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Yolk androgen deposition without an energetic cost for female rockhopper penguins: a compensatory strategy to accelerate brood reduction?

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Whether androgen deposition in eggs is physiologically costly for female birds has remained a crucial but unsolved question, despite a broad use of this assumption in functional studies. We tested whether females depositing high androgen concentrations experienced higher mass losses than females depositing low androgen concentrations. Analysing female body mass change during egg formation in rockhopper penguins (*Eudyptes chrysocome chrysocome*), we observed no energetic cost to androgen deposition. Nevertheless, lighter females laid eggs with higher yolk androgen concentrations. This relationship existed only for the second-laid egg (B-egg), but not for the first-laid egg (A-egg). Since the B-egg is usually the first to hatch and the only one to produce a fledging chick, we hypothesize that differential yolk androgen deposition may be an adaptive strategy for females to affect brood reduction.

Keywords: yolk testosterone; yolk androstenedione; female body mass; laying order

1. INTRODUCTION

Yolk androgens of birds influence the outcome of sibling competition through potential effects on embryonic developmental time and on chick begging behaviour, food competitiveness and growth rates (see review in Gil [1]). Since not all females deposit the same amount of these hormones in their eggs, Gil *et al.* [2] suggested that yolk androgen deposition had a cost, incurred either by the females or by their offspring. Functional studies often assume that yolk androgen allocation is costly for females [3,4], but the evidence so far contradicts this assumption. If androgen deposition *per se* is energetically costly, females in good condition should be able to deposit higher androgen levels [5]. In experiments manipulating the pre-

breeding diet, however, it has been shown that high-condition females do not lay eggs with higher yolk androgen concentrations than low-condition females do [6,7]. Similarly to experimental results, correlative data have also shown that high-condition females deposit a lower or equal (but not greater) concentration of androgen in their egg yolks compared with low-condition females [5,8]. However, female mass was measured only once (at any time before, during or even after egg formation) in these studies, which therefore did not test for the energetic cost of yolk androgen deposition, in terms of body mass change.

The cost hypothesis supposes that females experience energetic expenditure during egg formation in relation to the concentration of androgens they deposit in their eggs. Capital breeders acquire their resources in advance and store them endogenously or exogenously until they are needed to supply aspects of offspring production [9]. For these species, if androgen deposition *per se* is energetically costly, we expect females laying clutches of eggs high in yolk androgens to experience higher mass loss during egg formation than females laying clutches low in yolk androgens. Here, we test this prediction on wild rockhopper penguins (*Eudyptes chrysocome chrysocome*), analysing female body mass loss during egg formation according to maternal investment in yolk androgens across the laying sequence. Rockhopper penguin females fast from three weeks before laying until clutch completion. They are capital layers [10].

Rockhopper penguins exhibit brood reduction: two eggs are laid but only one chick usually fledges. They also present a reversed hatching asynchrony [11]: the second-laid egg (B-egg) hatches before the first-laid egg (A-egg). As a result, although both eggs frequently hatch, A-chicks generally die of starvation within days of hatching [12]. The reproductive value of A-eggs is therefore almost negligible, whatever the breeding conditions and the parents' ability to feed chicks. However, the survival and the reproductive value of B-eggs are more likely to vary according to the parents' abilities. Reducing yolk androgen concentrations with laying/hatching order has been proposed to be a tool for female birds to enhance brood reduction in favour of the first chick to hatch (hatching asynchrony adjustment hypothesis, [3]). Accordingly, female rockhopper penguins deposit higher androgen concentrations in B-eggs than in A-eggs [13]. Here, we test whether this difference between A- and B-eggs changes according to the female's future ability to feed chicks (measured as her body mass at clutch completion).

2. MATERIAL AND METHODS

(a) General

We conducted this study during October and November 2008 in a colony of 5000 pairs of rockhopper penguins on New Island, Falkland Islands (Malvinas). Males arrived at the colony first (early October) and established nest sites. Females arrived a few days later for pairing and copulation. After the arrival of the females, we visited study sites daily, initially to mark active nests and subsequently to monitor laying dates. The laying period ran from 25 October to 7 November. Clutch size was invariably two: a small A-egg and a large B-egg.

(b) Data collection

We captured and weighed the females of 60 different active nests once ($n = 15$), twice ($n = 26$) or three times ($n = 19$) at three different periods: after nest establishment ($n = 42$), the day they

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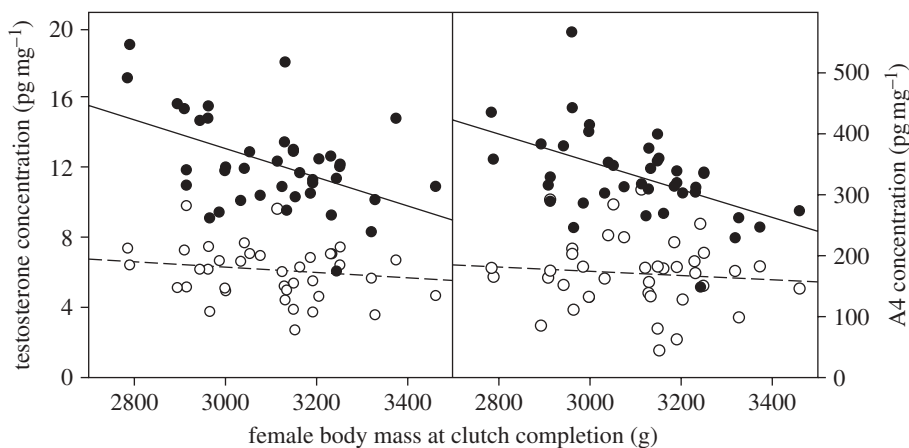


Figure 1. Yolk androgen concentrations in A-eggs (open circles, $n = 39$) and B-eggs (filled circles, $n = 39$) according to female body mass at clutch completion. Solid lines represent slopes significantly different from zero while broken lines represent non-significant slopes. Lines were obtained from a general linear model predicting androgen concentration from an interaction between egg category (A-egg or B-egg) and corrected female body mass.

laid A-eggs ($n = 43$) and the day they laid B-eggs (i.e. clutch completion, $n = 39$).

We collected both eggs of these nests the day they were laid ($n = 60$ A-eggs and 60 B-eggs). To avoid affecting the breeding success of the colony, we replaced the eggs from these nests with eggs found outside their own nest that we considered as lost by their original parents. After collection, we weighed the eggs, froze them whole and prepared them for hormone analyses according to Poisbleau *et al.* [14]. The procedures for extraction and radioimmunoassays of testosterone and androstenedione (A4), which are the two androgens present at the highest concentrations in bird yolk, are described in Poisbleau *et al.* [13].

(c) Statistical analysis

Statistical analyses were conducted with PASW STATISTICS v. 18.0. Female masses were corrected by adding the masses of any eggs already laid, to avoid the potential effect of a relationship between yolk androgen concentration and egg mass. Since indices do not provide more precise indicators of body condition than body mass alone [15], we did not control for structural size in the analyses. Time was defined relative to A-egg laying (day 0) such that captures before laying (i.e. after nest establishment) ranged from day -14 to day -1 and B-eggs were laid on days 4 and 5. Because of the strong correlation between testosterone and A4, the two hormones were analysed separately. To examine changes in female body mass during egg formation, we used linear mixed models predicting corrected female body mass from the yolk testosterone (or A4) concentration in their A- and B-eggs, each interacting with time. To account for the covariation between repeated measurements of the same female, we fitted random intercepts and slopes with respect to time in an unstructured covariance matrix. We also tested whether the deposition of hormones into the egg was related to the corrected mass of the female, using univariate general linear models in which the testosterone (or A4) concentration of an egg was predicted by the mother's mass at clutch completion, interacting with the egg category (A-egg or B-egg).

3. RESULTS

(a) Female body mass loss

Female body mass decreased significantly over the study period (linear mixed model as described, but omitting hormone variables $F_{1,36.833} = 1894.821$, $t_{36.833} = -43.530$, $p < 0.001$). However, this loss was not related to yolk androgen concentration in either A-eggs (testosterone: $F_{1,54.901} = 2.866$, $p = 0.096$; A4: $F_{1,31.178} = 2.096$, $p = 0.158$) or B-eggs (testosterone: $F_{1,50.572} = 1.560$, $p = 0.217$; A4: $F_{1,42.566} = 0.003$, $p = 0.957$). When total androgen amounts were used instead of concentrations, the effect of androgens on female body mass loss remained non-significant (electronic supplementary material).

(b) Androgen deposition as a function of female body mass at clutch completion

Egg category interacted significantly with corrected female mass at clutch completion (testosterone: $F_{1,74} = 4.444$, $p = 0.038$; A4: $F_{1,74} = 4.773$, $p = 0.032$), indicating that A- and B-eggs had significantly different slopes for androgen concentration with respect to female mass (figure 1). Post hoc tests indicated that androgen concentration was significantly negatively related to female mass in B-eggs (testosterone: $t_{74} = -3.717$, $p < 0.001$; A4: $t_{74} = -3.651$, $p < 0.001$) but not in A-eggs (testosterone: $t_{74} = -0.736$, $p = 0.464$; A4: $t_{74} = -0.561$, $p = 0.577$). Analyses for androgen deposition were repeated using total androgen amount (electronic supplementary material). Results were broadly similar, but the interactions between egg category and female body mass narrowly missed significance, indicating that androgen concentration was more closely related to female body mass than was androgen amount.

4. DISCUSSION

Females laying A- or B-eggs with high androgen levels (both concentrations and total amounts) did not suffer a significantly higher or lower mass loss during egg formation than females laying eggs with low androgen levels. These results do not support the idea that yolk androgen deposition has an energetic cost or, on the contrary, could be beneficial for females. They suggest that yolk androgen is not energetically costly for females to deposit and should not be considered as an initial investment for females. However, differences in yolk androgen deposition between and within clutches may embody trade-offs between other costs and benefits experienced by both parents and offspring. Most specifically, they may prepare the entire clutch for future environmental conditions, i.e. to the breeding conditions experienced by females ([16]; but see [17]).

Interestingly, yolk androgen levels in B-eggs were higher for females with low body mass, while in A-eggs, they were independent of female body mass. Yolk androgen levels were thus related to female body

mass only for the egg with the highest expected value, while the A-egg (which was not expected to give a fledging offspring) received yolk androgen levels unrelated to female body mass. In other words, since we verified that B-eggs had consistently higher androgen levels than A-eggs, we observed that the dimorphism in yolk androgen levels between the two eggs increased as female body mass decreased. Given that a female's body mass could indicate her ability to feed her chicks, this result could represent a compensatory strategy [5]. This compensatory strategy would enable a female in poor body condition to improve the quality of the chick with the higher expected value. More importantly, this difference in yolk deposition between eggs probably ensures a quicker brood reduction in females with low body mass. These two roles are not mutually exclusive but they are additive. Since in rockhopper penguins, females are the only parents feeding chicks during the first few weeks, we do not expect male body mass to have a strong impact on this mechanism.

In conclusion, this study provides, to our knowledge, the first conclusive evidence that yolk androgens (at least testosterone and A4) are not energetically costly for females to deposit. We also hypothesize that the difference in yolk androgen levels within a clutch may be adaptive by adjusting the clutch to the female's future ability to feed chicks. We finally suggest that females unable to bear the cost of feeding two chicks could deposit proportionally more androgens in the egg with the highest expected value and accelerate brood reduction at no initial energetic cost to herself. This, of course, would require active deposition of androgens into the eggs, an assumption that still needs to be tested and confirmed.

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