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Stress Response and the Value of Reproduction:
Are Birds Prudent Parents?

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ABSTRACT: In vertebrates, stressors such as starvation or predator attacks stimulate the rapid elevation of circulating glucocorticoid hormones, triggering physiological and behavioral responses that aid immediate survival but simultaneously inhibit reproduction. This stress response has been proposed to serve as a physiological mediator of life-history trade-offs: when the value of current reproduction is high relative to the value of future reproduction and survival, a mitigated stress response is expected to enable successful breeding and maximize fitness. Using phylogenetic comparative analyses, we investigated baseline and peak stress-induced plasma corticosterone levels during parental care in 64 bird species. We found that (1) species with a higher value of the current brood relative to future breeding mounted weaker corticosterone responses during acute stress, and (2) females in species with more female-biased parental care had weaker corticosterone responses. These results support the brood value hypothesis, suggesting that the stress response evolves as an adaptive basis for life-history strategies. Further, we found that (3) baseline corticosterone correlated positively with brood value and negatively with body mass, and (4) peak corticosterone was greater in species breeding at higher latitudes. The latter findings suggest that circulating corticosterone concentrations might be matched to the anticipated demands and risks during nesting.

Keywords: corticosterone, life history, brood value, evolution, comparative method, birds.

Introduction

According to life-history theory, animals are faced with trade-offs in the allocation of limited resources to different functions (Stearns 1992). The general reproductive effort model based on the cost of reproduction (Williams 1966) predicts that breeding individuals trade off their resources between current reproductive investment and their own survival, that is, future reproduction (Stearns 1992). Whether the individual should allocate more to current breeding or to survival depends on the relative importance of these two life-history components in maximizing fitness. For example, long-lived species are expected to behave as "prudent parents" and refrain from jeopardizing their own survival during reproduction (Drent and Daan 1980; Stearns 1992) because their lifetime reproductive success is primarily a function of adult survival rather than of seasonal fecundity (Williams 1966). In researching as to how animals can adopt such adaptive life-history strategies, growing attention is being paid to physiological mechanisms, as these may adjust life-history decisions to environmental conditions and perceived risks (Wingfield et al. 1998; Ricklefs and Wikelski 2002).

One potential mechanism underlying the regulation of reproductive effort may be the endocrine stress response. In vertebrates, stressors such as inclement weather, food shortage, or predator attacks activate the hypothalamic-pituitary-adrenal cortex (HPA) axis to elevate the circulating levels of glucocorticoid hormones (CORT) that promote physiological and behavioral changes to enhance immediate survival but to simultaneously inhibit reproduction (reviewed by Wingfield and Sapolsky 2003). High CORT elevations shift the animals into an "emergency life-history stage" in which they reduce or even abandon their parental activities (Wingfield et al. 1998). Thereby, the stress response redirects energy investment away from breeding, toward survival. Such a shift should be adaptive for "prudent parents" but less so when the value of current reproduction is high compared with the value of survival and future reproduction, such as in species with few breeding opportunities (Wingfield and Sapolsky 2003). Thus, the "brood value hypothesis" (Heidinger et al. 2006; Lendvai et al. 2007; Lendvai and Chastel 2008) proposes that

the stress response should be modulated as a function of the relative importance of current reproduction; for instance, when the value of current reproduction is relatively high, the stress response should be mitigated to ensure that the current breeding attempt is not compromised.

This hypothesis has been supported by a number of correlative studies in free-living birds. For example, the stress response was found to be attenuated when the chances for rebreeding were limited, such as in northerly breeding populations with shorter breeding seasons (e.g., Silverin et al. 1997; O'Reilly and Wingfield 2001; Breuner et al. 2003; but see Wingfield et al. 1995a) and in older individuals (Heidinger et al. 2006; but see Angelier et al. 2007b). A reduced stress response was also associated with highest parental investment in comparisons across different breeding phases (Meddle et al. 2003) and in the sex that invests more into offspring care (Wingfield et al. 1995b; O'Reilly and Wingfield 2001; Holberton and Wingfield 2003). Moreover, recent experimental manipulations of the brood value demonstrated flexible modulation of the stress response within individuals either up- or downward according to the value of current reproductive attempt (Lendvai et al. 2007; Lendvai and Chastel 2008). Parents' stress responses were higher when they had less valuable (i.e., smaller or poor-quality) broods than when they were tending more or better-quality chicks (Lendvai et al. 2007; Lendvai and Chastel 2008). Altogether, these results corroborate that the stress response may function as a physiological mediator for the adaptive allocation between current and future reproduction. Nevertheless, we still lack large-scale interspecific studies that would help to assess the generality of this pattern (Wingfield et al. 1995b).

It has been suggested that hormonal mechanisms that serve as physiological bases for life-history trade-offs may be targets of selection just like other phenotypic traits (Ketterson and Nolan 1992; Hau 2007). In support of this idea, recent comparative studies of androgen levels have repeatedly shown that testosterone profiles may evolve as species-specific characteristics, differing consistently among species according to the sexual and social challenges of their breeding system and ecology (Hirschenhauser et al. 2003; Goymann et al. 2004; Garamszegi et al. 2005; Ketterson et al. 2005; Møller et al. 2005; Bókony et al. 2008). For example, both sexes maintain higher testosterone levels in species with stronger intrasexual competition (Garamszegi et al. 2005; Ketterson et al. 2005). Similarly, one might expect the stress response to be matched to the species's breeding environment and life history, as a recent review strongly argued for the functional significance of plasma CORT titers per se (Williams 2008). Under this scenario, the brood value hypothesis predicts that species with relatively high importance of current reproduction should be selected to circulate lower levels of stress hormones.

In this study, we investigated the relationship between brood value and CORT level in birds at the interspecific level, using the phylogenetic comparative approach. This methodology has been successfully employed to detect evolutionary associations between various ecological and phenotypic traits, and recent studies have demonstrated the usefulness of this approach in identifying factors that influence the evolution of certain hormone levels (e.g., testosterone). Here we have compiled the most extensive data set of available CORT measures of avian species to investigate whether CORT levels may be viewed as species-specific traits and whether they may evolve in accordance with the relative importance of current reproduction. Specifically, we focused on two interspecific predictions of the brood value hypothesis. First, the stress response should be mitigated during nesting in species with higher brood value, that is, in which a single breeding attempt contributes a greater proportion of lifetime reproductive value because, for example, of limited opportunities to breed. Second, the sex differences in CORT levels should be inversely related to the sex bias in parental investment across species; that is, the stress response should be mitigated in the sex that invests more in the current brood.

Material and Methods

CORT Levels

We searched the primary literature published until May 2007 for data on circulating corticosterone concentrations of free-living birds, measured in the parental phase of breeding (incubation and/or chick rearing). We extracted two hormone levels (in ng mL^{-1}): (1) Baseline CORT level, that is, measured from the sample taken immediately after capture within 3 min (Romero and Reed 2005) or more if the study validated that CORT level had not begun to increase during that time interval (range 0–7 min; mean + SD = 3.11 + 1.12 min, $n = 275$). This measure is an approximation of the seasonal baseline level of CORT that the animals should maintain to be able to cope with the predictable demands of the current life-history stage (allostatic load; "state B," sensu Landys et al. 2006). (2) Peak CORT level, that is, the highest stress-induced level following capture and restraint for 5–70 min (mean + SD = 38.81 + 16.82 min, $n = 170$). This measure corresponds to the acutely elevated levels of CORT triggered by unpredictable perturbations that cannot be prepared for and may shift the animals into the "emergency life-history stage" (allostatic overload; "state C," sensu Landys et al. 2006). For both baseline and peak CORT levels, we used the mean (weighted by sample size) value of the different estimates when more than one CORT measurement was

available for a species. Whenever the data allowed, we collected CORT levels separately for the sexes and for the incubation and chick-rearing phases. However, we also calculated both baseline and peak CORT levels as averages for the whole parental period and for the two sexes because, for many species, only such combined measurements were available. We excluded CORT data measured in individuals that were subjected to experimental manipulations (e.g., testosterone implantation, brood-size manipulation) or extreme conditions (e.g., severe storms) that are expected or known to alter CORT levels. In total, we found CORT data for 64 bird species from 104 studies (table A1 in the online edition of the *American Naturalist*). Both CORT variables were $\log_{10}$ transformed to normalize distributions. Sex differences in CORT levels were expressed as $\log_{10}$ (male CORT/female CORT).

To test the reliability of species-specific CORT levels, we conducted several analyses. First, we tested whether CORT measures were related to the parameters of the hormone assays (sensitivity, inter- and intra-assay coefficients of variation [CVs], recovery, and accuracy) applied by different studies. We also tested whether CORT measures differed between studies that did or did not perform chromatographic separation of steroid phases prior to assay. Second, we tested whether CORT measures were influenced by sample size, handling time (i.e., the time elapsed from capture until the collection of blood sample), or breeding phase (i.e., incubation, chick rearing, or not specified). All of these analyses included study identity as a random factor to handle the repeated nature of data (several studies measured more than one species). Third, we tested the repeatability of CORT data measured in the breeding season by different studies for the same species, following Lessells and Boag (1987). Finally, because among-species differences might arise between different labs specializing in different species, we also assessed the differences attributable to different labs.

Life-History Variables

To quantify the importance of current reproduction across species, we collected data on life-history traits related to lifetime reproductive success, specifically on (1) average clutch size, that is, number of eggs laid in a single breeding attempt; (2) number of broods raised successively per season; and (3) average reproductive life span, that is, number of years over which offspring may be produced. Because direct data on average life span were not available for most species, we extracted data on the annual rate of adult mortality, preferably from capture-recapture studies based on large samples and/or long-term observations (for further details, see Liker and Székely 2005), and we estimated average reproductive life span as 1/mortality. This estimate

correlates strongly with known reproductive life spans ($r = 0.92$, $P < .001$, $n = 14$). By multiplying the above three variables, we calculated the number of offspring that can be produced during the lifetime of an average individual of the species. We expressed the value of current reproduction relative to the lifetime reproductive output of a species (henceforth, "brood value") as $\log_{10}$ (clutch size/[clutch size × broods per year × average reproductive lifespan]).

To calculate the sex differences in parental care, we scored the contribution of each sex to incubation, chick feeding, and brooding following Liker and Székely (2005): no contribution to care (score = 0), 1%–33% of care provided (score = 1), 34%–66% of care provided (score = 2), and 67%–100% of care provided (score = 3). Parental care of each sex was calculated as the sum of their scores for the three parental activities (range, 0–9). The difference between male and female care was used as an index for the sex bias in full parental care; that is, species with more female-biased care have smaller (more negative) values, whereas species with more male-biased care have larger (more positive) values.

We also considered two potentially confounding variables. First, it has been proposed that larger birds can rely on larger fat and/or protein reserves and thus may cope with stressors by using their energy stores instead of elevating CORT levels (Wingfield et al. 1995*b*). Second, CORT levels are known to vary latitudinally (e.g., Breuner et al. 2003), perhaps in part because of weather conditions (Romero et al. 2000). Therefore, we collected data on body mass (the average of male and female body mass in the breeding season) and the latitude of breeding (the absolute distance from the equator, averaged for those populations in which CORT levels had been measured). The complete data set and data sources are given in appendix A.

Phylogenetic Analyses

To investigate the evolutionary relationships between CORT levels and life-history variables, we used phylogenetic generalized least squares (PGLS) models (Pagel 1997, 1999). This approach controls for the nonindependence among species by incorporating a matrix of the expected covariances among species on the basis of their phylogenetic relationships (Martins and Hansen 1997; Pagel 1997, 1999), and it also estimates the importance of phylogenetic corrections in the data (Freckleton et al. 2002). We thus conducted all analyses by setting the degree of phylogenetic dependence (λ) to the most appropriate degree evaluated for each model by likelihood ratio statistics. To represent phylogenetic relationships among species, we compiled a composite tree using recent molecular phylogenies (app. B in the online edition of the *American Naturalist*). We

Table 1: Proportion of variance (η^2) explained by species differences and lab differences in corticosterone (CORT) measures

CORT	F	df	P	η^2
Baseline:				
Species	15.99	(61, 177)	<.001	.85
Lab	7.81	(14, 177)	<.001	.38
Peak:				
Species	6.83	(48, 89)	<.001	.79
Lab	5.79	(9, 89)	<.001	.37

set branch lengths proportional to the number of nodes, that is, gradual branch lengths, according to Nee's method (Maddison and Maddison 2006).

First, we tested the relationships of CORT levels with brood value, body mass, and breeding latitude in bivariate analyses. Then, we investigated the relative importance of these three predictors in multivariate analyses, comparing candidate models using the information theoretic approach (Burnham and Anderson 2002) based on the second-order Akaike's Information Criterion corrected for small sample size (AIC_c). We evaluated the candidate models by their relative Akaike weights (ω_i) and the predictors by the sums of their Akaike weights across all models that contain the given predictor (Σ); thus, inference was based on the entire set of plausible models. To test the effect of brood value on the magnitude of stress response across species, we used peak CORT level as a dependent variable and controlled for baseline CORT level as a covariate, and we also included any confounding variable identified for peak CORT level by the previous AIC_c analyses. We used this approach because differences in baseline CORT levels might reflect species differences in various components of the "CORT machinery" such as receptor affinities and binding proteins (Romero 2004). Thus, a given concentration of peak CORT level might have different impacts in species with very different baseline CORT concentrations.

To investigate the effects of sex differences in parental care, first we tested its relationship with sex differences in CORT levels. Then, to assess the contribution of male and female CORT in these relationships, we analyzed both sexes' CORT levels in relation to sex differences in parental care. Finally, we tested the effect of parental role differences on the magnitude of the stress response across species by using peak CORT level as a dependent variable and controlling for baseline CORT level (both for the two sexes separately and for the sex differences in CORT levels).

For all analyses, we used the R statistical computing environment (R Development Core Team 2006). All statistical tests were two-tailed, with a 95% confidence level. We followed the recent recommendations of Nakagawa (2004) and provided effect size estimates, the correlation effect size r sensu Cohen (1988), and the associated 95%

confidence intervals (CIs) to evaluate the strength and direction of each relationship in our multiple tests. Sample sizes differ across analyses because data are incomplete for various species. Because the PGLS method does not allow the graphical presentation of phylogenetically corrected data, we present figures that are based on raw species data.

Results

Reliability of CORT Measurements

The amount of measured CORT was not related to any of the assay parameters, that is, assay sensitivity (baseline: $F_{1,45} = 2.33$, $P = .134$; peak: $F_{1,13} = 0.51$, $P = .487$), intra-assay CV (baseline: $F_{1,105} = 0.52$, $P = .475$; peak: $F_{1,66} < 0.01$, $P = .952$), interassay CV (baseline: $F_{1,79} = 0.93$, $P = .338$; peak: $F_{1,51} = 1.49$, $P = .227$), and assay recovery (baseline: $F_{1,28} = 0.01$, $P = .912$; peak: $F_{1,22} = 0.81$, $P = .378$). Studies with and without chromatographic separation of steroid phases did not differ in their measured CORT concentrations (baseline: $F_{1,12} = 2.18$, $P = .166$; peak: $F_{1,5} = 0.15$, $P = .715$). There was no correlation between sample size and CORT measures (baseline: $F_{1,198} = 1.49$, $P = .224$; peak: $F_{1,108} = 0.10$, $P = .755$). Baseline CORT level was unrelated to handling time ($F_{1,168} = 2.64$, $P = .106$); although peak CORT level tended to increase with the time required to reach the

Table 2: Bivariate relationships of corticosterone (CORT) levels with brood value, body mass, and breeding latitude, and the magnitude of stress response in relation to brood value, controlled for breeding latitude

Model, dependent, predictor	r	Confidence limits of r Lower	Upper	P	λ	n
Bivariate models:						
Baseline CORT:						
Brood value	.32	.01	.57	.041	<.01	42
Body mass	−.28	−.50	−.02	.038	<.01	57
Breeding latitude	.13	−.14	.38	.337	<.01	57
Peak CORT:						
Brood value	.14	−.21	.45	.444	<.01	34
Body mass	.05	−.24	.33	.734	<.01	47
Breeding latitude	.38	.10	.60	.009	<.01	47
Multivariate model of stress response:						
Peak CORT:					<.01	33
Baseline CORT	.75	.55	.87	<.001		
Breeding latitude	.42	.08	.66	.020		
Brood value	−.37	−.63	−.03	.043		

Note: Brood value is defined as the relative importance of current reproduction. Stress response is expressed as the peak CORT level controlled for the baseline CORT level. Correlation effect sizes (r) with 95% confidence intervals have been adjusted by the degree of phylogenetic dependence (λ) in each phylogenetic generalized least squares model.

Table 3: Comparison of multivariate phylogenetic generalized least squares models for corticosterone (CORT) levels

CORT level, predictor	k	AIC_c	Δ_i	ω_i
Baseline ($n = 42$):				
Body mass	2	24.76	.00	.32
Brood value	2	25.00	.24	.28
Body mass + latitude	3	26.51	1.75	.13
Brood value + latitude	3	27.39	2.64	.09
Brood value + body mass	3	27.48	2.72	.08
Latitude	2	27.81	3.05	.07
Brood value + latitude + body mass	4	29.71	4.95	.03
Peak ($n = 34$):				
Latitude	2	−13.41	.00	.70
Brood value + latitude	3	−9.95	3.46	.12
Body mass + latitude	3	−9.94	3.47	.12
Brood value + latitude + body mass	4	−6.26	7.15	.02
Brood value	2	−6.17	7.24	.02
Body mass	2	−5.62	7.79	.01
Brood value + body mass	3	−2.80	10.61	<.01

Note: Akaike's Information Criterion corrected for small sample size (AIC_c), number of estimated parameters (k), AIC_c difference between the best model and each candidate model (Δ_i), and Akaike weight (a relative estimate of the probability that a given model is actually the best model in the model set; ω_i) are given for each candidate model. Models with $\Delta_i < 2$ are considered to have substantial support.

hormone peak ($F_{1,102} = 3.54$, $P = .063$), this tendency was largely dependent on one extremely high CORT value at 60 min (excluding this data point: $F_{1,101} = 2.23$, $P = .139$). There was no difference between incubating, chick-rearing, and overall breeding season CORT levels (baseline: $F_{1,174} = 0.64$, $P = .531$; peak: $F_{1,110} = 0.64$, $P = .530$). Repeatability was high for both baseline CORT ($R = 0.81$, $F_{31,47} = 11.39$, $P < .001$) and peak CORT ($R = 0.75$, $F_{16,25} = 8.19$, $P < .001$) levels, implying that variation within species is small compared with variation among species. The variance attributable to differences among species remained significant when we controlled for lab differences (table 1), with the effect of species being twice as great as the lab effect (table 1).

CORT and the Value of Reproduction

Baseline CORT level correlated positively with brood value (table 2; fig. 1*A*) and negatively with body mass (table 2). Breeding latitude was not related to baseline CORT level (table 2). In the multivariate model comparison (table 3), brood value ($\Sigma = 0.48$) and body mass ($\Sigma = 0.56$) received approximately equal support, whereas breeding latitude ($\Sigma = 0.32$) had a smaller impact on baseline CORT level.

Peak CORT level in itself was unrelated to brood value (table 2; fig. 1*A*) and body mass (table 2), but it correlated positively with breeding latitude (table 2). In the multivariate model comparison (table 3), peak CORT level was best explained by breeding latitude ($\Sigma = 0.96$), whereas brood value ($\Sigma = 0.17$) and body mass ($\Sigma = 0.16$) were each of far less importance.

To investigate the magnitude of stress response, we built a multivariate model for peak CORT level that included baseline CORT level (see "Material and Methods"), breeding latitude (see tables 2, 3), and brood value. After controlling for the potentially confounding effects of baseline CORT level and breeding latitude, peak CORT level correlated negatively with brood value (table 2; fig. 1*B*). The variance inflation factor was less than 1.3 for all variables in the latter model.

The sex differences in parental care correlated negatively with the sex differences in CORT level, such that the more caring sex tended to have a lower baseline CORT level (table 4; fig. 2*A*) and a lower peak CORT level (table 4; fig. 2*A*). The latter relationship remained significant when we controlled for the sex differences in baseline CORT level (table 4*B*). Neither male nor female baseline CORT level was related to sex differences in parental care (table 4; fig. 2*B*, 2*C*). Female but not male peak CORT level was greater in species with more male-biased care (table 4; fig. 2*B*, 2*C*). When we controlled for baseline CORT level, both male and female peak CORT levels correlated positively with the sex differences in parental care (table 4).

Discussion

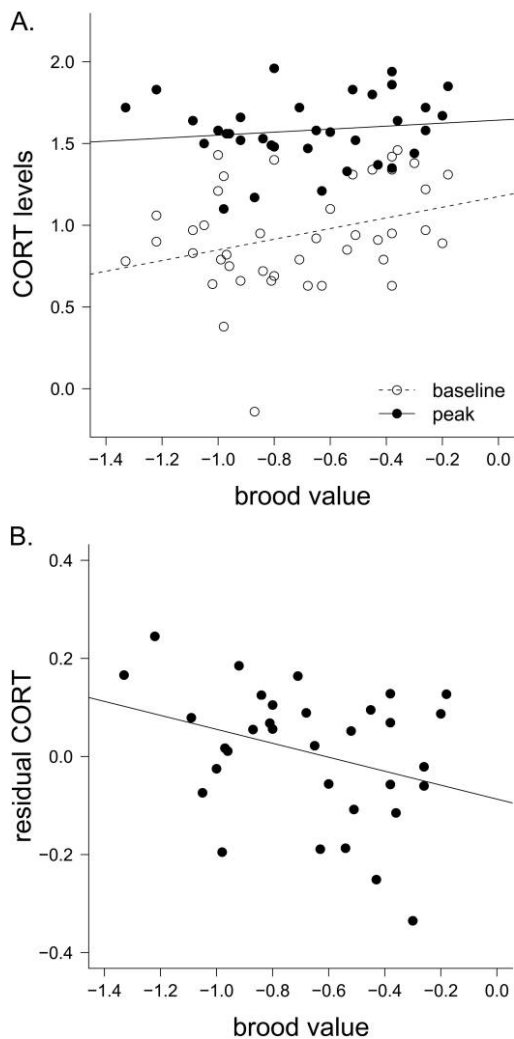Here we carried out the first large-scale study of avian stress hormones using modern phylogenetic comparative

Figure 1: *A,* Baseline and peak corticosterone (CORT) levels (ng mL^{-1}; log$_{10}$ transformed) in relation to brood value. *B,* Stress response, that is, peak CORT level controlled for baseline CORT level and breeding latitude (absolute distance from the equator), in relation to brood value. Brood value expresses the putative importance of current reproduction as log$_{10}$(clutch size/[clutch size × broods per year × average reproductive life span]).

analyses to test whether the species's CORT levels may be adaptively selected traits and whether these levels are related to the relative importance of current reproduction as predicted by life-history theory. We found that the species means of both baseline and peak CORT levels were highly repeatable across different studies that sampled different populations or applied different sampling and assay procedures. Furthermore, the influence of phylogeny (λ) was estimated to be close to 0 in most of our analyses. High repeatability and low phylogenetic dependence are both indicative of selection acting on CORT levels. Our

results are in accordance with previous comparative studies of other hormones (androgens; Garamszegi et al. 2005; Bókony et al. 2008) and imply that hormone levels are not phylogenetically conservative traits but rather evolve as species-specific attributes and may readily adapt to current conditions. Because CORT levels show high individual variation (e.g., Cockrem and Silverin 2002) that is at least partly heritable (Evans et al. 2006) and affects survival and reproduction (Romero and Wikelski 2001; Wingfield and Sapolsky 2003; Brown et al. 2005), they are likely to be subjects of selection. Our findings support the conclusion of artificial selection studies that plasma CORT levels themselves are functionally significant, adaptive characteristics of a species (reviewed by Williams 2008).

To investigate the relationship between the importance of current reproductive attempt and the magnitude of stress response, we focused on the stress-induced peak CORT levels while controlling for the interspecific differences in baseline levels that may reflect differences in other endocrine factors influencing the effect of hormone titers (Romero 2004). In doing so, we found two correlations that accord with the predictions of the brood value hypothesis at the interspecific level. First, higher brood value was associated with lower residual CORT level, suggesting that the stress response is mitigated in species with a life-history strategy that is characterized by fewer breeding opportunities and greater annual fecundity. This finding implies that the "CORT machinery" may evolve in response to the value of current reproduction, such that stress resistance may be a part of adaptation to a lifestyle with few breeding opportunities and robust responses to stress may be an adaptation for "prudent parents" with many breeding opportunities. In a previous comparative study, Wingfield et al. (1995*b*) found no association between life span and CORT response; however, CORT levels were then available for a smaller number of species, most of which were relatively short lived (two to three breeding seasons, on average). More than a decade after the seminal article of Wingfield et al. (1995*b*), the increasing number of published CORT data allowed us to include a greater diversity of species with a wider range of life spans (up to 44 years).

Second, sex differences in parental effort were inversely related to sex differences in residual CORT, such that the greater share the male takes in parental care, the higher the female's peak CORT level. Surprisingly, although we expected a similar inverse relationship in males, we found the opposite: male residual CORT level was higher in species with more male-biased care. The pattern observed in females is in accordance with the brood value hypothesis, and it corroborates former interspecific comparisons (Wingfield et al. 1995*b*; O'Reilly and Wingfield 2001; Holberton and Wingfield 2003) in a considerably larger sample

Table 4: Sex differences (SD) and sex-specific values of corticosterone (CORT) levels in relation to sex differences in parental care

Model, dependent, predictor	r	Confidence limits of r		P	λ	n
		Lower	Upper			
Bivariate models:[a]						
SD in baseline CORT	−.25	−.49	.02	.066	.02	54
SD in peak CORT	−.46	−.67	−.19	.002	<.01	43
Male baseline CORT	−.20	−.44	.07	.142	<.01	57
Male peak CORT	.05	−.24	.34	.721	<.01	46
Female baseline CORT	−.02	−.29	.24	.874	<.01	55
Female peak CORT	.37	.09	.60	.012	<.01	45
Multivariate models of stress response:						
SD in peak CORT:					<.01	42
SD in baseline CORT	.23	−.08	.50	.147		
SD in parental care	−.39	−.62	−.10	.011		
Male peak CORT:					<.01	45
Male baseline CORT	.79	.65	.88	<.001		
SD in parental care	.34	.05	.57	.025		
Female peak CORT:					<.01	44
Female baseline CORT	.79	.65	.88	<.001		
SD in parental care	.56	.32	.74	<.001		

Note: Greater values of parental care indicate more male-biased care. Correlation effect sizes (r) with 95% confidence intervals have been adjusted by the degree of phylogenetic dependence (λ) in each phylogenetic generalized least squares model.

[a] For all bivariate models, the predictor is SD in parental care.

of species ($n = 42$ vs. $n = 13, 4,$ and 3, respectively). Why males show a pattern that is so different from that of females is puzzling, as intraspecific studies demonstrated that CORT reduces parental behavior in both sexes (reviewed by Wingfield et al. 1998), and increased paternal investment is associated with reduced CORT levels in males (Meddle et al. 2003). However, male care is not always essential; that is, females of several biparental species can compensate for the loss of the male parent, which seems to be an important determinant of male hormonal responsiveness in breeding birds (Gill et al. 2008).

Our finding, that baseline CORT levels were higher in species with presumably higher importance placed on current reproduction, is unexpected in light of the brood value hypothesis. We suggest that this result might be explained by a recently proposed idea, the "preparative hypothesis" (Romero 2002), which is based on the permissive effects of CORT; that is, CORT prepares the organism to perform better under subsequent stress (Sapolsky et al. 2000). Therefore, it may be adaptive to circulate higher baseline CORT levels whenever the risk to be stressed is predictably high (Romero 2002). Although this hypothesis has not yet been tested directly, it appears to well explain the seasonal changes in vertebrate CORT levels, especially baseline levels (Romero 2002). We suggest that several lines of our results provide further indirect support for this idea. First, the positive correlation between baseline CORT level and

brood value might have arisen because parents of more valuable broods may be willing (and prepared) to make greater efforts and take more risks for their offspring (e.g., Hegner and Wingfield 1987; Montgomerie and Weatherhead 1988). Second, we found that larger species have lower baseline CORT levels, which might indicate that birds with more body reserves prepare for stressors by storing more energy instead of circulating more CORT. Although Wingfield et al. (1995*b*) found no interspecific relationship between body mass and peak CORT level, they did not investigate baseline CORT level, for which the preparative hypothesis is more relevant. Third, the reduced CORT response of less caring males may be due to the tendency for increased baseline CORT level in these males (see fig. 2*B*). It is possible that males have to face a higher risk of unpredictable stressors (such as social challenges, injuries, or infections) if they invest more in sexual competition (such as territory defense or the pursuit of extrapair copulations) than in parental care, and thus they are better prepared for these risks by having more elevated baseline CORT levels. In contrast, female baseline CORT level shows no hint of such a relationship (see fig. 2*C*), perhaps because polyandrous females still have to produce the eggs, and thus they might not be able to afford as high of elevations of baseline CORT level as males can if they are to reproduce successfully. Finally, we found higher peak CORT levels in species breeding at higher latitudes. Be-
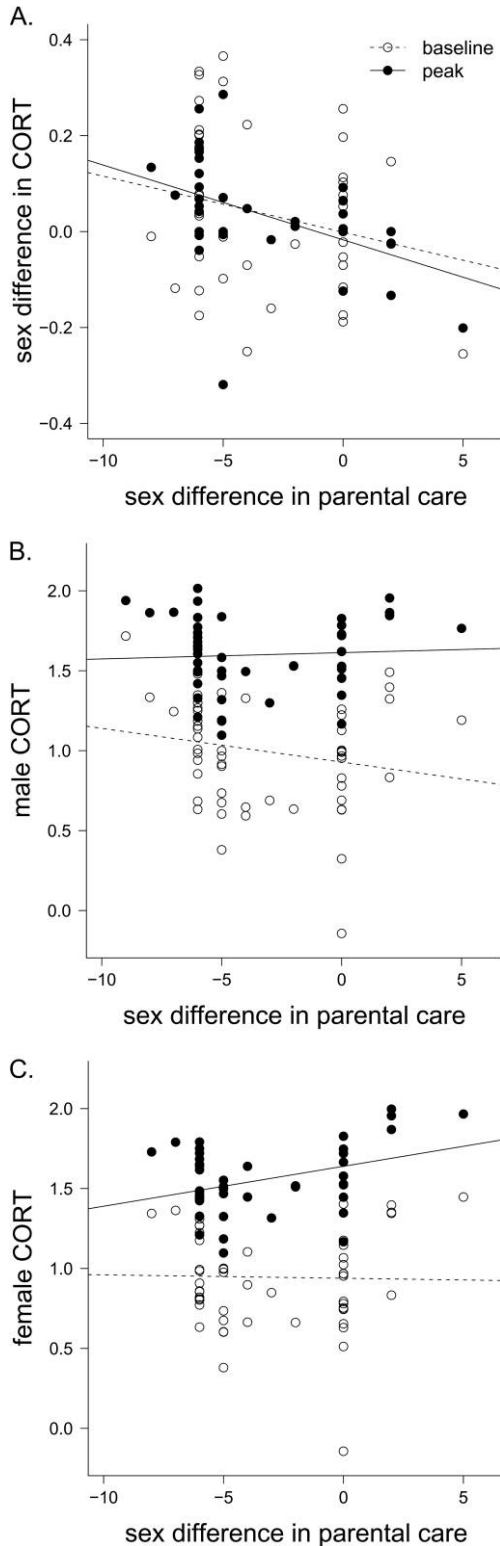
cause the preparative hypothesis predicts higher peak CORT levels when multiple stressors are more likely (Romero 2002), our result might reflect the effect of extreme weather conditions toward the poles (Romero et al. 2000). Alternatively, the latter result may be due to the higher energetic costs demanded by a colder climate (Romero 2002). These intriguing possibilities are all open to testing in future comparative studies and experiments.

As with all studies of plasma hormone levels, it should be borne in mind that the effect of circulating CORT may be either enhanced or attenuated by various internal factors such as the concentration and affinity of corticosterone-binding globulins (CBG) and CORT receptors in the target tissues (Romero 2004). Because of these complexities, hormone levels have been considered to be difficult to interpret as species-specific traits, and, up until now, phylogenetically controlled interspecific comparisons have used only some rank measures of CORT levels such as their qualitative differences across seasons (Romero 2002) or between high- and low-ranking individuals (Goymann and Wingfield 2004). In our study, we used plasma CORT concentrations per se as a proxy for the biological impact of CORT; therefore, the validity of our results and conclusions depend on the extent to which circulating CORT predicts biological impact. While the above-mentioned regulators of stress response may well differ across species (Breuner and Orchinik 2002), several lines of evidence suggest that hormone titers probably coevolve with downstream endocrine components, such that plasma levels may be viewed as a relatively easily measurable manifestation of the phenotypic variation in the underlying endocrine machinery that also determines responsiveness (reviewed by Williams 2008). Our findings provide further support for the functional significance of circulating CORT per se. First, despite the tremendous differences in technical details across studies and potentially in CBG or receptor levels across species, both baseline and peak measures of circulating CORT proved to be highly repeatable and reliable measures of the species, evolving almost without phylogenetic inertia. Second, these species-specific CORT levels show significant evolutionary relationships with relevant life-history traits that are known to influence intraspecific variation in CORT levels. Finally, the species levels of baseline and peak CORT are correlated with different ecological and life-history variables, conforming to the recent idea that baseline and peak CORT may be considered to be two different hormones because they interact with different receptors and have different physiological effects (Romero 2004). Nevertheless, there is still much noise in our data, as inferred from both the figures and the relatively wide effect-size estimates, which may be due at least in part to differences in downstream regulators. As knowledge on CBGs and CORT receptors becomes

Figure 2: Sex differences in parental care (greater values indicate more male-biased care) in relation to sex differences in corticosterone (CORT) levels ($\log_{10}$ [male CORT/female CORT]; *A*), to male CORT level (*B*), and to female CORT level (ng mL^{-1}; $\log_{10}$ transformed; *C*).

available for more and more species, forthcoming comparative studies should carefully assess the impact of these modulating factors on the interspecific variation in stress response.

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Capturing and bleeding Nuttall's white-crowned sparrows at Bodega Bay Marine Station, part of the University of California Reserve Systems (photographs by John C. Wingfield).