Evolution of Yolk Androgens in Birds: Development, Coloniality, and Sexual Dichromatism

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ABSTRACT: Current theory recognizes the adaptive value of maternal effects in shaping offspring phenotypes in response to selective pressures and vindicates the value of these traits in fostering adaptation and speciation. Yolk androgens in birds are a relatively well-known maternal effect and have been linked to adaptations related to development, coloniality life, and sexual selection. We tested whether interspecific patterns of yolk androgen levels (androstenedione and testosterone) were related to interspecific variation in development, sexual selection, and coloniality. First, we found no relationship between androgen levels and duration of development as reflected by incubation and nestling periods. However, androstenedione concentration was positively related to the relative duration of the incubation period and negatively related to the relative duration of the nestling period. These relationships were confirmed by analyses of phylogenetically independent contrasts. We suggest that androstenedione concentration may have evolved as a mechanism to shift the relative duration of development between the egg and nestling stages in response to selective pressures that differentially affect the duration of each stage. Second, neither plumage dichromatism nor mating system explained significant variation in yolk androgen levels after correction for similarity among species due to common descent. This finding indicates that sexual selection has not been an important selective pressure for this maternal effect. Third, we found a highly significant positive relationship between degree of breeding coloniality and concentration of androstenedione but not testosterone. These effects were confirmed in analyses of contrasts controlling for similarity due to common descent. Since the relationship with coloniality was different for each androgen, it is unlikely that increased levels of androgens in highly colonial species are a mere consequence of elevated androgen levels in mothers. Rather, our results suggest that high levels of androstenedione in eggs of colonial species are an adaptation to colony life, possibly related to the production of highly competitive phenotypes. In conclusion, from a comparative perspective, the results of this study support the role of maternal effects in promoting adaptation to certain environmental pressures.

Keywords: maternal effects, yolk androgens, testosterone, androstenedione, coloniality, dichromatism.

The study of adaptive maternal effects is concerned with the mechanisms that parents can use to improve offspring fitness (Mousseau and Fox 1998). A current topic of great interest is the role of maternal effects in modifying offspring phenotype in response to variation in environmental cues, thus providing mechanisms for transgenerational phenotypic plasticity. Current views regard plasticity as a key mechanism in promoting adaptation, not only at the population scale (Yeh and Price 2004) but also in a wider context, as a means of fostering speciation and macroevolutionary change (Robinson and Dukas 1999; Price et al. 2003; West-Eberhard 2003), as initially suggested by Baldwin (1896).

Female birds present extraordinary opportunities to study the evolutionary implications of maternal effects because their eggs contain a complex cocktail of resources for the offspring that can be easily measured and exper-

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imentally modified. A great variety of egg components, such as egg mass, antioxidants, hormones, and immune factors, has been shown to influence different components of offspring fitness (Schwabl 1993; Gasparini et al. 2001; Christians 2002; Saino et al. 2003; Hayward and Wingfield 2004; Biard et al. 2005). Hormones are especially interesting in this respect because they play a major role in organizing phenotypic differentiation and regulating physiological functions (Nelson 2000). The actions of a single hormone can affect many different functions within the organism (hormonal pleiotropy), resulting in complex trade-offs among life-history traits (Ketterson and Nolan 1999; Sinervo 1999).

Yolk androgens are the egg components that have received the greatest attention from research in recent years (for reviews, see Gil 2003; Groothuis et al. 2005*b*). Most research in this area has focused on three different aspects: effects of yolk androgens on development, influence of breeding coloniality on yolk androgen levels, and differential allocation of yolk androgen in relation to male attractiveness. We will briefly consider each of these aspects.

Development. Experimental research in several species belonging to different bird orders has identified a series of effects of yolk androgens in relation to growth. Thus, high levels of yolk androgens have been shown to speed up embryo development and nestling growth (Schwabl 1996; Eising et al. 2001), increase hatchling muscle mass (Lipar and Ketterson 2000), and induce a greater growth of ornaments (Strasser and Schwabl 2004). In terms of behavior, begging levels have been found to increase with high androgen levels (Schwabl 1996; Eising and Groothuis 2003).

However, other studies have shown that egg androgen injections can produce negative effects on growth or dose-dependent responses, suggesting stabilizing selection or phenotype-dependent effects (Sockman and Schwabl 2000; Rubolini et al. 2006). In addition, recent evidence in two different species shows that whether high levels of yolk androgens are beneficial or detrimental for nestlings depends on their sex (Saino et al. 2006; von Engelhardt et al. 2006), suggesting that females may be constrained in providing optimal levels of androgens to a mixed-sex brood. Benefits in terms of growth of high androgen levels can be costly, as shown by reductions in the immune responses of nestlings (Groothuis et al. 2005*a*; Müller et al. 2005) or by condition-dependent investment in females (Gil et al. 2004*b*, 2006*a*).

Breeding coloniality. The discovery that yolk testosterone levels increase with colony size in the house sparrow *Passer domesticus* (Schwabl 1997*a*) led Schwabl to propose that females could use this mechanism to prepare their offspring for the prevailing social conditions experienced by the maternal generation. Yolk androgen would thus be an adaptation to modify the behavioral phenotype of the offspring to match the levels of competition typical of the breeding density where that offspring developed. Further correlative studies (Reed and Vleck 2001; Groothuis and Schwabl 2002; Whittingham and Schwabl 2002; Mazuc et al. 2003; Pilz and Smith 2004) found a similar pattern in relation to breeding density, providing additional evidence for a role of yolk androgens as mechanisms of transgenerational phenotypic plasticity.

Sexual selection. Life-history theory predicts that females should invest higher amounts of resources when paired to attractive males (Burley 1988). In line with this prediction, experimental research has shown that eggs of females paired to males manipulated to be more attractive contained higher levels of egg androgens than eggs of females paired to males whose attractiveness had been reduced (Gil et al. 1999, 2004*a*, 2006*b*; but see Marshall et al. 2005; Navara et al. 2005). These differences have been taken to suggest that females pay a cost for higher androgen deposition that is compensated by nestling benefits or that only nestlings of high quality can reap the benefits of high androgen deposition (Gil et al. 1999). Two alternative hypotheses to explain these differences are (1) that females paired to attractive males increase yolk androgen to compensate for their reduced investment in paternal care (Groothuis et al. 2005*b*) and (2) that the fitness of the alternative phenotypes caused by differences in yolk androgens differs depending on the quality of the male. For instance, the bold and fast behavioral phenotypes that can been induced by high androgen levels (Daisley et al. 2005; Eising et al. 2006) may be especially advantageous for attractive males. Independent of these alternative hypotheses, patterns of differential allocation of yolk androgens in relation to attractiveness and the effects of these components in the development of secondary sexual characteristics (Strasser and Schwabl 2004; Eising et al. 2006) suggest a role of this maternal effect in processes of sexual selection.

Evidence about the current utility of a trait does not constitute proof of adaptation by itself (Harvey and Pagel 1991). Adaptation is the result of historical processes, and experiments in extant species cannot provide evidence of the role of a character in promoting adaptation. Comparative approaches are necessary to show that evolutionary transitions in the state of a trait under study are associated with transitions in the mechanisms or environments for which that trait is proposed to be an adaptation. In other words, mechanisms can evolve in response to changes in ecology and hence changes in the selection pressures that affect the physiological, anatomical, and behavioral underpinnings of traits (Schmidt-Nielsen 1997). Recent use of modern comparative methods in physiology has allowed scientists to distinguish between

species that have evolved specific mechanisms and species that have retained the same mechanisms due to their evolution in a common ancestor (Promislow et al. 1992). It is this ability to distinguish convergent evolution from retention of traits from a common ancestor that is the hallmark of modern comparative biology (Harvey and Pagel 1991). In the case of yolk androgens, a recent comparative study has produced evidence that between-species differences in yolk testosterone levels are related to differences in incubation period in the order Passeriformes (Gorman and Williams 2005). In this study, we have taken a more synthetic approach by considering the three different functional hypotheses that have been proposed at the species level to explain yolk androgen variation and testing their relevance across species in the whole Aves class. We hypothesized that if variation in yolk androgen concentration has played a role in the evolution of the duration of developmental periods or has evolved as an adaptation to colonial life and strength of sexual selection in birds, we should find a relationship between species-specific yolk androgen concentrations and measures of development, coloniality, and sexual selection. We predicted a negative relationship between androgen levels and duration of developmental periods, following Gorman and Williams (2005) and hence assuming that those negative effects on growth found in some experiments were idiosyncratic to certain species (Sockman and Schwabl 2000). We also predicted that yolk androgen levels should increase with increasing coloniality, sexual dichromatism, and extrapair paternity. To this end, we assayed two main yolk androgens, testosterone and androstenedione (hereafter T and A4, respectively), in a sample of 101 species of birds and related androgen concentration to species-specific measures of development, coloniality, and strength of sexual selection taken from the literature.

Material and Methods

Material

Freshly laid eggs were collected by us or by collaborators (see "Acknowledgments") from 101 species in several parts of the world (Europe, the Americas, and Africa), after official permits were obtained (for sample size, see table A1 in the online edition of the *American Naturalist*). Although it would have been desirable to obtain several full clutches from each species, sampling was constrained for many species by ethical and conservation considerations. On average, we obtained 6.7 eggs per species (SE = 1.02), which allowed tests of variance to be performed to assess within- and among-species variability. Eggs were frozen as soon as possible after collection and kept at −70°C until extraction and assays.

Extraction and Assays

For extraction, eggs were taken out of the freezer, allowed to defrost halfway, and dissected by separating the yolk from the white. Yolks were homogenized, and we took a small fraction, around 50 mg, which was weighed and transferred to a glass extraction tube. Extraction was performed by adding 3 mL of a mixture of diethyl and petroleum ethers, vortexing, centrifuging, and decanting the ether phase after snap-freezing. Ether phases were passed to a clean tube, dried under a stream of nitrogen, and dissolved in phosphate buffer (Gil et al. 2004*a*). Extraction recoveries were calculated on a random sample ($n = 20$) of yolk extractions by adding 1,000 counts per minute (CPM) of tritiated hormones, with values >90% for both T and A4. Given this high recovery rate, we considered it unnecessary to correct for sample-specific recovery rates.

Assays were conducted at two different labs, using slightly different protocols. At the Centre d'Etudes Biologiques de Chizé (CEBC), we ran custom assays, using antibodies from P.A.R.I.S. laboratories (Compiègne, France). Briefly, samples were incubated overnight at 4°C with about 6,000 CPM of ^{3}H-labeled hormone and a specific antibody. Bound and free fractions were separated by dextran-coated charcoal and centrifuged. A Packard 1600 liquid scintillation counter was used to count activity of the bound fractions. Cross-reactivity of T antibody was <2% for all androgens tested except 5α-dihydrotestosterone (DHT; 27%). Cross-reactivity of A4 antibody was <1% for all androgens tested (including those most susceptible to cross-reaction: 5α-DHT and T). All samples were assayed in duplicate. Intra-assay coefficients of variation were 5.2% and 13.6% for T and A4, respectively. Interassay coefficients of variation were 6.9% and 17.3% for T and A4. At the Universidad Complutense de Madrid (UCM), we used ^{125}I-labeled commercially available kits (Diagnostic Systems Laboratories, Webster, TX), based on antibody-coated tubes, which achieve separation of free and bound fractions by decanting. Reported cross-reactivities of the antibody used in the T assay kit (DSL-4000) were 5.8% for 5α-DHT, 2.3% for A4, and less than 0.5% for the rest of androgens tested. Reported cross-reactivities of the antibody used in the A4 assay kit (DSL-3800) were lower than 0.5% for all androgens tested. All samples were run in duplicate, and intra-assay coefficients of variation were 2.98% for T and 3.61% for A4. Interassay coefficients of variation were 17.7% for T and 8.38% for A4.

Pooling different interspecific hormone data from different labs is a common procedure in comparative analyses (Goymann et al. 2004; Gorman and Williams 2005; Ketterson et al. 2005) and assumes repeatability between labs. However, we tested this assumption explicitly for our study by running parallel hormone extractions and assays at the

CEBC and the UCM on the same samples ($n = 34$) from the same number of different species. The regression coefficients for the two androgens assayed at the two labs were highly significant and explained a large proportion of the variance (T: $F = 241.8$, df $= 1, 32$, $P < .001$, $r^2 = 0.88$; A4: $F = 365.7$, df $= 1, 32$, $P < .001$, $r^2 = 0.91$), thus allowing pooling of data from the two labs. Given this high consistency between the two assays differing in cross-reactivity to 5α-DHT (CEBC, 27% vs. UCM, 5.8%), we feel confident that our final estimates were not significantly affected by 5α-DHT. To allow pooling of data from both labs, we used the equations from the regressions between concentrations at the two labs to regress all data on UCM concentrations. After regression, there were no differences in androgen concentrations between the two laboratories (paired *t*-test for T and A4: $t = 0$, df $= 33$, $P = 1.0$). Repeatability estimates were high and significant (testosterone: 0.81; androstenedione: 0.64; df $= 1, 32$, both $P < .001$).

We ran a more stringent test on the repeatability of our estimates by comparing our data with previously published data (table B1 in the online edition of the *American Naturalist*) from a variety of labs that use different extraction and assay protocols. Although the number of species common to the two data sets is small, the regressions are highly significant and positive for the two androgens (T: $F = 14.08$, df $= 1, 8$, $P < .01$, $r^2 = 0.59$; A4: $F = 47.6$, df $= 1, 7$, $P < .001$, $r^2 = 0.85$), thus showing high repeatability across different studies and laboratory techniques (i.e., column separation vs. direct assay of ether extracted samples or variation in antibody cross-reactivity).

Comparative Data

Body mass, egg mass, and estimates of developmental periods were obtained by searching Brown et al. (1982–2004), Cramp et al. (1982–1994), Glutz von Blotzheim (1966–1997), and Poole and Gill (1992–2004). We used median values when a range of values was reported. Growth rates were taken from the data set available in Starck and Ricklefs (1998*a*). When several estimates were available, we calculated both species-specific mean and maximum growth rates.

Coloniality data were obtained from the same sources and were scored on a scale from 0 to 4, where 0 is solitary breeding in large, all-purpose territories, 1 is breeding in colonies that are maximally 2–10 pairs, 2 is breeding in colonies that are maximally 11–100 pairs, 3 is breeding in colonies that are maximally 101–1,000 pairs, and 4 is breeding in colonies that are maximally larger than 1,000 pairs. Coloniality data have been mapped onto the phylogeny in figure 1. We used maximum colony size instead of mean because this estimate is easier to obtain in the literature and because previous comparative analyses of coloniality have shown that maximum colony size is strongly positively correlated with mean colony size across species (Møller et al. 2001; Owens 2002). Additionally, Møller et al. (2001) showed in an analysis of coloniality and parasitism in the bird family Hirundinidae that maximum colony size explained 96% of the variance in mean colony size across 13 species, thus justifying the use of maximum colony size as an estimate of the degree of breeding sociality in comparative analyses.

Statistics and Comparative Analyses

Androgen concentrations, body mass, length of developmental periods, and T-cell immune response were log transformed to achieve normality. Since species-specific mean androgen values were calculated with substantial variation in sample sizes (see table A1), we used multiple regressions weighted by sample size to incorporate this variation in confidence in the analyses (Neter et al. 1996). We ran multiple regression models in SPSS software, using backward stepwise procedures to obtain final models where redundant or nonsignificant terms were dropped. In the results, whenever we give data on nonsignificant terms dropped from a given model, they correspond to those obtained when running the initial full model before deletion of nonsignificant terms. Collinearity was tested throughout and reported when tolerance estimates were lower than or equal to 0.2 (Tabachnick and Fidell 1996).

Measurements of yolk androgens can be expressed as either concentration (usually pg androgen per mg yolk) or as total content of androgens per egg (usually ng androgen per egg). We chose to use androgen concentration instead of total contents to maximize our sample size (data on yolk mass were not available for all species in the data set). However, for the 59 species for which yolk mass data were known, androgen concentration did not vary with proportion of yolk mass when egg mass was included in the model (effect of yolk proportion in the models for A4 concentration: $t = 0.66$, $P = .51$; for T concentration: $t = -0.39$, $P = .73$). Since body mass is an almost perfect correlate of egg mass ($F = 1{,}855.72$, df $= 1, 97$, $P < .001$, $r^2 = 0.95$, slope $= 0.81$ [0.02 SE]), any analysis of androgen concentration using body mass as a covariate would correct for a possible allometric effect of androgen concentration in relationship to egg size.

Because of common ancestry, comparative analyses based on species-specific data overestimate the number of independent observations, thus increasing the risk of statistical Type I errors (Harvey and Pagel 1991). In order to identify evolutionary independent comparisons, we used the method of independent contrasts (Felsenstein 1985) as implemented in the Macintosh-based software

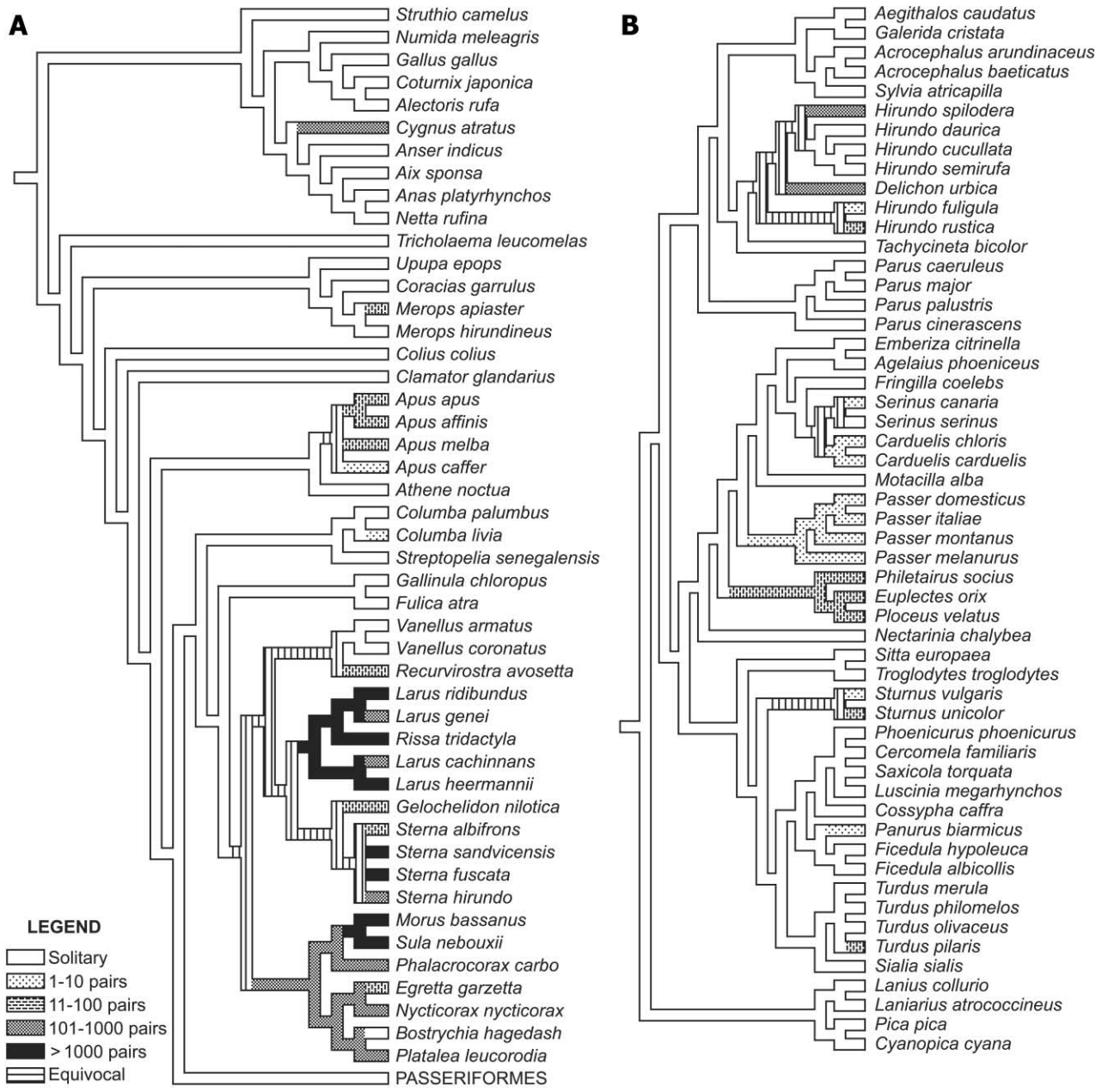

Figure 1: Phylogenetic relationships among species in the study. *A*, Nonpasseriforms; *B*, passeriforms. Breeding sociality data have been mapped on the phylogeny (see legend). See "Material and Methods" for sources.

CAIC, using the CRUNCH algorithm (Purvis and Rambaut 1995). To do this, we constructed a composite phylogeny of all species in our database (fig. 1) based on Sibley and Ahlquist (1990) and updated with recent phylogenies of a more limited range of taxa (Sheldon and Winkler 1993; Blondel et al. 1996; Crochet et al. 2000; Barker et al. 2001). All branches were assigned the same length, and analyses performed assuming uneven branch lengths produced similar results. Deleting contrasts with extreme residuals to test the robustness of the analysis did not change the results (Purvis and Rambaut 1995). Contrasts were analyzed by forcing regressions through the origin, because the dependent variable is expected not to change if there is no change in the independent variable (Harvey and Pagel 1991). In order to weight regressions for sample size in the analysis of contrasts, we calculated weights for each

contrast by calculating the mean sample size for the taxa immediately subtended by that node (C. N. Spottiswoode and A. P. Møller, unpublished manuscript) and log transforming this value to achieve normality. When measuring allometric relationships, we provide slopes calculated by reduced major axis regression (RMA) using the software RMA for Java platform (http://www.kimvdlinde.com/professional/rma.html). Major axis regression provides a more accurate estimation of slopes in phylogenetical data than standard least squares regression (Harvey and Pagel 1991).

Results

Yolk Androgen Descriptive Statistics and Allometry

Yolk androgen concentration varied widely among species: average T concentration = 10.32 pg/mg (SE = 1.05, range = 2.37–51.10); average A4 concentration = 34.52 pg/mg (SE = 3.81, range = 2.54–213.95). In terms of total contents, these values correspond to an average amount of 101.02 ng T per yolk (SE = 46.15, range = 0.62–4,112) and 561.95 ng A4 per yolk (SE = 177.15, range = 0.76–12,160). Between-species differences were large and significant for both androgen concentrations: T: $F = 14.41$, df = 100, 507, $P < .001$; A4: $F = 30.75$, df = 101, 545, $P < .001$.

Yolk T concentration was not significantly related to body mass ($F = 0.15$, df = 1, 99, $P = .69$, $r^2 = 0.01$, slope = −0.02 [0.04 SE]), but A4 concentration was strongly positively related to body mass ($F = 72.61$, df = 1, 99, $P < .001$, $r^2 = 0.42$, slope = 0.34 [0.04 SE]). Across species, there was no relationship between yolk concentration of the two androgens ($F = 1.45$, df = 1, 99, $P = .23$, $r^2 = 0.00$, slope = 0.09 [0.08 SE]).

Analysis of independent contrasts by regressions through the origin confirmed the lack of a significant relationship between yolk T concentration and body mass ($F = 1.92$, df = 1, 86, $P = .17$, $r^2 = 0.01$, slope = 0.08 [0.06 SE]) and the positive relationship between yolk A4 concentration and body mass ($F = 6.06$, df = 1, 86, $P = .02$, $r^2 = 0.06$, slope = 0.17 [0.07 SE], RMA slope = 1.02 [0.10 SE]) found in the analyses using species-specific data. The relationship between yolk A4 and mass thus follows an isometric relationship, implying that the increases in yolk A4 are proportional to body mass. There was a positive relationship between contrasts of concentrations of the two androgens; changes in yolk A4 concentration were strongly positively related to changes in yolk T concentration ($F = 19.35$, df = 1, 86, $P < .001$, $r^2 = 0.17$; slope = 0.54 [0.12 SE]).

Developmental Mode

We obtained yolk androgen data for six categories from the classification of developmental modes proposed by Nice (1962): precocial 2, precocial 3, precocial 4, semiprecocial, semialtricial 1, and altricial. Since these categories are assumed to represent a continuum of development (Starck and Ricklefs 1998*b*), we used regression analysis considering developmental mode as a meristic variable (Sokal and Rohlf 1995). The yolk T concentration did not vary in relation to developmental mode ($F = 0.01$, df = 1, 99, $P = .98$, $r^2 = 0.01$). However, yolk A4 concentration was strongly linked to developmental mode, and the best model to express this relationship was provided by a cubic term of A4 ($F = 32.98$, df = 2, 98, $P < .001$, $r^2 = 0.40$). Highest levels of A4 were found at the intermediate parts of the continuum (precocial 4 and semiprecocial species from Rallidae and Laridae in our sample), whereas altricial species (e.g., Passeriformes, Columbiformes, Apodiformes) had the lowest concentrations (fig. 2*A*). Correcting for body mass by including it as a covariate did not substantially modify the results ($F = 6.51$, df = 2, 98, $P = .002$, $r^2 = 0.12$), although the percentage of explained variance dropped significantly, and the two extremes of the continuum (altricial and precocial) were found to have similar levels of A4 for their body size (fig. 2*B*). It is not possible to perform a phylogenetic analysis of this relationship because developmental mode varies little within large taxonomic groups.

Development: Incubation and Nestling Periods

Incubation and nestling periods were strongly positively correlated (species-specific data: $F = 299.38$, df = 1, 96, $P < .001$, $r^2 = 0.75$, slope = 1.45 [0.08 SE]; contrasts: $F = 96.76$, df = 1, 86, $P < .001$, $r^2 = 0.52$, slope = 1.48 [0.15 SE]). Therefore, analyses were initially performed on a first principal component (PC1) of the two parameters, thus testing whether yolk androgens were related to common variance in developmental period (common variance explained by PC1 = 94%). Subsequent analyses were done for incubation and nestling period separately, including the other corresponding measurement as a covariate, so as to explore specific variance of each developmental stage. In the remainder of the article, we will refer to these variables as relative incubation period and relative nestling period, respectively.

The final model for developmental period (PC1) included the positive effect of body mass, while concentration of neither yolk androgen was retained (table 1; fig. 3*A*). Thus, developmental period increased with increasing body mass, as expected. The final model for relative incubation period revealed a positive effect of A4 concen-

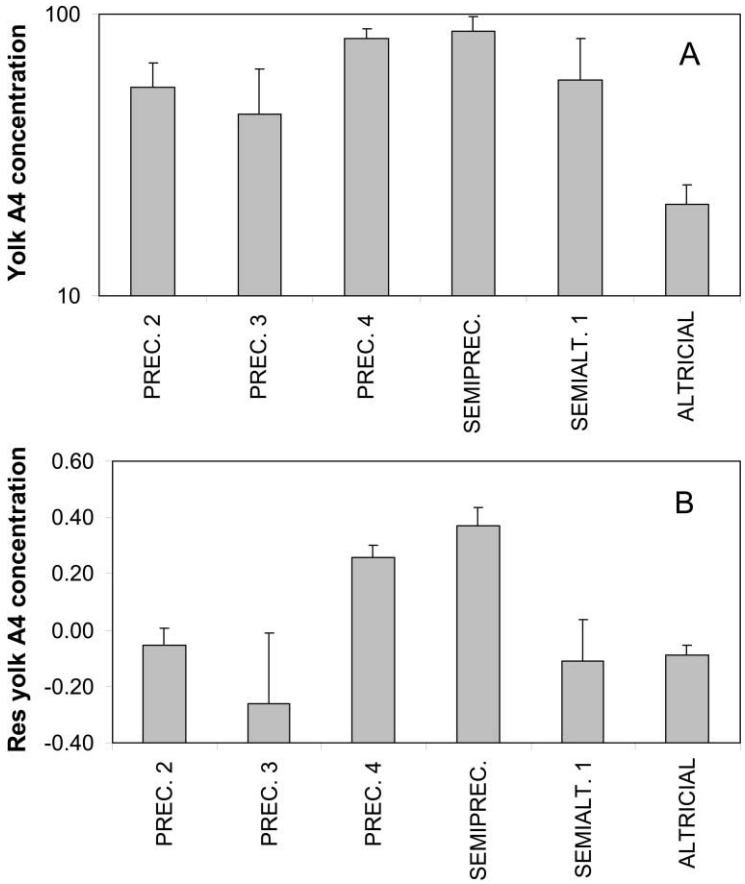

Figure 2: Differences among developmental modes in yolk A4 concentration, either as direct concentrations on a log scale (*A*) or as residuals from the regression on body mass (*B*). See "Material and Methods" for definition of developmental modes. Data show means + 1 SE.

tration (fig. 3*B*). In this case, body mass was dropped and nestling period remained, probably because of the high level of collinearity (tolerance = 0.2) between these two variables in the model. When we ran additional models with either of these two variables, final statistics for A4 concentration were almost identical, showing that the effects of body mass and nestling period were interchangeable (data not shown). The final model for relative nestling period included the independent and positive effects of body mass and incubation period and a negative effect of A4 yolk concentration (fig. 3*C*). This means that species with relatively higher levels of yolk A4 for their body mass had relative shorter nestling periods for the duration of incubation.

Corresponding analyses using multiple regression of independent standardized contrasts through the origin confirmed previous analyses using species-specific data (table 1). The final model for contrasts of developmental period (PC1) retained only the positive effect of contrasts of body mass as a predictor. Contrasts of yolk A4 concentration were positively related to contrasts of relative incubation period (fig. 3*E*) and were negatively related to contrasts of relative nestling period (fig. 3*F*).

Passeriformes: A Special Case?

We made a further, separate examination of the relationship between the duration of incubation and the concentration of yolk androgen in the order Passeriformes to test the results of a previous study (Gorman and Williams 2005). The final model for developmental period (table 2) retained only T yolk concentration, which showed a negative effect similar to that reported by Gorman and Williams (fig. 4*A*). The corresponding analyses for relative incubation and nestling periods resulted in models that dropped the effect of both yolk androgen concentrations. A regression analysis of independent contrasts of developmental period resulted in a model that did not retain any of the introduced variables. The negative effect of yolk

Table 1: Results from multiple linear regressions between duration of developmental periods and yolk androgen concentrations, weighting regressions by sample size

Dependent variable and parameter	Coefficient (SE)	*T*	*P*
Species-specific data:			
Developmental period (PC1):			
Intercept	−2.06 (.13)	−15.50	<.001
Body mass	1.06 (.07)	16.27	<.001
[Yolk T concentration]	−.26 (.16)	−1.62	.11
[Yolk A4 concentration]	.28 (.16)	1.71	.09
Relative incubation period:			
Intercept	.52 (.03)	15.32	<.001
Yolk A4 concentration	.11 (.02)	7.05	<.001
Nestling period	.40 (.03)	15.14	<.001
[Yolk T concentration]	−.01 (.02)	−.26	.79
[Body mass]	.02 (.01)	1.78	.08
Relative nestling period:			
Intercept	−.23 (.15)	−1.53	.13
Yolk A4 concentration	−.15 (.04)	−3.87	<.001
Body mass	.12 (.03)	4.67	<.001
Incubation period	1.28 (.15)	8.66	<.001
[Yolk T concentration]	−.04 (.04)	−1.05	.29
Independent contrasts:			
Developmental period (PC1):			
Body mass	.84 (.10)	8.25	<.001
[Yolk T concentration]	.19 (.21)	.91	.36
[Yolk A4 concentration]	−.16 (.17)	−.89	.37
Relative incubation period:			
Yolk A4 concentration	.04 (.02)	2.17	.03
Nestling period	.35 (.04)	9.93	<.001
[Yolk T concentration]	.01 (.03)	.35	.72
[Body mass]	.02 (.02)	.87	.39
Relative nestling period:			
Yolk A4 concentration	−.09 (.04)	−2.34	.02
Body mass	.15 (.03)	5.11	<.001
Incubation period	1.07 (.16)	6.72	<.001
[Yolk T concentration]	.02 (.05)	.46	.64

Note: Parameters in brackets were dropped from the final model, and their statistics refer to the first model. Global statistics for final models based on species are as follows: developmental period (PC1): $F = 264.70$, df $= 1, 96$, $P < .001$, $r^2 = 0.73$; relative incubation period: $F = 259.41$, df $= 2, 95$, $P < .001$, $r^2 = 0.842$; and relative nestling period: $F = 146.05$, df $= 3, 94$, $P < .001$, $r^2 = 0.812$. Global statistics for final models based on contrasts are as follows: developmental period (PC1): $F = 68.08$, df $= 1, 86$, $P < .001$, $r^2 = 0.442$; relative incubation period: $F = 52.86$, df $= 2, 85$, $P < .001$, $r^2 = 0.542$; and relative nestling period: $F = 51.02$, df $= 3, 84$, $P < .001$, $r^2 = 0.642$.

T was no longer significant (fig. 4*B*), in contrast to the results of Gorman and Williams (2005). Similarly, contrasts of neither yolk androgen concentration were found to predict relative incubation or nestling periods.

Coloniality and Sexual Selection

Two main processes have been hypothesized to have driven yolk androgen deposition: coloniality and sexual selection. We tested whether these processes have shaped species-specific differences in yolk androgen concentrations by running stepwise multiple regression models for each yolk androgen, including colony size, dichromatism, and mating system as predictors as well as body mass and the complementary yolk androgen concentration.

The final model for yolk T concentration (table 3) retained the positive effect of yolk A4 and the positive effect of dichromatism; dichromatic species have higher yolk T concentrations than monochromatic species. Neither mating system nor coloniality (fig. 5*A*) were retained as sig-

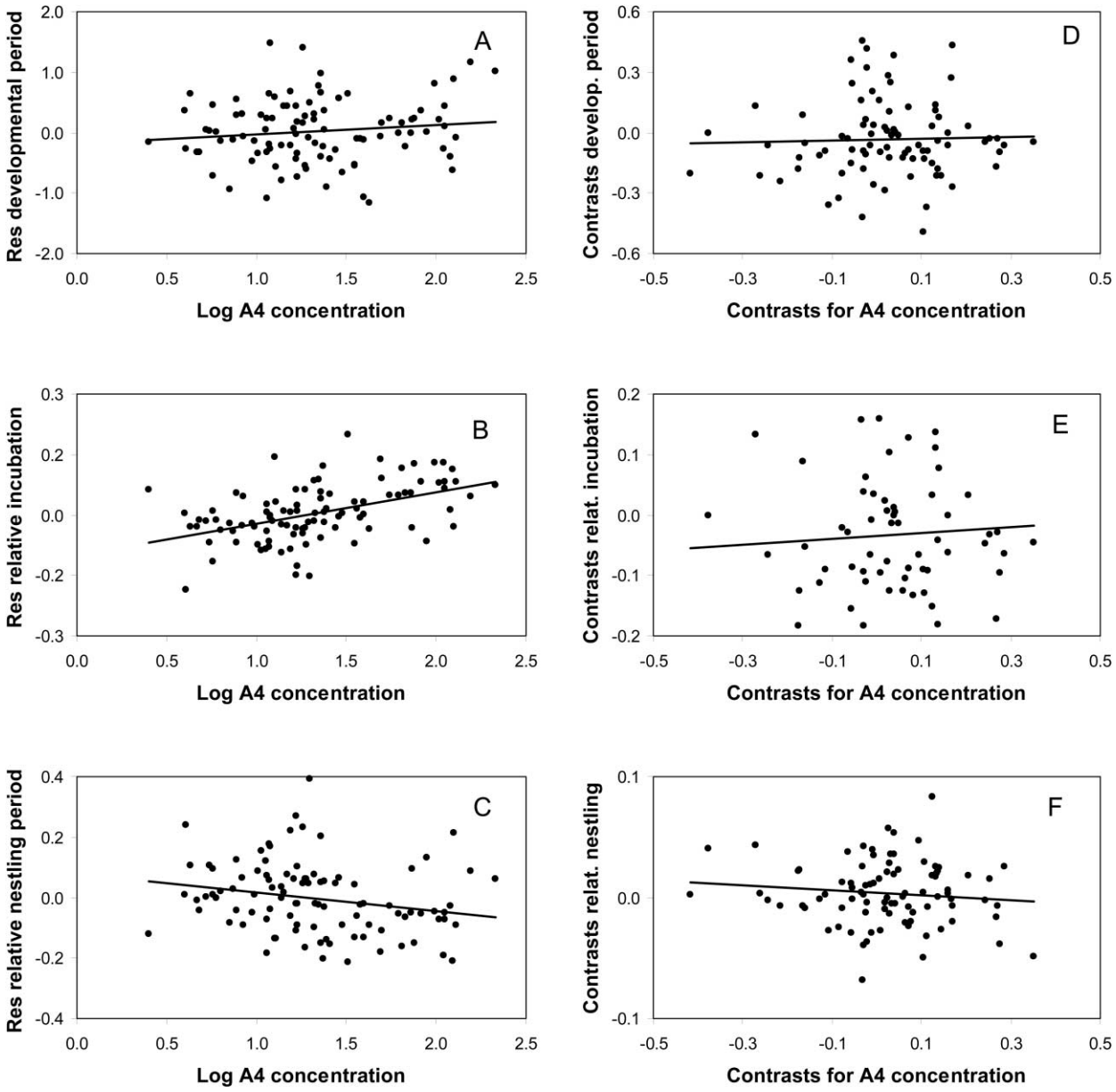

Figure 3: Relationship between yolk A4 concentration and the duration or period of developmental periods corrected for the remainder of the covariates of the models shown in table 1. *A*, *B*, and *C* represent the relationships for species-specific data for developmental duration (PC1), and relative incubation and nestling periods, respectively. *D–F* represent the corresponding relationships for independent contrasts. The lines are the linear regression lines.

nificant predictors of yolk T concentration. The analysis using independent contrasts retained only the positive effect of yolk A4 concentration on yolk T concentration, and none of the effects of dichromatism, mating system, or coloniality (fig. 5*B*) were statistically significant.

The same analysis for yolk A4 concentration retained the positive effects of body mass and coloniality (fig. 5*A*; table 4) and dropped mating system and dichromatism as predictors. The positive effect of yolk T concentration, although significant in the initial model, was not retained by the stepwise procedure. Multiple regression of independent contrasts through the origin revealed that body mass, coloniality (fig. 5*B*), and yolk T concentrations were significant predictors of yolk A4 concentration. Both analyses thus showed that species with larger colony sizes had higher levels of yolk A4 but not of yolk T.

Table 2: Results from multiple linear regressions between developmental periods and yolk androgen concentrations within the order Passeriformes, correcting for body mass and weighting yolk androgen data by sample size

Dependent variable and parameter	Coefficient (SE)	*T*	*P*
Species-specific data:			
Developmental period (PC1):			
Intercept	−.40 (.11)	−3.42	.001
Yolk T concentration	−.31 (.11)	−2.72	.009
[Yolk A4 concentration]	−.17 (.17)	−1.01	.37
[Body mass]	.16 (.14)	1.16	.25
Relative incubation period:			
Intercept	.81 (.08)	10.08	<.001
Nestling period	.27 (.06)	4.18	<.001
[Yolk T concentration]	−.03 (.02)	−1.60	.11
[Yolk A4 concentration]	.01 (.03)	.78	.93
[Body mass]	.20 (.02)	.94	.35
Relative nestling period:			
Intercept	.17 (.25)	.68	.50
Incubation period	.93 (.21)	4.18	<.001
[Yolk T concentration]	−.04 (.04)	−1.08	.28
[Yolk A4 concentration]	−.06 (.05)	−1.12	.27
[Body mass]	.10 (.04)	.26	.79
Independent contrasts:			
Developmental period (PC1):			
[Yolk T concentration]	.56 (.16)	.35	.72
[Yolk A4 concentration]	−.18 (.16)	−1.14	.25
[Body mass]	.15 (.15)	.95	.34
Relative incubation period:			
Nestling period	.29 (.08)	3.48	.001
[Yolk T concentration]	−.06 (.03)	−.23	.81
[Yolk A4 concentration]	−.01 (.03)	−.03	.97
[Body mass]	−.02 (.03)	−.80	.44
Relative nestling period:			
Incubation period	.71 (.20)	3.48	.001
[Yolk T concentration]	.03 (.04)	.63	.53
[Yolk A4 concentration]	−.05 (.04)	−1.17	.25
[Body mass]	.07 (.03)	1.86	.07

Note: Parameters in brackets were dropped from the final model, and their statistics refer to initial models. Global statistics for final models based on species are as follows; developmental period (PC1): $F = 7.37$, df $= 1, 52$, $P = .009$, $r^2 = 0.11$; relative incubation period: $F = 17.52$, df $= 1, 52$, $P < .001$, $r^2 = 0.24$; and relative nestling period: $F = 17.52$, df $= 1, 52$, $P < .001$, $r^2 = 0.24$. Global statistics for final models based on contrasts are as follows; developmental period (PC1, all terms dropped): $F =$ not applicable, df $= 0, 48$, $P =$ not applicable, $r^2 = 0.00$; relative incubation period: $F = 12.14$, df $= 1, 47$, $P < .001$, $r^2 = 0.19$; and relative nestling period: $F = 12.14$, df $= 1, 47$, $P < .001$, $r^2 = 0.19$.

Coloniality and Development: A Confounding Effect on A4?

Since several selective pressures associated with colony size, such as higher parasite load or competition for resources, could influence developmental patterns, it is important to consider whether the increase of A4 yolk levels in colonial species that we found was explained by its effect on relative nestling periods only or, alternatively, that coloniality explains additional variance in yolk A4 levels. To do so, we included coloniality in the models of relative developmental periods shown in table 1 as well as its interaction with yolk A4 and checked whether these terms were kept in the final model. The results show that neither coloniality nor its interaction with yolk A4 was related to the relative duration of the incubation period, so the final model remained the same as in table 1. However, in the case of relative nestling period, the final model retained a marginally significant effect of coloniality ($t = 1.68$, df $= 4, 93$, $P = .095$, slope $= 0.02$ [0.01 SE]), which led to an increase in the effect size of yolk A4 concentration ($t =$

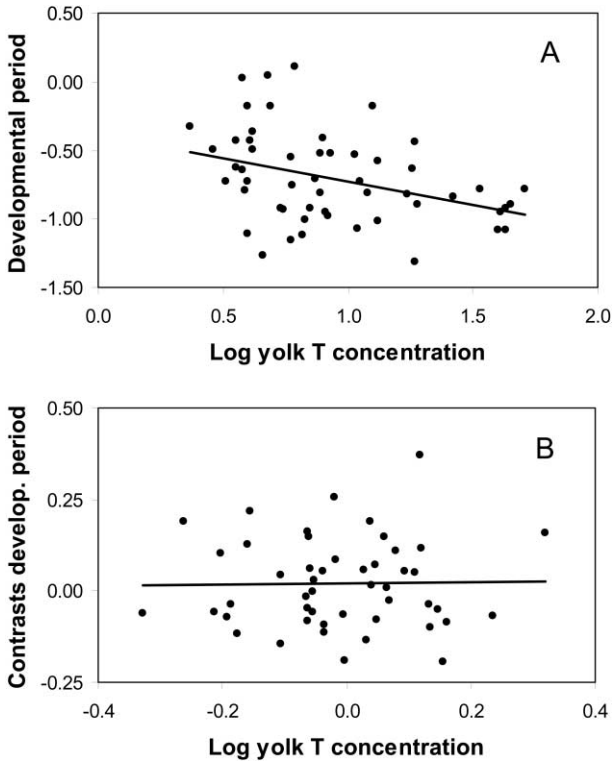

Figure 4: Relationship between yolk T concentration and residual developmental period (PC1) in Passeriformes, using species-specific data (*A*) or independent contrasts (*B*). The lines are the linear regression lines from table 2.

-4.25, df $= 4, 93$, $P < .001$, slope $= -0.17$ [0.04 SE]). The interaction term between coloniality and yolk A4 was not significant, confirming that both effects are additive. Analysis of independent contrasts confirmed the previous analyses; coloniality was not retained in the final model of relative incubation period, but it was kept as an additive predictor for relative nestling period, strengthening the effect of yolk A4 concentration (coloniality: $t = -3.31$, df $= 4, 83$, $P = .001$, slope $= 0.04$ [0.01 SE]; yolk A4: $t = -3.24$, df $= 4, 83$, $P = .002$, slope $= -0.12$ [0.04 SE]).

Discussion

Modes of Development

The mode of development in birds has traditionally been described as a series of categories ranging from altricial to precocial (Nice 1962) that today is considered to represent a continuum, with the possible exception of the altricial group, which might constitute a different category (Starck and Ricklefs 1998*b*). Differences in relative yolk content underline some of these differences in developmental mode (Sotherland and Rahn 1987). Our data show that yolk androgens are unlikely to be responsible for these differences in development. First, we found no differences in yolk T concentration across developmental modes. Second, although yolk A4 varied significantly with developmental mode, two extremes of developmental modes (the truly altricial and precocial species) had similar levels of yolk A4. This result is consistent with our finding a lack of a relationship between relative yolk content and egg androgen concentration (see "Material and Methods").

Some intermediate categories in developmental mode, such as the precocial 4 and semiprecocials (Rallidae and Laridae here), had higher levels of yolk A4 than the rest, and this may indicate a specific functional relationship within those taxa. The only characteristic that is unique for these groups is the fact that, despite being precocial in terms of motor development and being feathered at hatching, nestlings are fed by parents as in altricials (Nice 1962). Given that research on gulls (semiprecocials) has been particularly successful in demonstrating a positive relationship between begging behavior and both yolk androgen and nestling androgen levels (Ros et al. 2002; Eising and Groothuis 2003), we can speculate that elevated levels of A4 in these groups may be related to the need to develop strong begging levels in nidifugous species. However, the lack of a general pattern in yolk androgen levels along the altricial-precocial continuum speaks against a role of this maternal effect in the evolution of developmental modes in birds.

An interesting pattern is the positive relationship between body mass and yolk A4 concentration but not yolk

Table 3: Results from multiple linear regressions examining potential predictors of egg T concentration, correcting for body mass and yolk A4 and weighting data by sample size

Basis of analysis, parameter	Coefficient (SE)	*t*	*P*
Species-specific data:			
Intercept	.59 (.11)	5.20	<.001
Yolk A4 concentration	.16 (.08)	2.16	.03
Dichromatism	.21 (.06)	3.42	.001
[Coloniality]	.04 (.03)	1.41	.16
[Mating system]	−.12 (.08)	−1.42	.16
[Body mass]	−.09 (.06)	−1.53	.13
Independent contrasts:			
Yolk A4 concentration	.35 (.08)	4.57	.001
[Dichromatism]	.02 (.05)	.38	.70
[Coloniality]	.02 (.03)	.75	.45
[Mating system]	−.01 (.05)	−.26	.79
[Body mass]	−.04 (.06)	−.66	.51

Note: Parameters in brackets were dropped from the final model, and their statistics refer to initial models. Global statistics for the final model based on species: $F = 6.67$, df $= 2, 97$, $P = .002$, $^2 = 0.10$. Global statistics for the model based on contrasts: $F = 20.94$, df $= 1, 91$, $P < .001$, $r^2 = 0.18$.

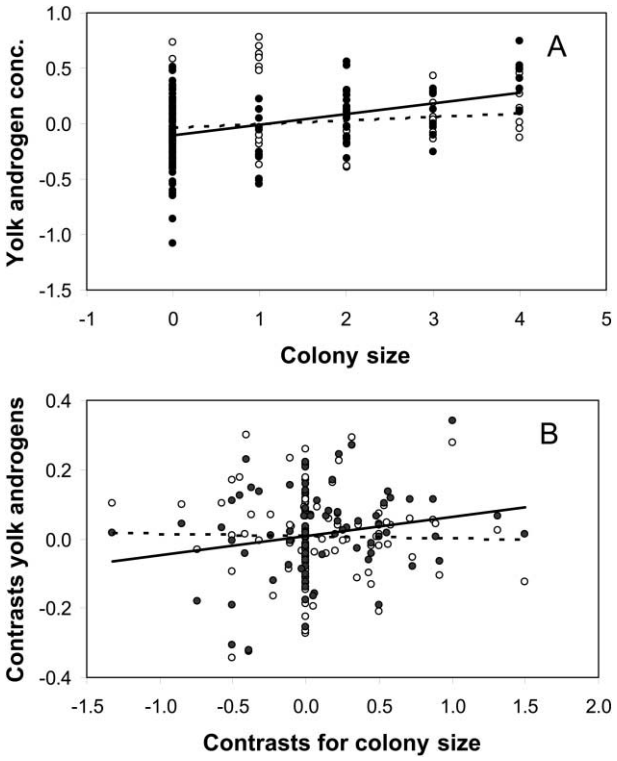

Figure 5: Relationship between yolk androgen concentrations and coloniality, using species-specific data (*A*) and independent contrasts (*B*). The A4 concentration data is shown by solid circles and solid lines and T data by open circles and dashed lines. Regression lines derive from models in tables 3 and 4.

T concentration. In this case, the positive pattern may suggest that embryos or nestlings require a concentration of A4 proportional to their body size. This might relate to other mass-related functional correlates of embryonic development, such as functional maturity of organs (Starck and Ricklefs 1998*b*). Additionally, it has been suggested that the development of large nestlings may require relatively higher levels of androgens and that the best option to avoid toxic effects of T would be to supply embryos with higher A4 levels (Groothuis and Schwabl 2002). Because A4 is a precursor of T, embryos could convert A4 into T progressively, avoiding damage by high levels of T. However, this question would require specific knowledge on the physiological pathway of A4 through development in birds, which currently is lacking (see next section for a more detailed consideration of this issue).

Developmental Periods

Several experiments on the effect of egg androgens in development within species have suggested that increases in yolk androgen are related to shorter development during both embryonic and nestling periods (Schwabl 1996; Eising et al. 2001; Pilz et al. 2004; Tschirren et al. 2005), although several other studies provide either negative evidence on this respect (Sockman and Schwabl 2000; Navara et al. 2005; Rubolini et al. 2006) or evidence for antagonistic effects depending on embryo sex (Saino et al. 2006; von Engelhardt et al. 2006). We predicted that if yolk androgen levels have had a role in shaping the evolution of developmental periods in birds, the duration of developmental periods should be correlated with egg androgen concentration across species. We found that patterns were different for different components of the duration of development. A general component of developmental duration (PC1), accounting for common variance in incubation and nestling periods, was found to be not significantly related to either T or A4 concentration across species. However, A4 concentration was positively related to the relative duration of the incubation period and negatively related to the relative duration of the nestling period. These patterns were confirmed by analyses of independent contrasts, thus showing that evolutionary transitions in the relative duration of developmental periods have coevolved with modifications of yolk A4 levels.

A first conclusion from these results is that since neither androgen was related to common variance in developmental duration, increases or reductions in general developmental time across species have not been promoted by changes in egg androgen concentrations. Only body size was a significant predictor of the general duration of development (PC1), a well-known relationship explained by cellular and physiological constraints on the time

Table 4: Results from multiple linear regressions examining potential predictors of egg A4 concentration, correcting for body mass and yolk T and weighting data by sample size

Basis of analysis, parameter	Coefficient (SE)	*t*	*P*
Species-specific data:			
Intercept	.56 (.08)	7.31	<.001
Body mass	.37 (.04)	9.10	<.001
Coloniality	.08 (.02)	3.70	<.001
[Yolk T concentration]	.18 (.09)	2.08	.04
[Mating system]	.13 (.07)	1.79	.08
[Dichromatism]	−.83 (.06)	−1.32	.19
Independent contrasts:			
Body mass	.14 (.06)	2.34	.02
Coloniality	.06 (.03)	1.99	.04
Yolk T concentration	.47 (.11)	4.17	<.001
[Mating system]	.06 (.06)	.93	.35
[Dichromatism]	−.06 (.06)	−.92	.35

Note: Parameters in brackets were dropped from the final model, and their statistics refer to initial models. Global statistics for the final model based on species: $F = 65.26$, df = 2, 97, $P < .001$, $r^2 = 0.56$. Global statistics for the model based on contrasts: $F = 11.42$, df = 3, 89, $P < .001$, $r^2 = 0.25$.

needed to mature and develop (Ricklefs 1969; Ricklefs and Starck 1998). However, the percentage of variance explained by this variable was not overwhelming (70% in the species data set, 40% in the contrasts). This leaves ample margin for adaptations to time-dependent mortality rate, sibling competition, or adult survival rates, as shown in previous studies (Williams 1966; Martin 2002; Remes and Martin 2002; Lloyd and Martin 2003). Contrary to our predictions, yolk androgens were not significantly related to variation in developmental period, and thus we can rule out a role of this maternal effect in shaping global developmental duration in response to ecological factors (Martin et al. 2001; Martin 2002; Remes and Martin 2002).

Second, the opposing patterns of A4 in relation to incubation and nestling periods, corrected for common variance in developmental duration, may suggest a trade-off in the determination of developmental periods. In other words, an evolutionary transition toward higher A4 concentration may have led to an increase in relative incubation period and a reduction in relative nestling period. In this sense, A4 concentration could act as a mechanism to allow switching the duration of development between the egg and nestling stages in response to stage-specific selection pressures. For instance, the relative duration of the incubation period has been shown to respond to variation in parasitism-driven mortality (Møller 2005), showing that nestling mortality due to parasitism is higher for species with relatively short incubation periods. Similarly, variation in parasite prevalence between species shows that short incubation periods are associated with higher prevalence of blood parasites, suggesting that the development of the immune system may be compromised if a minimum developmental period is not allowed (Ricklefs 1992).

Yolk A4 could influence the trade-off between duration of incubation and nestling periods by two non-mutually-exclusive mechanisms: a reduction in the nestling period through increased begging or an increase in the incubation period through a retarding effect on embryo development. Since the effects of A4 by itself have not been investigated, it is difficult to disentangle these possibilities. However, we can use evidence from experiments in which injections of an androgen cocktail with a high A4/T ratio have been used, assuming that these results pertain largely to the effects of A4. A reduction in nestling period through begging is consistent with experimental work in the black-headed gull, in which chicks hatching from androgen-injected eggs have been shown to beg more strongly than control chicks (Eising and Groothuis 2003). The second possibility meets with contradictory evidence; data show that androgen injections can induce both accelerated pre-embryonic development (Eising et al. 2001) in the black-headed gull and retarded growth (Sockman and Schwabl 2000) in the American kestrel (*Falco sparverius*). There is additional evidence of growth inhibition by A4 under certain circumstances in other organisms (McGivern et al. 1996; Dlugonski and Wilmanska 1998). Since the action of A4 could depend on the conversion to T by the enzyme 17β-hydroxysteroid dehydrogenase (17HSD; Horton and Tate 1966), it is likely that its effects depend on the availability of this enzyme (Dlugonski and Wilmanska 1998). Although 17HSD is present and functional in the developing avian embryo (Bruggeman et al. 2002), it is not known whether species differ in 17HSD availability. Additionally, A4 can also be converted to estrogen or to 5α-DHT if the necessary enzymes are present, thus limiting our predictions on the action of A4 unless the precise enzymatic environment of the embryo is known. However, our comparative data underline the possibility of a largely retarding activity of A4 on embryo development. It is possible that higher yolk A4 levels might evolve as a response to increased parasite pressure, by increasing chick incubation periods and thus allowing a more effective development of the immune system (Ricklefs 1992; Møller 2005).

We tested the relationship between the duration of developmental periods and yolk androgen concentration separately in the Passeriformes because a previous study had examined this relationship in the group (Gorman and Williams 2005). Although we found a negative relationship between yolk T and developmental period similar to that reported by Gorman and Williams, this relationship was not robust to a phylogenetic correction, in contrast to what was reported in their study. Although our sample size is much larger, it is possible that further examination of this question within the order Passeriformes with a larger sample size might modify this picture, providing taxon-specific functions of yolk T in incubation periods. However, since a functional link between yolk T and development has been proposed in many species across many orders of birds, both altricial and precocial, our data are evidence against a role of yolk T in the evolution of differences in developmental periods across birds.

Sexual Selection

Female birds have been shown to increase yolk androgen concentration when mated to attractive males or exposed to attractive ornaments (Gil et al. 1999, 2004*a*, 2006*b*; but see Marshall et al. 2005; Navara et al. 2006). These findings emphasize the link between sexual selection and maternal effects and allow us to predict that species with intense sexual selection should deposit more yolk androgens in their eggs. Our analysis did not support this prediction. We tested two different correlates of intensity of sexual selection: sexual dichromatism and mating system (monogamy vs. polygyny). The relationship between dichro-

matism and sexual selection dates back to Darwin (1871) and has been validated in recent studies that show higher rates of extrapair paternity, speciation, and polygyny in dichromatic than in monochromatic species (Björklund 1990; Møller and Birkhead 1994; Barraclough et al. 1995; Owens and Hartley 1998). We found that although yolk T levels were higher in dichromatic than in monochromatic species, this difference was no longer significant in an analysis of independent contrasts. This suggests that the difference obtained in the species-specific data set was due to sampling bias or clustering of specific values in particular taxa. Mating system was not significantly related to either yolk androgen concentration. These results speak against a general role of sexual selection in shaping yolk androgen levels across species despite the fact that a number of experimental studies have provided intraspecific evidence of such an effect (Gil et al. 1999, 2004*a*, 2006*b*; Tanvez et al. 2004). However, we would like to emphasize that sexual dichromatism and social mating system provide only approximate surrogates of the intensity of sexual selection and that future analyses using other components may prove fruitful.

Coloniality

Our comparative study of a taxonomically wide range of avian species showed a positive relationship between yolk A4 levels and breeding coloniality. This result was confirmed by analysis of independent contrasts, which revealed that transitions to coloniality have coevolved with increases in yolk A4 concentration over evolutionary time. Yolk T concentration was not significantly related to coloniality. Although this is surprising, given the positive correlation between the concentrations of the two androgens, this strengthens the conclusions of our previous results on development by suggesting a differential effect of the two androgens.

A positive intraspecific relationship between colony size and yolk androgens has been found in three different species of birds, for both A4 and T in the European starling and the American coot (Pilz and Smith 2004; Reed and Vleck 2001, respectively) and for T in the house sparrow (Schwabl 1997*a*). However, a recent study in a Spanish population of the barn swallow *Hirundo rustica* detected no differences in yolk A4 despite large variation in colony size (Gil et al. 2006*b*). Positive relationships between group size and yolk androgens have been hypothesized to be an adaptation to shape the physical and behavioral offspring phenotype to the conditions experienced by the mother (Schwabl 1997*a*). More explicitly, this would be expected because offspring hatching from eggs with high androgen levels should be more competitive, develop faster, grow larger ornaments, and show higher dominance and a proactive behavioral phenotype (Schwabl 1993; Lipar and Ketterson 2000; Strasser and Schwabl 2004; Daisley et al. 2005; Eising et al. 2006).

This scenario would require that offspring choose a social environment for reproduction similar to that of their parents. Indeed, there is evidence suggesting significant heritability of social breeding environment (Brown and Brown 2000; Møller 2002) and nonrandom dispersal between patches of a similar degree of sociality (Brown et al. 2003). Such choice could be due to the effects of quantitative genes or, more likely, a maternal effect (Møller 2002).

The positive relationship between coloniality and yolk A4 concentration could be interpreted in at least two different functional ways. First, since we previously showed that yolk A4 is related to the relative duration of the incubation and nestling periods, it could be possible that yolk A4 levels are elevated in colonial birds to mediate these changes in the duration of the relative nestling period. Alternatively, the relationship may be due to an additional effect of A4 on phenotype unrelated to growth. We tested this by adding coloniality as a predictor in the model of developmental period, together with yolk A4 concentration and the interaction between coloniality and A4 concentration, so as to control for their common variance. We found that coloniality was not a significant predictor of the relative duration of incubation, implying that the relationship between yolk A4 and coloniality was not linked to development. Although coloniality was positively related to the relative duration of the nestling period, this effect was additive to that of yolk A4 concentration, again suggesting that yolk A4 and coloniality are not linked through the influence of yolk A4 on developmental time. Therefore, the data indicate that the increased yolk A4 deposition found in colonial birds is more likely related to the production of competitive phenotypes that would be advantageous in highly social groups (Schwabl 1997*a*).

An alternative explanation to these adaptive views is that increased androgen levels in the eggs of colonial species or individuals are an unselected consequence of increased androgen levels in female plasma (Pilz and Smith 2004; Groothuis et al. 2005*b*). Since androgens in females have two targets of action (female and embryo; Staub and De Beer 1997), it is possible that females may be unable to control their yolk androgen deposition if plasma androgen levels are increased due to increased competition or other reasons. This should not matter if there is a positive relationship between the androgen optima of the two targets across different situations (this would be a case of coadaptation). However, since the relationship between androgen optima is highly unlikely to be perfect, it follows that androgen yolk deposition could be maladaptive if females cannot control this transfer. Coloniality implies the need

to defend nests sites and protect clutches and offspring from rival individuals. Such aggressive behavior, which is typically restricted to males in most territorial birds, is also performed by females in colonial species (Burger et al. 1980; Stenhouse et al. 2004). Two lines of evidence suggest that the physiological basis for this behavioral shift is partially due to an increase in female T concentration. First, females of colonial species had higher T levels than those of solitary species (Møller et al. 2005), even after correction for the positive covariance between male and female T levels (Ketterson et al. 2005; Møller et al. 2005). Second, a recent study in the cliff swallow *Tachycineta bicolor* showed that females in large colonies had higher T levels than females in small colonies (Smith et al. 2005). This strengthens previous evidence for a role of this hormone in regulating competitive behavior in females (Cristol and Johnsen 1994; Langmore et al. 2002). Therefore, increased yolk androgen in colonial birds could be interpreted as an epiphenomenon of increased androgens in females.

However, if there was a passive transfer of androgens from mother to yolk, we would expect a positive relationship between yolk T and coloniality, and this pattern was not found to be significant. This lack of relationship with T levels, in the face of an increase in yolk A4 levels, could fit with previous suggestions that A4 could be used by embryos as a source of active androgens (T and DHT) without exposing itself to the toxic effects of a high T dose (Groothuis and Schwabl 2002), but further research should ascertain the precise metabolic pathways of maternal hormones in the embryo.

Conclusions and Future Directions

Modern evolutionary perspectives consider that maternal effects can induce adaptive patterns and speciation events in a context of reproductive isolation (Mousseau and Fox 1998; Wade 1998). Our results suggest that evolutionary transitions in the relative duration of developmental periods and in the incidence of coloniality in birds have coevolved with modifications of yolk A4 levels. Because variation in A4 levels within species has effects on the development of physiology and behavior, we propose that our data are evidence for a role of such maternal effects inducing phenotypic plasticity as a result of differences in certain selective pressures among species.

In addition, our results provide evidence in support of a differential action of yolk A4 and T in avian development and behavior. The most parsimonious assumption so far had been to suppose that the effects of yolk A4 and T were similar. However, the differential effect of yolk A4 on developmental periods and the contrasting influences of coloniality on the two androgens suggest that each androgen has specific actions. Future research should consider the specific mechanisms of the two androgens and their differential metabolic pathways with respect to immunity and development.

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