

# Hormonal Correlates of Siblicide in Galápagos Nazca Boobies

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Nazca boobies (*Sula granti*) show unconditional obligate siblicide immediately after hatching, reducing the typical two-egg clutch size to one. We studied body mass changes and levels of testosterone (T), corticosterone (CORT), and progesterone (P) for A-chicks (dominant, first hatched), B-chicks (subordinate, second hatched), and singletons, during the first 7 days after hatching, when siblicide normally occurs. Mass increase with age was higher for A-chicks than for singletons and B-chicks. This exaggerated the existing developmental advantage of A- over B-chicks that is due to hatching asynchrony. In nests with two chicks, CORT titer was significantly higher in B-chicks than in A-chicks. During ontogenetic development, CORT decreased with age for A-chicks, but did not change for singletons. P showed qualitatively similar ontogenetic changes to CORT, remaining unchanged for A-chicks but increasing for singletons. Thus, both CORT and P levels were lower for A-chicks than for singletons, and both hormones varied inversely with body mass. Overall, T levels did not differ between different categories of chicks. However, one B-chick in the process of reversing the dominance relationship with its older, but weakened, sibling had significantly elevated T. We suggest that CORT and P are regulated to promote exaggerated mass gain in socially challenged A-chicks, facilitating siblicide. Whether T induces aggressiveness during short time intervals of intense sibling rivalry needs further attention. © 2001

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Low hatching success in some bird species selects for the production of extra “insurance” eggs, in spite of their cost, to increase the probability of hatching at least one egg (Dorward, 1962; Clifford and Anderson, in press). These species frequently lay and hatch two eggs per clutch, but persistent attacks by one hatchling on the other prevent fledging of more than one chick (Mock and Parker, 1997). Hatching intervals are long in these species, giving the first hatched A-chick a size and developmental advantage over the second hatched chick, facilitating obligate siblicide (Rowe, 1947; Cash and Evans, 1986; Anderson, 1989). Although the insurance egg hypothesis and food limits on brood size (e.g., Nelson, 1978, for obligately siblicidal boobies) provide a satisfactory ultimate explanation for obligate siblicide behavior, regulation at the proximate level is largely unknown. We hypothesize that steroid hormones mediate differential developmental changes between chicks, as well as siblicidal behavior itself, in Nazca boobies (*Sula granti*), an obligately siblicidal seabird (Anderson, 1989).

Testosterone (T) and related androgens are often involved in aggressive behavior of vertebrate animals, and many bird species have high levels of circulating sex steroids (Marler, Peters, and Wingfield, 1987; Schwabl, 1993; Hau, Wikelski, Soma, and Wingfield, 2000; Ros, 1999). According to the *challenge hypothesis* (Wingfield, Hegner, Dufty, and Ball, 1990), hormonal induction of aggressive behavior by T should occur when the risk of social competition is high and reverse when the risk is low. Social behaviors are also influenced by hormones other than androgens, including progesterone (P) and corticosterone (CORT). P, for example, may organize the phenotypic expression of

aggression and morphology (Moore and Crews, 1986; Lindzey and Crews, 1993).

In addition to influencing aggressive behavior itself, P and CORT may be involved in body mass regulation (Schneider and Wade, 1987; Cherel, Mauget, Lacroix, and Gilles, 1994). Body mass typically influences the outcome of aggressive competitions among nestling birds (Mock and Parker, 1997) and these hormones may account for the observed increase in body mass of Nazca booby A-chicks over singletons. CORT levels can increase after aggressive encounters and in response to stress (Harvey, Phillips, Rees, and Hall, 1984; Romero, Ramenofsky, and Wingfield, 1997). CORT levels may also increase as a result of food competition (Schwabl, Ramenofsky, Schwabl-Benzinger, Farner, and Wingfield, 1988) or food deprivation and are correlated with submissive behaviors in subordinate (B) chicks in blue-footed boobies (Nunez de la Mora, Drummond, and Wingfield, 1996; Ramos-Fernandez, Nunez de la Mora, Wingfield, and Drummond, 2000).

In this paper we investigate endocrine levels of obligately siblicidal Nazca booby chicks during the period of siblicide (A-chick age, 0–7 days). Partly extending the logic of the challenge hypothesis (Wingfield *et al.*, 1990), we predicted (i) that CORT and P are indirectly involved in siblicide through an increase in the developmental advantage (mass) of first hatched chicks, while (ii) immediate siblicidal behavior is directly regulated by high T levels.

Nazca boobies hatch at long intervals (typically 5–6 days), and the A-chick attacks the B-chick by pushing it out of the nest, within days of its hatching, resulting in the B-chick's death through starvation or heat exposure (Anderson, 1989). The rapid siblicide is due in part to the long hatching interval causing dramatic size differences (Anderson, 1989), but other factors also promote the aggression. Interspecific cross-fostering experiments indicate that Nazca booby hatchlings are inherently more aggressive than are closely related blue-footed booby hatchlings (Lougheed and Anderson, 1999), which do not show obligate siblicide among hatchlings. Nazca booby hatchlings also apparently alter their growth trajectories facultatively in response to a sibling's presence, gaining mass more quickly than singleton hatchlings until siblicide occurs. Siblicide occurs, on average, at B-chick age 1.8 days. Due to interyear variability, two-egg clutches comprise 43–66% of all clutches (Anderson, 1990). Both eggs hatch in 29–52% of two-egg clutches, therefore 12–34% of clutches produce two hatchlings (Anderson, 1990). Both chicks and parents have regu-

latory roles in brood reduction (Lougheed and Anderson, 1999). Parent Nazca boobies facilitate the ejection of the B-chick by laying eggs at long intervals, perhaps using flat nest sites (Anderson, 1995), and failing to interfere in acute sibling rivalry (Anderson, 1989). Despite the overwhelming competitive advantage of A-chicks, they die before their sibling in about 5% of two-chick broods due to accidents and starvation (Anderson, 1990).

The Nazca boobies of the Galápagos have traditionally been considered masked boobies (*Sula dactylatra granti*), but recent analyses of morphological and breeding data (Pitman and Jehl, 1998) and of mtDNA differentiation (Friesen *et al.*, submitted for publication) support elevation of the Galápagos and nearby populations to species status. In this paper we follow the nomenclature of Pitman and Jehl (1998) and refer to these birds as Nazca boobies.

## METHODS

We studied a colony of ca. 10,000 Nazca boobies at Punta Cevallos, Isla Española (89° 37' W, 1° 23' S) in the Galápagos Islands during December 1998 as part of a long-term study of breeding biology. Nests were checked daily to record date of hatching and siblicide (as described before in, e.g., Anderson, 1989). We referred to a chick's day of hatching as age 0 and categorized chicks of ages 0–3 days as "young" and those of 4–6 days of age as "old." With one exception (see below), no B-chick survived beyond 3 days of age. Our sample included a total of 11 sibling pairs (A- and B-chick both alive), 12 young and 9 old singletons, 6 young A-chicks (A-chick with a B-egg), and 7 old chicks with an egg that did not hatch (referred to as "secondarily single" chicks). Each individual was sampled only once, so that all young chicks are different individuals from old chicks. Young B-chicks and old A-chicks were always taken from the same two-chick nests; otherwise, all chicks were taken from different nests. Young singletons were on average 0.8 ( $\pm 1$  SD) days old, young A-chicks 0.5 ( $\pm 0.5$ ) days, and young B-chicks 0.5 ( $\pm 0.5$ ) days. Old singletons, A-chicks, and secondarily single chicks all had on average an age of 5.2 days ( $\pm 0.6$ ,  $\pm 0.7$ , and  $\pm 0.5$  days, respectively). We excluded one sibling pair in which the 5-day-old A-chick sibling died as a result of being expelled from the nest by its 1-day-old B-chick sibling from all analyses except of T. The sex of the chicks was not determined.

In all cases, chicks were carefully removed from the nest and blood samples were obtained by puncturing the superficial brachial wing vein with a 26-gauge needle. Blood was collected in heparinized microcapillaries. The procedure was completed within 3 min. Chicks were weighed with spring scales (precision, 2 g) and then quickly returned to the nest. Blood was kept cool (4°C) until centrifugation at  $588 \times 10^2$  m/s for 4 min. Plasma was separated and treated with 10  $\mu$ L of B-propiolactone solution to destroy viruses, according to U.S. import regulations for avian blood. Plasma was then stored at  $-20^\circ\text{C}$  and transported on dry ice to the University of Illinois for hormone analysis.

We took 100–300  $\mu$ L blood (maximally 1.5% of chick mass) for the determination of hormones. We used 15  $\mu$ L of plasma for analyses of CORT and the remaining plasma for P and T. Plasma levels of CORT were determined with an indirect radioimmunoassay (RIA) as described in Wingfield and Farner (1975; Hau, Wikelski, and Wingfield, 1997). Plasma levels of P and T were determined by RIA after separation of hormones on chromatography columns. In short, aliquots of about 100  $\mu$ L of plasma were equilibrated with 2000 cpm of  $^3\text{H}$ -labeled hormone overnight at 4°C, extracted with dichloromethane (CORT) or anhydrous ethyl ether (P, T), dried in a 40°C water bath under nitrogen gas, and redissolved in 550  $\mu$ L of buffer. Samples were allowed to equilibrate with buffer overnight at 4°C. Fractions of 200  $\mu$ L were taken for duplicates used in the RIA as described previously (e.g., Wingfield and Farner, 1975; T antibody, T3003, and P antibody, P-1604, both Wien Laboratories, Succasunna, NJ 07876; CORT antibody, B3-163 Endocrine Sciences, Calabasas, CA 91301). Fractions (100  $\mu$ L) were directly counted for the determination of recovery. Mean ( $\pm$ SE) recovery was  $62 \pm 2.2\%$  for T,  $80 \pm 1.8\%$  for CORT, and  $53 \pm 2.0\%$  for P. Two 400- $\mu$ L aliquots of distilled water (water blanks) and a total of four 400- $\mu$ L aliquots containing 0.15, 0.25, or 0.50 ng of non-radioactive hormone standards were taken through the whole assay procedure to estimate non-specific interference, assay accuracy, and intra-assay variation. Blanks were below the detection limit, and the accuracy of the hormone standards was 6% for T, 12% for P, and 9% for CORT. The intra-assay variation between two RIAs was 5% for T and 12% for P. The assay sensitivity was at 0.18 ng/mL for T, 3 ng/mL for CORT, and 0.20 ng/mL for P.

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search, and adheres to NIH standards for the use of animals in research.

### Statistical Analysis

Data were processed and analyzed with SPSS for Windows (SPSS Inc., Chicago), using a General Linear Model (GLM) to determine overall variance within each data set. Posthoc least squares difference (LSD) tests were used to analyze differences between chicks of different type. Significance of tests was accepted at the  $\alpha = 0.05$  level. The potential effect of body mass on hormone levels was tested by including body mass as a covariate; no such effect was detected except for CORT in young chicks (see below).

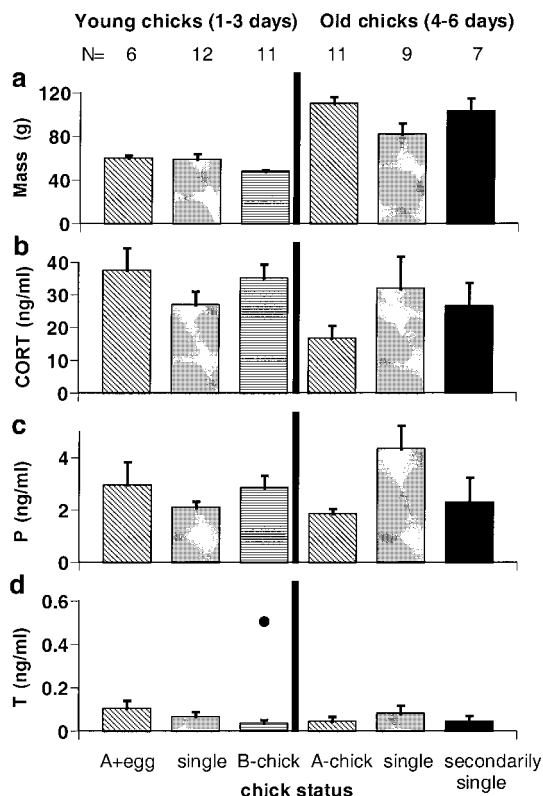
## RESULTS

### Body Mass

The body masses of chicks of different age and status differed significantly (Fig. 1a; GLM,  $F(4, 51) = 19.8$ ,  $P < 0.001$ ; for chick type,  $P = 0.04$ ; for age,  $P < 0.001$ ; for chick type (A, single)  $\times$  age (young, old),  $P = 0.03$ ). Posthoc tests showed that young B-chicks were significantly smaller than young A-chicks and singletons (LSD posthoc tests,  $P < 0.01$  in both cases) but young A-chicks and singletons did not differ. Note that young A-chicks shared a nest with an egg, not a hatchling. Among old chicks, the mass of A-chicks and of secondarily single chicks exceeded that of singletons (LSD posthoc tests,  $P < 0.05$ ). Secondarily single chicks (those chicks that had a potential challenge indicated by an egg) had a body mass similar to that of old A-chicks.

### Corticosterone

As for body mass, chicks of different status and age showed significant differences in their levels of plasma CORT. Among young chicks, singletons tended to have lower CORT levels than A- or B-chicks, although this was only significant if we included body mass as a covariate (for young chicks only: GLM,  $F(5, 29) = 4.8$ ,  $P = 0.004$ ; for chick type,  $P = 0.02$ ; for mass,  $P < 0.001$ ; for interaction of mass and chick type,  $P = 0.03$ ). Old chicks did not differ in CORT levels between chick types (Fig. 1b). While CORT decreased with age in A-chicks (LSD posthoc tests,  $P = 0.02$ ), it remained at the same level in young and old singletons, explaining the significant interaction term. In



**FIG. 1.** Differences between chicks of different ages and status for mass (a), corticosterone (b), progesterone (c), and testosterone (d). Standard error bars represent means  $\pm$  95% confidence intervals. The left side of the dividing bar represents young chicks and the right side represents old chicks. Sample size is indicated in (a). The filled circle in (d) refers to the single case of a B-chick that expelled its older, A-chick sibling from the nest.

two-chick clutches, B-chicks had significantly higher CORT than their older A-chick sibling (LSD posthoc tests,  $P = 0.05$ ).

### Progesterone

Overall, P showed a pattern similar to that of CORT, differing between chicks of different age and status (Fig. 1c; GLM,  $F(4, 51) = 3.5$ ,  $P = 0.01$ ; for chick type (A, single)  $\times$  age (young, old),  $P = 0.004$ ). P level did not differ among young chicks. While old and young A-chicks did not differ, singleton P levels increased with age (LSD posthoc tests,  $P = 0.02$ ). P of secondarily single chicks had similar levels to those of old A-chicks.

### Testosterone

We found no significant difference in T between chicks of different type and age (Fig. 1d). However, in

the single case of a B-chick that was sampled in the process of reversing the preexisting dominance relationship with its older, but weakened, A-chick sibling, the B-chick had a markedly elevated T level, about five times that of any other chick (0.59 ng/mL).

## DISCUSSION

Nazca booby chicks showed endocrine changes consistent with the hypothesis that steroid hormones may be involved in the regulation of fatal social interactions. Siblicide may be facilitated in two ways: first, A-chicks may increase their body mass beyond “normal” growth trajectories to increase their developmental advantage over B-chicks. Second, during the very act of siblicide, chicks may boost their aggressiveness through the secretion of T, as predicted by the challenge hypothesis (Wingfield *et al.*, 1990). We found hormonal differences between double and single chicks that were consistent with this “developmental advantage” hypothesis. In addition, there was encouraging evidence pointing to an involvement of T during siblicide. However, our data do not yet allow us to determine whether testosterone is necessary for siblicide to occur.

### The Developmental Advantage Hypothesis

A-chicks increased in body mass beyond what would be expected for singletons (Fig. 1a). This result supports and extends results previously found by Anderson (unpublished data). A disproportionate body mass increase in A-chicks could be due to parents supplying more food to a two-chick brood and the A-chick outcompeting its size-disadvantaged, younger sibling. However, secondarily single chicks showed similar body mass increases without having a second chick in the nest. Thus, it is likely that the increase in mass depends more on the A-chick demanding more food from the parents in the face of pending competition by a chick, rather than on direct competition with its sibling. In addition, A-chicks could also change their physiological body mass regulation when facing a sibling challenge. A-chicks could then allocate energy directly into achieving a high body mass while keeping growth in body length at the “normal” rate. This scenario is likely given that long bone (humerus) length trajectories are similar between A-chicks and singletons, but their body mass differs significantly during the time of siblicide (Anderson, unpublished data). Mass asymmetry is

likely to be more important in the outcome of siblicidal attacks than is general size asymmetry (Anderson, 1989).

Consistent with the developmental advantage hypothesis and with the hypothesis that hormones mediate such developmental body mass differences, age-related changes in CORT and P differed between socially challenged A-chicks and singletons. For both hormones, the change in titer with age was more positive in singletons than in A-chicks. Both hormones have been implicated in the regulation of body mass and social interactions (Schneider and Wade, 1987; Cherel, Robin, Walch, Karmann, Netchitailo, and Maho, 1988; Smith, Wingfield, and Veit, 1993; Cherel *et al.*, 1994; Schoech, Mumme, and Wingfield, 1996). In blue-footed booby chicks, a sibling-tolerant congener of Nazca boobies, CORT has been shown to increase when chicks were starved (Nunez de la Mora *et al.*, 1996).

In Nazca booby nests with two chicks, the higher baseline levels of CORT in B-chicks compared to A-chicks is consistent with our hypothesis that B-chicks will be stressed by the presence of A-chicks. The high levels of CORT in the B-chicks may be a direct consequence of food or social competition in the nest: Larger A-chicks outcompete B-chicks either by physically repressing them or by depriving them of food. This possibly leaves the B-chicks frustrated and malnourished. However, considering that young A-chicks also had high CORT levels, another interpretation is that CORT is intrinsically higher in clutches with two chicks and CORT is not socially modulated. These two hypotheses are testable and presently under investigation.

Similar to our results, Ramos-Fernandez *et al.* (2000) found no significant difference in CORT titers of same age blue-footed booby chicks and 17–26% higher levels for younger subordinate chicks than for older dominant chicks. Additionally, Nunez de la Mora *et al.* (1996) found significantly higher (109%) baseline levels of CORT in subordinate blue-footed booby chicks than in dominant or single chicks. However, the overall higher CORT in Nazca booby chicks (17–38 ng/mL, current study) compared to blue-footed booby chicks (6–15 ng/mL, Nunez de la Mora *et al.* (1996) and 17–24 ng/mL, Ramos-Fernandez *et al.* (2000)) is not clearly understood. It is possible that Nazca booby chicks had higher CORT levels because they were sampled during a low food year (La Nina oceanographic conditions in 1998) or that intrinsic levels of CORT are indeed higher for obligatory siblicidal species than for facultative siblicidal species.

Contrary to the current study, some studies have found high levels of CORT and P to increase body mass. For example, Holberton (1999) found an increase in body mass and fat reserves concurrent with elevated CORT during stress in the yellow-rumped warbler. Also, in rats, P has been found to affect lipoprotein lipase, an enzyme in fat cells that mediates the amount of triglycerides taken up from the blood, thus increasing fat storage (Gray and Wade, 1979). Furthermore, Schwabl (1999) found that CORT levels differed between nestling and fledglings, as well as with hatching order in canaries. However, there was no relationship with body mass. Even though, as seen in these studies, CORT and P are not always involved in an increase in body mass, we have strong correlative evidence for that to be the case in Nazca boobies during siblicide. Experimental studies with hormone implants and blockers are currently under way to confirm causal effects of CORT and P in body mass change.

In other studies, CORT and P have been shown to be inversely correlated with body mass. For example, high P may decrease body fat in pregnant golden and Siberian hamsters (Schneider and Wade, 1987). Cherel *et al.* (1994) found that King penguins with low body mass have higher P due to an increased mobilization of body protein. The penguins also show low CORT levels with high body mass, while CORT increases in times of mass loss (Cherel *et al.*, 1988).

Based on these data we hypothesize that CORT and P are directly involved in the regulation of body mass, as both are inversely correlated with changes in body mass. A-chicks may decrease the plasma levels of these hormones during ontogeny to assure success during siblicide. Secondarily single chicks may exhibit intermediate levels (between old A-chicks and old singletons) due to the potential challenge indicated by an egg when young.

### *The Challenge Hypothesis*

So far we have no unequivocal indication that T differs significantly between chicks in a way predicted by the challenge hypothesis: T should increase as aggression between siblings escalates (Wingfield *et al.*, 1990). It is possible that siblicide does not require T because siblicidal aggression is not regulated by T, which may be the case in some situations (i.e., Hunt, Wingfield, Astheimer, Buttemer, and Hahn, 1995). Furthermore, the existing developmental advantage of A-chicks over B-chicks may be sufficient to facilitate siblicide without additional T-induced aggression.

However, in one unusual case of a B-chick outcompeting its A-chick sibling, blood was taken during the aggressive phase. A high T level was found, as expected, in the B-chick, indicating that secretion of T may be a direct, but short duration, response to a social challenge. As such, the hormonal reaction of siblicidal Nazca boobies (T secretion) may be different from sibling-tolerant blue-footed boobies. In blue-footed booby chicks, T was undetectable during a starvation experiment conducted by Nunez de la Mora *et al.* (1996) to test the challenge hypothesis. Also in blue-footed booby chicks, T levels were low and variable, with no significant difference found between age group or status in a study done by Ramos-Fernandez *et al.* (2000). Additionally, during experimental pairings of unrelated chicks, T remained undetectable, despite increased aggression (Ramos-Fernandez *et al.*, 2000). However, if adaptive T induction is in fact a short duration phenomenon, then a meaningful test of the challenge hypothesis in both blue-footed and Nazca boobies requires sampling right at the time of siblicide. T may only be secreted during very specific times to boost persistence in birds facing short periods of high aggression (as suggested by Wikelski, Hau, and Wingfield, 1999, for tropical birds).

Our data offer the following working hypotheses: both low CORT and low P may be involved in the significant body mass increase of socially challenged chicks, i.e., of A-chicks that had a B-chick (or an egg) in their nest. If the difference in body mass between A- and B-chicks is sufficiently large, it is conceivable that no additional hormone action is necessary for the A-chick to expel the B-chick from the nest. However, if one chick must repeatedly and persistently fight against the other chick, then we expect that T levels will also be increased, according to the challenge hypothesis.

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