Are yolk androgens adjusted to environmental conditions? A test in two seabirds that lay single-egg clutches

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1. Introduction

Mothers possess considerable capacity to adjust their offspring’s phenotypes to prepare them to meet the challenges posed by the environment in which they develop ("adaptive maternal effects"; Mousseau and Fox, 1998). The deposition by oviparous vertebrates of maternal androgens into egg yolks is one facet of maternal investment that has received considerable attention recently (Groothuis et al., 2005a). Androgens are steroid hormones that can have long term, beneficial effects on offspring behavior, growth and development (Schwabl, 1993; Eising et al., 2006). However, yolk androgens also could impose long term costs including reduced growth, survival and fecundity, and impairment of immune function (Sockman and Schwabl, 2000; Groothuis et al., 2005b; Rubolini et al., 2007). In addition, testosterone-enhanced traits often cited as beneficial, such as elevated growth rates and enhanced begging, are unlikely to be beneficial in all contexts (Kilner, 2001; Metcalfe and Monaghan, 2001; Uller and Olsson, 2003).

Given that suite of potential fitness costs and benefits, selection could operate on females to allocate androgens to offspring in a manner that maximizes fitness. If so, one prediction we can make is that the optimal strategy will vary with environmental conditions (Schwabl, 1996; Verboven et al., 2003). To date, most avian research on maternal androgens has focused on patterns of within-clutch allocation of yolk androgens in species that lay multi-egg clutches, and their consequences for sibling competition (Schwabl, 1993; Reed and Vleck, 2001; Müller et al., 2004). Due to the tight focus on sibling competition, we still know little about how females might allocate yolk androgens in direct response to environmental conditions (Groothuis et al., 2005a). Studies on species that lay single-egg clutches, in which sibling competition is not a factor, could help to fill the void.

The single-egg clutch is a characteristic life-history feature of offshore-feeding seabirds (Lack, 1968). Survival and growth of seabird chicks is tightly linked to food availability, which fluctuates dramatically over a range of temporal and spatial scales (Schneider and Duffy, 1985). Faced with such extreme environmental variation, and with brood reduction unavailable as a primary response to food shortages for all but a few species (Braun and Hunt, 1983), long-lived oceanic birds might be expected to make strong annual adjustments in maternal yolk androgen allocation. Specifically, we...
can hypothesize either (1) that early-laying (high quality) mothers will elevate yolk androgens in years of poor food availability, despite the associated risks, in order to enable their offspring to induce more feedings from parents through physiological and behavioral mechanisms. In many species, including several species of Alcidae (Harding et al., 2002; Litzow and Piatt, 2003; Gjerdrum, 2004), seabird chicks are able to induce changes in the rate at which their parents provision them (Hamer and Hill, 1994; Phillips and Croxall, 2003); or alternatively, (2) that early-laying mothers will reduce yolk androgen levels in years of poor food availability, when parents will not be able to keep up with the nutritional demands of fast-growing chicks (Benowitz-Fredricks and Kitaysky, 2005).

To test these hypotheses about androgen allocation, we measured concentrations of androstenedione [A4] and testosterone [T] in the yolks of the single-eggs laid by two alcid, Cassin’s auklets Psycorphalus alleuticus and rhinoceros auklets Cerorhincus monocerata, in each of 3 years. Our study site, Triangle Island, lies within the area influenced by the California Current, an extremely variable marine ecosystem in which feeding conditions and thus timing and success of seabird breeding vary dramatically among years (AINLEY AND BOEKELHEIDE, 1990; BERTRAM ET AL., 2001; SYDENMAN ET AL., 2006). That was true during our 3 year study, which bridged the transition from a “cool and high productivity” oceanic period to a “warm and low productivity” oceanic period, and which affected the phenology and availability of prey for both of our study species (MACKAS ET AL., 2007). We restricted our analyses to eggs laid early in the season, because early-laying females tend to be the older, more experienced members of seabird populations (DEFOREST AND GASTON, 1996; PYLE ET AL., 2001), those in better body condition (GASTON AND HIPFNER, 2006), and most likely to be employing the most effective strategies relative to environmental variation. To provide context, in each year we simultaneously monitored the timing and success of breeding of control groups of early layers, i.e. females of comparable ability to those whose eggs we removed.

2. Methods

2.1. Study site and field methods

The study was conducted at Triangle Island, British Columbia, Canada (50°52’N, 129°05’W) during the 2002–2004 breeding seasons. In each year, burrows of both species were checked every 2 days. Ten Cassin’s auklet eggs and 10 rhinoceros auklet eggs were collected when first found, weighed (±0.1 g on an electronic balance), and then frozen immediately at −10 °C. All eggs were collected prior to the population-wide median laying date, and under permit from Simon Fraser University (Animal Care Permit) and the Canadian Wildlife Service (Migratory Bird Collection Permit); Breeding phenology and success were monitored on adjacent plots of the colony as outlined in Hipfner et al. (2004). The early-laying individuals on these plots served as control birds for the females from which eggs were collected as they are similar in laying date, and nest site features.

2.2. Hormone analysis

In the lab, the eggs were thawed and yolk and albumen separated. Homogenized yolks were aliquoted for hormone analysis, then re-frozen at −80 °C until assay at the University of Alaska Fairbanks. Yolk aliquots (average 1.3 g) were thawed and homogenized with 3 ml distilled water using a tissue grinder. A yolk pool was used to validate that dilutions of yolk, and charcoal-stripped yolk spiked with hormone, were parallel to the standard curve. Approximately 0.02 g of yolk/water mix (9 mg yolk) was equilibrated with 2000 cpm of radiolabeled testosterone and androstenedione to assess the percent of hormone recovered from the extraction process, and correct final values for recovery percent.

Steroids were extracted using the following version of the protocol used by Schwabl (1993): Samples were extracted twice with 4 ml diethyl ether, dried under continuous nitrogen flow in a water bath at 40 °C, reconstituted in 1 ml 90% ethanol, and frozen for 24 h at −70 °C. The ethanol was decanted and dried using nitrogen. Androgens were then separated using column chromatography. Samples were reconstituted in 0.5 ml 10% ethyl-acetate in iso-octane, and added to short columns of diatomaceous earth, with a water trap and glycol phase. Androstenedione, testosterone and corticosterone were eluted in ethyl-acetate in iso-octane (following protocols described in Schwabl, 1993); however corticosterone levels were undetectable and so are not discussed further. We conducted separate radio-immunoassays for each species (Wingfield and Farner, 1975; Wingfield et al., 1991). Antibodies for testosterone and androstenedione were obtained from Wein Laboratories, Inc. (Concord, MA). For rhinoceros and Cassin’s auklet, average testosterone recovery was 71% and 68%, respectively, average androstenedione recovery for both species was 66%, and post-extraction coefficient of variability among duplicates for both hormones in both assays was 2%.

2.3. Statistical analysis

Data met the requirements for parametric statistical analyses. For each species, concentrations of A4 and T were compared across years, using egg mass and laying date as covariates, and tested for parallelism of slopes with year*egg mass interaction terms in a multivariate ANCOVA using SAS (v. 6.12) proc glm. Univariate F-tests were generated for each androgen, as well as Wilks’ lambda tests for each effect. For each species, mass of sampled eggs was compared using ANOVA with year as the predictor variable. Descriptive statistics were generated for breeding parameters of pre-median lay date nests, including hatching, fledging, and net reproductive success; as eggs were collected from pre-median lay date nests, these females are of comparable age. We compared, hatching, fledging, and overall breeding success across years by contingency table analysis.

3. Results

Median laying dates varied by about 1 week across the 3 years of study, being earliest in 2002 (Cassin’s auklet) or 2004 (rhinoceros auklet), and latest in 2003 in both species (Table 1). The proportion of control, early-laying pairs that raised chicks to fledging varied weakly among years in Cassin’s auklets (Wald $\chi^2$ = 4.47, $P = 0.09$), but very strongly in rhinoceros auklets (Wald $\chi^2$ = 11.07, $P = 0.004$). Most of the variation in breeding success was due to hatching success, as fledging success was consistently high (Table 1). Fledging masses varied among years (Table 1) in Cassin’s auklets (ANOVA, $F_{3,71} = 5.69, P = 0.005$), being lowest in 2003, but not in rhinoceros auklets (ANOVA, $F_{2,59} = 1.87, P = 0.16$). Collectively, the results presented in Table 1 indicate that environmental conditions for breeding for both species were better in 2002 and 2004 than in 2003.

Egg mass varied little among years (one-way ANOVA, year $R^2<0.01$ in both species). The overall variation in both [A4] and [T] was marked: in Cassin’s auklet yolks, both [A4] (mean = 569, 28 ng g$^{-1} \pm 90.15$ 95%CI; range = 141.5–1091.5 ng g$^{-1}$) and [T] (mean = 12.9 ng g$^{-1} \pm 2.22$ 95%CI; range = 3.6–28.6 ng g$^{-1}$) varied eightfold over 3 years. In rhinoceros auklet yolks, [A4] (mean = 837.03 ng g$^{-1} \pm 82.52$ 95%CI; range = 486.3–1386.9 ng g$^{-1}$) varied almost threefold, below.

Table 1: Timing of breeding for all females, and success of breeding for females that laid on or prior to the median laying date in 2002–2004

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Median lay date</th>
<th>No. eggs</th>
<th>% Hatched</th>
<th>% Fledged</th>
<th>% Success</th>
<th>Mean ±SD fledging mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassin’s auklet</td>
<td>2002</td>
<td>31 Mar–4 Apr</td>
<td>43</td>
<td>72</td>
<td>97</td>
<td>70</td>
<td>162 ±17</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>9–12 Apr</td>
<td>23</td>
<td>78</td>
<td>94</td>
<td>74</td>
<td>152 ±15</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>2–5 Apr</td>
<td>36</td>
<td>89</td>
<td>100</td>
<td>89</td>
<td>169±15</td>
</tr>
<tr>
<td>Rhinoceros auklet</td>
<td>2002</td>
<td>5–9 May</td>
<td>38</td>
<td>63</td>
<td>96</td>
<td>61</td>
<td>312±26</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>7–11 May</td>
<td>24</td>
<td>46</td>
<td>91</td>
<td>42</td>
<td>294±34</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>2–6 May</td>
<td>30</td>
<td>93</td>
<td>93</td>
<td>87</td>
<td>298±40</td>
</tr>
</tbody>
</table>

All eggs analysed for androgens were collected on or before the median lay date in each year.
and [T] (mean = 22.82 ng g⁻¹ ± 3.10 95%CI; range = 6.7–39.0 ng g⁻¹) almost sixfold. There were strong, positive correlations between [A4] and [T] in both Cassin’s (r = 0.70, P < 0.001) and rhinoceros (r = 0.81, P < 0.001) auklet eggs (Fig. 1).

Contrary to expectation, little of the variation in [A4] and [T] could be explained by year, nor by egg mass, lay date or year × egg mass effects (Fig. 2; ANCOVA, all P > 0.4), except for year and year × egg mass interaction in Cassin’s auklets, which were marginally non-significant (year: \( F_{2,10} = 3.39, P = 0.07 \); year × egg mass: \( F_{2,10} = 3.68, P = 0.06 \)). Post-hoc univariate ANCOVAs of Cassin’s auklet androgens showed no support for the year or year × egg mass interaction (both \( R^2 < 0.3, P > 0.4 \)). Addressing our main hypothesis very directly, single factor ANOVAs showed that year effects were not important determinants of [A4] or [T] in either species (for Cassin’s auklet, both \( R^2 < 0.09 \); for rhinoceros auklet, both \( R^2 < 0.14 \); all P > 0.14).

4. Discussion

Despite marked interannual variation in environmental conditions (Mackas et al., 2007), which affected the timing and success of seabird breeding (Hipfner et al., in press; Table 1), we found that the very marked, 3–8 fold interindividual variation in yolk androgen concentrations (A4 and T) in early-laid Cassin’s and rhinoceros auklet eggs could not be attributed to year effects (Fig. 2). That result is not consistent with the hypothesis that females in these species strategically allocate yolk androgens to their single offspring in response to environmental variability. If we found no interannual variability in conjunction with very low interindividual variability, it would be difficult to ascertain whether auklet yolk androgen deposition was unaffected by interannual differences, or whether our subset of high-quality females were simply able to deposit some optimum level of androgens regardless of environmental conditions. However, because interindividual variability was so high, optimization by high-quality early breeders seems unlikely to explain the lack of interannual differences.

One possible explanation for the lack of interannual variation in yolk androgen concentrations is that the interannual variation in environmental conditions prior to laying was insufficient to trigger such a physiological response in females. However, we consider that most unlikely. As mentioned, Triangle Island lies within a highly variable marine ecosystem, and a variety of measures indicate that early-season conditions varied dramatically along the British Columbia coast in 2002–2004, with repercussions across trophic levels (Mackas et al., 2007). Our study years spanned the transition from “cold and productive” to “warm and less productive” oceanic periods, which featured dramatically different ocean phenomenologies (e.g. 3–4 week variation in the timing of the annual zooplankton biomass peak Mackas et al., 2007). In our study species, that was reflected in the observed variation in laying dates and especially hatching success; two sensitive measures (Ainley and Boekelheide, 1990; Bertram et al., 2001). Moreover, carbon and nitrogen isotope signatures of the same yolks assayed in our study indicate different foraging conditions during egg production across our study years for both species (Hipfner et al., in press; Hipfner, MacFarlane-Tranquilla and Addison, unpublished manuscript). It may be that environmental variation is of little consequence to yolk androgen allocation strategies in the absence of sibling competition.

Concurrent experimental studies on relaying propensity in the same two species at Triangle Island found that this breeding parameter, like yolk androgen concentration, was not modified in response to environmental conditions (90–100% relaying rates among early layers; Hipfner et al., in press). Combined with the observations on interannual variation in breeding success (Table 1; and 0–80% among experimental relayers), these data collectively suggest that Cassin’s and rhinoceros auklets employ a fixed-investment strategy in producing their single-egg clutch, then adjust their investment as appropriate later in the reproductive effort (Table 1). In particular, we note that the marked interannual variation in hatching success likely reflects that widely variable proportions of pairs abandoned eggs. Whether the lack of responsiveness to pre-laying conditions is typical of all species that lay single-eggs, including other long-lived seabirds, remains to be determined.

If it is true that auklets and other oceanic seabirds employ such a fixed-investment strategy related to yolk androgens, then there should be little selection for offspring to respond to variations in androgen levels. Investigation into the effects of yolk androgens on offspring phenotype in auklets and other single-egg clutch species will be important tests of adaptive hypotheses for androgen allocation.

Why do yolk androgen concentrations vary so dramatically within a population breeding at the same time and place? Such issues lie at the heart of understanding phenotypic plasticity and...
natural selection (Williams et al., 2004; Williams, 2008). For example, several factors, including mate age (Michl et al., 2005) and quality (Gil et al., 1999, 2006; Tanvez et al., 2004), developmental stress (Gil et al., 2004), and frequency of intraspecific aggressive encounters (Wittingham and Schwabl, 2002) have been shown to influence circulating and yolk androgen concentrations, and may cumulatively contribute variability in the concentration of yolk androgens that a female deposits, independently of environmental conditions. There is increasing evidence that yolk androgen deposition levels are highly repeatable within females (Tobler et al., 2007; Eising et al., 2008), and this suggests that intrinsic factors may be more important than conditions measured here.

However, as noted previously (Williams et al., 2004), the high interindividual variability in yolk androgens is difficult to reconcile with the notion that the strategic maternal allocation of these hormones to egg yolks is important to offspring fitness, a notion for which there is considerable evidence in avian species that lay multi-egg clutches (Groothuis et al., 2005a). Yet not all studies have found beneficial effects of yolk androgens on offspring fitness (Navara et al., 2005), and effects on maternal fitness, which may be as important or even more so, have been little studied to date (Navara et al., 2006). Moreover, the question of whether females in oviparous vertebrates have the capacity to strategically allocate hormones to yolk, independently of their own hormonal state at the time, remains largely unresolved (Birkhead et al., 2000; Williams et al., 2004; but see Rhen et al., 2006). Eggs may in fact act as androgen sinks, enabling mothers to maintain optimal circulating levels (Navara et al., 2006). Clearly, a complete understanding of yolk androgen allocation strategies will require a better understanding of the ultimate drivers and physiological mechanisms involved in their transfer from female to offspring. At present, it remains difficult to determine whether yolk androgen allocation is an adaptation, an adaptation, or even a constraint (Retterson and Nolan, 1999; Navara et al., 2006).

To summarize, results of our study of Cassin’s and rhinoceros auklets, two species of long-lived oceanic birds that inhabit a highly variable environment and lay single-egg clutches (Groothuis et al., 2005a), are not consistent with the hypothesis that females allocate yolk androgens in direct response to oceanographic environmental conditions for breeding. Instead, we found that in both species the more competent females in the population, i.e. early layers, made a relatively fixed investment in offspring at the egg stage, then adjusted effort later in the breeding episode. These results have potentially important implications to studies of optimal maternal investment and yolk allocation strategies.

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