

Feather corticosterone levels are not correlated with health or plumage coloration in juvenile house finches

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Stressful developmental conditions can have both short- and long-term effects on animal physiology and behaviour, but studies on this topic are rarely conducted in the wild and, if so, largely focus on only the first few weeks of life. To fill this gap, we tested developmental links between early-life stress and the physiology of wild-caught juveniles later during development. Specifically, we examined potential associations between feather corticosterone levels of hatchling house finches (*Haemorrhous mexicanus*) and several phenotypic and physiological traits measured several months later in juveniles. We assessed four indices of health (oxidative damage to lipids, innate immunity, intestinal parasite infection intensity and plumage colour) and two morphological traits (body mass and tarsus length) in juveniles. Feather corticosterone content was not related to any of the juvenile traits later in development. Our results suggest that physiological variables can change rapidly during ontogeny, such that stress hormone levels in juvenile feathers could be uncoupled from the real stress levels experienced by nestlings. Instead, juvenile physiology might be more dependent on current environmental conditions than on early-life conditions (i.e. environmental matching), and this may limit the effects on fitness of poor early-developmental conditions.

ADDITIONAL KEYWORDS: body size – carotenoid pigmentation – *Haemorrhous mexicanus* – immunity – oxidative stress – parasitism – steroids.

INTRODUCTION

The conditions that individuals experience during early life can have effects on their growth, physiology and behaviour that can persist into adulthood (Roff, 1992; Mousseau & Fox, 1998; Metcalfe & Monaghan, 2001; Larcombe *et al.*, 2017). Many of these early-life experiences are stressful (e.g. parasite burden, sibling competition, food shortages), and these are often accompanied by an increased secretion of stress hormones released by the

hypothalamic–pituitary–adrenal axis of the endocrine system (e.g. glucocorticoids; Harris & Seckl, 2011; Crespi *et al.*, 2012). These stress hormones can affect short-term behaviour and physiology in developing animals (Blas, 2006; Grava *et al.*, 2013) and can alter the organization of morphological, physiological, neurological and behavioural traits throughout life (Love & Williams, 2008; Spencer & MacDougall-Shackleton, 2011; Farrell *et al.*, 2015). For example, developmental stress has been suggested to increase oxidative stress and affect ageing in a wide variety of organisms, including mice (Gibson, Garratt & Brooks, 2015), fish (Kishi, 2014), snakes (Bronikowski & Vleck, 2010) and humans (de Rooij & Roseboom,

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2013). In addition, elevated levels of stress hormones or exposure to environmental stressors during development can have short-term effects on growth and immune responses (Saino *et al.*, 2003; Martin *et al.*, 2005; Loiseau *et al.*, 2008) and long-term effects on immunity (Berghänel *et al.*, 2016; Danese & Lewis, 2017) and parasite resistance (Devevey *et al.*, 2010).

In recent years, birds have emerged as popular subjects for investigating how the early developmental environment shapes the phenotype, mainly because the distinct egg stage in their development allows for relative ease in separating pre-hatch vs. post-hatch effects (Crino & Breuner, 2015). Although it is relatively easy to measure levels of stress hormones from eggs (i.e. Hayward & Wingfield, 2004), studies on long-term effects of post-hatch developmental stress have lagged behind, mainly because measuring glucocorticoid levels during development in wild animals can be challenging because of the rapid nature of stress responses (Romero & Reed, 2005) and the possible confounding effect of handling stress (Romero & Romero, 2002; Hamalainen *et al.*, 2014). A method for measuring levels of the stress hormone corticosterone (CORT) deposited in growing feathers was developed a decade ago to overcome this problem (Bortolotti *et al.*, 2008). Corticosterone is deposited into feathers throughout feather growth and thus its levels in plumage reflecting individual differences in stress level and adrenocortical response (i.e. integrating baseline level, magnitude and time course of stress responses, and number/types of stressors experienced) over several weeks (Bortolotti *et al.*, 2008; Lattin *et al.*, 2011). Prior avian studies have shown that feather CORT levels respond to experimental manipulations of food availability (Will *et al.*, 2014) and brood size (Lodjak *et al.*, 2015). Most studies of feather CORT have been done on adult birds; therefore, more studies on juveniles are needed to understand the long-term effects of developmental stress (Martinez-Padilla *et al.*, 2013).

Here, we collected feathers grown during the first few weeks of life from young male and female house finches (*Haemorrhous mexicanus* Müller, 1776) during their first adult moult to study retrospectively the stress levels that these birds experienced during development, while assessing the physiological condition of the birds at this time (i.e. several months after hatching). We tested for relationships between feather CORT levels and four indices of current health state (oxidative damage to lipids, innate immunity, intestinal parasite infection intensity and plumage colour intensity) and two morphological traits (body mass and tarsus length).

As stressful conditions experienced during the first few weeks of life can have long-term negative effects on parasite resistance and immunity (Stjernman, Raberg & Nilsson, 2008; Kriengwatana *et al.*, 2013), we predicted that birds with higher feather CORT levels

would show decreased innate immunity and resistance to intestinal parasites. Developmental stress can also lead to accelerated ageing (Bronikowski & Vleck, 2010; Gibson *et al.*, 2015); therefore, we predicted that birds with higher feather CORT levels would have more oxidative damage. If developmental stress levels affect condition during the first adult moult, we would expect associations between the ornamental colour of a bird's first adult plumage and juvenile feather CORT levels, because sexual coloration is hypothesized to reflect individual quality (von Schantz *et al.*, 1999; Garratt & Brooks, 2012). This association could be either positive (a recent study in house finches indicated that redder adult male house finches had higher levels of feather CORT; Lendvai *et al.*, 2013) or negative (if higher stress levels interfere with feather pigmentation). Likewise, for morphological measurements (body mass, tarsus length and body condition), we could expect either positive or negative relationships with feather CORT levels, because elevated stress levels during development impair growth in some organisms (reviewed by Crino & Breuner, 2015), but accelerate growth in others (Coslovsky & Richner, 2011; Berghänel *et al.*, 2016).

MATERIAL AND METHODS

FIELD METHODS

A full description of the methods is available as [Supporting Information](#). From 13 to 20 September 2012, we used hanging basket traps and ground Potter traps baited with sunflower seeds (Giraudeau, Toomey & McGraw, 2012) to capture 74 moulting hatch-year house finches (37 females and 37 males) on the Arizona State University campus (Tempe, AZ, USA). At capture, birds were completing their first pre-basic plumage moult. Juvenile plumage was distinguished from basic female plumage by the broad buffy proximal edges of upper surfaces of secondaries, tertials and greater and median secondary coverts (Badyaev, Belloni & Hill, 2012). We determined body mass (to the nearest 0.1 g with a digital scale), tarsus length (to the nearest 0.1 mm with digital callipers) and body condition (calculated as mass/length residuals). We also collected 150 µL of whole blood from the alar vein with heparinized capillary tubes. Blood was centrifuged (10 000g for 3 min) and the plasma saved at -80 °C for later analysis of innate immunity and oxidative damage to lipids (see below). Source data are available as [Supporting Information](#).

MEASUREMENT OF FEATHER CORTICOSTERONE CONTENT

We collected the four outer wing covert feathers of the juvenile plumage to quantify corticosterone levels. The CORT content were measured using

radioimmunoassay (RIA), following the method described by [Lendvai *et al.* \(2013\)](#). The results were corrected by feather mass (as described by [Lendvai *et al.*, 2013](#)), because the length of the covert feathers could be determined less reliably than their mass, and because the four feathers from the same individuals were pooled before extraction. Feather CORT corrected by feather mass was unrelated to sample mass. The intra-assay coefficient of variation was 4.27%.

QUANTIFYING COCCIDIAN PARASITES

We assessed the coccidian infection intensity of each bird from faecal samples collected in the afternoon and evening (i.e. after 16:30 h), which is when coccidian oocysts are shed, via standard faecal-float and microscope-evaluation methods ([Brawner, Hill & Sundermann, 2000](#); [Giraudeau *et al.*, 2014](#)). Coccidians inhabit the gut lining of birds and are thought to disrupt nutrient uptake, thereby affecting the health and appearance of birds ([Brawner *et al.*, 2000](#); [Pap *et al.*, 2009](#)). The coccidian oocyst load was estimated with a light microscope on an integer scale from zero to five, as follows: 0, no oocysts present; 1, 1–10 oocysts present; 2, 11–100 oocysts present; 3, 101–1000 oocysts present; 4, 1001–10 000 oocysts present; and 5, $\geq 10 000$ oocysts present.

AGGLUTINATION AND LYSIS ASSAY

We used the haemolysis–haemagglutination assay to assess the strength of innate immune responses. Agglutination of foreign red blood cells is a measure of the concentration of natural antibodies (produced before antigen exposure) that assist in foreign particle removal and complement-mediated lysis. Lysis assesses the ability of the plasma to destroy foreign cells by rupturing them ([Matson, Ricklefs & Klasing, 2005](#)). We followed the protocol developed by [Matson *et al.* \(2005\)](#), which was then modified by [Moeller, Butler & DeNardo \(2013\)](#) and [Butler *et al.*, \(2013\)](#). Details of this method are available as [Supporting Information](#). Two persons independently scored the plates blind to sample identity. We previously showed that their lysis and agglutination scores were highly repeatable for both agglutination and lysis ([Davies *et al.*, 2015](#)).

MEASUREMENT OF LIPID PEROXIDATION

Following the description by [Giraudeau *et al.* \(2014\)](#), we used a commercially available kit (Oxi-Tek TBARS assay kit; ZeptoMetrix Corp., Buffalo, NY, USA) to assess oxidative damage to lipids in the form of the concentration of malondialdehydes (MDA) from plasma. The thiobarbituric acid reactive substances (TBARS)-based colorimetric method is widely used

in ecological studies because it is convenient, simple and low cost, but it is criticized for the inherent problems of data specificity (reactivity towards other compounds other than MDA) and variability ([Halliwell & Gutteridge, 2007](#)), suggesting caution in interpreting results obtained with this method. Details of this method are available as [Supporting Information](#). Sample concentrations are expressed as nanomoles per millilitre of MDA equivalents. Higher values correspond to greater oxidative damage.

CAROTENOID-BASED COLORATION

At capture, we digitally photographed each male to measure the expression of ornamental plumage coloration, following the methods published by [Giraudeau *et al.* \(2014\)](#). Using a Canon PowerShot SD1200S (Lake Success, NY, USA), we took two separate photographs of the breast of each bird against a neutral grey-board, using identical distance from camera to object, shutter, exposure and flash settings for each photograph. Digital images (JPEG, 3648 × 2736 pixels) were imported into Adobe Photoshop (San Jose, CA, USA) to determine the plumage hue.

STATISTICS

All statistical analyses were carried out using Statistica software (StatSoft, Tulsa, OK, USA). We used separate analyses of covariance (ANCOVAs) to test for the effects of feather CORT, sex and their interaction on the physiological and morphological traits measured. To eliminate the possible problem of collinearity, we tested whether feather CORT levels differed for sexes. This was not the case [mean for males 6.99 ± 0.73 (SE), mean for females 7.44 ± 0.68 (SE), *t*-test $P = 0.66$, $t = -0.44$]. We \log_{10} -transformed the TBARS and hue data to normalize them. No transformation could normalize the coccidia and immunity data, so we ranked them ([Conover & Iman, 1981](#)) before analyses. Given that CORT analyses were run on two plates, we included plate number as a cofactor in all analyses. This factor was significant for the hue model only, and was therefore removed together with the non-significant sex × feather CORT interaction from all other final analyses. Full models ([Table S1](#)) and source data ([Table S2](#)) are presented in [Supplementary Material](#).

RESULTS

The mean SEM value for feather corticosterone was 7.23 ± 4.23 pg/mg, minimum 0.70 pg/mg and maximum 22.21 pg/mg. Feather corticosterone levels were not significantly correlated with any of the

variables measured (Table 1, Fig. 1). There were no intercorrelations between variables (all $P > 0.1$) except for agglutinations scores that were correlated with oxidative damage levels ($F_{1,43} = 7.68$, $P = 0.0082$) and lysis scores ($F_{1,44} = 61.60$, $P = 10^{-6}$) and hue values that were correlated with coccidian levels ($F_{1,28} = 5.74$, $P = 0.023$) and oxidative damage levels ($F_{1,33} = 4.17$, $P = 0.049$). The only trait that was affected by the sex of the bird was body condition, which was lower for females (Table 1, Fig. 1).

DISCUSSION

Here, we examined possible relationships between CORT levels in juvenile feathers and morphological and physiological measurements several months after fledging in hatch-year house finches. Feather CORT content was not related to four indices of health (oxidative damage to lipids, innate immunity, intestinal parasite infection intensity and plumage color intensity) or to body mass, tarsus length or body condition.

Our negative results seem to suggest that house finches display a significant amount of developmental plasticity, growing out of the effects of varying stress hormone levels during the nestling period by the time they have moulted into their first adult plumage. Previous studies have shown that nestling feather CORT is related to condition when the latter is measured concurrently with CORT secretion in feathers. For example, strong associations between feather CORT and nestling body condition have been shown to exist in tree swallows (*Tachycineta bicolor*;

Harms *et al.*, 2010) and black kites (*Milvus migrans*; López-Jiménez *et al.*, 2016) and between fledging success and feather CORT levels in northern flickers (*Colaptes auratus*; Gow & Wiebe, 2014) and tree swallows (Fairhurst *et al.*, 2013).

Another possibility is that CORT levels might be uncoupled from the stress levels experienced by nestlings, so that the physiological condition of the birds would not be related to their feather CORT at the nestling age. For example, a recent study on European starlings (*Sturnus vulgaris*) showed that there was no difference in feather CORT between nestlings with unpredictable access to food and those with continuous access, indicating that feather CORT might not always detect ecologically relevant stressors in the nestling period (Fischer, Rao & Romero, 2017). Young birds may be limited in their abilities to perform many of the adult-like responses to overcome stressful situations and, as a consequence, an adult-like adrenocortical response to stress might expose chicks to chronic CORT elevations, with potentially deleterious consequences for development, without any rapid benefits in terms of survival (Sims & Holberton, 2000; Kitaysky *et al.*, 2003; Blas *et al.*, 2006).

This lack of connection between condition and the level of stress responses could be mediated by, for example, plasma corticosteroid binding globulins, which can regulate the general availability of steroid to tissues (Malisch & Breuner, 2010), or the number of CORT receptors on the target cells (Lattin, Waldron-Francis & Romero, 2013). Accordingly, some non-precocial nestlings have been shown to display very low stress responses at the beginning of the nestling period, but the typical adrenocortical pattern of fully

Table 1. Results of ANCOVAs examining the effects of sex and feather corticosterone levels on various physiological and morphological variables

Dependent variable	Factor	d.f.	F	η^2	P -value
Tarsus length	fCORT	1,68	2.99	0.04	0.088
	Sex	1,68	0.42	0.006	0.52
Body condition	fCORT	1,68	0.004	<0.0001	0.95
	Sex	1,68	10.26	0.13	0.002*
Oxidative damage (TBARS)	fCORT	1,66	3.43	0.007	0.07
	Sex	1,66	3.4	0.002	0.07
Immunity (agglutination)	fCORT	1,42	0.24	0.005	0.63
	Sex	1,42	0.68	0.01	0.41
Immunity (lysis)	fCORT	1,44	0.00008	0.0002	0.99
	Sex	1,44	3.22	0.09	0.08
Coccidia infection	fCORT	1,56	0.15	0.01	0.7
	Sex	1,56	0.12	0.0006	0.91
Colour (hue)	fCORT	1,32	0.02	0.0006	0.89
	Assay number	1,32	6.13	0.16	0.019*

Abbreviations: fCORT: feather corticosterone; TBARS, thiobarbituric acid reactive substances. η^2 denotes effect sizes in the models. *Statistically significant values.

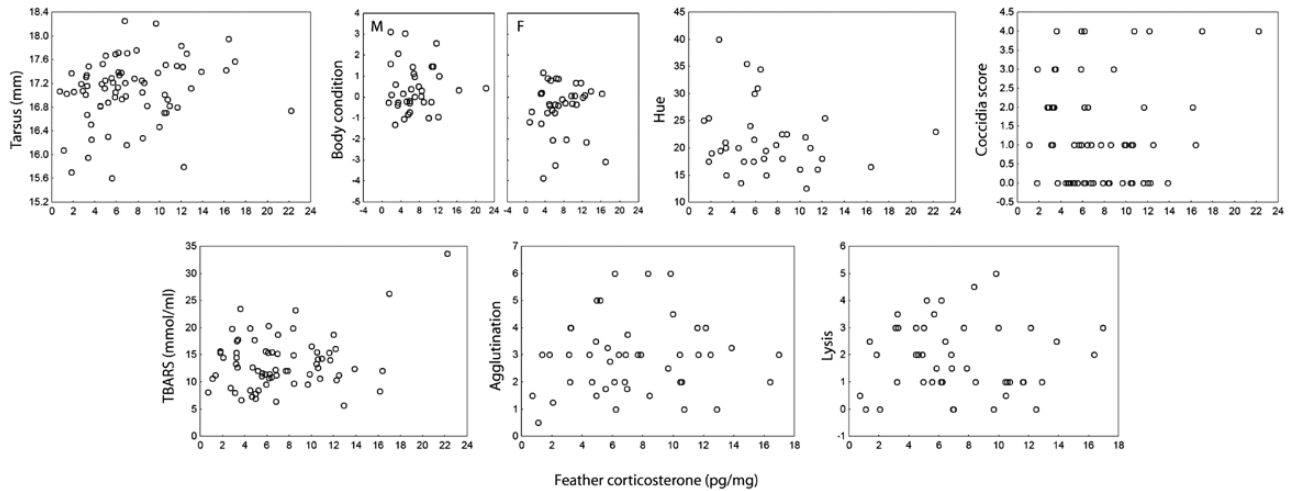


Figure 1. Associations between the levels of corticosterone deposited in the feathers during the nestling age and traits related to physiological condition measured during first adult moult in male (M) and female (F) house finches. Feather hue was measured only for male birds, because females generally lack carotenoid-based coloration.

developed birds near fledging (Magellanic penguin, *Spheniscus magellanicus*, Walker, Wingfield & Boersma, 2005; white storks, *Ciconia ciconia*, Blas *et al.*, 2006; American kestrel, *Falco sparverius*, Love & Williams, 2008).

Given that we measured only birds that had reached the fledgling phase, selective disappearance of birds experiencing high levels of stress (and, as a result, a lower health state) during the nestling period cannot be ruled out. Additional data on feather corticosterone levels and survival until the juvenile stage are necessary to gain a full understanding of the ecological meaning of juvenile feather corticosterone levels.

In conclusion, we found that natural variation in feather CORT levels during development were uncoupled from physiological and morphological parameters measured several months later in life. This is one of the first studies to test the impact of developmental stress on the physiology later in life in wild animals in their natural environment. We propose the following hypotheses to explain these negative results: (1) birds in our study population might display high levels of developmental plasticity and, as they approach maturation, their physiological condition might be uncoupled from the levels of stress experienced during the nestling period; (2) stress hormone levels in nestlings might be uncoupled from the stress levels that the nestlings experience; and (3) natural conditions allow environmental matching that might limit the effects on fitness of poor developmental conditions. Future studies on the associations between nestling feather corticosterone levels, reproductive success, behaviour and lifespan in wild animals might considerably add to our

knowledge about the lifetime effects of developmental stress.

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REFERENCES

- Badyaev AV, Belloni V, Hill GE. 2012. House finch (*Haemorrhous mexicanus*), version 2.0. In: Rodewald PG, ed. *The birds of North America*. Ithaca, NY, USA: Cornell Laboratory of Ornithology.
- Badyaev AV, Martin TE. 2000. Sexual dimorphism in relation to current selection in the house finch. *Evolution* **54**: 987–997.

- Berghänel A, Heistermann M, Schülke O, Ostner J. 2016.** Prenatal stress effects in a wild, long-lived primate: predictive adaptive responses in an unpredictable environment. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20161304.
- Blas J, Baos R, Bortolotti GR, Marchant TA, Hiraldo F. 2006.** Age-related variation in the adrenocortical response to stress in nestling white storks (*Ciconia ciconia*) supports the developmental hypothesis. *General and Comparative Endocrinology* **148**: 172–180.
- Blas J, Bortolotti GR, Tella JL, Baos R, Marchant TA. 2007.** Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 8880–8884.
- Bortolotti GR, Marchant TA, Blas J, German T. 2008.** Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Functional Ecology* **22**: 494–500.
- Brawner WR, Hill GE, Sundermann CA. 2000.** Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male House Finches. *The Auk* **117**: 952–963.
- Bronikowski A, Vleck D. 2010.** Metabolism, body size and life span: a case study in evolutionarily divergent populations of the garter snake (*Thamnophis elegans*). *Integrative and Comparative Biology* **50**: 880–887.
- Butler MW, Stahlschmidt ZR, Ardia DR, Davies S, Davis J, Guillette LJ Jr, Johnson N, McCormick SD, McGraw KJ, DeNardo DF. 2013.** Thermal sensitivity of immune function: evidence against a generalist-specialist trade-off among endothermic and ectothermic vertebrates. *The American Naturalist* **181**: 761–774.
- Conover WJ, Iman RL. 1981.** Rank transformations as a bridge parametric and nonparametric statistics. *The American Statistician* **35**: 124–129.
- Coslovsky M, Richner H. 2011.** Predation risk affects offspring growth via maternal effects. *Functional Ecology* **25**: 878–888.
- Crespi EJ, Williams TD, Jessop TS, Delehanty B. 2012.** Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Functional Ecology* **27**: 93–106.
- Crino OL, Breuner CW. 2015.** Developmental stress: evidence for positive phenotypic and fitness effects in birds. *Journal of Ornithology* **156**(Suppl 1): 389–398.
- Danese A, Lewis SJ. 2017.** Psychoneuroimmunology of early-life stress: the hidden wounds of childhood trauma? *Neuropsychopharmacology* **42**: 99–114.
- Davies S, Behbahaninia H, Giraudeau M, Meddle SL, Waites K, Deviche P. 2015.** Advanced seasonal reproductive development in a male urban bird is reflected in earlier plasma luteinizing hormone rise but not energetic status. *General and Comparative Endocrinology* **224**: 1–10.
- De Rooij SR, Roseboom TJ. 2013.** The developmental origins of ageing: study protocol for the Dutch famine birth cohort study on ageing. *BMJ Open* **3**: e003167.
- Devevey G, Bize P, Fournier S, Person E, Christe P. 2010.** Testing the predictive adaptive response in a host-parasite system. *Functional Ecology* **24**: 178–185.
- Fairhurst GD, Marchant TA, Soos C, Machin KL, Clark RG. 2013.** Experimental relationships between levels of corticosterone in plasma and feathers in a free-living bird. *The Journal of Experimental Biology* **216**: 4071–4081.
- Farrell TM, Morgan A, Sarquis-Adamson Y, MacDougall-Shackleton SA. 2015.** Effects of early-developmental stress on growth rates, body composition and developmental plasticity of the HPG-axis. *General and Comparative Endocrinology* **222**: 134–143.
- Fischer CP, Rao R, Romero LM. 2017.** Exogenous and endogenous corticosterone in feathers. *Journal of Avian Biology* **48**: 1301–1309.
- Garratt M, Brooks RC. 2012.** Oxidative stress and condition-dependent sexual signals: more than just seeing red. *Proceedings of the Royal Society B: Biological Sciences* **279**: 3121–3130.
- Gibson AB, Garratt M, Brooks RC. 2015.** Experimental evidence that litter size imposes an oxidative challenge to offspring. *The Journal of Experimental Biology* **218**: 3911–3918.
- Giraudeau M, Chavez A, Toomey M, McGraw KJ. 2015.** Effects of carotenoid supplementation and oxidative challenges on physiological parameters and carotenoid-based colouration in an urbanization context. *Behavioral Ecology and Sociobiology* **69**: 957–970.
- Giraudeau M, Mousel M, Earl S, McGraw K. 2014.** Parasites in the city: degree of urbanization predicts pox-virus and coccidial infections in house finches (*Haemorrhous mexicanus*). *PLoS One* **9**: e86747.
- Giraudeau M., Toomey MB, McGraw KJ. 2012.** Can House Finches (*Carpodacus mexicanus*) use non-visual cues to discriminate the carotenoid content of foods? *Journal of Ornithology* **153**: 1017–1023.
- Gow EA, Wiebe KL. 2014.** Determinants of parental care and offspring survival during the post-fledging period: males care more in a species with partially reversed sex roles. *Oecologia* **175**: 95–104.
- Grava T, Fairhurst GD, Avey MT, Grava A, Bradley J, Avis JL, Bortolotti GR, Sturdy CB, Otter KA. 2013.** Habitat quality affects early physiology and subsequent neuromotor development of juvenile black-capped chickadees. *PLoS One* **8**: e71852.
- Halliwell B, Gutteridge JMC. 2007.** *Free radicals in biology and medicine, 4th edn.* Oxford: Oxford University Press.
- Hämäläinen A, Heistermann M, Fenosoa ZS, Kraus C. 2014.** Evaluating capture stress in wild gray mouse lemurs via repeated fecal sampling: method validation and the influence of prior experience and handling protocols on stress responses. *General and Comparative Endocrinology* **195**: 68–79.
- Harms NJ, Fairhurst GD, Bortolotti GR, Smits JE. 2010.** Variation in immune function, body condition, and feather corticosterone in nestling Tree Swallows (*Tachycineta bicolor*) on reclaimed wetlands in the Athabasca oil sands, Alberta, Canada. *Environmental Pollution* **158**: 841–848.

- Harris A, Seckl J. 2011.** Glucocorticoids, prenatal stress and the programming of disease. *Hormones and Behavior* **59**: 279–289.
- Hayward LS, Wingfield JC. 2004.** Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *General and Comparative Endocrinology* **135**: 365–371.
- Hill GE. 1990.** Female house finches prefer colorful males: sexual selection for a condition-dependent trait. *Animal Behaviour* **40**: 563–572.
- Kishi S. 2014.** Using zebrafish models to explore genetic and epigenetic impacts on evolutionary developmental origins of aging. *Translational Research* **163**: 123–135.
- Kitaysky AS, Kitaiskaia EV, Piatt JF, Wingfield JC. 2003.** Benefits and costs of increased levels of corticosterone in seabird chicks. *Hormones and Behavior* **43**: 140–149.
- Kriengwatana B, Wada H, Macmillan A, MacDougall-Shackleton SA. 2013.** Juvenile nutritional stress affects growth rate, adult organ mass, and innate immune function in zebra finches (*Taeniopygia guttata*). *Physiological and Biochemical Zoology* **86**: 769–781.
- Larcombe SD, Herborn KA, Alexander L, Arnold KE. 2017.** Dietary antioxidants in life-history trade-offs: differential effects of α -tocopherol supplementation on blue tit *Cyanistes caeruleus* mothers and offspring during reproduction. *Biological Journal of the Linnean Society* **122**: 313–328.
- Lattin CR, Reed JM, DesRochers DW, Romero LM. 2011.** Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. *Journal of Avian Biology* **42**: 247–252.
- Lattin CR, Waldron-Francis K, Romero LM. 2013.** Intracellular glucocorticoid receptors in spleen, but not skin, vary seasonally in wild house sparrows (*Passer domesticus*). *Proceedings of the Royal Society B: Biological Sciences* **280**: 20123033.
- Lendvai ÁZ, Giraudeau M, Németh J, Bakó V, McGraw KJ. 2013.** Carotenoid-based plumage coloration reflects feather corticosterone levels in male house finches (*Haemorhous mexicanus*). *Behavioral Ecology and Sociobiology* **67**: 1817–1824.
- Lessells CM, Boag PT. 1987.** Unrepeatable repeatabilities: a common mistake. *The Auk* **104**: 116–121.
- Lodjak J, Mägi M, Rooni U, Tilgar V. 2015.** Context-dependent effects of feather corticosterone on growth rate and fledging success of wild passerine nestlings in heterogeneous habitat. *Oecologia* **179**: 937–946.
- Loiseau C, Sorci G, Dano S, Chastel O. 2008.** Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows. *General and Comparative Endocrinology* **155**: 101–108.
- López-Jiménez L, Blas J, Tanferna A, Cabezas S, Marchant T, Hiraldo F, Sergio F. 2016.** Ambient temperature, body condition and sibling rivalry explain feather corticosterone levels in developing black kites. *Functional Ecology* **30**: 605–613.
- Love OP, Williams TD. 2008.** Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre- and post-natal developmental stress. *Hormones and Behavior* **54**: 496–505.
- Malisch JL, Breuner CW. 2010.** Steroid-binding proteins and free steroids in birds. *Molecular and Cellular Endocrinology* **316**: 42–52.
- Martin LB II, Gilliam J, Han P, Lee K, Wikelski M. 2005.** Corticosterone suppresses cutaneous immune function in temperate but not tropical House Sparrows, *Passer domesticus*. *General and Comparative Endocrinology* **140**: 126–135.
- Martínez-Padilla J, Mougeot F, García JT, Arroyo B, Bortolotti GR. 2013.** Feather corticosterone levels and carotenoid-based coloration in Common Buzzard (*Buteo buteo*) nestlings. *Journal of Raptor Research* **47**: 161–173.
- Matson KD, Cohen AA, Klasing KC, Ricklefs RE, Scheuerlein A. 2006.** No simple answers for ecological immunology: relationships among immune indices at the individual level break down at the species level in waterfowl. *Proceedings of the Royal Society B: Biological Sciences* **273**: 815–822.
- Matson KD, Ricklefs RE, Klasing KC. 2005.** A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental and Comparative Immunology* **29**: 275–286.
- McGraw KJ, Hill GE. 2000.** Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **267**: 1525–1531.
- McGraw KJ, Stoehr AM, Nolan PM, Hill GE. 2001.** Plumage redness predicts breeding onset and reproductive success in the House Finch: a validation of Darwin's theory. *Journal of Avian Biology* **32**: 90–94.
- Metcalfe NB, Monaghan P. 2001.** Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution* **16**: 254–260.
- Moeller KT, Butler MW, DeNardo DF. 2013.** The effect of hydration state and energy balance on innate immunity of a desert reptile. *Frontiers in Zoology* **10**: 23.
- Mousseau TA, Fox CW. 1998.** The adaptive significance of maternal effects. *Trends in Ecology & Evolution* **13**: 403–407.
- Pap PL, Vágási CI, Czirják GA, Titilincu A, Pinteá A, Barta Z. 2009.** Carotenoids modulate the effect of coccidian infection on the condition and immune response in moulting house sparrows. *The Journal of Experimental Biology* **212**: 3228–3235.
- Roff DA. 1992.** *The evolution of life histories. Theory and analysis*. New York: Chapman & Hall.
- Romero LM, Reed JM. 2005.** Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **140**: 73–79.
- Romero LM, Romero RC. 2002.** Corticosterone responses in wild birds: the importance of rapid initial sampling. *Condor* **104**: 129–135.

- Saino N, Suffritti C, Martinelli R, Rubolini D, Møller AP. 2003.** Immune response covaries with corticosterone plasma levels under experimentally stressful conditions in nestling barn swallows (*Hirundo rustica*). *Behavioral Ecology* **14**: 318–325.
- Sims CG, Holberton RL. 2000.** Development of the corticosterone stress response in young Northern Mockingbirds (*Mimus polyglottos*). *General and Comparative Endocrinology* **119**: 193–201.
- Spencer KA, MacDougall-Shackleton SA. 2011.** Singing to impress: the importance of developmental stress. *Behavioral Ecology* **22**: 14–15.
- Stjernman M, Raberg L, Nilsson JA. 2008.** Long-term effects of nestling condition on blood parasite resistance in blue tits (*Cyanistes caeruleus*). *Canadian Journal of Zoology* **86**: 937–946.
- Von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H. 1999.** Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society B: Biological Sciences* **266**: 1–12.
- Walker BG, Wingfield JC, Boersma PD. 2005.** Age and food deprivation affects expression of the glucocorticosteroid stress response in Magellanic penguin (*Spheniscus magellanicus*) chicks. *Physiological and Biochemical Zoology* **78**: 78–89.
- Will AP, Suzuki Y, Elliott KH, Hatch SA, Watanuki Y, Kitaysky AS. 2014.** Feather corticosterone reveals developmental stress in seabirds. *The Journal of Experimental Biology* **217**: 2371–2376.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Results of ANCOVAs examining the effects of sex and feather corticosterone levels on various physiological and morphological variables. η^2 denotes effect sizes in the models.

Table S2. Source data.