phase ($P < 0.05$ for all; fig. 1*b*). This effect was observed irrespective of what early feeding treatment (H or L) birds had previously experienced. Therefore, these results suggest that nutritional conditions during the juvenile phase can significantly increase growth rates if they are different from nutritional conditions during the early phase.

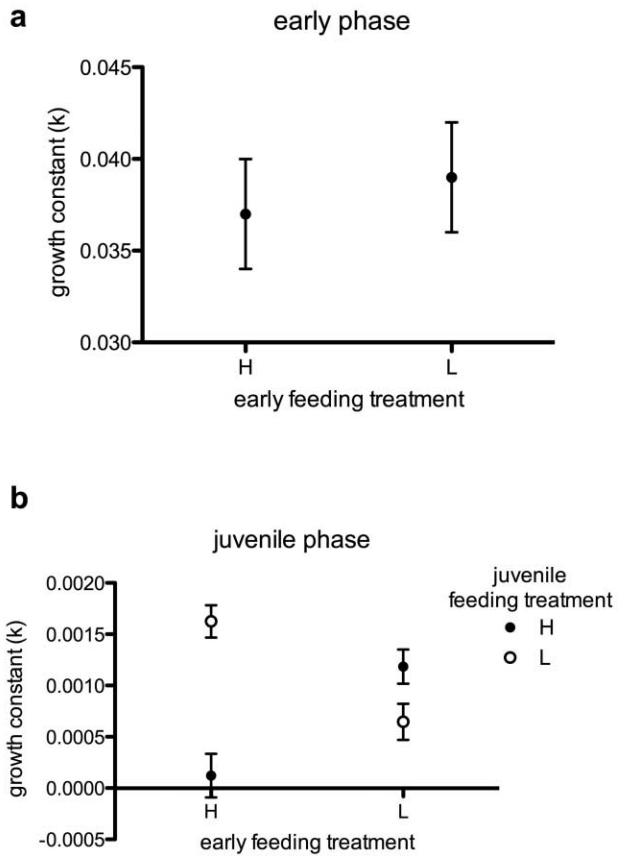

Figure 1. Feeding treatment had a significant effect on growth rates during the juvenile phase but not during the early phase (*a*). During the juvenile phase, the main effect and interaction revealed that birds that experienced low feeding (L) juvenile conditions grew faster than birds in high feeding (H) juvenile conditions. Birds that experienced juvenile conditions that were different from early conditions also grew faster than birds that experienced the same early and juvenile conditions (*b*). Error bars represent SEM.

Body Fat

Experimental treatment during development had significant immediate and enduring sex-specific effects on body composition. Body fat was significantly influenced by the main effects of juvenile feeding treatment ($F_{1,134} = 13.36$, $P < 0.001$; n for H = 15 and L = 18; fig. 2*a*) and age ($F_{4,134} = 12.97$, $P < 0.001$). The covariate of nonfat mass was also significant ($F_{1,134} = 19.383$, $P < 0.001$), where birds with greater nonfat mass also had greater fat mass ($t_{(134)} = 4.40$, $P < 0.001$). In addition, body fat was significantly influenced by the interactions of early feeding treatment × age ($F_{4,134} = 3.02$, $P = 0.020$), where birds that experienced H early feeding treatment had more body fat than birds that experienced L early conditions at PHD 600 ($P = 0.003$; fig. 2*a*). The interactions of sex × age and juvenile feeding treatment × sex × age were also significant ($F_{4,134} = 2.86$, $P = 0.026$ and $F_{4,134} = 2.68$, $P = 0.034$, respectively), so we ran separate linear mixed models for each sex with juvenile feeding treatment, age, and juvenile feeding treatment × age as fixed effects and report the results below.

Female body fat was significantly influenced by juvenile feeding treatment and age (juvenile $F_{1,77} = 7.28$, $P = 0.009$, n for H = 9 and L = 9; age $F_{4,77} = 5.53$, $P = 0.001$). Females had more body fat if they experienced L juvenile feeding treatment ($P = 0.009$). Females also had significantly more fat at PHD

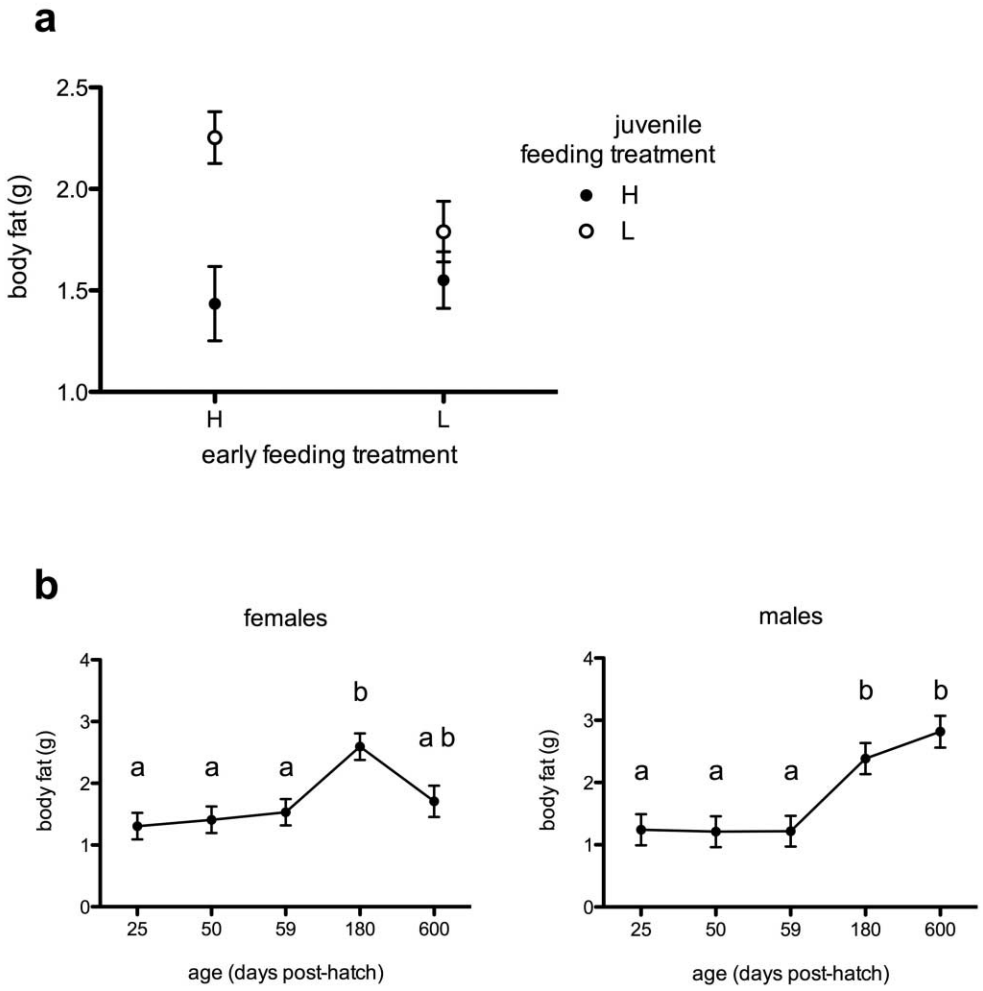

Figure 2. Feeding treatment had a significant effect on body fat. Birds that experienced low feeding (L) juvenile conditions had more body fat, and birds that experienced high feeding (H) early and L juvenile conditions had the greatest amount of body fat (*a*). Regardless of feeding treatment, both males and females had more body fat as adults (posthatch day [PHD] 180); however, only males continued to have greater body fat at PHD 600 (*b*). Means were adjusted for total mass minus body mass as a covariate, and error bars represent SEM.

180 compared to PHD 25 ($P = 0.001$), PHD 50 ($P = 0.004$) and PHD 600 ($P = 0.015$; fig. 2*b*).

Male body fat was significantly influenced by juvenile feeding treatment and age (juvenile $F_{1,64} = 17.08$, $P < 0.001$, n for H = 6 and L = 8; sex $F_{4,64} = 8.78$, $P < 0.001$) as well as the interaction between these variables (juvenile feeding treatment × age $F_{4,64} = 3.20$, $P = 0.019$). Similar to females, males that experienced L juvenile feeding treatment tended to have more body fat. Males also tended to have more fat as adults than during development ($P < 0.05$ for all except for the comparison between PHD 180 and PHD 59, where $P = 0.051$). The main effect of juvenile feeding treatment appeared to be driven by the interaction of juvenile feeding treatment × age, as males that had experienced L juvenile feeding treatment only had more body fat at PHD 600 ($P < 0.001$; fig. 2*b*).

Overall these results indicate that the effects of nutritional conditions during development persist into adulthood, reappearing much later in life, when birds are almost 2 yr old. These effects appear to be dependent on period of development, as H early and L juvenile feeding treatment seem to increase body fat stores. We also see sex-specific effects that depend on age. As young adults (PHD 180), both males and females had increased body fat compared to during development (PHD 25, 50, 59); however, only males appeared to maintain this increased body fat as older adults (PHD 600).

Adult Body Mass

Our results suggest that adult body mass at PHD 600 was affected by treatment conditions during development in a sex-specific manner. Adult body mass was significantly influenced by juvenile feeding treatment ($F_{1,22} = 6.64$, $P = 0.017$, n for H = 14 and L = 16) but not by early feeding treatment or sex (early $F_{1,22} = 0.24$, $P = 0.631$, n for H = 14 and L = 16; sex $F_{1,22} = 0.019$, $P = 0.892$). Birds that experienced L juvenile feeding treatment weighed more than birds that experienced

H juvenile feeding treatment (fig. 3*a*). Adult body mass was also significantly affected by the interaction of juvenile feeding treatment × sex and early feeding treatment × juvenile feeding treatment × sex ($F_{1,22} = 13.27$, $P = 0.001$ and $F_{1,22} = 5.31$, $P = 0.031$, respectively). To examine the three-way interaction we ran separate linear mixed models for each sex with early feeding treatment, juvenile feeding treatment, and early feeding treatment × juvenile feeding treatment as fixed effects and report the results below.

Female adult body mass was significantly affected by the interaction of early feeding treatment × juvenile feeding treatment ($F_{1,12} = 5.90$, $P = 0.032$) but not by early or juvenile feeding treatments separately (early $F_{1,12} = 0.035$, $P = 0.856$, n for H = 9 and L = 7; juvenile $F_{1,12} = 0.84$, $P = 0.377$, n for H = 8 and L = 8). Pairwise comparisons of the interaction indicated that females that experienced H early and H juvenile feeding treatments weighed significantly more than females that experienced H early and L juvenile feeding treatments ($P = 0.026$; fig. 3*b*).

Male adult body mass was significantly affected by juvenile feeding treatment ($F_{1,10} = 13.57$, $P = 0.004$, n for H = 6 and L = 8) but not by early feeding treatment or the interaction between the two variables (early $F_{1,10} = 0.20$, $P = 0.662$, n for H = 5 and L = 9; early × juvenile $F_{1,10} = 1.15$, $P = 0.310$). Males that experienced L juvenile feeding treatment weighed significantly more than males that experienced H juvenile feeding treatment (fig. 3*b*).

In summary, these findings indicate that nutritional stress during development can have long-lasting and sex-specific consequences for body mass. Adult females that were switched from H to L feeding treatment were lighter than females that were maintained on H feeding treatment. On the contrary, adult males that experienced L juvenile feeding treatment were heav-

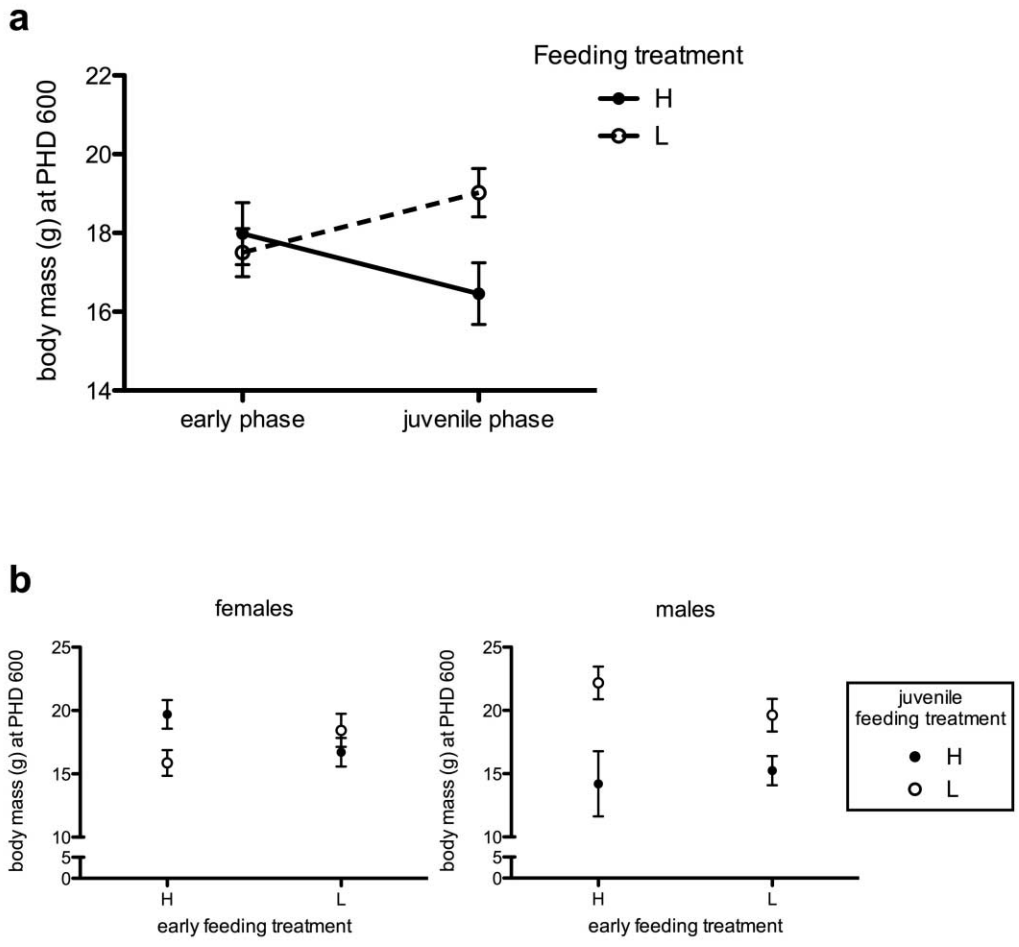

Figure 3. Feeding treatment had a significant and sex-specific effect on adult body mass (posthatch day 600). Birds that experienced low feeding (L) juvenile feeding had higher adult body mass than birds that experienced high feeding (H) juvenile feeding treatment (*a*). Female body mass was significantly influenced by the interaction of early and juvenile feeding treatments, where adult females that experienced H early and H juvenile feeding treatment were heavier than adult females that experienced H early and L juvenile feeding treatments. Male body mass was significantly influenced only by juvenile feeding treatments, where adult males that experienced L juvenile feeding treatment were heavier than adult males that experienced H juvenile feeding treatment. Error bars represent SEM.

ier than males that experienced H juvenile feeding treatment, regardless of previous early feeding treatments.

Adult Organ Mass

Examination of organ mass indicated that treatment during development significantly affected adult liver mass. Liver mass covaried significantly with body mass ($F_{1,20.49} = 17.28$, $P < 0.001$) and was affected by the interaction of juvenile feeding treatment × sex ($F_{1,20.62} = 5.81$, $P = 0.025$; fig. 4*b*) but not by early feeding treatment, juvenile feeding treatment, or sex (early $F_{1,4.91} = 0.036$, $P = 0.858$, n for H = 14 and L = 16; juvenile $F_{1,5.91} = 0.32$, $P = 0.595$, n for H = 14 and L = 16; sex $F_{1,20.48} = 0.012$, $P = 0.918$; fig. 4*a*). Pairwise comparisons of the juvenile feeding treatment × sex interaction indicated that females that experienced L juvenile feeding treatment had larger liver masses than females that experienced H juvenile feeding treatment ($P = 0.044$; fig. 4*b*). Liver mass of males was not significantly affected by juvenile feeding treatment. Liver mass covaried significantly with body mass ($F_{1,20.49} = 17.28$, $P < 0.001$), which suggests that the observed differences in liver mass are likely not attributable to differences in overall body mass.

Adult kidney mass was not significantly affected by early feeding treatment, juvenile feeding treatment, or sex (early $F_{1,21} = 1.59$, $P = 0.221$, n for H = 14 and L = 16; juvenile $F_{1,21} = 0.12$, $P = 0.733$, n for H = 14 and L = 16; sex $F_{1,21} = 2.40$, $P = 0.136$; fig. 4*a*). However, kidney mass may have been affected by the interaction of juvenile feeding treatment × sex, but this was not statistically significant ($F_{1,21} = 4.21$, $P = 0.053$). Kidney mass also covaried significantly with body mass ($F_{1,21} = 17.20$, $P < 0.001$) suggesting that this trend for feeding treatment to affect kidney mass is not likely due to differences in body mass.

Adult heart mass was not significantly affected by feeding treatment or sex (early $F_{1,4.35} = 1.20$, $P = 0.330$, n for H = 14 and L = 16; juvenile $F_{1,4.91} = 0.819$, $P = 0.408$, n for H = 14 and L = 16; sex $F_{1,18.17} = 0.064$, $P = 0.803$; fig. 4*a*). Adult pancreas mass was also not affected by feeding treatment or sex (early $F_{1,3.32} = 1.63$, $P = 0.284$, n for H = 13 and L = 15; juvenile $F_{1,4.09} = 0.062$, $P = 0.284$, n for H = 13 and L = 15; sex $F_{1,17.113} = 0.505$, $P = 0.487$; fig. 4*a*).

Basal Metabolic Rate

MR and RQ were not significantly affected by nutritional manipulations. The results of the linear mixed models for BMR and RQ are presented in table 1.

Innate Constitutive Immune Function

Feeding treatment during development significantly affected the ability of components in blood to kill microbes when subjects were adults. Ability of blood to kill *Candida albicans* was significantly affected by juvenile feeding treatment ($F_{1,25} = 9.68$, $P = 0.005$, n for H = 15 and L = 18; fig. 5*a*) but not by early feeding treatment or sex (early $F_{1,25} = 0.716$, $P = 0.405$, n for H = 16 and L = 17; sex $F_{1,25} = 0.55$, $P = 0.465$). Birds that experienced L juvenile feeding treatment showing higher antimicrobial activity than birds that experienced H juvenile feeding treatment (fig. 5*a*).

Ability of blood to kill *E. coli* was significantly affected by the interaction of early feeding treatment × juvenile feeding treatment ($F_{1,22} = 8.45$, $P = 0.008$) but not by early feeding treatment, juvenile feeding treatment, or sex (early $F_{1,22} = 1.51$, $P = 0.232$, n for H = 14 and L = 16; juvenile $F_{1,22} = 2.68$, $P = 0.116$, n for H = 13 and L = 17; sex $F_{1,22} = 0.25$, $P = 0.620$). Birds that experienced H early and L juvenile feeding treatment showed greater antimicrobial activity than birds that experienced constant H or L conditions throughout the early and juvenile phases ($P = 0.007$ and 0.002, respectively; fig. 5*b*). Overall, these results suggest that L juvenile feeding treatment may lead to greater innate immune function in adulthood.

Humoral Immune Function

Feeding treatment did not seem to affect adult humoral immune function. Analysis of hemagglutination after exposure to SRBC revealed no significant main effect or interaction of the early or juvenile feeding treatment (early $F_{1,26} = 0.19$, $P = 0.664$; juvenile $F_{1,26} = 0.61$, $P = 0.443$; data not shown).

Relationship between Growth and Immune Function

Our results suggest that there is a relationship between growth rate during a specific period of time and immune function, Ability of blood to kill *E. coli* in adulthood covaried significantly with growth during the juvenile phase ($F_{1,25} = 4.69$, $P = 0.04$). Faster growth during this interval was associated with greater ability of blood to kill *E. coli* in adulthood ($t_{(25)} = 2.17$, $P = 0.04$, $N = 30$; fig. 6). However, neither the ability of blood to kill *C. albicans* or level of SRBC hemagglutination in adulthood covaried significantly with early or juvenile growth rates. Therefore, these results suggest that faster growth during development can be positively correlated with specific arms of the immune system.

Discussion

Our study demonstrates that growth, body composition, and the immune system can be differentially affected by the period at which developing organisms face adverse environments. We found that the developmental trajectory of zebra finches during the juvenile phase may be particularly sensitive to unfavorable environmental conditions, whereby birds may preferentially invest in traits such as increased somatic growth and organ mass, fat deposition, and immune function that may prepare them for suboptimal adult conditions. In the broader perspective, these results provide further evidence of phenotypic programming, where conditions during development may modify or

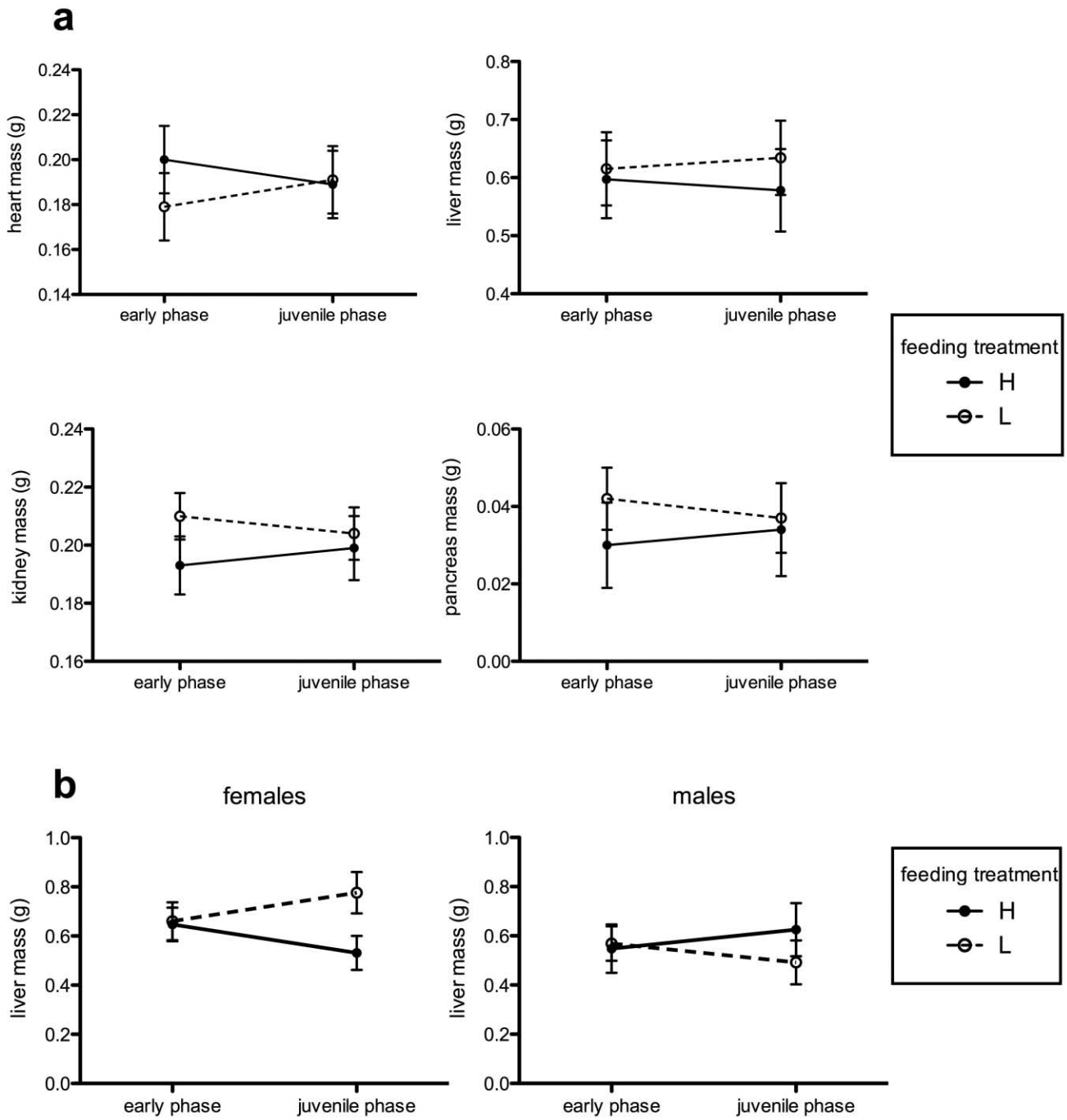

Figure 4. There was no main effect of feeding treatment on heart, liver, kidney, or pancreas mass (*a*). However, feeding treatment during phase 2 had a significant effect only on adult liver mass at posthatch day 600 (*a*). This effect was likely driven by females, as females that experienced low feeding (L) juvenile feeding treatment had larger liver masses than females that experienced high feeding (H) juvenile feeding treatment (*b*). Means were adjusted for body mass as a covariate, and error bars represent SEM.

guide development toward a particular phenotype that is better adapted for a particular environment (Monaghan 2008).

Our results also highlight the importance of the developmental period at which stress is experienced in determining the nature of the relationship between growth and immune function. Developmental correlations between such traits will depend on the timing of the stressor and on changes in the susceptibility of developing systems to the stressor (Spencer and MacDougall-Shackleton 2011). Previous studies on the long-term effects of early developmental environments on adult

Table 1: Fixed effects table for linear mixed models of BMR and RQ

Source, df (num, den)	*F*	*P*
Intercept:		
1, 24	.298	.590
1, 24	50.517	.000
Early feeding treatment (EFT):		
1 ,24	1.623	.215
1 ,24	1.519	.230
Juvenile feeding treatment (JFT):		
1, 24	.104	.750
1, 24	1.470	.237
Sex:		
1, 24	.450	.509
1, 24	.066	.799
EFT × JFT:		
1, 24	.218	.645
1, 24	.000	.988
EFT × sex:		
1, 24	.225	.640
1, 24	.507	.483
JFT × sex:		
1, 24	.010	.922
1, 24	.064	.802
EFT × JFT × sex:		
1, 24	.195	.663
1, 24	1.158	.293
Body mass:		
1, 24	14.194	.001
1, 24	2.618	.119

Note. Upper values are for the basal metabolic rate (BMR) and lower values are for the respiratory quotient (RQ). In this table we have abbreviated early and juvenile feeding treatments as EFT and JFT, respectively. Main feeding treatment effect sample sizes: EFT (H = 16 and L = 17) and JFT (H = 15 and L = 18). num, den = numerator, denominator, respectively.

immune function were inconsistent, with some suggesting that poor early environments enhance immune activity, while others suggested the opposite (e.g., Naguib et al. 2004; Stjernman et al. 2008; Tschirren et al. 2009). This inconsistency reveals the complex nature of the relationship between growth and development of immune function: there may be developmental periods when organisms must face a trade-off between growth and immune function, but there may also be other periods when both processes are enhanced or impaired by a stressor. Furthermore, the trade-off or enhancement may affect the different arms of the immune system to varying degrees. Although our data do not support the hypothesis that there is a trade-off between early growth and immune function (Lochmiller and Deerenberg 2000), it does suggest that faster growth at particular developmental periods may be associated with enhancement of specific aspects of immune function. Our findings indicate that faster growth during the juvenile period leads to better adult innate immune responses. Organisms may invest in innate immune defenses (especially when growing up in unfavorable conditions) because these defenses are thought to be inexpensive to develop and maintain (Lee 2006) and vital for determining survival and fitness (Lochmiller and Deerenberg 2000). With poor conditions during the juvenile phase (i.e., after nutritional independence) resulting in faster growth, increased immune defenses, and elevated adult body fat, our results point to a life-history strategy where experiencing impoverished environments later in development primes the organism to favor immune defenses that are crucial for survival but that are nonspecific and inexpensive to maintain in order to invest resources into other physiological processes that increase fitness (perhaps reproduction). As poor maternal environments can result in greater offspring parasite resistance potentially due to increased maternal investment (Boots and Roberts 2012), a future question of interest is whether developmental stress can have a transgenerational effect: birds that invested more in immune function because they experienced developmental stress may also invest more in the immune response of their offspring.

Nutritional deficits during development have been shown to

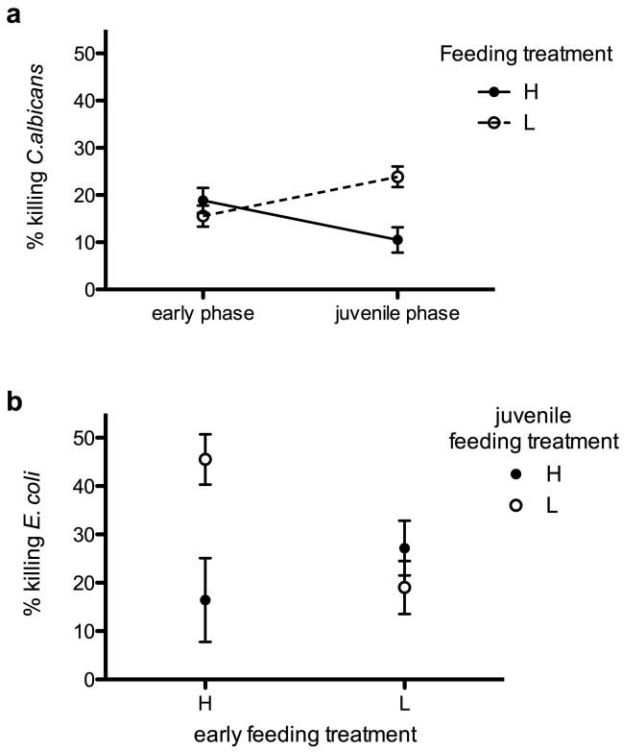

Figure 5. Feeding treatment had significant effects on adult microbial killing ability at posthatch day 150. Birds that experienced low feeding (L) juvenile feeding treatment killed more *Candida albicans* relative to birds that experienced high feeding (H) juvenile feeding treatment (*a*). The interaction between early and juvenile feeding treatment also significantly affected ability to kill *E. coli*. Birds that experienced H early and L juvenile feeding treatments killed more *E. coli* than birds that experienced H early and H juvenile or L early and L juvenile feeding treatments.

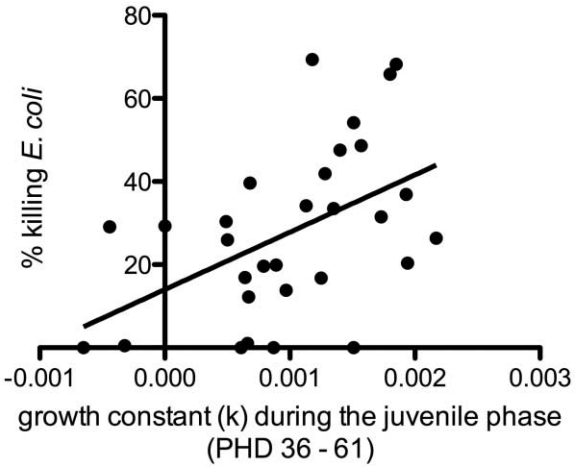

Figure 6. Simple regression showing the relationship between growth during the juvenile phase and *E. coli* killing capacity. Birds that grew faster during the juvenile interval also killed more *E. coli* as adults.

impair the rate of cell multiplication, alter cell composition and size (see Desai et al. 2005), and affect organ morphology (Rinaudo and Wang 2011). In our study, birds that experienced L feeding treatment were able to compensate—and even exceed relative to birds that experienced H feeding treatment—for some of the disadvantages incurred during unfavorable environmental conditions, although once again, the results were dependent on the period at which stress was experienced. Regardless of quality of conditions experienced before, females that experienced poor conditions after nutritional independence tended to have greater liver mass (and perhaps also kidney mass) as adults, suggesting that poor conditions during this developmental period may alter investment in mass of particular organs. However, work by Desai et al. (1996, 2005) indicates that poor nutritional conditions during development can have immediate (i.e., immediately after treatment) repercussions on organ mass. Thus, whether developing organisms will preferentially invest more in organ size and growth during periods of resource scarcity requires further investigation.

Reports of effects of nutritional stress on metabolic rate in birds have been inconsistent. While some researchers have found that poor developmental conditions elevate metabolic rates (e.g., Verhulst et al. 2006; Criscuolo et al. 2008; Schmidt et al. 2012), other researchers, including ourselves, have found no effect (e.g., Krause et al. 2009). These inconsistencies may be dependent on species and type of developmental stress employed. For example, Verhulst et al. (2006) manipulated brood sizes of zebra finches, Criscuolo et al. (2008) manipulated dietary protein intake of zebra finches, and Schmidt et al. (2012) manipulated quantity of food intake of song sparrows. Krause et al. (2009), who used similar manipulations as Criscuolo et al. (2008) but found no effect of their treatment on resting metabolic rate in zebra finches, suggest that effects of early nutritional treatment may resurface under stressful conditions in adulthood. Thus, in addition to any variation introduced by different treatment methods, measures of metabolic rate may also be sensitive to current nutritional status.

The experimental manipulation did not produce the expected differences in growth rates in the early phase, even though results from other studies employing the same experimental methods have indirectly (through differences in mass) suggested this to be the case (e.g., Lemon 1993; Spencer et al. 2003; Zann and Cash 2008). Possible explanations for the lack of differences in growth rate in early life are (1) parents buffered their offspring from poor nutritional conditions at the expense of self-maintenance, (2) the treatment conditions were not stressful enough, or (3) body mass is strongly genetically determined. The first explanation is plausible, as studies have found that parents may buffer the costs of reduced resource availability to the offspring (Mauck and Grubb 1995; Moreno et al. 1999) or themselves (Saino et al. 1999; Takahashi et al. 2003; Ardia 2005), or they may share the costs equally with offspring (Gaston and Hipfner 2006). Future experiments should therefore monitor parental condition in order to avoid the confounding effects of parental care. The second explanation is improbable as the juvenile feeding treatment affected adult liver mass, as discussed above. More likely, subjects were affected by treatment, but this was not reflected in body mass (the third explanation). Although there is literature suggesting that body mass of nestlings is determined to a large degree by environmental factors (e.g., van Noordwijk et al. 1988; Christie et al. 2000), there are also instances where adult body mass appears to be determined to a great degree by genetics. One such example is from Schmidt et al. (2012), where adult body mass of song sparrows captured from the wild and hand-raised from PHD 3 was significantly correlated to body mass of their free-living fathers, even though father and offspring presumably did not share similar developmental and adult environments.

In conclusion, the results from this study provide support for phenotypic programming, where an interaction of early and late developmental conditions influences multiple aspects of adult physiology. Previous research has suggested that very early life conditions can shape adult phenotypes and that in addition to environmental quality, environmental instability may be an important factor in determining fitness of the adult organism (Hales and Ozanne 2003; Wells 2007; Monaghan 2008). Our findings add to this growing body of research by suggesting that phenotypic programming can also occur in later stages of development, hence this factor should be taken into consideration in future experiments.

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Literature Cited

Ardia D.R. 2005. Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows. J Anim Ecol 74:517–524.

Birkhead T.R., F. Fletcher, and E.J. Pellat. 1998. Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. Behav Ecol Sociobiol 44:179–191.

———. 1999. Nestling diet, secondary sexual traits and fitness in the zebra finch. Proc R Soc B 266:385–390.

Boots M. and K.E. Roberts. 2012. Maternal effects in disease resistance: poor maternal environment increases offspring resistance to an insect virus. Proc R Soc B 279:4009–4014, doi:10.1098/rspb.2012.1073.

Brommer J.E. 2004. Immunocompetence and its costs during development: an experimental study in blue tit nestlings. Proc R Soc Lond B 271:S110–S113.

Brzęk P. and M. Konarzewski. 2007. Relationship between avian growth rate and immune response depends on food availability. J Exp Biol 210:2361–2367.

Buchanan K.L., S. Leitner, K.A. Spencer, A.R. Goldsmith, and C.K. Catchpole. 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. Proc R Soc B 271:2381–2386.

Butler M.W. and K.J. McGraw. 2011. Past or present? relative contributions of developmental and adult conditions to adult immune function and coloration in mallard ducks (*Anas platyrhynchos*). J Comp Physiol B 181:551–563.

Calder W.A. 1964. Gaseous metabolism and water relations of the zebra finch, *Taeniopygia castanotis*. Physiol Zool 37:400–413.

Chin E.H., O.P. Love, A.M. Clark, and T.D. Williams. 2005. Brood size and environmental conditions sex-specifically affect nestling immune response in European starling *Sturnus vulgaris*. J Avian Biol 36:549–554.

Christians J.K. 1999. Controlling for body mass effects: is part-whole correlation important? Physiol Zool 72:250–253.

Christie P., A.P. Møller, N. Saino, and F. De Lope. 2000. Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, *Delichon urbica* (the house martin). Heredity 85:75–83.

Criscuolo F., P. Monaghan, L. Nasir, and N.B. Metcalfe. 2008. Early nutrition and phenotypic development: "catch-up" growth leads to elevated metabolic rate in adulthood. Proc R Soc B 275:1565–1570.

Deerenberg C., V. Arpanius, S. Daan, and N. Bos. 1997. Reproductive effort decreases antibody responsiveness. Proc R Soc B 264:1021–1029.

De Coster G., S. Verhulst, E. Koetsier, L. De Neve, M. Briga, and L. Lens. 2011. Effects of early developmental conditions on innate immunity are only evident under favourable adult conditions. Naturwissenschaften 98:1049–1056.

De Kogel C.H. 1997. Long-term effects of brood size manipulation on morphological development and sex-specific mortality of offspring. J Anim Ecol 66:167–178.

Desai M., N.J. Crowther, A. Lucas, and C.N. Hales. 1996. Organ-selective growth in the offspring of protein restricted mothers. Br J Nutr 76:591–603.

Desai M., D. Gayle, J. Babu, and M.G. Ross. 2005. Permanent reduction in heart and kidney organ growth in offspring of undernourished rat dams. Am J Obstet Gynecol 193:1224–1232.

Fair J.M., E.S. Hansen, and R.E. Ricklefs. 1999. Growth, developmental stability and immune response in juvenile Japanese quails (*Coturnix coturnix japonica*). Proc R Soc B 266: 1735–1742.

Gaston A.J. and J.M. Hipfner. 2006. Adult Brunnich's guillemots *Uria Iomvia* balance body condition and investment in chick growth. Ibis 148:106–113.

Gerson A.R. and C.G. Guglielmo. 2011. House sparrows (*Passer domesticus*) increase protein catabolism in response to water restriction. Am J Physiol 300:R925-R930.

Guglielmo C.G., L.P. McGuire, A.R. Gerson, and C.L. Seewagen. 2011. Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. J Ornithol 152(suppl. 1):75–85.

Hales C.N. and S.E. Ozanne. 2003. The dangerous road of catch-up growth. J Physiol 547:5–10.

Hoi-Leitner M., M. Romero-Pujante, H. Hoi, and A. Pavlova. 2001. Food availability and immune capacity in serin (*Serinus serinus*) nestlings. Behav Ecol Sociobiol 49:333–339.

Krause E.T., M. Honarmand, J. Wetzel, and M. Naguib. 2009. Early fasting is long lasting: differences in early nutritional conditions reappear under stressful conditions in adult female zebra finches. PLoS ONE 4:e5015.

Lee K.A. 2006. Linking immune defenses and life history at the levels of the individual and the species. Integr Comp Biol 46:1000–1015.

Lemon W. C. 1993 The energetics of lifetime reproductive success in the zebra finch *Taeniopygia guttata*. Physiol Zool 66: 946–963.

Liebl A.L. and L.B. Martin II. 2009. Simple quantification of blood and plasma antimicrobial capacity using spectrophotometry. Funct Ecol 23:1091–1096.

Lighton J.R.B. 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, New York.

Lindström J. 1999. Early development and fitness in birds and mammals. Trends Ecol Evol 14:343–348.

Lochmiller R.L. and Deerenberg C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87–98.

Matson K.D., R.E. Ricklefs, and K.C. Klasing. 2005. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Dev Comp Immunol 29:275–286.

Mauck R.A. and T.C. Grubb Jr. 1995. Petrel parents shunt all experimentally increased reproductive costs to their offspring. Anim Behav 49:999–1008.

Mauck R.A., K.D. Matson, J. Philipsborn, and R.E. Ricklefs. 2005. Increase in the constitutive innate humoral immune system in leach's storm-petrel (*Oceanodroma leucorhoa*)

chicks is negatively correlated with growth rate. Funct Ecol 19:1001–1007.

McGraw K.J. and D.R. Ardia. 2005. Sex differences in carotenoid status and immune performance in zebra finches. Evol Ecol Res 7:251–262.

Metcalfe N.B. and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16:254–260.

Millet S., J. Bennett, K.A. Lee, M. Hau, and K.C. Klasling. 2007. Quantifying and comparing constitutive immunity across avian species. Dev Comp Immunol 31:188–201.

Monaghan P. 2008. Early growth conditions, phenotypic development and environmental change. Phil Trans R Soc B 363:1635–1645.

Moreno J., S. Merino, J. Potti, A. Leon, and R. Rodriguez. 1999. Maternal energy expenditure does not change with flight costs or food availability in the pied flycatcher (*Ficedula hypoleuca*): costs and benefits for nestlings. Behav Ecol Sociobiol 46:244–251.

Morton M.L., M.E. Pereya, and L.F. Baptista. 1985. Photoperiodically induced ovarian growth in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*) and its augmentation by song. Comp Biochem Physiol 80:93–97.

Naguib M., K. Riebel, A. Marzal, and D. Gil. 2004. Nestling immunocompetence and testosterone covary with brood size in a songbird. Proc R Soc B 271:833–838.

Palacios M.G., J.E. Cunnick, D. Vleck, C.M. Vleck. 2009. Ontogeny of innate and adaptive immune defense components in free-living tree swallows, *Tachycineta bicolor*. Dev Comp Immunol 33:456–463.

Råberg L., M. Stjernman, D. Hasselquist. 2003. Immune responsiveness in adult blue tits: heritability and effects of nutritional status during ontogeny. Oecologia 136:360–364.

Ricklefs R.E. 1968. Patterns of growth in birds. Ibis 110:419–451.

Rinaudo P. and E. Wang. 2011. Fetal programming and metabolic syndrome. Annu Rev Physiol 74:3.1–3.24.

Saino N., S. Calza, P. Ninni, and A.P. Møller. 1999. Barn swallows trade survival against offspring condition and immunocompentence. J Anim Ecol 68:999–1009.

Schmidt K.L., E.A. MacDougall-Shackleton, and S.A. MacDougall-Shackleton. 2012. Developmental stress has sex-specific effects on nestling growth and adult metabolic rates but no effect of adult body size or body composition in song sparrows. J Exp Biol 215:3207–3217.

Soler J.J., L. de Neve, T. Pérez-Contreras, M. Soler, and G. Sorci. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. Proc R Soc B 270:241–248.

Spencer K. A., K.L. Buchanan, A.R. Goldsmith, and C.K. Catchpole. 2003 Song as honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). Horm Behav 44:132–139.

Spencer K.A. and S.A. MacDougall-Shackleton. 2011. Indicators of development as sexually selected traits: the developmental stress hypothesis in context. Behav Ecol 22:1–9.

Stambaugh T., B.J. Houdek, M.P. Lombardo, P.A. Thorpe, and D.C. Hahn. 2011. Innate immune response development in nestling tree swallows. Wilson J Ornithol 123:779–787.

Stjernman M., L. Råberg, J.-A. Nilsson. 2008. Long-term effects of nestling condition on blood parasite resistnace in blue tits (*Cyanistes caeruleus*). Can J Zool 86:937–946.

Takahashi A., Y. Watanuki, K. Sato, A. Kato, N. Arai, J. Nishikawa, and Y. Naito. 2003. Parental foraging effort and offspring growth in Adelie penguins: does working hard improve reproductive success? Funct Ecol 17:590–597.

Tella J.L., M.G. Forero, M. Bertellotti, J.A. Donazar, G. Blanco, and O. Ceballos. 2001. Offspring body condition and immunocompetence are negatively affected by high breeding densities in a colonial seabird: a multiscale approach. Proc R Soc B 268:1455–1461.

Tschirren B., A.N. Rutstein, E. Potsma, M. Marriette, and S.C. Griffith. 2009. Short- and long-term consequences of early developmental conditions: a case study on wild and domesticated zebra finches. J Evol Biol 22:387–395.

van Noordwijk A.J., J.H. van Balen, and W. Scharloo. 1988. Heritability of body size in a natural population of the great tit (*Parus major*) and its relation to age and environmental conditions during growth. Genet Res 51:149–162.

Verhulst S., M.J. Holveck, and K. Riebel. 2006. Long-term effects of manipulated natal brood size on metabolic rate in zebra finches. Biol Lett 2:478–480.

Wells J.C.K. 2007. Environmental quality, developmental plasticity, and the thrifty phenotype: a review of evolutionary models. Evol Bioinform 3:109–120.

Zann R. and E. Cash. 2008. Developmental stress impairs song complexity but not learning accuracy in non-domesticated zebra finches (*Taeniopygia guttata*). Behav Ecol Sociobiol 62: 391–400.