Developmental conditions have intergenerational effects on corticosterone levels in a passerine

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ABSTRACT

The developmental environment can have powerful, canalizing effects that last throughout an animal’s life and even across generations. Intergenerational effects of early-life conditions may affect offspring phenotype through changes in the hypothalamic-pituitary-adrenal axis (HPA). However, such effects remain largely untested in altricial birds. Here, we tested the impact of maternal and paternal developmental conditions on offspring physiology and morphology in the zebra finch (Taeniopygia guttata). Specifically, we exposed one generation (F1) to elevated corticosterone (CORT) during development and quantified the impact on offspring (F2) phenotype. We predicted that intergenerational effects would be apparent through effects of parental developmental treatment on offspring body mass, growth, body condition, body composition, and CORT levels. We found an intergenerational impact on CORT levels, such that F2 birds reared by CORT-treated fathers had higher baseline CORT than F2 birds reared by control fathers. This result shows the potential for intergenerational effects on endocrine function, resulting from developmental conditions. We found no effect of parental treatment on F2 body mass, size, or body condition, but we found that the body mass and tarsus length for offspring and parent were correlated. Our study demonstrates the subtle effects of developmental conditions across generations and highlights the importance of distinguishing between maternal and paternal effects when studying intergenerational effects, especially for species with biparental care.

1. Introduction

Early developmental conditions can have lifelong consequences, and there is growing evidence that these effects can persist across generations (Champagne, 2008; Burton and Metcalfe, 2014). Such intergenerational effects enable heritable adaptations to arise without changes to the genome, allowing organisms to rapidly adapt to locally relevant environmental changes (Fuxjager et al., 2019; Danchin et al., 2011; Sentis et al., 2018; Broggi et al., 2016). Parents can be potential powerful drivers of intergenerational effects by adaptively programming their offspring for anticipated environmental conditions (Guerrero-Bosagna et al., 2018; Uller et al., 2013). For example, parents can alter the developmental outcomes of their offspring through changes in their behaviour or maternal yolk constituents they provide to the developing egg (Groothuis et al., 2005; Aplin et al., 2015; Banerjee et al., 2012; Mariette and Buchanan, 2016). Recent evidence suggests that intergenerational effects are common, but the number of species and systems that have been examined is small (Burton and Metcalfe, 2014), and the underlying mechanisms are poorly tested.

Glucocorticoid hormones represent one possible mechanism by which intergenerational effects can be mediated, either through direct transfer of hormones into the yolk or through effects on parental behaviour (Hayward and Wingfield, 2004; Crossin et al., 2012; Ouyang et al., 2013). Secretion of glucocorticoid steroid hormones is one the primary components of the hypothalamic-pituitary-adrenal (HPA)-axis (Sapolsky, 2002). The HPA-axis determines individual physiological response to environmental changes by integrating nervous system and endocrine responses (Selye, 1936; McEwen, 1998). The HPA-axis is activated to cope with a range of environmental changes, from predation to harsh environmental conditions, and to redirect resources away from nonessential functions to those relevant to immediate survival (Clinchy et al., 2013; Crino et al., 2020; Bize et al., 2010). The developmental environment can program HPA-axis function later in life (Schmidt et al., 2014; Buchanan et al., 2003; Spencer et al., 2009), but it is unclear to...
what extent these changes can persist across generations. Animals exposed to elevated levels of glucocorticoids during development often respond to perturbations with elevated baseline and stress-induced glucocorticoid secretion as adults (Honarmand et al., 2010; Hayward and Wingfield, 2004, but not always, e.g. Love and Williams, 2008, Crino et al., 2014a). In addition, there are cases where prenatal exposure to elevated CORT (Coturnix japonica, Zimmer et al., 2013) and maltreatment during early development (Sula granti, Grace and Anderson, 2018) have resulted in depressed glucocorticoid levels later in life. Exposure to glucocorticoids during development can affect many other traits that influence fledging survival (e.g. growth, Hayward and Wingfield, 2004, and begging rate, Loiseau et al., 2008), and it has been shown to decrease growth and final adult size and body condition in zebra finches (Taeniopygia guttata, Kraft et al., 2019). Although the relationships between glucocorticoids and fitness are highly variable and dependent on the life history of the species (Schoenle et al., 2021), baseline and peak glucocorticoids can affect both survival and reproductive success in wild animals (Blas et al., 2007, Ethan Pride, 2005, reviewed in Bonier et al., 2009, Schoech et al., 2011). For these reasons, long-term changes to the HPA-axis may affect fitness, directly through changes in glucocorticoid secretion or indirectly through associated changes in growth or body size, ultimately impacting on survival.

The impact of developmental environment on body condition, metabolism, and HPA-axis function and subsequent epigenetic modifications have been best studied in mammals (reviewed in Meany, 2001, Weaver et al., 2004, Gluckman et al., 2009). Studies in precocial bird species also demonstrate that the developmental conditions experienced by parents can affect the HPA-axis function of their offspring in the subsequent generation (Goerlich et al., 2012; Ericsson et al., 2016; Zimmer et al., 2017). For example, Japanese quail females that have been exposed to elevated corticosterone (CORT; the predominant avian glucocorticoid) during development sire offspring with a modified CORT level response to capture-handling-stress (Zimmer et al., 2017). There are clear differences in the developmental modes of altricial and precocial bird species (Starch and Ricklefs, 1998; Martin, 1987), and this is likely to affect how intergenerational effects can be mediated. For example, the HPA-axis and many essential motor skills are developed earlier (even before hatching) in precocial birds compared to altricial birds (Wada, 2008; Starck and Ricklefs, 1998). Compared to altricial bird species, precocial species have predominantly maternal care and more parental care is provided during the pre-hatching period. In contrast, altricial bird species tend to have more extensive biparental care, and altricial nestlings are more dependent on post-hatching parental care for survival. The developmental environment may therefore be more strongly influenced by both parents in altricial compared to precocial species. In altricial birds, the brood size in which the mother was raised has been shown to affect body size and body condition in nestlings and sub-adult birds (Alonso-Alvarez et al., 2007; Naguib and Gil, 2005). In addition, the effects are sometimes sex-specific, as shown in zebra finches where female offspring were specially affected by the brood size in which their mother was raised (Naguib and Gil, 2005). However, no study on altricial birds has tested if developmental conditions in one generation can affect offspring CORT secretion across generations, and very few have examined the potential intergenerational effects of developmental conditions on avian morphology. Furthermore, the possible interaction between maternal and paternal effects on offspring morphology and HPA-axis function remains to be tested.

Here, we tested whether exposure to elevated CORT during development in one generation (F1) affects morphology and HPA-axis function in the subsequent generation (F2). We exposed a generation of zebra finch nestlings (F1) to elevated levels of CORT. After they reached adulthood, we allowed them to breed and measured their offspring’s (F2) baseline and peak CORT levels and body mass, size, and condition. We predicted that F1 CORT treatment would affect F2 growth and morphology, and that maternal and paternal effects would interact such that parental treatments would have additive or synergistic effects. In line with previous studies on post-natal stress in precocial bird species (Ericson et al., 2016), we predicted that CORT-treated parents (F1) would produce offspring (F2) with higher baseline and/or peak CORT levels, compared to control parents (F1). We also predicted that parental treatment would have a negative effect on offspring body condition and growth, as this is the effect that we have observed within a generation of elevated CORT during development (Kraft et al., 2019). If CORT treatment in F1 parents affects offspring HPA-axis function and morphology in passerines, it would indicate that developmental conditions can have long-term effects on a range of traits important for survival and fitness in altricial birds.

2. Methods

2.1. F1 treatment

This study was conducted at Deakin University, Geelong, Australia. In 2015, we dosed nestling zebra finches (F1) with a CORT or control treatment (methods as per Kraft et al., 2019). Within each clutch, the first nesting was randomly assigned to the CORT or control treatment group, and then treatments were alternated for each subsequent nesting. Treatment lasted from 5 to 18 days post-hatching. CORT-treated nestlings were fed two oral boluses twice per day (approximately 5 h ± 1 h apart) consisting of a CORT solution (0.25 mg/ml, Sigma Aldrich) dissolved in peanut oil, for total daily dose of 12.5 μg of CORT (see “high-dose nestlings” in Kraft et al., 2019). Control nestlings were fed the peanut oil vehicle only on an identical feeding schedule (Fig. 1). For method details, and the resulting effects on F1 physiology, see the supplementary materials and Kraft et al. (2019).

2.2. Breeding experiment

We conducted a breeding experiment from November 2017 to December 2018 with 76 F1 birds (Fig. 1). Breeding birds were housed in two rooms (3 × 2 × 2 m; Room one: NControl = 9 females, 10 males; NCORT = 9 females, 10 males; Room two: NControl = 9 females, 10 males; NCORT = 9 females, 10 males) and were given free choice of mates. Halfway through the breeding experiment (April 2018), we swapped all females between the two rooms to allow all F1 birds access to all potential partners. A total of 164 F2 nestlings from 45 nests were produced over the course of the experiment (F1 treatment groups: N = 21 ControlFather/ControlMother; N = 21 ControlFather/CORTMother; N = 22 CORTFather/ControlMother; N = 21 CORTFather/CORTMother). All birds were housed at a 14:10 light/dark cycle at 20 °C (±1 °C) with 50% humidity, and were provided with ad libitum commercial seed diet, water, cuttlefish, grit, and cucumber. Breeding birds also received a daily provision of supplemental food consisting of hardboiled egg and spinach. Breeding birds were provided with 24 wooden nest boxes per room and nesting material (shredded burlap and dry grass) as needed. At age 70 days post-hatching (±5 days), F2 birds were moved to sex separated cages (100 × 50 × 50 cm) until blood samples were collected.

2.3. F2 morphometric and body composition measurement collection

When the F2 nestlings were 10 and 20 days post-hatching, we measured tarsus length to the nearest 0.01 mm using digital callipers and weighed nestlings to the nearest 0.01 g. We collected body composition data using a quantitative magnetic resonance (QMR) body composition analyzer (EchoMRI-B, Echo Medical Systems, Houston, USA). We quantified total fat, wet lean mass (non-structural tissues such as muscle), total water (free water and water contained in lean mass), and free water (water not bound to tissues, e.g. inside bladder). The sex of the nestlings was determined when sexually dimorphic traits became apparent (after 60 days of age, Zann, 1996). Once the birds reached adulthood, we captured all individuals and blood sampled them
At this time, we also weighed and measured the birds as above. To estimate body fat reserves in adult birds, we calculated the scaled mass index values (see supplementary materials). The scaled mass index (hereafter SMI) is a condition index that accounts for errors associated with the dependency between length and body mass measurements (Peig and Green, 2010; Peig and Green, 2009). We have previously shown that this is an effective way of estimating body fat in the same cohort of adult zebra finches used in this experiment (Kraft et al., 2019).

2.4. Blood sample collection

Blood samples were collected to measure CORT levels. The day before we collected blood samples, we housed birds in cages (50 × 50 × 50 cm) overnight and collected blood between 0800 and 1100 h the following day. We collected an initial sample within three minutes of disturbing birds (baseline) by puncturing the alar vein with a 26.5 g needle and collecting 50–75 μl of blood with heparinized micro-hematocrit tubes. We then held birds in opaque cloth bags until a second (peak) blood sample was collected 15 min (±1 min) after initial disturbance. We collected blood samples from 82 F2 birds (N = 38 females; N = 41 males) between 87 and 117 days post-hatching (N = 10 to 11 birds per treatment group per sex, except N = 8 females with two control parents).

2.5. Corticosterone assays

We quantified CORT levels from raw plasma with Enzyme Immunoassay (EIA) kits (Cat No. ADI 900-097, Enzo Life Sciences). An external standard of 500 pg/ml was run on every plate and used to calculate inter-plate variation. All samples and standards were run in triplicate at half volume to the protocol standard (Crino et al., 2017). Plates were read on a Varioskan LUX microplate reader at 405 nm corrected at 580 nm. Levels of CORT were determined from a seven-point standard curve ranging from 20,000 to 3.91 pg/ml. Intra- and inter-plate variation was 7.19% and 11.29% respectively. Samples that were below the detection limit (N = 14 out of 82 baseline samples) were assigned the value of 0.02 ng/ml.

2.6. Statistics

All statistical analyses were performed using R version 3.5.2 and the lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), and MuMIn (Barton, 2009) packages. We examined residual plots and skewness-kurtosis plots when choosing the distribution to use for each model. We used linear mixed models to analyse the influence of parental treatment groups on F2 body mass, growth, body condition (SMI value), tarsus, and relative fat mass and lean mass. We assessed growth by
calculating the difference in mass between ages 10 and 20 days post-hatching, and we used this variable to test for effects on growth. We used generalized linear mixed models with gamma distribution (log link function) to analyse the impact of parental treatment on F2 CORT levels. We included F2 sex, brood size, and the interaction between brood size and sex in full models with maternal and paternal treatment. Brood size and the time to collect blood (only CORT models) were used as covariates. We included the three-way interactions between parental treatments (paternal and maternal treatment) and sex, as well as parental treatments and brood size, to test if parental treatments interact with F2 developmental conditions or sex (all models used are reported in Supplementary materials Table S1). Parental pair ID was included as a random factor to account for relatedness effects in the F2 generation. We created several reduced models with only biologically plausible interactions. We performed model selection using the model.sel function from the MuMn package (the full model selection is reported in the Supplementary materials Table S2). When the top model was the null model, we only reported this model in the results. When there were several similarly weighted candidate models, we used 4 delta AICc (corrected Akaike information criterion) as the cut-off point for the top models (Symonds and Moussalli, 2011). We then used the model.avg. function from the MuMn package to average the top models. For the dependent variables where we used model averaging, we reported the full results and conditional model average results. In full model averaging, it is assumed that all variables are included in every model, but in some models the corresponding coefficient is set to zero. In conditional model average (sometimes “subset” model average), only the models that contain the factor in question are considered when producing parameter and error estimates for that factor. There is a risk of conditional average biasing results away from zero (Bartoń, 2009), which is why we included both modes of model averaging.

Finally, we tested if parental and offspring morphology were related by correlating F1 (Kraft et al., 2019) and F2 morphology. We used Spearman's signed rank correlation to correlate relative fat and lean mass, body mass, and tarsus length from mothers and fathers with the same data from offspring at age 20 days post-hatching. We also correlated the SMI value, body mass, and tarsus length in adulthood in the same fashion. As we did not have a complete data set of parental data, the resulting sample sizes were Noffspring = 76 offspring, 7 fathers, 11 mothers; Nadult = 103 offspring, 20 fathers, 23 mothers. Because we correlated offspring morphological values with both maternal and paternal values and separated the data according to parental treatments, we corrected for multiple comparisons using the p.adjust function and false discovery rate for four comparisons.

### 3. Results

When testing the influence of parental treatment on offspring baseline CORT levels, there were four models that performed similarly to the top model (Table 1). We found that F2 birds from CORT-treated fathers had higher baseline CORT when we used conditional and full model averaging (Fig. 2A, Paternal treatment: estimate = 0.819, standard error = 0.314, z = 2.569, P = 0.010). Within the F2 generation there was a sex difference, as F2 males had higher baseline CORT than F2 females (Fig. 2B, Sex: estimate = 0.840, standard error = 0.317, z = 2.611, P = 0.009). For peak CORT, there were five models with similar performance (Table 1). When we performed full model averaging there was no effect of sex, but when we used conditional model averaging males had higher peak CORT than females (Fig. 2C, Sex: estimate = 0.311, standard error = 0.150, z = 2.044, P = 0.041). Brood size was included in several top models but did not affect peak or baseline CORT in any model averaging results. See the Supplementary materials Table S3 for the full summary of the model average. For all other tested variables, the most parsimonious top model was the intercept-only model (Table 1).

In total, we quantified body composition of 145 10-day old F2 nestlings, 131 20-day old F2 nestlings, and 102 adult F2 birds. We found no effect of parental treatment on F2 mass, tarsus, relative fat or lean mass, or SMI value, at any of the ages we tested (Fig. 3), as the null model performed the best in model comparisons for these variables. Also, we found no evidence of intergenerational effects on F2 growth from ages 10 to 20 days, suggesting that F2 growth and body composition were determined either by genetic factors and/or recent food availability, rather than parental CORT treatment. See the Supplementary materials Table S2 for the complete model selection results.

In line with the conclusion that genes and the current environment are important for determining F2 morphology, we found significant influence of parental morphology on offspring morphology. For parents and offspring at age 20 days post-hatching, heavier CORT-treated mothers fledged heavier offspring (Fig. 4A, Spearman correlation, ρ = 0.551, P = 0.002). In contrast, heavier control fathers fledged lighter offspring (Fig. 4B, Spearman correlation, ρ = −0.643, P < 0.001). In adulthood, control mothers with longer tarsi had offspring with longer

### Table 1

The top models from AICc model selection identifying the variables best describing variation in for all F2 traits tested. For baseline and peak corticosterone, we used model averaging of reported models to quantify which variables best described trait variation. All models for baseline and peak CORT also included sampling time.

| F2 trait            | Age | Fixed factors                  | df  | Log-likelihood | AICc | ΔAICc | Weight |
|---------------------|-----|--------------------------------|-----|----------------|------|-------|--------|
| Mass 10             | Null model |                            | 3   | −211.15        | 428.50 | 0.00  | 0.27   |
| Rel. fat mass 10     | Null model |                            | 3   | 276.97         | −547.70 | 0.00  | 0.94   |
| Rel. lean mass 10    | Null model |                            | 3   | 218.57         | −430.90 | 0.00  | 0.94   |
| Tarsus 10            | Null model |                            | 3   | −191.83        | 389.90  | 0.00  | 0.29   |
| Mass 20              | Null model |                            | 3   | −147.40        | 301.90  | 0.00  | 0.23   |
| Rel. fat mass 20     | Null model |                            | 3   | 277.05         | −547.90 | 0.00  | 0.98   |
| Rel. lean mass 20    | Null model |                            | 3   | 261.45         | −516.70 | 0.00  | 0.98   |
| Tarsus 20            | Null model |                            | 3   | −94.62         | 195.50  | 0.00  | 0.62   |
| Growth 10 to 20      | Null model |                            | 3   | −180.91        | 368.00  | 0.00  | 0.22   |
| Baseline CORT Adult  | Pat. Treatment + Sex |                    | 6   | −44.66         | 102.40  | 0.00  | 0.36   |
| Baseline CORT Adult  | Pat. Treatment + Brood size + Sex |                        | 7   | −44.30         | 104.10  | 1.68  | 0.16   |
| Baseline CORT Adult  | Pat. treatment + Brood size |                  | 7   | −44.33         | 104.20  | 1.73  | 0.15   |
| Baseline CORT Adult  | Mat. Treatment + Pat. Treatment + Sex |                       | 7   | −44.65         | 104.80  | 2.37  | 0.11   |
| Peak CORT Adult 6    | Brood size + Sex |                        | 6   | −240.86        | 494.80  | 0.00  | 0.27   |
| Peak CORT Adult 5    | Sex |                        | 5   | −242.89        | 496.60  | 1.73  | 0.11   |
| Peak CORT Adult 5    | Brood size |                        | 5   | −243.12        | 497.00  | 2.19  | 0.09   |
| Peak CORT Adult 7    | Mat. Treatment + Brood size + Sex |                     | 7   | −240.86        | 497.20  | 2.39  | 0.08   |
| Peak CORT Adult 4    | Pat. Treatment + Brood size + Sex |                    | 7   | −240.86        | 497.20  | 2.39  | 0.08   |
| Peak CORT Adult 4    | Null model |                        | 4   | −244.51        | 497.50  | 2.70  | 0.07   |
| Mass Adult 3         | Null model |                        | 3   | −125.55        | 257.50  | 0.00  | 0.43   |
| SMI Adult 3          | Null model |                        | 3   | −103.96        | 214.20  | 0.00  | 0.29   |
| Tarsus Adult 3       | Null model |                        | 3   | −69.30         | 144.90  | 0.00  | 0.62   |
Baseline CORT (ng/ml) | Peak CORT (ng/ml) 
--- | --- 
Control | 10 | 0 
Patal treatment | 20 | 2 

**Fig. 2.** Boxplots of A) F2 baseline CORT levels with paternal treatment B) F2 baseline CORT levels with F2 sex and C) F2 peak CORT levels with F2 sex. The effect of sex on peak CORT (C) was significant when using conditioned (but not full) model averaging. N = 82 birds, 35 parental pairs. *P < 0.05.

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4. Discussion

Previous work has indicated that intergenerational effects are common across animal taxa (Burton and Metcalfe, 2014). We show for the first time in an altricial bird species that elevated levels of CORT during development in the parental generation affected CORT levels in their offspring. Specifically, we found that F2 offspring reared by CORT-treated fathers had higher baseline CORT levels than F2 offspring reared by control fathers, regardless of maternal treatment. We also found that males had higher baseline CORT than females. There was a possible effect of sex on peak CORT as well, but this effect was only significant when using conditional model averaging and may not be biologically relevant. We demonstrate that some traits are more strongly influenced by intergenerational effects than others; paternal CORT treatment affected F2 CORT levels, but did not affect F2 morphology.

In contrast to our prediction, we found no influence of the developmental environment of parents on any measure of offspring growth or morphology. Variation in phenotypic traits is determined by genetic control, environmental influences and the interaction between these effects (Via and Lande, 1985). Morphology may have been less affected by parental CORT treatment because morphological traits have relatively high heritability in passerines (e.g. body mass Woodgate et al., 2013, and tarsus length Riddington and Gosler, 1995), and the birds had ad libitum access to food. We found that parent and offspring tarsus length and body mass were correlated (Fig. 4). However, the morphological traits were only correlated for some ages and treatments, possibly because the sample sizes were limited for this analysis. These relationships suggest that offspring morphology is more dependent on genetic control and current environmental conditions than parental developmental environment.

The lack of a clear effect of parental treatment on offspring morphology is in marked contrast with two previous studies on zebra finches, which documented sex-specific intergenerational effects of the brood size the mother was raised in on the body size (Naguib and Gil, 2005) and body condition (Alonso-Alvarez et al., 2007) of their offspring. The reasons for these differences are not clear and may in part be due to the difference in the experimental treatment, the timing of measurements of the offspring, or the strain of birds used. While the sample size Naguib and Gil (2005) used was similar to ours and they used wild-derived birds, they tested for effects on juvenile offspring rather than nestlings or adults. Alonso-Alvarez et al. (2007) observed effects of parental brood size on offspring body condition (SMI value) in nestlings, but their sample size was almost twice the size of ours and they used domesticated zebra finches (Alonso-Alvarez et al., 2006). Also, we tested for effects of parental treatment on QMR-derived relative fat mass in nestlings as an estimate of body condition, and relative fat mass is not closely correlated to the most commonly used condition indices innestling zebra finches (Kraft et al., 2019). Furthermore, neither of these studies tested for effects in adulthood, which is when we observed effects of parental treatment.

F2 males had higher baseline CORT than females. We also found a possible effect of sex on peak CORT, with males having higher peak CORT levels than females. These sex differences in CORT levels had no apparent link to treatment and so may represent a genuine example of variation in HPA axis function between the sexes. Breeding males have been found to have higher CORT levels than breeding females in several avian species (Wingfield et al., 1995). Also, male birds have higher baseline and peak CORT than females across species and seasons, as shown in a recent meta-analysis (Casagrande et al., 2018). Previous research in zebra finches has found mixed results as to differences baseline CORT between the sexes, with some finding that males have
higher CORT levels than females (Wada et al., 2008, but some found no sex difference, Jimeno et al., 2017). One study found that baseline CORT levels were higher in females, but the sample sizes in this study were small (N = 4 or 5 per treatment group, Khan and Robert, 2013). Also, several studies have found sex-specific effects of environmental perturbations during development and in adulthood (Schmidt et al., 2012; Jimeno et al., 2017). Hence, our finding that males have higher baseline CORT levels is in line with much of the research to date.

In this study, paternal developmental CORT treatment resulted in elevated baseline CORT in their offspring (Fig. 2.A), and this could be mediated through altered paternal behaviour. As we did not measure parental behaviour, we can only speculate on how variation in parental care could mediate the effects observed on F2 CORT levels. Zebra finches exhibit biparental care, with both parents contributing equally to incubation and nestling provisioning (Gilby et al., 2011). Males can vary in the amount of parental care they provide (e.g. provisioning, Gilby et al., 2011, and incubating, Zann and Rossetto, 1991) based on the clutch size, offspring quality, and certainty of paternity (Dixon et al., 1994; Mock et al., 2005; Michael et al., 2007). Previous research has shown that early-life CORT exposure can affect foraging behaviour, dominance behaviour, and social interactions between offspring and parent in zebra finches (Crino et al., 2014b; Spencer and Verhulst, 2007; Boogert et al., 2014). Such differences in behaviour may have affected parental provisioning, which could influence offspring CORT levels as early-life nutritional conditions can affect CORT levels post-nutritional independence in zebra finches (Kriengwatana et al., 2014). Paternal treatment may have affected parental synchronicity and pair bonding in the F1 birds, and this is an important aspect of parental care (Mariette and Griffith, 2012, Ouyang et al., 2014, Prior and Soma, 2015, Crino et al., 2014). Great tit pairs (Parus major) that start a breeding season with more similar baseline CORT levels are more likely to remain paired across years, and pairs that remained together became more similar in their hormone profiles (Ouyang et al., 2014). In zebra finches, juveniles that have been exposed to a CORT-treatment during development exhibit altered social foraging behaviour (Farine et al., 2015), and this could potentially influence the parenting behaviour of the F1 fathers even after their offspring have fledged. The effect of paternal treatment may reflect differences in parenting success due to CORT-mediated pair bonding, or differences in the care provided by fathers. Future studies should parse out these effects with brood size manipulation and/or cross-fostering treatments, as well as measuring parental behaviour.

While differences in male parenting behaviour is a possible mechanism for the observed effects on F2 baseline CORT, there are many ways males could potentially affect the development of their offspring. For example, parental CORT treatment may have affected offspring CORT levels through epigenetic inheritance (Curley et al., 2011). Alternatively, fathers can directly influence the development of their offspring through ejaculate quality (Evans et al., 2019; White et al., 2008; Preston et al., 2015). Paternal effects could also be a result of males indirectly affecting offspring development via the mother. In birds, mothers transfer hormones as well as nutrients and antioxidants to the egg, but fathers have less influence over variation in egg quality (Grootveld et al., 2008). Elevated maternal CORT during ovulation can increase yolk CORT, which in turn may affect offspring CORT secretion and phenotype (Hayward and Wingfield, 2004; Sockman and Schwabl, 2001). Fathers could indirectly affect offspring through maternal investment, as female

![Fig. 3. A) Body mass, B) tarsus, C) relative fat mass and D) lean mass, and E) SMI value, across life-history stages in F2 birds. Black lines represent the mean, and coloured points represents individuals from the different treatment groups. Bars represent mean ± standard error. Green = control father, control mother; yellow = control father, CORT-treated mother; orange = CORT-treated father, control mother; red = CORT-treated father, CORT-treated mother. N_{10 days} = 145 nestlings, 37 parental pairs; N_{20 days} = 131 nestlings, 37 parental pairs, N_{adults} = 102 birds, 37 parental pairs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)
birds generally invest more in offspring when paired with an attractive partner (Horváthová et al., 2012). We did not measure maternal investment in this study, but there is some evidence from previous studies that maternal investment is affected by male quality in zebra finches. For example, zebra finch nestlings reared by CORT-treated fathers had improved body condition, likely because females increased their parental effort when paired with a CORT-treated male (Crino et al., 2014). We did not find any effect on F2 body condition, and higher baseline CORT levels has previously been shown to be negatively associated with higher body condition in zebra finches within a generation (Crino et al., 2018). Although we did not find effects of parental treatment on offspring morphology, it is still possible for parental CORT treatment to affect offspring fitness. For example, parental treatment could cause changes to offspring behaviour, and the potential resulting consequences for offspring fitness can vary depending on the environment experienced by the offspring (reviewed in Sheriff et al., 2017).

Some authors have hypothesised that parents can adaptively program their offspring to survive in a challenging environment that is similar to their own (Zimmer et al., 2017; Mosseau and Fox, 1998). This requires accurate anticipation of environmental circumstances to allow offspring to be matched appropriately. It is possible that the observed intergenerational effects on offspring CORT reflect this type of parental programming. Whether such programming is beneficial to fitness depends on the costs and benefits of elevated baseline CORT, as well as environmental predictability. Several avian studies have reported negative fitness consequences of high levels of baseline CORT, but other studies have found no relationship or even a positive relationship (reviewed in Bonier et al., 2009). Furthermore, a recent meta-analysis showed that there is an overall negative relationship between reproductive success and elevated baseline CORT in bird species (Schoenle et al., 2021). In male zebra finches, high baseline CORT is associated with lower courtship success (Wada et al., 2008). In wild zebra finches, increased levels of baseline and peak CORT have been linked to lower body condition, which is in turn linked to lower testosterone levels in males (Crino et al., 2018). This suggests that there is a condition-mediated trade-off between HPA-axis and hypothalamic-pituitary-gonadal-axis activity (Wingfield and Sapolsky, 2003). Zebra finch females that have experimentally increased CORT levels lay larger clutches with greater embryonic mortality and smaller nestlings (Khan et al., 2016). Our results show that there are carry-over effects of parental developmental CORT treatment, and previous findings indicate that these effects could have negative long-term consequences for offspring fitness. To date, there is mixed evidence as to the extent to which parental effects are adaptive (Uller et al., 2013), and this may to

Fig. 4. The correlation between F1 parental and F2 offspring values for A, B) body mass at age 20 days, C) tarsus length in adulthood, D) body mass in adulthood. N20 days = 76 offspring, 7 fathers; Nadult = 103 offspring, 20 fathers, 23 mothers. F1 treatment group is shown by colour (green = control, red = CORT). *P < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
seasonal glucocorticoid changes depend on reproductive investment? A comparative approach in birds. Integr. Comp. Biol. 58, 739–750.
Champagne, F.A., 2008. Epigenetic mechanisms and the transgenerational effects of maternal care. Front. Neuroendocrinol. 29, 386–397.
Clinchy, M., Sheriff, M.J., Zanette, L.Y., 2013. Predator-induced stress and the ecology of fear. Funct. Ecol. 27, 56–65.
Crino, O.L., Prather, C.T., Driscoll, S.C., Good, J.M., Breuner, C.W., 2014. Developmental stress increases reproductive success in male zebra finches. Proc. Biol. Sci. 281, 1–8.
Crino, O.L., Driscoll, S.C., Breuner, C.W., 2014a. Corticosterone exposure during development has sustained but not lifelong effects on body size and total and free corticosterone responses in the zebra finch. Gen. Comp. Endocrinol. 196, 121–129.
Crino, O.L., Driscoll, S.C., Ton, R., Breuner, C.W., 2014b. Corticosterone exposure during development improves performance on a novel foraging task in zebra finches. Anim. Behav. 91, 27–32.
Crino, O.L., Buchanan, K.L., Tromp, L., Mainwaring, M.C., Griffith, S.F., 2017. Stress reactivity, condition, and foraging behavior in zebra finches: effects on boldness, exploration, and sociality. Gen. Comp. Endocrinol. 244, 101–107.
Crino, O.L., Jensen, S.M., Buchanan, K.L., Griffith, S.F., 2018. Evidence for condition mediated trade-offs between the HPA- and HPG-axes in the wild zebra finch. Gen. Comp. Endocrinol. 259, 189–198.
Crino, O.L., Driscoll, S.C., Brandl, H.B., Buchanan, K.L., Griffith, S.F., 2020. Under the weather: Corticosterone levels in wild nestlings are associated with ambient temperature and wind. Gen. Comp. Endocrinol. 285, 113247.
Croix, G.T., Trathan, P.N., Phillips, R.A., Gorman, K.B., Dawson, A., Sakamoto, K.Q., Williams, T.D., 2012. Corticosterone predicts foraging behavior and parental care in macaronsi penguins. Am. Nat. 180, E21–E41.
Curley, J.P., Mashoudh, R., Champagne, F.A., 2011. Epigenetics and the origins of paternal effects. Horm. Behav. 59, 306–314.
Danchin, E., Charmantier, A., Champagne, F.A., Mesoudi, A., Pojol, B., Blanchet, S., 2011. Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. Nat. Rev. Genet. 12, 475–486.
Dixon, A., Ross, D., O’Malley, S.L.C., Burke, T., 1994. Paternal investment inversely related to degree of extra-pair paternity in the red-bunting. Nature 371, 678–700.
Eisen, H., Heinkranz, R., Belc, B., Jud, A., Fassina, R., Menken, J., 2006. An experimental manipulation of life-history trajectories and resistance to oxidative stress. Evolution 60, 1913–1924.
Alonso-Alvarez, C., Bertrand, S., Dorion, F.C., 2007. Sex-specific transgenerational effects of early development conditions in a passerine. J. Biol. Linn. Soc. 91, 469–474.
Aplin, L.M., Farine, D.R., Morand-Ferron, J., Cockburn, A., Thornton, A., Sheldon, B.C., 2015. Experimentally induced innovations lead to persistent culture via conformity in wild birds. Nature 518, 538–541.
Barnerjee, S.B., Arterbery, A.S., Fergus, D.J., Adkins-Regan, E., 2012. Deprivation of maternal care has long-lasting consequences for the hypothalamic—pituitary—adrenal axis of zebra finches. Proc. Biol. Sci. 279, 779–786.
Barto ´t, K., 2009. MuMin: Multi-Model Inference. R Package.
Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.
Bize, P., Stocker, A., Jenni-Eiermann, S., Gasparini, J., Roulin, A., 2010. Sudden weather deterioration but not brood size affects baseline corticosterone levels in nestling alpine swifts. Horm. Behav. 58, 591–598.
Blas, I., Bertolotti, G.R., Yella, J.S., Iano, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. PNAS 104, 8880–8884.
Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009. Do baseline glucocorticoid predict fitness? TREE 24, 634–642.
Boogert, N.J., Farine, D.R., Spencer, K.A., 2014. Developmental stress predicts social network position. Biol. Lett. 10, 20140561.
Brogly, J., Sorgier, R.C., Figuerola, J., 2016. Transgenerational effects enhance specific immune response in a wild passerine. PeerJ 4, 1–18.
Buchanan, K.L., Spencer, K.A., Goldsmith, A.R., Catchpole, C.K., 2003. Song as an honest signal of past developmental stress in the European starling (Sturnus vulgaris). Proc. Biol. Sci. 270, 1149–1156.
Burton, T., Metcalfe, N.B., 2014. Can environmental conditions experienced in early life influence future generations? Proc. Biol. Sci. 281, 20140311.
Casagrande, S., Zsolt Garamszegi, L., Goymann, W., Donald, J., Francis, C.D., Fuxjager, M.J., Huisk, J.F., Johnson, M.A., Kircher, B., Knapp, R., Martin, L.B., Miller, E.T., Schonle, L.A., Vitousek, M.N., Williams, T.D., Hau, M., 2018. Do some extent be due to a lack of testing under ecologically relevant conditions. The fitness consequences of adaptive programming are dependent on the conditions experienced by the parent as well as the offspring (Mousseau and Fox, 1998). In our experiment, both parents and offspring were kept under matched ad libitum food conditions, meaning that parents and offspring should have experienced similar environmental conditions. Still, these conditions may not have been particularly demanding compared to the natural environment. To determine the adaptive nature of these intergenerational effects, future studies need to test parental effects across matched and mismatched conditions in ecologically relevant settings, and across life-history stages in wild animals.
In conclusion, we found effects of parental developmental treatment on baseline CORT secretion in F2 offspring but no effect on F2 growth, condition, or adult morphology. These results suggest that endocrine functions may be more susceptible to intergenerational effects compared to morphological traits in captive zebra finches. Our data also suggest that fathers are more important in mediating this effect than mothers, and this raises questions about the possible underlying mechanisms. Future studies should quantify how intergenerational effects are mediated by both maternal and paternal effects, the possible mechanisms, and the relevance of such effects in wild populations.

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