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Hormonal correlates of siblicide in Nazca boobies: support for the Challenge Hypothesis

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Abstract

The androgen hormone testosterone (T) mediates vertebrate aggression in many contexts and according to the Challenge Hypothesis is up-regulated during social challenges. While originally applied to challenges experienced by breeding adults, we show for the first time that T is similarly up-regulated during deadly sibling aggression in young birds. When two nestling Nazca boobies hatch, one—usually the older chick—virtually always kills the other chick by pushing it from the nest. We compared concentrations of T, dehydroepiandrosterone (DHEA; a precursor of T), and corticosterone (Cort; a stress hormone) of chicks at various stages. T was elevated during fights in both chicks in two-chick broods, but not before and after fights, and not in chicks lacking a nest mate. DHEA was elevated 1 day after hatching and declined with age but appeared not to vary in concert with aggression. Cort did not vary across fighting and nonfighting periods. In conjunction with an earlier study [Tarlow, E.M., Wikelski, M., Anderson, D.J., 2001. Hormonal correlates of siblicide in Galápagos Nazca boobies. Horm. Behav. 40:14–20], these results indicate that T is temporarily up-regulated around the time of fights, as predicted by the Challenge Hypothesis. Our data suggest a general role for T during challenges at any time in life, not just during breeding.

Keywords: Aggression; Challenge Hypothesis; Hormonal regulation; Testosterone; DHEA; *Sula granti*

Introduction

Sex steroids, most notably testosterone (T), have been implicated in the regulation of aggression in many vertebrate species (Schwabl, 1993; Soma et al., 1999; Wingfield et al., 1987), including birds. How exactly T mediates aggression is still under debate, but one way aggression is enhanced by T is through its stimulation of the arginine vasotocin system, which itself has been shown to mediate aggression (Plumari et al., 2004). High circulating T levels have been shown to be associated with costs to the individual (Wingfield et al., 2001), such as increased energetic costs that come with increased activity levels (Marler et al., 1995; Wikelski et al., 1999a,b). Because maintaining high T levels entails various costs (Ketterson et al., 1996, Schoech et al., 1999), T should be elevated only when needed, a prediction made by the Challenge Hypothesis (Wingfield et al., 1990). The Challenge Hypothesis originally explained differences in T level between male birds of polygynous species that are not involved in parental care and males of monogamous species, which trade off aggressive behavior with parental behavior (Wingfield et al., 1990). Monogamous males respond to a territory intruder by temporarily up-regulating T, while polygynous males can maintain consistently high T (Wingfield et al., 1990). Few studies have tested the Challenge Hypothesis in other contexts, even though young organisms regularly show aggressive behavior as well. In young birds, support is limited to one study of black-headed gull chicks that temporarily elevated T levels during conspecific territory defense (Ros et al., 2002). Another form of aggression in young birds, lethal attacks by siblings (siblicide), has been well documented in several species (pelicans, kittiwakes,

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gulls, boobies, and various raptors; Mock and Parker, 1997), but its endocrinological basis is not well understood. Siblicide is an obvious candidate for testing the Challenge Hypothesis because battling siblings could control aggressive behavior with T using temporary up-regulation of T to minimize its negative effects (Ketterson et al., 1996). In this paper, we test the applicability of the Challenge Hypothesis to obligate siblicide in a seabird, the Nazca booby (*Sula granti*).

To date, studies of siblicidal Nazca boobies (Tarlow et al., 2001), and closely related blue-footed boobies (*Sula nebouxii*; Nuñez-de la Mora et al., 1996, Ramos-Fernández et al., 2000), have failed to show detectable plasma T levels, even when aggression was experimentally induced (Nuñez-de la Mora et al., 1996, Ramos-Fernández et al., 2000). In the earlier investigation of Nazca boobies, we observed one of the sampled chicks attacking its sibling immediately before sampling and that exceptional bird had a T level that was five times that of other chicks (Tarlow et al., 2001). If T is only elevated temporarily around the time of attacks, the Challenge Hypothesis could explain the apparent absence of T in siblicidal chicks that are not attacking at the moment of sampling. To test the relevance of the Challenge Hypothesis to siblicidal behavior, we have therefore focused on gathering appropriately timed samples that were taken during aggression.

Nazca boobies often lay two-egg clutches, with the second egg acting as insurance against the failure of the first egg (Clifford and Anderson, 2001a,b, 2002). If both eggs hatch, the first chick kills the second chick within a few days by pushing it from the nest scrape (Anderson, 1989). Due to hatching asynchrony, chicks in these broods typically differ in age by 4–7 days (Anderson, 1989), giving first-hatching “A-chicks” an overwhelming size and developmental advantage over second-hatching “B-chicks” (Anderson, 1989). Furthermore, while B-chicks show some aggression, A-chicks direct many more attacks at their sibling (median = 72.5) than they receive (median = 3.5; unpublished data). After expulsion, the B-chick dies from starvation, exposure, or predation, unless it finds its way back into the nest: of 1901 two-chick broods that we studied between 1985 and 2001, two chicks attained juvenile plumage in only one nest (1/1901 = 0.0005; Humphries et al., unpublished data). The regularity of siblicide in Nazca boobies, and the extreme nature of this social challenge, makes this species an appropriate model to study the proximate mechanisms underlying aggression, specifically in relation to the Challenge Hypothesis.

Because maintaining a high T concentration can be costly, we were also interested in levels of dehydroepiandrosterone (DHEA), a hormone that can be rapidly converted to T (Hau et al., 2004; Soma and Wingfield, 2001; Vanson et al., 1996). DHEA has been suggested to mediate aggression in territorial wintering adult birds that have low T (Soma and Wingfield, 2001; Soma et al., 2002; Hau et al., 2004) and could be involved in sudden periods of siblicidal aggression in young birds as well. Finally, we measured levels of corticosterone (Cort), a stress hormone that may aid in providing energy for activity (Nelson, 2000) such as aggression. Because DHEA is a precursor of T, we expected to see the DHEA level of siblicidal chicks decline over time due to conversion to T during aggression. As an indicator of stress, Cort level should be highest during aggression.

Hypotheses

For A-chicks in two chick broods, the Challenge Hypothesis predicts elevated T during aggression, but not when they have no nest mate shortly after hatching and 2 days after siblicide. Similar reasoning applies to B-chicks, which attack their nest mate (Tarlow et al., 2001; Clifford et al., unpublished data) and sometimes survive a sick sibling (24/1973 = 0.012 of two-chick broods; Humphries et al., unpublished data). However, since an A-chick represents a greater social challenge to a B-chick than vice versa (because the A-chick has a size and developmental advantage), the Challenge Hypothesis may be taken to predict a higher up-regulation of T level in B-chicks than in A-chicks during aggression. As a control, we included “secondarily single” chicks (from a two-egg nest with only one hatch) in the study (Table 1).

Application of the Challenge Hypothesis to the B-chick’s situation requires the assumption that hyperaggressive behavior produced by a comparatively high T level allows B-chicks to take advantage of a weak sibling when that rare opportunity presents itself. This assumption is incorrect if B-chicks are so inherently overmatched that up-regulation of T would provide no benefit to the B-chick and could even provoke aggression from the A-chick. We therefore considered an alternative “Overmatched Competitor” Hypothesis, assuming that aggression by the B-chick is futile and that A-chicks sometimes predecease B-chicks. This hypothesis predicts down-regulation of T in B-chicks to avoid incurring any costs associated with higher T concentrations and resulting aggressive behavior. Under the Overmatched Competitor Hypothesis, a B-chick avoids aggressive interactions and survives by default only if its sibling dies.

Table 1
Predictions for T, DHEA, and Cort under the Challenge Hypothesis

Hormone	Comparisons
T	$B_{sibl} > B_{d1}$
	$A_{sibl} > A_{d1}, A_{sec}, A_{post}$
	$B_{sibl} > A_{sibl}$
DHEA	$B_{d1}, A_{d1} > B_{sibl}, A_{sibl}, A_{sec}, A_{post}$
Cort	$B_{sibl}, A_{sibl} > B_{d1}, A_{d1}, A_{sec}, A_{post}$

Symbols correspond to A- and B-chicks sample on day 1 (A_{d1}, B_{d1}), A- and B-chicks sampled during siblicidal attacks (A_{sibl}, B_{sibl}), secondarily single A-chicks (A_{sec}) age-matched to aggressive A-chicks, and siblicidal A-chicks resampled 2 days after siblicide (A_{post}).

Materials and methods

We studied Nazca booby hatchlings in December 2000 and January 2001 at Punta Cevallos, Isla Española, Galápagos Islands (89°37′ W, 1°23’ S), the site of our long-term research on this species. For this study, we monitored 70 nests with two-egg clutches, from which 47 were used for blood samples and 23 for behavioral observations. Nests were checked daily for hatched eggs and scheduled blood samples. We monitored 11 two-chick broods after hatch of the second chick for siblicidal attacks, watching these nests closely each day by patrolling among them continually during daylight hours until we observed the A-chick eject the B-chick from the nest. Because only one researcher (EDF) collected blood samples, we took blood sequentially from B- and then A-chicks, rather than simultaneously. Upon observing ejection of the B-chick, we obtained a blood sample from the B-chick (B_{sibl}) and returned it to the nest. We observed that nest continuously until the next ejection, which occurred within 10 min in all 11 nests, and then immediately removed the A-chick for a blood sample (A_{sibl}). This sampling sequence avoided the potential problem of the reverse sequence, in which handling of the A-chick (the principal aggressor) might have influenced its subsequent attacks and compromised our ability to sample B-chicks during the next attack. It is important to note that the B-chick often temporarily returns to the nest after being forced out by the A-chick (Humphries et al., unpublished data), so this sequence of events is not abnormal. Furthermore, we monitored the nests until the B-chick was scavenged to verify that our siblicide samples were all collected during the final ejection bout. We also sampled A-chicks again 2 days after the B-chick’s death (A_{post}, $n = 11$). From other broods, A- and B-chicks ($n = 12$ for each group) provided blood samples on the day after hatching (A_{d1}, B_{d1}) for comparison of baseline hormone levels. Secondarily single A-chicks (A_{sec}), those sharing a nest with a failed egg, were sampled on a day that age-matched them to an A-chick during an attack ($n = 12$). All field procedures were approved by the Wake Forest University Institutional Animal Care and Use Committee.

One hundred to 200 μl of blood were taken for analysis of sex steroid hormones and Cort was collected in less than 3 min in heparinized microcapillary tubes. Samples were then centrifuged in the field at 6000 rpm for 4 min. The plasma was treated with 75% ethanol and kept at ambient temperature for 2–3 weeks before being taken to Wake Forest University. Samples were kept at −20°C until delivery on dry ice to Princeton University, where they were analyzed. Plasma levels of T, DHEA, and Cort were assayed with radioimmunoassay after the separation of each hormone on a chromatography column (Wingfield and Farner, 1975; Wikelski et al., 1999a,b; full methodology is described in Tarlow et al., 2001, 2003). Detection limits were 0.04 ng/ml for T and DHEA and 2.0 ng/ml for Cort. Twenty microliters of tritiated hormone was added to each sample to determine recoveries. Recoveries were as follows (mean ± SD): for T, 83 ± 5%; for DHEA, 58 ± 7%; for Cort, 78 ± 8%.

Because we based our predictions on a large body of data collected from 1985 to 2001, we verified that A- and B-chick interactions were typical of other years (Clifford et al., unpublished data; Humphries et al., unpublished data) by recording aggressive interactions between chicks at 23 nests during 2-h observations. Chicks strike one another with closed-beaked jabs as well as open-beaked pushes, two aggressive behaviors that were combined in analyses. We did not collect behavioral data at nests observed for siblicide blood samples since sampling could have affected behaviors.

Nonparametric tests were used because the data failed the test of homogeneity of variances. Furthermore, because some of the samples are nonindependent (A- versus nest mate B-chicks during attacks, and siblicidal A-chicks during attacks versus 2 days after siblicide), each pairwise comparison was conducted separately. Each comparison between independent samples was a univariate Mann–Whitney U test, and those between dependent samples were Wilcoxon paired sign tests. To correct for increased probability of Type I errors from multiple comparisons, we used the false discovery rate method (Benjamini and Hochberg, 1995; Curran-Everett, 2000; Garcia, 2003). The procedure requires comparisons be ordered by decreasing P values and then compared to the α_{crit}, denoted d_i, beginning with the largest P value. The d_i is calculated by dividing the specific comparison i by the total number of comparisons n and then multiplying by the false discovery rate (the expected proportion of null hypotheses mistakenly rejected (Benjamini and Hochberg, 1995; Curran-Everett, 2000). If the achieved significance level is less than the d_i for a given comparison, then the null hypothesis is rejected for that comparison and all remaining comparisons (Benjamini and Hochberg, 1995, Curran-Everett, 2000).

Results

Behavior

Of 23 nests providing 2 h of behavioral data each in this study, A-chicks struck B-chicks significantly more times than B-chicks struck A-chicks (A-chick median: 2 attacks/h, range 0–12; B-chick median: 0 attacks/h, range 0–7; Wilcoxon-matched pairs: $T = 10.5$, $df = 23$, $P = 0.0007$). These interactions were comparable to those seen in other breeding seasons, where A-chicks attacked more than B-chicks (Clifford et al., unpublished data; Humphries et al., unpublished data).

Testosterone

As predicted by the Challenge Hypothesis, both A- and B-chicks had significantly higher T levels during attacks than newly hatched A- and B-chicks did, respectively (Fig. 1A, Table 2). A-chicks also had significantly higher T levels during attacks than they did 2 days postsiblicide (Fig. 1A, Table 2); B-chicks were not available for sampling after siblicide due to predation and scavenging. Finally, T levels of secondarily single A-chicks (controls with no opportunity for aggression) were significantly lower than those of age-matched siblicidal A-chicks but similar to those of A-chicks at day 1 and 2 days postsiblicide (Fig. 1A, Table 2).

B-chicks had significantly higher levels of T than A-chicks did during aggression (Fig. 1A, Table 2), as predicted by the Challenge Hypothesis but not by the Overmatched Competitor Hypothesis. B-chicks also had higher T levels 1 day after hatching than A-chicks did (Fig. 1A, Table 2).

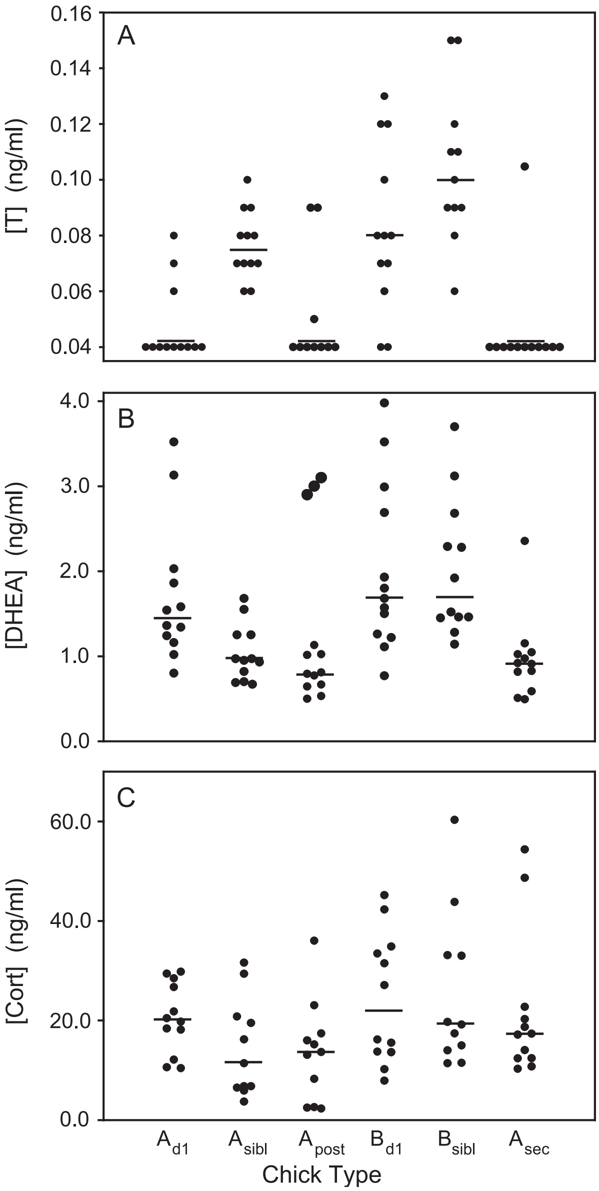

Fig. 1. Plasma testosterone (A), DHEA (B), and Cort (C) concentrations for all chick types described in Table 2. Horizontal lines indicate medians; each point shows the hormone concentration of an individual chick.

DHEA

Under the assumption that DHEA is converted to T when needed, we predicted that DHEA level should decrease with age within two-chick broods. Newly hatched A-chicks, which did not yet have a hatched sibling, did have significantly higher DHEA levels than siblicidal A-chicks during attacks, but levels in hatchling B-chicks did not differ from those taken during attacks (Fig. 1B, Table 2). A-chick levels during attacks and 2 days after siblicide did not differ (Fig. 1B, Table 2). DHEA level of A-chicks declined significantly from day 1 until 2 days postsiblicide (Fig. 1B, Table 2), indicating an overall decline in that hormone's level due principally to the significant decline between day 1 and siblicide. DHEA level in nonaggressive secondarily single A-chicks did not differ from that of age-matched siblicidal A-chicks (Fig. 1B, Table 2).

A- and B-chicks did not differ in DHEA level at day 1, but during aggression B-chicks had significantly more DHEA than did A-chicks (Fig. 1B, Table 2).

Cort

We found no significant differences (all P values were >0.05) in Cort in any of the comparisons listed in Table 2 (Fig. 1C).

Discussion

Testosterone

In three other studies of boobies, plasma T levels outside periods of aggression were low and did not differ among chicks (Nuñez-de la Mora et al., 1996, Ramos-Fernández et al., 2000, Tarlow et al., 2001). None of these studies attempted to synchronize blood sampling with aggression, and we suggested that T might nonetheless mediate aggression if T varies on a short time scale (Tarlow et al., 2001). By closely monitoring two-chick Nazca booby broods, we were able to collect blood during siblicidal aggression and to compare plasma hormone levels during aggression to those from chicks sampled before siblicide (day 1 A- and B-chicks) and afterward (A-chicks 2 days after siblicide). Supporting the idea that T is elevated only during aggression, we found that siblicidal A-chicks had significantly higher T levels during attacks than on day 1, and that A-chick T level returned to baseline 2 days postsiblicide (Fig. 1A). Furthermore, T level of secondarily single chicks (sharing original clutch size, status, and age, but not aggressive behavior, with siblicidal A-chicks) matched that of day 1 and postsiblicidal A-chicks but was significantly lower than that of A-chicks during attacks (Fig. 1A). Our refinement of the Challenge Hypothesis predicted that the T level of chicks at the precise time of aggression would be higher than

Table 2
Post hoc comparisons of T and DHEA serum concentrations of chicks from two-egg nests

T						DHEA					
Comparison	*U*/*T*	*z*	df	*P*	d_i	Comparison	*U*/*T*	*z*	df	*P*	d_i
$\mathrm{B_{d1}}$–$\mathrm{B_{sibl}}$	65.0	0.40	12,12	0.69	0.05	$\mathrm{A_{Sibl}}$–$\mathrm{A_{post}}$	30.0	0.27	11	0.79	0.05
$\mathrm{A_{sec}}$–$\mathrm{A_{d1}}$	61.5	0.61	12,12	0.54	0.044	$\mathrm{A_{d1}}$–$\mathrm{B_{d1}}$	63.0	0.52	12,12	0.60	0.04
$\mathrm{A_{sec}}$–$\mathrm{A_{post}}$	55.0	0.68	12,11	0.50	0.039	$\mathrm{A_{sibl}}$–$\mathrm{A_{sec}}$	59.0	0.75	12,12	0.45	0.03
$\mathrm{B_{d1}}$–$\mathrm{B_{sibl}}$	30.0	2.42	12,12	**0.015**	0.033	$\mathrm{A_{sec}}$–$\mathrm{A_{post}}$	49.0	1.05	12,11	0.30	0.03
$\mathrm{A_{Sibl}}$–$\mathrm{A_{post}}$	4.0	2.58	11	**0.010**	0.028	$\mathrm{A_{Sibl}}$–$\mathrm{A_{post}}$	9.0	2.13	11	0.033	0.02
$\mathrm{A_{d1}}$–$\mathrm{B_{d1}}$	27.0	2.60	12,12	**0.006**	0.022	$\mathrm{A_{d1}}$–$\mathrm{A_{sibl}}$	28.0	2.54	12,12	**0.011**	0.02
$\mathrm{A_{sibl}}$–$\mathrm{B_{sibl}}$	1.0	2.85	12	**0.003**	0.017	$\mathrm{A_{sec}}$–$\mathrm{A_{d1}}$	20.0	3.00	12,12	**0.003**	0.01
$\mathrm{A_{d1}}$–$\mathrm{A_{sibl}}$	12.5	3.44	12,12	**0.0006**	0.011	$\mathrm{A_{sibl}}$–$\mathrm{B_{sibl}}$	0.0	3.06	12	**0.002**	0.01
$\mathrm{A_{sibl}}$–$\mathrm{A_{sec}}$	12.0	3.46	12,12	**0.0005**	0.006	$\mathrm{A_{d1}}$–$\mathrm{A_{post}}$	6.0	3.69	12,11	**0.0002**	0.006

For comparisons of A-chick samples collected serially over time ($\mathrm{A_{sibl}}$–$\mathrm{A_{post}}$), and siblings during attacks ($\mathrm{A_{sibl}}$–$\mathrm{B_{sibl}}$), Wilcoxon's matched pairs tests were used, yielding the *T* statistic with a single term for degrees of freedom. For the other comparisons, not involving a within-individual design, a Mann–Whitney *U* test was used, yielding the *U* statistic with two terms for degrees of freedom. Comparisons are presented sorted by *P* value, and d_i was calculated using the false discovery method. Statistically significant *P* values, as identified by the false discovery method ($P < d_i$; see Materials and methods), are shown in bold.

levels of those not exhibiting this type of behavior. Our results, which found high T during aggression, and those of Tarlow et al. (2001), which found no detectable T when sampling at random times from two-chick broods, support this prediction. Experimental hormone implants can confirm whether T up-regulation directly mediates siblicidal behavior in Nazca booby A-chicks, a result that would ultimately confirm the Challenge Hypothesis.

During attacks, B-chick T level was significantly higher than that of B-chicks at day 1, adding further support for the Challenge Hypothesis. Comparing siblings revealed that B-chicks had significantly higher T levels than A-chicks, both during aggression (as predicted by our interpretation of the Challenge Hypothesis and not by the Overmatched Competitor Hypothesis) and when aggression was absent on day 1 (not predicted by the Challenge Hypothesis). We argued that A-chicks represent a larger social challenge to B-chicks than B-chicks do to A-chicks, which may explain why B-chicks have higher T than A-chicks at both day 1 and during attacks. A-chicks pay little cost by allowing only occasional, temporary up-regulation of T because an attack by the B-chick when the A-chick's T is down-regulated has little chance of affecting the A-chick, given a healthy A-chick's inherent competitive advantage. However, the challenge perceived by a hatchling B-chick, that of a larger, older siblicidal nest mate, may be so extreme that B-chicks are selected to maintain continually up-regulated T, and at even higher levels than those of A-chicks, so that they are ready to respond as best they can with even further up-regulation when an A-chick attacks. Under this interpretation, we did not measure the baseline T level of B-chicks because B-chicks could have perceived a sibling's presence and partially up-regulated their T level by the time we sampled them on day 1. If the baseline of B-chicks in the absence of a competitor was the same as that of A-chicks, then the scope of up-regulation by B-chicks considerably exceeded that of A-chicks. Alternatively, B-chicks could hatch at a higher baseline (perhaps due to maternal manipulation of egg contents; see below) that is indicated by their day 1 sample. In that case, A-chick up-regulation would exceed that of B-chicks. We can discriminate between these two possibilities in the future with blood samples from B-chicks (hatching from second eggs) lacking siblings.

Another possibility to explain differences in T levels between chick types involves our sampling method. While B-chicks were sampled immediately after expulsion from the nest, A-chicks were sampled when B-chicks were expelled from the nest a second time. It is conceivable that the later aggressive interaction is not as challenging because it is already obvious to the A-chick that it can eject the B-chick, and the A-chick partially down-regulated its T level in the min between B-chick and A-chick sampling.

DHEA

To produce such rapid increases in circulating T, we reasoned that presiblicidal chicks might maintain a supply of precursor to T for conversion (Hau et al., 2004; Soma and Wingfield, 2001; Vanson et al., 1996). Because DHEA blood concentrations were an order of magnitude higher than those of T, conversion of only small proportions of the DHEA pool could be required to account for the observed T concentrations during siblicide. Clearly, the DHEA pool could provide an abundant supply of T precursor, on a molecule-for-molecule basis; however, it remains unresolved whether the DHEA pool actually affects T level in Nazca booby chicks during siblicide.

DHEA level declined with age in A-chicks from two-chick broods and also in secondarily single chicks. We interpret these results to indicate that first-hatching chicks from two-egg clutches hatch with similar DHEA levels, which undergo an invariant ontogenetic decline (Nelson, 2000) that is also observed in chicks from single-egg clutches (Ferree et al., unpublished). We failed to detect a similar change in DHEA between B-chicks on day 1 and during siblicide, but B-chicks are expelled only a few days after hatching, which perhaps is not enough time to reveal an age-related decline.

While relative levels of DHEA do not suggest a direct influence on aggression, DHEA levels in Nazca booby chicks were higher than those reported in studies of territorial adult birds (Fig. 1B; Soma et al., 2002; Soma and Wingfield, 2001; Hau et al., 2004) and showed similar patterns with T. In territorial, nonbreeding song sparrows and spotted antbirds, DHEA levels were several times greater than T levels during simulated territory intrusions (song sparrow, 0.8 ng/ml DHEA versus <0.1 ng/ml T; Soma et al., 2001, 2002; spotted antbird, 0.6 ng/ml DHEA versus <0.1 ng/ml T; Hau et al., 2004). Furthermore, DHEA implants (to 3 ng/ml) in song sparrows increased song output as well as T level (to 0.33 ng/ml) during territory intrusions while DHEA itself declined as intrusions proceeded (Soma et al., 2002). Both studies indicated that DHEA is involved in nonbreeding aggression by providing a precursor to T, as we have suggested.

Cort

We predicted that Cort levels would be elevated during stressful periods, such as sibling aggression. In several species, Cort increases during times of food shortage (Nuñez-de la Mora et al., 1996, Kitaysky et al., 1999), perhaps as a mechanism to induce feeding (begging in chicks, Kitaysky et al., 2001; feeding in migrants, Tsipoura et al., 1999). Cort level also is correlated with social status, but not in a consistent manner: dominant wild dogs show high Cort (Creel et al., 1997), while subdominant mice, rats, and primates have higher levels (Nelson, 2000). In experimental studies, the relationship between Cort and aggression is also relatively unclear. In tree sparrows, Cort implants did not affect T level or aggression (Astheimer et al., 2000); and in breeding greylag geese, T and Cort were affected by status and pair-bond challenges independently of each other (Hirshenhauser et al., 2000).

We predicted that aggressive A- and B-chicks would have higher Cort levels than chicks would on day 1 and than older, nonaggressive A-chicks (secondarily single A-chicks and A-chicks 2 days after siblicide) would. We also predicted that B-chicks, at a competitive disadvantage to their sibling, would have higher Cort levels than their siblings would on day 1 and during aggression. Instead, we found no difference among chicks in Cort levels (Fig. 1C), indicating that plasma Cort was probably not involved in mediating aggressiveness during our study. However, it is also conceivable but not tested here that levels of Cort binding globulins (CBGs) or Cort receptors differ between chick types (Breuner and Orchinik, 2001).

In three other studies on boobies, Cort level was higher in subordinates (B-chicks) than in dominants (A-chicks) (Nuñez-de la Mora et al., 1996, Ramos-Fernández et al., 2000, Tarlow et al., 2001), although in our initial investigations of Nazca boobies, chicks were not sampled during aggression (Tarlow et al., 2001). However, the nonsignificant trend in this study of higher Cort levels in both day 1 and siblicidal B-chicks than in corresponding A-chicks matches that in previous studies. Future manipulations that also take into account nutritional status could help clarify the role of Cort in sibling competition in boobies.

Few other studies investigated Cort in hatchling birds but all documented robust stress responses in chicks (Love et al., 2003b; Müllner et al., 2004; Sockman and Schwabl, 2001). However, hatching order may or may not effect baseline Cort (no effect in kestrels; Sockman and Schwabl, 2001; hatching order effect in canaries and kestrels; Love et al., 2003a; Schwabl, 1999). The long-term consequences of elevated Cort during early ontogeny are largely unclear but could be linked to differential survival as documented for hoatzin chicks in tourist-exposed areas (Müllner et al., 2004).

How is T up-regulated?

Behavioral research to date has shown that steroid hormone levels can be influenced by maternal effects as well as by social cues. In our study, while A- and B-chicks appear to up-regulate T during siblicide, we also found that B-chicks had higher T levels than A-chicks both 1 day after hatching and during aggression. The high level of T in B-chicks compared to A-chicks might be due to greater up-regulation by the B-chick itself in response to a competitor, or possibly a result of manipulation by the mother via the egg contents (Schwabl, 1993), such that B-chicks have a higher baseline T level. However, it should be clear that hormones in eggs are probably not directly linked to plasma hormones after hatching. It is expected that hormones in eggs have more of an organizational role on posthatching hormone levels (Schwabl, 1996). In canaries and American kestrels, for example, levels of maternally derived T increase in successive eggs (Schwabl, 1996; Sockman and Schwabl, 2000); while in storks and cattle egrets, first-laid eggs had elevated testosterone (Sasvari et al., 1999; Schwabl and Mock, 1997). The characteristics of the latter two species could be related to frequent sibling aggression seen in these species, with high androgen levels possibly facilitating efficient reduction in brood size by the elder chick. Our results raise the possibility that mothers allocate more androgen hormones to the otherwise disadvantaged B-chick. Evaluation of the androgen contents of Nazca booby eggs is now needed to test this idea.

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