Individual variation in baseline and stress-induced corticosterone and prolactin levels predicts parental effort by nesting mourning doves

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Individual variation in baseline and stress-induced corticosterone and prolactin levels predicts parental effort by nesting mourning doves

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A B S T R A C T

Endocrine systems have an important mechanistic role in structuring life-history trade-offs. During breeding, individual variation in prolactin (PRL) and corticosterone (CORT) levels affects behavioral and physiological processes that drive trade-offs between reproduction and self-maintenance. We examined patterns in baseline (BL) and stress induced (SI; level following a standard capture-restraint protocol) levels of PRL and CORT for breeding mourning doves (Zenaida macroura). We determined whether the relationship of adult condition and parental effort to hormone levels in wild birds was consistent with life-history predictions. Both BL PRL and BL CORT level in adults were positively related to nestling weight at early nestling ages, consistent with the prediction of a positive relationship of hormone levels to current parental effort of adults and associated increased energy demand. Results are consistent with the two hormones acting together at baseline levels to limit negative effects of CORT on reproduction while maintaining beneficial effects such as increased foraging for nestling feeding. Our data did not support predictions that SI responses would vary in response to nestling or adult condition. The magnitude of CORT response in the parents to our capture-restraint protocol was negatively correlated with subsequent parental effort. Average nestling weights for adults with the highest SI CORT response were on average 10–15% lighter than expected for their age in follow-up visits after the stress event. Our results demonstrated a relationship between individual hormone levels and within population variation in parental effort and suggested that hormonal control plays an important role in structuring reproductive decisions for mourning doves.

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Introduction

The central theme of the study of life-history evolution has been to elucidate trade-offs among different components of fitness and to determine the environmental factors that influence these trade-offs (Stearns, 1992). An important component of life-history study has been focused on understanding the physiological mechanisms that underlie trade-offs (Zera and Harshman, 2001; Ricklefs and Wikelski, 2002). Within this context, endocrine systems are of special interest because of the importance of hormones in orchestrating behavioral and physiological shifts between life-history stages and in structuring individual variation in investment (e.g., survival versus reproduction) within stages (Ketterson and Nolan, 1992; Finch and Rose, 1995).

Prolactin (PRL) and corticosterone (CORT) have been shown to have major pleiotropic functions in vertebrate physiology that include actions related to reproductive effort (Bole-Feysoy et al., 1998; Sapolsky et al., 2000). Therefore, they are ideal hormones to look at in the context of structuring life-history trade-offs. Both baseline (BL) and stress-induced (SI; in response to acute stressors) levels of PRL and CORT have demonstrated central roles in behavior and physiology during avian reproduction (Silver and Cooper, 1983; Horseman and Buntin, 1995; Buntin, 1996; Wingfield et al., 1998; Romero, 2002; McEwen and Wingfield, 2003; Landys et al., 2006; Angelier and Chastel, 2009).

Although there is a strong body of evidence for understanding how PRL and CORT levels vary between species and among different periods within the life history of individuals (Horseman and Buntin, 1995; Romero, 2002), less attention has been given to individual variation within populations during a single life-history stage (Williams, 2008). During reproduction, individuals must balance investment in current and future reproduction (Williams, 1966) and are likely to vary their reproductive effort in response to their current state, environmental conditions, and future opportunities (McNamara and Houston, 1996). Endocrine systems have the potential to play an important role in orchestrating decisions to optimize life-history decisions by assimilating input regarding current conditions and adjusting physiology and behavior to optimize investment (Finch and Rose, 1995). In this case, we expect individuals to vary BL and SI levels of PRL and CORT in response to their current condition and in parallel with parental effort.

PRL plays an important role during reproduction for Columbiformes (i.e., doves and pigeons; Silver and Cooper, 1983; Horseman and Buntin,
by stimulating behavioral and physiological actions involved in the crop-milk feeding of nestlings. Crop milk consists of regurgitated cells of the crop epithelium that have been transformed into a high nutrient source of food for young (Davies, 1939). In mourning doves (Zenaida macroura), crop milk is primarily fed during the first half of the nestling period from hatch to 4–5 days of age, with the proportion of crop milk in feedings declining during the late nestling period (Mirarchi, 1993). In addition to crop-milk production, during reproduction PRL has been shown to positively affect regurgitation behavior, broodiness, and other parental behaviors (Wang and Buntin, 1999) and promote changes in the immune system (Barriga Ibars et al., 1997). In several bird species, PRL level has been shown to decline in response to acute stressors (reviewed in Chastel et al., 2005; Angelier and Chastel, 2009). The factors affecting and the consequences of the avian PRL stress response have received little study. PRL response to stress was suppressed in breeding black-legged kittiwakes (Rissa tridactyla) when compared to non-breeding birds (Chastel et al., 2005), and in older individuals in a population of snow petrels (Pagodroma nivea) where reproductive output increases with age (Angelier et al., 2007a), indicating that modulation of the PRL stress response may be important in maintaining parental effort in birds.

Traditionally, studies of CORT have focused on BL level as an indicator of negative effects of stress. However, the concept of allostatics has emerged as a framework for structuring hypotheses based on life-history theory and endocrine processes (McEwen and Wingfield, 2003; Landys et al., 2006). Allostatics is defined as changes in physiological state that promote the maintenance of stability (i.e., homeostasis) across changing environments and conditions. Allostatics is important in maintaining energy balance across different life-history stages and will vary among individuals with respect to their current condition and to the level of reproductive investment. Glucocorticoid concentration (McEwen and Wingfield, 2003; Landys et al., 2006) has been proposed to be central in allostatic processes by facilitating behavioral and physiological changes to match current energy demands of individuals.

Within this framework, predictions can be made about how CORT should vary among and within life-history stages for free-living birds. BL and SI CORT levels promote different physiological states and thus are predicted to have different effects on reproductive behavior (Landys et al., 2006). Avian species show a general increase in BL CORT levels during reproduction (Romero, 2002), indicating that increasing BL CORT level may promote behaviors that meet the increased energy demands across different life-history stages and will vary among individuals with respect to their current condition and to the level of reproductive investment. Glucocorticoid concentration (McEwen and Wingfield, 2003; Landys et al., 2006) has been proposed to be central in allostatic processes by facilitating behavioral and physiological changes to match current energy demands of individuals.

The potential for SI CORT secretion to affect the life-history trade-off between the immediate needs of an individual for survival and their investment in reproductive output has led to a focus on factors that cause individuals to modulate their stress response (Wingfield et al., 1998; Romero, 2002; Wingfield and Sapolsky, 2003). There is mixed evidence that birds suppress their CORT response to acute stressors during breeding (Romero, 2002; Wingfield and Sapolsky, 2003) or that different populations of the same species vary their response to reflect different conditions during breeding (Kitaysky et al., 2007; Shultz and Kitaysky, 2008). Differences among species in reproductive value of offspring predict differences in SI CORT responses (Bókony et al., 2009), indicating that life-history predictions may be important in driving variation in the response. An alternative approach to investigation is to examine individual variation in the stress response to determine if responses are modulated in response to life-history trade-offs (Lendvai et al., 2007; Williams, 2008).

Columbiformes have served as a model for laboratory studies of avian endocrine regulation (e.g., Riddle et al., 1933; Horsem and Buntin, 1995), making the taxon an ideal model for examining the relationship between endocrine function and life-history trade-offs within wild populations. Laboratory studies show that BL levels of both hormones are important in determining reproductive behavior (Horsem and Buntin, 1995; Koch et al., 2002). In addition, SI changes in response to acute stressors (i.e., increases in CORT secretion and decreases in PRL level) may help mediate the immediate costs of the stress event and have indirect effects on reproductive behavior by reducing investment in current reproductive effort (Wingfield et al., 1998; Chastel et al., 2005; Angelier and Chastel, 2009).

We examined whether BL and SI levels of PRL and CORT in mourning doves fit six predictions or hypotheses based on life-history theory and our current understanding of the function of both horomones during reproduction. When examining individual variation in BL level, we focused on two different components of investment: adult weight as a measure of available endogenous resources and nestling weight as a measure of parental effort in the current nesting attempt. The first prediction we made was that if hormone levels were driven by adult condition, structuring prioritization of adult maintenance over reproductive output, lighter adults would suppress BL PRL because less energy would be available to transfer to feeding young and would increase BL CORT level to meet the increased energy demands on the adult. Our second prediction was that alternatively, if CORT plays a primary role in promoting hyperphagia and weight gain as hypothesized by Koch et al. (2002, 2004), we would expect to see a positive relationship between CORT and adult weight. Our third and fourth predictions were that if reproduction was prioritized in hormone function, parental effort of adults at the same reproductive stage would be positively correlated with PRL, promoting greater feeding of young, and that CORT level would be elevated to meet the greater energy demand of higher feeding rates.

SI CORT levels are expected to lead to a different physiological state than those typical of BL levels by mechanisms such as binding to different receptors (Landys et al., 2006), promoting an “emergency life-history stage” where self-maintenance is prioritized over reproduction (Wingfield et al., 1998). Likewise, some evidence suggests that the magnitude of decrease in PRL in response to an acute stressor may be negatively related to current and subsequent investment in breeding (Chastel et al., 2005; Angelier et al., 2007c; Angelier and Chastel, 2009). Therefore, our fifth prediction was that the magnitude of SI responses of both hormones would be smallest when adults were heaviest and likely to maintain their reproductive effort in the face of greater stress and when nestlings were heavier and thus parental effort had been high. Finally, our sixth prediction was that the magnitude of SI responses would be inversely related to subsequent parental effort.

Methods

Data collection

Samples were collected as part of a larger study on reproductive ecology of mourning doves in central Iowa, USA, between May and August of 2005 and 2006. Nests were found by systematic searches of trees and shrubs at four sites characterized by a mix of trees and grassland that were located within a matrix of intense agricultural land-use (i.e., primarily corn and soybean row-crops). Clutch size for mourning doves is two eggs. Nests were monitored on a regular basis (~3–5 days between visits, every 1–2 days near the predicted hatch date to determine hatching date) until the young either fledged or the nest was destroyed by predation or weather events. Eggs were candled to determine their age ±1 day for all nests found during the
incubation period (Hanson and Kossack, 1963). Nestling age was determined based on observations of hatching and the hatch day was considered to be day 1.

We collected blood samples from adult females and adult males to measure plasma concentrations of PRL and CORT. Both adults contribute to the incubation and feeding of the young. Males generally take incubation and brooding duties during the day (∼1000 to ∼1800 h), while females are present on the nest from early evening to mid-morning (∼1800 to ∼1000 h the following day). Males were sampled between 1150 and 1600 h (X = 13:55) while females were sampled from 1820 to 2030 h (X = 19:11). Adults were trapped by flushing birds off their nest into a mist-net placed adjacent to the nest site. Birds were immediately removed from the net and a blood sample of 250–500 μl was collected from the brachial vein to measure BL hormone concentrations. A second sample was taken following a standard capture–handle–restraint protocol (Wingfield et al., 1992).

Birds were handled to take measurements after the first sample was collected, then birds were restrained by placing them securely in a cotton bag until 30 min had passed from the initial capture, at which time the second blood sample was collected. Samples were immediately placed in a microcentrifuge tube and placed in ice. Samples were centrifuged within 8 h of capture to separate plasma from cell, and plasma samples were stored in a −80 °C freezer until hormone levels were assayed. Measurements of nestling weight (±0.1 g) were taken at the time that adults were trapped and at follow-up visits to the nest site to check the nest's status. Measurements of adult weight (±0.1 g) and wing length (±0.5 mm) were taken. Samples were cross-sectional with only a single sample from an adult within a single nesting attempt, although in some cases both the male and the female were caught from the same nest. Only one bird was sampled more than once, during two nesting attempts that occurred more than 1 year apart.

For adult weight (AW), we calculated residual adult weight in grams when plotted versus wing chord length, a measure of size-independent weight (Johnson et al., 1985). When adults were relatively heavy compared to birds of a similar size, values were positive, while birds that were light for their size had negative values.

As a measure of parental effort, we calculated the residual weight for nestlings from a spline regression for nestling weight plotted versus nestling age because mean weight gain was non-linear with respect to age. This served as a measure of relative growth in weight and an indirect measure of parental effort where we assumed differences in weight gain were dependent on differences in parental feeding. We calculated the average value of the residual weight for both nestlings in a nest and used that as an index to residual nestling weight (NW).

We also wanted to examine the effect of adult hormonal stress responses to capture on parental effort following the capture. To measure this, we calculated how our measure of residual nestling weight, NW, changed from the time of adult capture to subsequent follow-up visits. We calculated the difference in NW on the date the adult was captured (time 1) and NW on the date the nest was last visited (time 2; nestlings age 7 to 11 days) as a measure of the change in nestling weight (ΔNW). Thus, if at time 1 nestlings within a nest averaged 2 g heavier than the mean for young at that age and if at time 2 nestlings within a nest averaged 3 g lighter than the average for that age, the value of ΔNW for that nest would be −5 g. This served as a measure of how investment in reproduction changed in response to the stress event.

Birds were captured under all appropriate federal and state permits, and all procedures were reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee.

Hormone assays

Concentrations of plasma CORT and PRL were determined by double antibody radioimmunoassay (RIA). Samples were run in triplicate whenever sufficient plasma was available. Separate assays were run for each hormone in the 2 years. Intra-assay variation for CORT assays was 11.1% and 10.3% for 2005 and 2006 samples, respectively, and for PRL assays was 7.0% and 9.4% for the 2 years. Assays were run 1 year apart and we did not have an accurate measure of inter-assay variability because replicate samples from the first set of assays were not reliable after being stored for this long. We controlled for assay/year in all analyses; however, we were unable to distinguish annual effects from assay effects in the analyses.

CORT concentrations were measured using a commercially available immunoassay kit (ImmunoChem™ Double Antibody Corticosterone 125I RIA Kit, Catalog No. 07-120103; MP Biomedicalals, LLC, Solon, Ohio USA). The kit has been previously verified for parallelism and recovery of exogenous CORT using mounting dove plasma samples (Washburn et al., 2002). We followed the protocol outlined by Washburn et al. (2002), except we diluted plasma samples in a 1:25 ratio rather than a 1:50 ratio to maximize precision in measurements for both low (BL) and high (SI) samples.

PRL was measured using an avian prolactin double antibody RIA following the protocol for iodination and PRL assay of Vleck et al. (2000). Chicken prolactin for iodination (AFP-4444B) and reference standards (AFP-10328B) as well as rabbit anti-chicken prolactin antibodies (AFP-151040789) were obtained from Dr. A. F. Parlow and the National Hormone and Peptide Program of the National Institute of Diabetes and Digestive and Kidney Diseases. Secondary antibodies were obtained from Antibodies, Inc. (catalog # 51-155-0005). We used 20-μl aliquots of plasma in the assay. The assay was verified for mounting doves for parallelism by serially diluting plasma samples ranging in volume from 80 to 5 μl and testing whether slopes differed between standards and diluted samples plotted against the percent binding (p = 0.79). BL hormone values were determined from the initial sample, while SI levels were calculated as the difference between the initial sample and the sample taken 30 min after the initial capture.

Statistical analyses

Our data sets were largely observational with the only manipulation being the capture-restraint protocol used to simulate stress. Thus, observations of hormone levels were not in response to randomly assigned treatments. For each specific prediction about a relationship of BL and SI hormone levels to different combinations of explanatory variables, we developed a set of alternative multiple regression models and compared them with Akaike’s information criterion (AIC). Model selection included all possible combinations of main effects and interaction described below. This statistic is a measure of model parsimony that combines the negative log-likelihood of the model with a penalty for the number of parameters in the model. Model selection using this information-theoretic philosophy is an appropriate and commonly used approach for observational studies (Burnham and Anderson, 1998).

After a best model was determined for each analysis, we examined each of the regression parameter estimates to assess their importance by computing confidence intervals and inferring that there was strong support for the importance of the parameter if the 95% confidence interval did not include 0. For each response variable, we present the covariates included in the best model, R² values for the model, and parameter estimates along with 95% confidence intervals.

We only included samples for BL measurements that were collected within 4 min of the time of initial capture to minimize effects of handling time (Romero and Reed, 2005). Even within this time frame, CORT levels can be elevated and therefore we included an effect of handling time in all analyses of baseline CORT levels. We corrected BL CORT level to a standard handling time of 1.5 min, the smallest handling time in our study, and below the time at which plasma CORT is expected to start significantly increasing in concentration after an initial stressor (Romero and Reed, 2005). In addition to...
the handling time effect, for all models we also included an effect for assay/year in all analyses (see Hormone assays).

To test predictions, we examined how parents’ hormone levels varied in response to adult weight and nestling weight (AW and NW). We included covariates for nesting age, AW, and NW. We included two-way interactions between age and AW and between age and NW because the relationship between adult and nestling state may have changed as nestlings grew older and adults shifted from crop-milk feeding with endogenous resources to seed feeding with exogenous resources.

In examining how SI hormone levels related to changes in parental effort after the stress event (ΔNW), we included covariates for SI hormone levels, sex, and their interaction, with ΔNW as the response variable. We included only nests where one adult was captured because we relied on measurements after the initial capture and therefore the effect of capturing the other adult was likely to have direct effect on measurements of the nestlings. However, to verify that results were independent of the sample, we also ran models with samples from nests where both adults were captured.

Our objectives in this study were not related to sex-specific hypotheses about hormonal relationships, and therefore we chose to analyze male and female data together. We made the null assumption that no difference between the males and the females occurred because both sexes play important and relatively equal roles in incubation and feeding of young. Previous studies of Columbiformes find similar patterns in hormone profiles for PRL and CORT for both sexes (Horsman and Buntin, 1995; Washburn et al., 2002), and exploratory analysis with our data set found that overall mean values of both BL and SI hormone levels differed little between the sexes (Table 1). To test this assumption for patterns in individual variation as opposed to means, we compared the best model from the initial analysis to one in which all effects differed by sex to determine whether there was support for sex-specific patterns in hormone levels.

In addition to the multiple regression analysis, we used path analysis to examine relationships among BL hormone levels and adult weight and nestling weight (Olobatuyi, 2006). This allowed for a more explicit estimation of relationships among parameters and a comparison of alternative models for how the parameters related to each other (Grace, 2008). Hormone levels were adjusted for handling time in the case of CORT and for assay effects by adjusting to the mean between years/assays. We estimated path coefficients based on the correlation matrix for the covariates to generate standardized path coefficients. Alternative models were compared using AIC, and fit of models to the overall correlation matrix was assessed using a χ² goodness-of-fit test, which is a test for significant correlation among variables not explained by the model. Models that fit the data well were those with low AIC values and non-significant χ² values.

Results

Hormone levels, nesting weights, and adult weights

We captured 30 male and 35 female birds during the 2 years that were used in subsequent analyses of variation in PRL and CORT among individuals during the nesting period. Ages of nestlings at the time of capture ranged from 1 to 7 days. Mean hormone levels differed little between males and females or between the late and early period (Table 1).

The best model describing BL PRL (R² = 0.58) included an effect of nesting age (−0.23; 95% CI = ±0.49), NW (1.24 ± 0.72), and their interaction (−0.26 ± 0.16). Additional parameters included in competing models with low ΔAIC values all had confidence intervals that widely overlapped 0, suggesting the best model included the important parameters for this data set. BL PRL level was positively related to NW at early nestling ages while the relationship disappeared at later nestling ages (Fig. 1A). There was some evidence suggesting patterns for BL PRL differed among the sexes. When the best model was compared to a model in which parameters differed between the sexes, the ΔAICc value was 0.56 with three additional parameters. This model showed that the positive relationship between NW and BL PRL occurred for both sexes; however, the effect was larger for females than males.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>All samples (n = 65)</th>
<th>Female (n = 35)</th>
<th>Male (n = 30)</th>
<th>Early (n = 35; nestlings ≤ 4 days)</th>
<th>Late (n = 30; nestlings ≥ 5 days)</th>
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<tbody>
<tr>
<td>Baseline CORT</td>
<td>3.78 (1.66)</td>
<td>3.86 (1.79)</td>
<td>3.72 (1.57)</td>
<td>3.60 (1.61)</td>
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<td>30 min CORT</td>
<td>29.44 (11.51)</td>
<td>31.03 (11.18)</td>
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<td>28.93 (11.82)</td>
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<td>25.66 (11.43)</td>
<td>27.17 (11.12)</td>
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<td>25.33 (11.54)</td>
<td>26.01 (11.51)</td>
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<td>Baseline PRL</td>
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<td>25.46 (4.93)</td>
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<td>30 min PRL</td>
<td>20.37 (4.85)</td>
<td>20.16 (4.88)</td>
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<td>21.57 (5.57)</td>
<td>19.09 (3.61)</td>
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<td>Stress-induced PRL</td>
<td>−4.18 (3.51)</td>
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<td>−4.34 (3.95)</td>
<td>−3.89 (3.65)</td>
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All values are presented in ng·ml⁻¹.
The best model for SI PRL included only an intercept term, and therefore our analysis did not support a relationship between the PRL stress response and nesting or adult weight.

The best model for BL CORT ($R^2 = 0.37$) included effects for nestling age (0.25; 95% CI = ±0.24), NW (0.47 ± 0.35), and their interaction (−0.08 ± 0.07). The handling time effect was consistent with other studies, with CORT levels increasing 0.90 ng·ml$^{-1}$·min$^{-1}$ (±0.66). Similar to our results for BL PRL, we found that BL CORT was positively related to NW during the early nestling period, while the relationship disappeared once young reached 6 days of age (Fig. 1B). The next best model included an additional effect of AW and its interaction with age ($\Delta$AICc = 1.2) with CORT positively related to AW and the effect declining with age. However, support for this effect was not strong with confidence intervals of parameter interactions overlapping 0. There was little support for a difference in patterns between the sexes. The sex-specific version of the best model had a $\Delta$AICc value of 4.56 with wide overlap of 0 for all confidence intervals of parameter interactions with sex.

The best model for SI CORT ($R^2 = 0.13$) included NW; however, the confidence interval overlapped 0 ($-0.48; 95\% CI = \pm 0.54$). The model with only the assay/year effect and none of our effects of interest fit nearly as well as the best model ($\Delta$AICc = 1.0). Thus, we could not demonstrate conclusive support for a relationship between SI CORT and NW.

To further examine the relationship among the variables, we compared three path models for BL hormone levels and a null model that included no interactions among the parameters (Fig. 2, Table 2). Because relationships between hormone levels and NW appeared to differ between the early nestling period and the later period, we fit separate models for data from nests where young were ≤4 days old and those where young were ≥5 days old (n = 35 and 30, respectively). Model 1 was our full model with all hypothesized relationships that included effects of AW on PRL and CORT as well as NW, correlation between PRL and CORT, and effects of PRL and CORT on NW. Model 2 was based on results from our regression models where the effect of AW on CORT and PRL was eliminated from the full model. Finally, Model 3 was based on results from Koch et al. (2002, 2004), which indicate that CORT elevation may occur secondarily to PRL elevation to promote hyperphagia by adults. For this model, we specified PRL to have a direct effect on NW and CORT. CORT was specified to have a direct effect on AW leading to weight gain and AW subsequently affected NW. Prior to fitting models, we eliminated year/assay effects for both hormones and corrected CORT for handling time, using residual values in the analysis.

During the early period, both the null model and Model 2 had a significant lack of fit for the correlation matrix, indicating significant relationships among variables were left out of the model (Table 2). Model 3 fit the data well, while being more parsimonious than Model 1. This was reflected in the model ranks with Model 3 having the lowest AIC value. Path coefficients for the early period are shown in Fig. 2. For Model 3, CORT was positively related to PRL (Fig. 3).

![Fig. 2. Estimates of standardized path coefficients for models including nesting weight (NW), adult weight (AW), and baseline prolactin (PRL) and corticosterone (CORT) level for nests ≤4 days after hatch. Solid lines represent effects that did not include 0 in the confidence intervals (CI). Dashed lines represent effects that did include 0 in the 95% CI. Dotted lines represent effects that included 0 in the 90% CI. Results indicate the full model (model 1) was over-specified and the best fit was for model 3. These results corroborate laboratory results for ring doves (Koch et al., 2002, 2004) that indicate CORT elevation in response to PRL elevation stimulates hyperphagia to meet the energetic needs of crop milk feeding. PRL level had a direct effect on NW while CORT was related to NW during the early nestling period, while the relationship between baseline CORT and PRL levels of adult mourning doves caring for nestlings 2–4 days old. Estimates are corrected for handling time (1.5 min) and assay/year effects (average of 2 years). Levels were positively correlated (r = 0.43) during this period.](image-url)

![Fig. 3. Relationship between baseline CORT and PRL levels of adult mourning doves caring for nestlings 2–4 days old. Estimates are corrected for handling time (1.5 min) and assay/year effects (average of 2 years). Levels were positively correlated (r = 0.43) during this period.](image-url)

<table>
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<td>2</td>
<td>0.26</td>
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We used AIC to assess the relative fit of models with lower values representing a better fit. In addition, we used a $\chi^2$ goodness-of-fit test to determine whether there was significant unexplained variation with significant p-values indicating a lack of fit for the model. During the early period, the best fit was for model 3, which is consistent with CORT increasing in response to PRL elevation to promote hyperphagia. During the late period, there was little evidence for a relationship among the parameters with the null model having the lowest AIC value and a strong probability that the correlation structure among variables could occur by chance.

Table 2 Comparison among alternative path models for the relationship among CORT, PRL, AW, and NW during the early (≤4 days) and late (≥5 days) nestling period.

Fig. 2. Estimates of standardized path coefficients for models including nesting weight (NW), adult weight (AW), and baseline prolactin (PRL) and corticosterone (CORT) level for nests ≤4 days after hatch. Solid lines represent effects that did not include 0 in the 95% CI, dashed lines represent effects that did include 0 in the 90% CI, and dotted lines represent effects that included 0 in the 90% CI. Results indicate the full model (model 1) was over-specified and the best fit was for model 3. These results corroborate laboratory results for ring doves (Koch et al., 2002, 2004) that indicate CORT elevation in response to PRL elevation stimulates hyperphagia to meet the energetic needs of crop milk feeding. PRL level had a direct effect on NW while CORT was related to NW during the early nestling period, while the relationship between baseline CORT and PRL levels of adult mourning doves caring for nestlings 2–4 days old. Estimates are corrected for handling time (1.5 min) and assay/year effects (average of 2 years). Levels were positively correlated (r = 0.43) during this period.
we made predictions about how both adult weight, a measure of current adult state, and nestling weight, a measure of parental effort by adults, would be correlated to hormone levels within individuals.

Our results are consistent with predictions for BL PRL and CORT, regulating the acquisition of resources through feeding and the transfer of resources through provisioning of young. The positive relationship of BL PRL to parental effort indicates that PRL levels positively affect feeding rates of young, leading to faster growth of nestlings when adults have higher levels. Our results are not consistent with the elevation of BL CORT in response to an energy deficit in adults, and instead our path analysis suggests that at early ages elevated BL CORT levels may promote adult weight gain which in turn is positively related to nestling weight gain. A positive correlation between BL CORT levels and adult weight and the positive relationship between BL CORT levels and nestling weight during the early nestling period indicate that BL CORT promoted additional energy transfer to nestlings. Past studies of ring doves indicate the mechanism by which CORT affects this process is through promotion of increased feeding by adults (Koch et al., 2002, 2004). CORT can function to promote feeding in birds (Astheimer et al., 1992; Koch et al., 2002; McEwen and Wingfield, 2003; Landys et al., 2004, 2006; Löhmus et al., 2006; Angelier et al., 2007b,c). Thus, BL CORT may indirectly increase parental effort, by increasing feeding rates where additional acquired resources are transferred to the young to increase growth rates.

Previous studies as well as our path analysis results indicate that PRL and CORT may act together to steer resources to greater investment in nestlings during early reproduction. Koch et al. (2002) demonstrated that in ring doves, experimental elevation of PRL levels caused increased CORT levels. They suggested that elevation in CORT may promote increased feeding necessary for provisioning young (Koch et al., 2002, 2004). We observed a similar correlation in BL PRL and CORT levels during the early nestling period when crop-milk feeding is greatest for mourning doves.

Hormonal controls of life-history decisions are likely to be characterized by pleiotropic effects because of the multiple effects associated with changes in levels of a single hormone (Sinervo and Svennson, 1998). This can result in constraints on selection on hormone systems (Ketterson and Nolan, 1992, 1999; Ricklefs and Wikelski, 2002). It has previously been suggested that potential negative effects on reproduction due to CORT elevation may be minimized by upregulation of other physiological actions that promote parental effort. Koch et al. (2002) suggested that increased PRL levels counteract negative effects of increasing BL CORT level during reproduction, leading to beneficial effects of CORT by increasing foraging. They hypothesized that this may explain inconsistencies in the relationship between BL CORT level and parental effort in other studies. Our study lends further support to their hypothesis by confirming that PRL and CORT are correlated and both positively related to parental effort. In contrast Angelier and Chastel (2009) review studies that indicate that CORT may have negative effects on PRL levels; however, they focus on CORT levels typical of stress-induced levels. Further studies in which individual variation in both PRL and CORT are determined along with experimental manipulation of multiple hormones will be needed to determine whether the hypothesized relationship we propose among baseline PRL, CORT, and parental effort is supported for non-Columbiformes. Other factors such as receptor actions (Landys et al., 2004), binding proteins (Love et al., 2004; Shultz and Kitaysky, 2008), or alternative physiological mechanisms may act similarly to PRL to minimize negative effects of CORT on reproduction and may vary among species. The potential for changes in hormone levels to act on multiple physiological and behavioral processes makes the simultaneous study of multiple hormones important in understanding hormone/life-history relationships (Angelier et al., 2009).

Much of the focus on hormonal control in avian life-history trade-offs has focused on variation in the CORT response to unpredictable stressors (Wingfield et al., 1998; Romero, 2002; McEwen and
The rapid increase in CORT level significantly above BL level that occurs in response to acute stressors has been hypothesized to help facilitate a transition into an “emergency life-history stage” where self-maintenance is prioritized with respect to other investment including investment in reproduction (Wingfield et al., 1998). The original hypothesized effects on reproduction of this transition were largely based on laboratory studies where CORT level has been manipulated. More recently studies in which CORT level was manipulated in wild populations of birds to mimic SI level have demonstrated effects of strongly elevated CORT on reproductive parameters (Silverin, 1998; Criscuolo et al., 2005; Angelier et al., 2009); however, surprisingly little attention has been given to whether natural variation in the CORT stress response is related to subsequent parental effort in wild populations.

Our results are important in demonstrating that there is a strong relationship between natural variation in the SI CORT response in mourning doves and subsequent parental effort. We found a wide degree of variation in the SI CORT response among individuals ranging from increases of 14 to 59 ng·mL⁻¹ over BL CORT level. Consistent with the prediction that the magnitude of the SI CORT response would have negative effects on parental effort, we found that residual weight of nestlings for individuals that maintained CORT level near BL level did not change after the stress event. Nestling growth declined significantly when adults exhibited a strong SI CORT response with declines up to 15% in age-specific residual weight for nestlings of adults with the greatest SI CORT response. These results support the assertion that modulation of SI CORT response at levels exhibited in natural settings can have a relevant role in relating life-history trade-offs between self-maintenance and reproduction in natural populations and are consistent with recent results for female house sparrows (Passer domesticus; Lendvai and Chastel, 2008).

Because of the potential negative effects of the stress response on parental effort (Wingfield et al., 1998; Romero, 2002; McEwen and Wingfield, 2003), we predicted individuals would vary their response with respect to the current value of offspring and their own condition. We hypothesized that SI level of PRL and CORT would vary in response to both adult weight and nestling weight. However, we did not find a relationship of either SI CORT or SI PRL level to the condition of either adults or nestlings. Processes governing the PRL stress response and the effects of the response are not well understood in birds. At least one previous study indicates that breeding birds may modulate the decline in PRL level when facing acute stressors when compared to non-breederes (Chastel et al., 2005), indicating that the PRL stress response may have important consequences for breeding birds. The CORT stress response is better studied in birds, but ambiguity exists in whether and when birds modulate the stress response (Shultz and Kitaysky, 2008; Romero, 2002; Wingfield and Sapolsky, 2003). Given the dramatic effects of SI CORT level on subsequent parental effort we found in this study, it is somewhat surprising that we did not find evidence that parents modulated the response in relation to their own current conditions or that of their young. In common murres (Uria aalge), SI CORT levels are best predicted by food availability the previous month, indicating that there may be a lag in the time between environmental stimuli that affect individuals and the upregulation of CORT production (Kitaysky et al., 2007). This stands in contrast to recent results for house sparrows (P. domesticus) where the SI CORT response was negatively related to reproductive value of offspring (Lendvai et al., 2007; Lendvai and Chastel, 2008) and to differences among species where birds with greater investment in young have smaller SI responses (Bókony et al., 2009).

Conclusions

The importance of hormonal control of life-history decisions has been recognized in many vertebrate populations (Silver and Cooper, 1983; Ketterson and Nolan, 1992; Finch and Rose, 1995; Horsemann and Buntin, 1995; Sinervo and Svensson, 1998; Wingfield et al., 1998; Wang and Buntin, 1999; Romero, 2002; Landys et al., 2006; Bókony et al., 2009). Research in this area has concentrated on three levels of inquiry: how differences in endocrine levels among species relate to variation in life-history characteristics of the species, how levels vary among life-history stages within a population, and how levels vary among individuals of a population within a single life-history stage. Our knowledge of the effects of individual variation in hormone levels among individual life-history stages and its affect on life-history trade-offs is much more limited than the first two levels (Williams, 2008), and this level was the focus of our study. Our results are consistent with individual variation being a potentially fruitful level of focus for the study of endocrinology in wild populations.

Although we demonstrate that individual variation in hormones was correlated with parental effort, we did not determine factors that lead to individual variation in hormone levels. An important component to improving our understanding of the role that hormonal control plays in reproductive plasticity in birds will be determining whether birds vary their hormonal responses in response to their environment rather than the variation being fixed among individuals. Given that endocrine systems have characteristics allowing organisms to respond to environmental influence (e.g., CORT and the HPA axis; Sapolsky et al., 2000) and have been shown to be responsive to environmental input (e.g., Romero, 2002; Lendvai et al., 2007; Bradshaw, 2007; Kempenaers et al., 2008; Lendvai and Chastel, 2008), we suspect that individual variation observed in this study includes both fixed and plastic components.

Given the importance of individual variation in optimizing life histories (McNamara and Houston, 1996; Piersma and Drent, 2003), our results are important in demonstrating that individual variation in hormone levels is correlated with parental effort by adult mourning doves. We found that individual variation in both BL CORT and BL PRL levels was positively correlated to parental effort early in the nestling period. Similarly, we found that individual variation in SI CORT response was related to subsequent parental effort.

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Aske, J., 1992. Life-history trade-offs is much more limited than the first two levels (Williams, 2008).