Behav Ecol Sociobiol (2008) 62:617–625
DOI 10.1007/s00265-007-0486-z

ORIGINAL PAPER

Sex-specific effects of yolk-androgens on growth of nestling American kestrels

Keith W. Sockman · Jack Weiss · Michael S. Webster · Vanessa Talbott · Hubert Schwabl

Received: 16 April 2007 / Revised: 25 June 2007 / Accepted: 25 August 2007 / Published online: 22 September 2007

Abstract Maternally derived androgen hormones concentrate in avian egg yolks as the yolks grow on the female's ovary, possibly forming a basis for important maternal effects in birds. In the American kestrel (*Falco sparverius*), experimental elevation of yolk androgens in the first-laid egg of a clutch (a-egg) to the concentrations found naturally in a clutch's later-laid eggs reduces the growth rate of a-egg nestlings compared to controls. These findings, together with discoveries from other species that the effects of yolk androgens on growth of female nestlings may differ from their effects on growth of male nestlings, raise the hypothesis that natural changes in yolk-androgen concentrations with laying order are ultimately due to a difference between the sexes in their yolk-androgen sensitivity and between early- and late-laid eggs in their sex ratio. By re-analyzing previously published data and adding to the analysis data from previously unanalyzed blood samples used for sex determination, we investigated possible sex-specific effects of yolk-androgens in the context of a potential sex-biased laying order in free-living American kestrels. We used a multi-level, mixed model with a Gompertz function to analyze growth of nestlings hatching from a-eggs that were control-treated or in which we experimentally elevated yolk-androgen concentrations shortly after laying to the higher concentrations naturally found in later-laid eggs. We discovered that male nestlings were more susceptible than female nestlings to growth inhibition by yolk-androgen elevation but did not find a bias in sex ratio with respect to laying order. Together, these findings do not support the above hypothesis. However, they are consistent with the hypothesis that sex differences in yolk-androgen sensitivity enable mothers to economically tune reproductive effort to an individual offspring's reproductive value, which can vary more for one sex than the other.

Keywords Maternal effects · Reproductive value · Sex allocation · Sex ratio · Sibling differences

Communicated by J. Graves

K. W. Sockman (✉)
Department of Biology, University of North Carolina,
Chapel Hill, NC 27599-3280, USA
e-mail: kws@unc.edu

J. Weiss
Curriculum in Ecology, University of North Carolina,
Chapel Hill, NC 27599-3275, USA

M. S. Webster · V. Talbott · H. Schwabl
School of Biological Sciences, Center for Reproductive Biology,
Washington State University,
Pullman, WA 99164-4236, USA

Introduction

Maternal effects shape the behavior and general phenotype of offspring, often enhancing maternal fitness in the process (Bernardo 1996). Maternally derived androgen hormones concentrate in avian egg yolks as the yolks grow on the female's ovary, possibly forming the basis for important maternal effects in birds (Sockman et al. 2006). In many bird species, maternally derived yolk androgens increase in concentration with respect to the within-clutch order of laying (hereafter, laying order; e.g., Schwabl 1993; Sockman et al. 2001). Experimental elevation of yolk androgens in first-laid eggs of a clutch (a-eggs) to the approximate concentrations that occur naturally in later-laid eggs elevates growth rates of nestling canaries (*Serinus canaria*; Schwabl 1996) and black-headed gulls (*Larus ridibundus*; Eising et al. 2001) hatching from a-eggs but reduces growth rates of nestling American kestrels (*Falco sparverius*; Sockman

and Schwabl 2000) hatching from a-eggs. These and other findings have hence raised the hypothesis that within-clutch variation in yolk-androgen concentrations modifies growth-rate differences among sibling nest-mates and contributes to the optimization of parental reproductive effort (Schwabl et al. 1997; Sockman et al. 2006).

In studies such as those described above, where the goal is to elucidate the functional basis for yolk-androgen deposition, the experimental elevation of androgen concentrations in a-eggs with naturally low concentrations has been the sensible approach. It has allowed investigators to produce concentrations similar to those that occur naturally in later-laid eggs, whereas the alternative — experimentally removing androgens from later laid eggs — has not been feasible. These studies are difficult to interpret, however, because their results may reflect some aspect of a-eggs, which differs from later-laid eggs and interacts with yolk androgens to influence nestling growth (Sockman and Schwabl 2000; Sockman et al. 2006). For instance, in lesser black-backed gulls (*Larus fuscus*), yolk-androgen and yolk-antioxidant concentrations are, respectively, lower and higher in a-eggs than in later-laid eggs (Royle et al. 2001). Because antioxidants may protect offspring from oxidative stress induced by the effects of yolk-androgens on growth and metabolism (Royle et al. 2001), the effects of experimentally elevated yolk-androgen concentrations in a-eggs might be lost or even detrimental in late-laid eggs with naturally elevated yolk-androgen concentrations. In the kestrel, a similar pattern of yolk-antioxidant deposition would not explain the growth-reducing effects of high yolk-androgen concentrations in a-eggs, but perhaps some other idiosyncrasy of the a-egg does.

Embryonic sex is potentially one such factor that can vary with respect to laying order, can interact with yolk-androgens to influence offspring growth, and therefore might challenge the validity of the several studies that assume differences between eggs with respect to laying order are not relevant to the effects of yolk androgens. It is well known that embryonic sex can vary with laying order in some species (e.g., Badyaev et al. 2002a, b). Moreover, recent publications might suggest the presence of sex-specific sensitivity to yolk androgens. In black-headed gulls, pharmacological treatment of eggs with an antagonist of the androgen receptor elevates growth of male nestlings and inhibits growth of female nestlings (Müller et al. 2005). Inferring the effects of yolk androgens from pharmacological manipulations targeted at the androgen receptor is risky because it assumes high specificity of the drug and that yolk-androgens act via androgen receptors, as opposed to an estrogen receptor after aromatization of testosterone (e.g., Balthazart et al. 1996; Ball et al. 2002). In addition, pharmacologically antagonizing the androgen receptor would interfere with androgens not only of maternal (yolk) origin but also of embryonic and nestling origin. Still, these findings are intriguing and suggest that sensitivity to yolk androgens might be sex-specific. Indeed, in zebra finches (*Taeniopygia guttata*), experimentally elevated yolk-androgen concentrations enhance the growth rates of female nestlings but reduce it in males (von Engelhardt et al. 2006), whereas in barn swallows (*Hirundo rustica*), experimentally elevated yolk-androgen concentrations enhance the growth rate of males but reduce it in females (Saino et al. 2006).

To our knowledge, no study has demonstrated sex-specific effects of yolk-androgens at natural levels in the context of a potential sex-biased laying order. If early-laid eggs tend to be one sex and that sex is more sensitive than the other to yolk androgens, then early-laid eggs may be protected from elevated yolk androgens by a natural pattern in which concentration increases with laying order (Rubolini et al. 2006). To investigate this possibility, we determined the effects of elevated yolk-androgen concentrations on growth of male versus female nestlings hatching from a-eggs and the effects of laying order on sex ratio in American kestrels.

Materials and methods

Field work, species, and experiment procedure

This study made use of data on nestling growth that were collected as part of a previously published experiment (Sockman and Schwabl 2000). The widespread availability of molecular sexing techniques and recent advances in statistical modeling enabled us to add to these data newly collected data on the sex of individual nestlings and a more sophisticated method of analysis. Details of most field methods are published elsewhere (Sockman and Schwabl 2000). Briefly, in Spring 1999, we checked nest boxes in eastern Washington state, USA (46°44′N, 117°10′W) every 1–3 days and marked new eggs when found. Individual American kestrels lay at approximately 2-day intervals until the clutch is complete. Incubation and nestling periods, each approximately 30 days, follow, after which nestlings fledge (Balgooyen 1976; Sockman and Schwabl 1998, 2001a).

In our experimental procedure, we were careful to ensure that the final concentrations of a-egg androgens (endogenous plus injected) were close to those that can occur naturally in later-laid eggs. The maximal concentrations of yolk-testosterone and yolk-androstenedione we have measured in this American kestrel population are 36.5 and 1,291.9 pg/mg yolk, respectively. Assuming a yolk mass of 3.45 g (mean mass of eight yolks), this translates to maxima of 0.13 μg testosterone and 4.46 μg androstenedione per yolk. Within a few days of clutch completion (within the first 10% of the incubation period, on average), we injected either 50 μl sesame oil (control) or 0.1 μg testosterone and 4 μg androstenedione dissolved together in 50 μl sesame oil (androgen-treated) into the yolk of a-eggs (estimated mean

endogenous yolk-testosterone and yolk-androstenedione contents of 0.03 and 0.62 μg/yolk), mimicking in them the higher maternal doses of each of these androgens found in c and d eggs (Sockman and Schwabl 2000; Sockman et al. 2001). Like many birds species, kestrels are prone to nest abandonment due to disturbances during laying. Delaying egg injection until we were sure the clutch was complete, which often required waiting a few days past clutch completion, minimized this problem. Throughout the laying season, we alternated between androgen and control injections. We determined which nestling hatched from each egg based on the coloring of downy plumage after application of food dye allowed to seep through the pipped area of nearly hatched eggs. We marked nestlings for individual identification and determined body mass every 5 days from ages 0–25 days.

Blood collection and sex identification

As part of another study (Sockman and Schwabl 2001b), we had collected a blood sample from nestlings' wing veins beginning at age 5 days, and we had stored separated red blood cells at −20°C. Although kestrel nestlings can be sexed by age 15 days based on plumage (Bird 1988), having blood samples before that enabled us to sex those that died between age 5 and 15 days.

For molecular sex-assessment, we amplified an intron of the sex-linked CHD gene, using primers 1237L and 1272H, as described by Kahn et al. (1998). We extracted template DNA from blood samples via phenol-chloroform protocols (see Westneat 1990). We conducted PCR amplification in 10 μl reactions with 1 μl diluted DNA template (approximately 50 ng), 0.15 mM dNTPs, 0.5 μM primers (each), 2.0 mM $MgCl_2$, approximately 2.5 units of *Taq* polymerase, and 1X buffer (Promega). Each reaction consisted of 30 cycles of denaturation at 95°C for 60 s, annealing at 56°C for 60 s, and extension at 72°C for 45 s. We electrophoresed PCR products on a 3% agarose gel stained with ethidium bromide and scored the gel visually; females showed two bands (W-linked and Z-linked copies of the CHD gene), whereas males showed only a single band (Z-linked only).

Analyses

In analyses of nestling growth, we used only those nests for which we unequivocally knew the identity of the nestling hatching from the injected egg and that the injected egg was the a-egg. This was a longitudinal study, in that the masses of individual birds were measured repeatedly over time. Coupled with observations on different birds, this yielded a hierarchically structured data set, with observations nested within individuals. Because some of our subjects died during the course of the study, our data structure was unbalanced. To account for observational heterogeneity and the lack of balance, we used mixed-effects models, which readily handle unbalanced and missing data and allow all observational units to contribute information to the analysis (Pinheiro and Bates 2000). A mixed-effects model accounts for observational heterogeneity by including random effects that permit regression coefficients to vary randomly across individuals.

Although animal growth can appear linear over short time intervals, for longer time spans growth is typically nonlinear, often showing a hyperbolic or sigmoidal pattern (Ricklefs 1968; Ricklefs 1973). One can use highly parameterized polynomial models for such data, but appropriate nonlinear models, such as the Gompertz function, produce more interpretable results and are frequently used to model growth in birds (Aggrey 2002; de Freitas 2005; Sengül and Kiraz 2005; Nahashon et al. 2006), particularly when the data are hierarchically structured and unbalanced and thus when they warrant a mixed-effects model (Wang and Zuidhof 2004).

We used the self-starting Gompertz function, SSgompertz, from the software package R (R Development Core Team 2004) that uses the following parameterization,

$$\text{mass} = a_0 \exp\left(-b_0 b_1^{\text{age}}\right),$$

after first determining that the correlations between the parameters a_0, b_0, and b_1 for our data were all low (less than 0.6 in magnitude). We derived some understanding of the parameterization by plotting different Gompertz curves, in which one parameter was allowed to vary while the others were held fixed at means obtained from separate fits of the Gompertz function to individual birds (Fig. 1). These plots led to the following parameter interpretations: a_0 primarily affects the asymptote and therefore the final mass; b_0 primarily affects the hatching mass (y intercept) and indirectly the growth rate to some extent; and b_1 primarily affects the location of the inflection point and also the growth rate to some extent.

Mixed-effects models for hierarchical designs are also called multilevel models. A multilevel model for our data has two levels: one level to describe how the mass of an individual bird changes over time and a second level to describe how these changes vary across individuals. At level 1, we used separate Gompertz equations to describe the growth of individual birds. At level 2, we expressed the individual Gompertz parameters—a_0, b_0, and b_1 in our model above—as linear functions of predictors with values that vary among the individual birds. Thus at level 2, each growth parameter at level 1 had its own linear regression equation with an intercept, additional parameters corresponding to the included predictors (e.g., yolk-androgen treatment, sex, treatment × sex interaction), and a random effect to

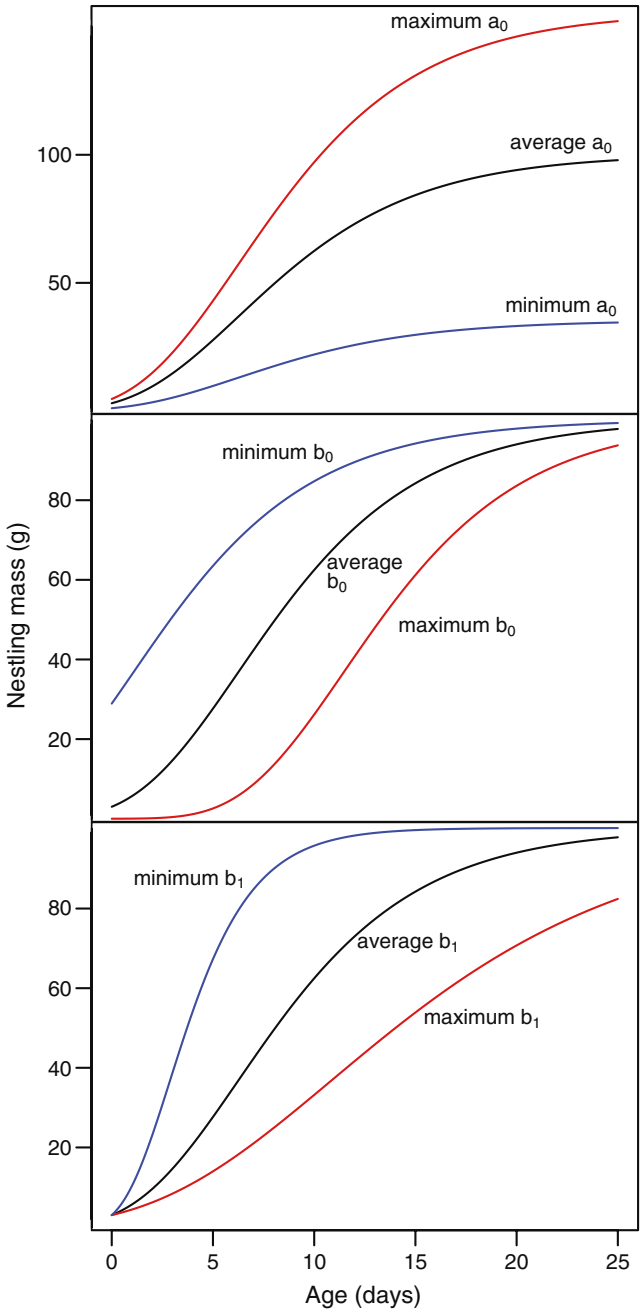

Fig. 1 Plotting of different Gompertz curves, in which one parameter was allowed to vary while the others were held fixed at means obtained from separate fits of the Gompertz function to individual American kestrels (*Falco sparverius*)

account for whatever residual heterogeneity remained. The best multilevel model will not necessarily contain a level-2 random effect or predictor for each level-1 parameter. We chose to introduce a random effect in the equation for a_0 because plots of interval estimates for each parameter (not shown) indicated that a_0 varied between individuals much more than the other parameters did.

Plots of the level-1 Pearson residuals from preliminary model constructions revealed a violation of the assumption of normality, as well as the presence of heteroscedasticity and temporal autocorrelation. Because the pattern shown by the heteroscedasticity suggested that the variance was an increasing function of the mean, we modeled the response as arising from a gamma distribution, using Proc NLMIXED of SAS 9.1 (SAS Institute Inc 2004). This resulted in well-behaved residual plots that exhibited neither heteroscedasticity nor temporal correlation.

We finished the formulation of the model by assessing the need for predictors in the level 2 equations. Individual models were estimated using maximum likelihood and the sets of models obtained were compared using a bias-corrected Akaike Information Criterion (AIC_c; Burnham and Anderson 2002).

The analysis of sex ratio relative to laying order presented analytical challenges as well, insofar as the response is dichotomous and the predictor of interest, laying order, is hierarchically nested within clutch (Krackow and Tkadlec 2001). Because laying order is ordinal, we treated it as an ordered factor and created four categorical variables for the regression whose components are polynomial contrasts corresponding to a linear, quadratic, cubic, and quartic trend. We fitted a mixed effects logistic regression with a random intercept assigned to each nest using the lmer function of the lme4 package of R and Adaptive Gaussian quadrature to obtain maximum likelihood estimates of the parameters.

Results

Regardless of yolk treatment (control or androgen), the injected egg hatched in all nests in which at least one egg (injected or not) hatched. Three uninjected eggs from two control nests and one uninjected egg from an androgen-treated nest failed to hatch. Validation of molecular sexing was confirmed by agreement between molecular and plumage-based sex in all 65 cases in which we had both markers.

The AIC_c best-ranked model was one in which the Gompertz parameters a_0 and b_0 were affected by sex, treatment, and their interaction, while the parameter b_1 varied only with sex (Table 1). From this, we conclude that hatching mass, growth rate, and maximum size were each affected by these factors (Fig. 2a–e). Wald tests for individual parameters confirmed that the highest-order effects in each of the level-2 equations of this model (sex × treatment interaction for a_0 and b_0; sex main effect for b_1) were each relatively reliable (Table 2).

Figure 2(a–e) suggested that the effect of the sex-by-treatment interaction was of a very specific sort. Males under yolk-androgen treatment tended to grow more slowly and achieve smaller size than individuals in the other three groups. Therefore, we conducted a post hoc examination of this particular contrast by dichotomizing the four levels of

Table 1 Level-2 equations for the parameters of a Gompertz function with a gamma-distributed response modeling growth of free-living nestling American kestrels (*Falco sparverius*)

Level-2 model					
a_0	b_0	b_1	K	Loglikelihood	AIC_c
$a_0 \sim 1+u_1$	$b_0 \sim 1$	$b_1 \sim 1$	5	575.0	585.8
$a_0 \sim \text{sex}+u_1$	$b_0 \sim 1$	$b_1 \sim 1$	6	572.0	585.1
$a_0 \sim \text{treatment}+u_1$	$b_0 \sim 1$	$b_1 \sim 1$	6	564.7	577.8
$a_0 \sim \text{sex}+\text{treatment}+u_1$	$b_0 \sim 1$	$b_1 \sim 1$	7	560.7	576.3
$a_0 \sim \text{sex}\|\text{treatment}+u_1$	$b_0 \sim 1$	$b_1 \sim 1$	9	554.2	574.1
$a_0 \sim \text{sex}\|\text{treatment}+u_1$	$b_0 \sim \text{sex}$	$b_1 \sim 1$	9	550.0	570.5
$a_0 \sim \text{sex}\|\text{treatment}+u_1$	$b_0 \sim \text{sex}+\text{treatment}$	$b_1 \sim 1$	10	543.7	566.8
$a_0 \sim \text{sex}\|\text{treatment}+u_1$	$b_0 \sim \text{sex}\|\text{treatment}$	$b_1 \sim 1$	11	539.5	565.2
$a_0 \sim \text{sex}\|\text{treatment}+u_1$	$b_0 \sim \text{sex}\|\text{treatment}$	$b_1 \sim \text{sex}$	12	535.5	564.0
$a_0 \sim \text{sex}\|\text{treatment}+u_1$	$b_0 \sim \text{sex}\|\text{treatment}$	$b_1 \sim \text{treatment}$	12	537.9	566.5
$a_0 \sim \text{sex}\|\text{treatment}+u_1$	$b_0 \sim \text{sex}\|\text{treatment}$	$b_1 \sim \text{sex}+\text{treatment}$	13	534.7	566.0
$a_0 \sim \text{sex}\|\text{treatment}+u_1$	$b_0 \sim \text{sex}\|\text{treatment}$	$b_1 \sim \text{sex}\|\text{treatment}$	14	534.5	568.8
$a_0 \sim \text{maleandro}+u_1$	$b_0 \sim 1$	$b_1 \sim 1$	6	558.0	571.1
$a_0 \sim 1+u_1$	$b_0 \sim \text{maleandro}$	$b_1 \sim 1$	6	570.0	583.1
$a_0 \sim \text{maleandro}+u_1$	$b_0 \sim \text{maleandro}$	$b_1 \sim 1$	7	542.2	557.7
$a_0 \sim \text{maleandro}+u_1$	$b_0 \sim \text{maleandro}$	$b_1 \sim \text{maleandro}$	8	542.1	560.1
$a_0 \sim \text{maleandro}+u_1$	$b_0 \sim \text{maleandro}$	$b_1 \sim \text{sex}$	8	541.2	559.2
$a_0 \sim \text{maleandro}+u_1$	$b_0 \sim \text{maleandro}$	$b_1 \sim \text{treatment}$	8	542.2	560.1

Equations compare the effects of including a predictor for sex, one for yolk-androgen treatment, and one dichotomizing androgen-treated males and all others (maleandro) *post hoc*. a_0, b_0, and b_1 refer to the function's parameters. All equations include an intercept. Thus, the level-2 model $b_0 \sim \text{sex}$ is a regression model for the Gompertz parameter b_0 that includes an intercept and a dichotomous indicator of sex. K denotes the number of estimated parameters. u_1 denotes a random effect drawn from a normal distribution with mean 0 and variance τ^2. sex|treatment = sex + treatment + sex × treatment interaction, 1=intercept.

the sex × treatment interaction into androgen-treated males versus all others (i.e., a pool of androgen-treated females, control females, and control males). The best model (i.e., that with the lowest AIC_c) was one which allowed males from androgen-treated eggs to differ from all others in their values of a_0 and b_0 but not b_1 (Fig. 2f, Table 1). Wald tests supported the reliability of the effect of this dichotomized variable (Table 2).

In our analysis of offspring sex ratio, we found that the quadratic trend across laying order was relatively reliable (P=0.039; Fig. 3). None of the other trends reliably differed from 0 (each P>0.2). Despite this, it was important to determine if laying order should even be included in the modeling of sex-ratio variation, as we did for sex, yolk-androgen treatment, and their interaction in the models described above. Therefore, we fitted a model without egg order (only an intercept) and used this "null" model to carry out a likelihood ratio test of the variable construct "egg order." This was essentially a test of whether or not egg order (as formulated using all four trend contrasts simultaneously) was needed in the model. We found that the addition of the factor "laying order" to a null model with only an intercept did not improve the model's fit relative to its number of parameters (likelihood ratio test P>0.2; $AIC_{intercept}$=91.6, $AIC_{intercept,laying\ order}$=93.7). Hence, there was no compelling reason to include the term laying order in the model describing variation in sex ratio.

Discussion

Experimentally elevating yolk-androgen concentrations in the a-egg to the levels naturally found in later-laid eggs did not appear to affect the growth rates of female kestrel nestlings but reduced growth rates of male nestlings, relative to controls. Thus, it seems likely that the effects of yolk androgens reported previously in American kestrels (Sockman and Schwabl 2000) are principally due to a growth-inhibiting action specifically on males.

The proximate causes of sex differences in androgen sensitivity are unknown but could reflect sexually dimorphic mechanisms of growth and differentiation, such as a sex difference in the timing or location of androgen-receptor expression or, in the event that yolk androgens are aromatized, estrogen-receptor expression. Little is known about sexual dimorphism in the development of androgen- or estrogen-receptor expression. Godsave et al. (2002) investigated androgen-receptor expression in developing zebra finches but found no sex differences in the areas of their primary interest (syringeal and hindbrain regions). Still, it is possible

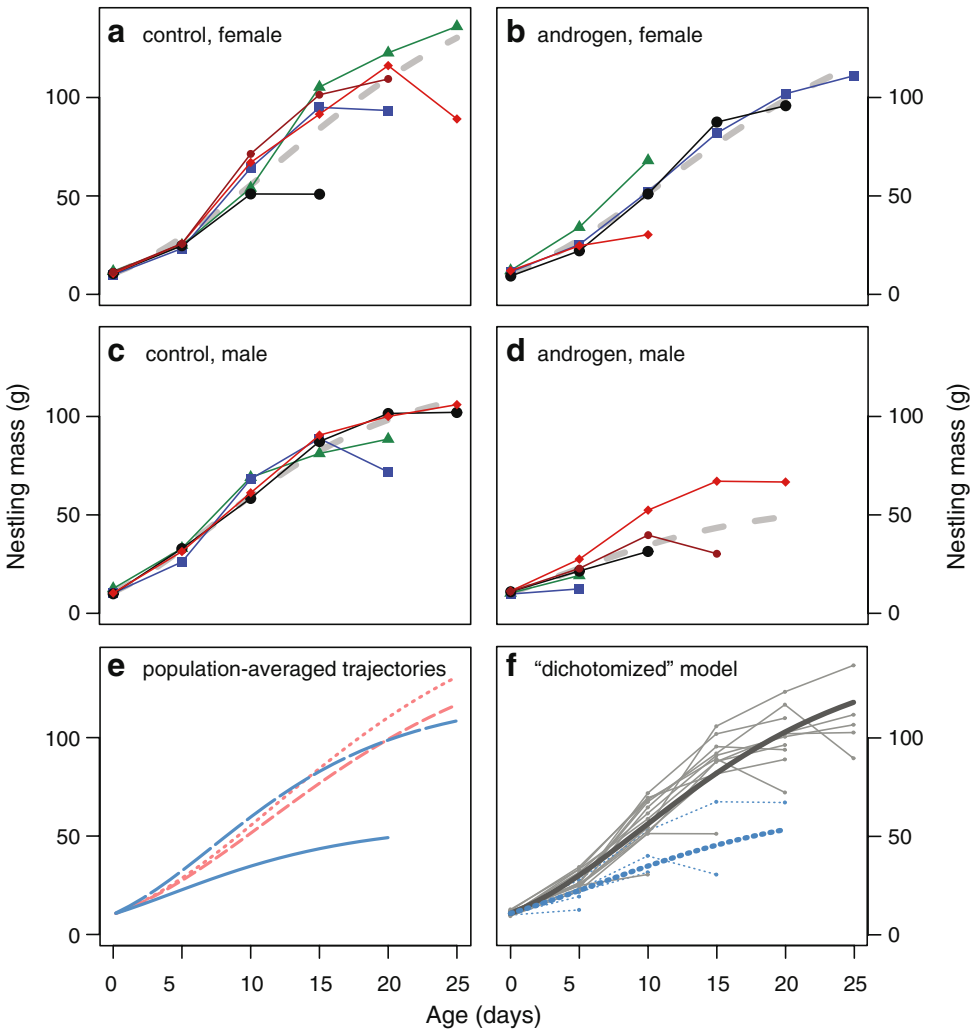

Fig. 2 Individual (each symbol type in a-d) and population-averaged (thick lines in a-d) growth trajectories in nestling American kestrels (*Falco sparverius*): **a** control, female; **b** androgen, female; **c** control, male; **d** androgen, male; **e** population-averaged trajectories from a-d (solid line: androgen, male; long-dashed line: control, male; short-dashed line: androgen, female; dotted line: control, female); **f** "dichotomized" model (thin lines: individual trajectories; thick lines: population-averaged trajectories; dotted lines: androgen, male; solid lines: all other individuals)

that the sexes differ in the timing of androgen-receptor expression elsewhere, such as in some areas that might directly mediate body growth. In addition, sex differences in timing and location of estrogen-receptor expression might mediate sex-specific effects of aromatized yolk androgens.

In contrast to the growth inhibiting effects we find, androgens are widely acknowledged for their anabolic effects on vertebrate growth (Herbst and Bhasin 2004). However, the assumption of a general anabolic role is based almost entirely on studies of species with male-larger sexual size-dimorphism (Cox and John-Alder 2005). The American kestrel is a species with reverse sexual size-dimorphism; females are larger (Anderson et al. 1993). Testosterone promotes growth in some reptile species with normal sexual size-dimorphism but inhibits it in some with reverse sexual size-dimorphism (e.g., Crews et al. 1985; Cox and John-Alder 2005). This raises the possibility that the male growth-inhibiting effects of yolk androgens in American kestrels regulates, in part, the species's normal pattern of reverse sexual size-dimorphism in which males are smaller than females. Androgen mediation of reverse sexual size-dimorphism is putatively based on life-history trade-offs between androgen-induced growth and androgen-induced activity (Cox and John-Alder 2005); however, how significant, growth-inhibiting variation in activity might manifest in nest-bound young is not clear. Still, this hypothesis, as it pertains to nest-bound birds exposed to yolk androgens, merits further examination.

Several previous studies, including two on the American kestrel (Sockman and Schwabl 2000; Sockman et al. 2001), have shown increases in yolk-androgen concentrations with laying order (e.g., Schwabl 1993; Eising et al. 2001; Royle et al. 2001; Pilz et al. 2003; Tschirren et al. 2004). One possible reconciliation of this pattern of yolk-androgen deposition with the results presented here is that variation across laying order in both sex ratio and in yolk-androgen deposition optimizes exposure of the sexes to maternal hormones (e.g., Badyaev et al. 2002a). Females may protect male eggs from the detrimental effects of yolk androgens by adjusting yolk-androgen deposition and sex ratio across

Table 2 Parameter estimates for the AIC_c-best models (Table 1) on growth of free-living nestling American kestrels (*Falco sparverius*)

Parameter	Estimate	Standard error	*t* values	*P* values
Model: AIC Best *a priori* Model from Table 1				
a_0: intercept	155.480	24.285	6.40	<0.0001
a_0: sex	−98.870	25.633	−3.86	0.001
a_0: treatment	21.348	17.108	1.25	>0.20
a_0: sex|treatment	42.863	20.438	2.10	0.050
b_0: intercept	2.693	0.168	15.99	<0.0001
b_0: sex	−1.010	0.236	−4.28	0.0005
b_0: treatment	0.133	0.159	0.84	>0.20
b_0: sex|treatment	0.606	0.237	2.56	0.020
b_1: intercept	0.915	0.009	106.02	<0.0001
b_1: sex	−0.031	0.015	−2.00	0.061
τ^2	58.405	39.870	–	–
$\log\phi$	3.699	0.174	21.30	<0.0001
Model: AIC Best *post hoc* Model from Table 1				
a_0: intercept	144.650	13.445	10.76	<0.0001
a_0: maleandro	−76.913	12.335	−6.24	<0.0001
b_0: intercept	2.618	0.093	28.06	<0.0001
b_0: maleandro	−0.766	0.171	−4.48	0.0003
b_1: intercept	0.904	0.007	122.08	<0.0001
τ^2	76.236	52.506	–	–
$\log\phi$	3.623	0.175	20.65	<0.0001

The Parameter column denotes the name of the parameter of the Gompertz function followed by the name of the regressor that was included in that parameter's level-2 equation. Sex was coded 0 for female, 1 for male. Treatment was coded 0 for androgen, 1 for control. The predictor maleandro was coded 1 for males hatching from androgen-treated eggs and 0 for all others. Wald test statistics (*t*, *P*) were based on 18 degrees of freedom. Conventions and symbols as in Table 1.

the laying cycle. Because the a-egg differs from all others in its yolk-androgen concentrations and because yolk androgens inhibit growth of nestlings hatching from the a-egg, we expected a-eggs to differ from others in sex ratio; but, our evidence for this in kestrels is limited, as we found no compelling evidence that laying order affects sex ratio. Any reality to the quadratic trend in sex ratio with respect to laying order (Fig. 3) suggests possible sex biases for laying orders 3 and 5, but not for the a-egg. Nonetheless, a slight sex-biased laying order in our study may have been partially obscured by

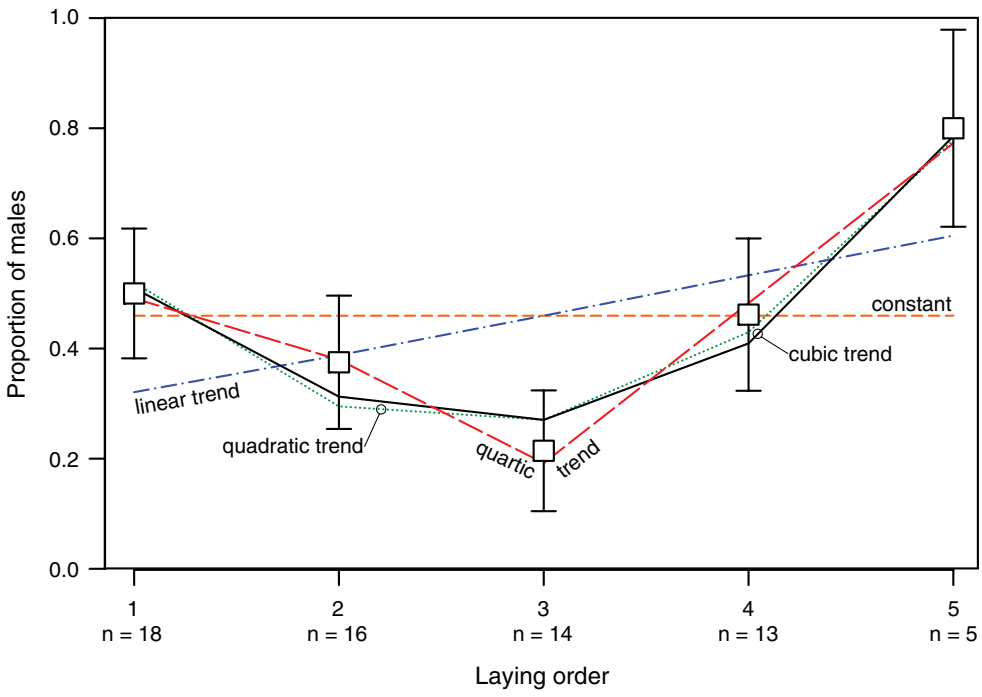

Fig. 3 Sex ratio (mean ± S.E.M.) with respect to laying order in nestling American kestrels (*Falco sparverius*)

our relatively small sample sizes or by the possibility of an effect of a sex-by-laying order interaction on early mortality.

Another possibility is that a-eggs differ from later-laid eggs in some other attributes. A-eggs may lack protective substances that, in later-laid eggs, are present in combination with high yolk androgens. However, to our knowledge, no study so far has shown increases with laying order of any egg components other than androgens. Conversely, a-eggs may contain substances that are less concentrated in later-laid eggs that have naturally elevated androgen levels and that, in combination with high androgens, are detrimental to males. We know of no such substances that decline in concentrations with laying order. In black-backed gulls (Royle et al. 2001), barn swallows (Saino et al. 2002), and zebra finches (Williamson et al. 2006), concentrations of carotenoid and vitamins decline with laying order. However, carotenoids are thought to defray, not exacerbate costs (in the form of production of radical oxygen species) of elevated metabolism, which one would expect from androgen exposure (Royle et al. 2001). Therefore, if protective components such as yolk-antioxidants decline or do not vary with laying order, then the growth-reducing effects of yolk androgens in nestling kestrels hatching from a-eggs may apply to later-laid eggs and influence males in particular. Given this possibility, a function of elevated yolk-androgen concentrations in later-laid eggs in the kestrel may be to handicap male nestlings under certain conditions described below.

Assuming sex ratio does not vary with laying order, how can our observations that yolk androgens inhibit male growth be reconciled with adaptive increases in yolk androgens with laying order, and therefore, under what conditions would yolk androgens function to handicap the growth of nestlings? A possible answer to this question might be found in the Offspring Value Hypothesis for yolk-androgen deposition, which was recently described in detail (Sockman et al. 2006) and a corollary of which we briefly describe here. In most species, male fitness is thought to be more conditional than female fitness (Trivers and Willard 1973). Thus, the economics of reproduction would favor a positive correlation between parental condition and investment in male offspring (Clutton-Brock 1986). During our study, nestling mortality was unusually high, regardless of yolk treatment (Sockman and Schwabl 2000), and food seemed to be scarce or of low quality (K.W. Sockman, personal observation), suggesting that environmental and possibly parental and nestling conditions were poor. Because male fitness can vary more than female fitness in the American kestrel (Smallwood and Smallwood 1998), we propose that sex differences in sensitivity to elevated yolk-androgen concentrations enable parents to economically tune reproductive effort to the conditional value of individual offspring. Offspring value will be a product of the interaction between offspring sex and offspring condition, the latter of which is unpredictable at the time of yolk-androgen deposition but which is influenced by nestling provisioning (Sockman et al. 2006). This hypothesis predicts a positive correlation between parental condition (and hence nestling provisioning) and the effects of yolk androgens on growth of male young, with the effects of yolk androgens changing from detrimental to neutral to favorable as parental condition and nestling provisioning increases (Sockman et al. 2006). A definitive test of this hypothesis has yet to be conducted in any species and requires the controlled, experimental manipulation of both yolk-androgens and parental condition or nestling provisioning to determine their interactive effects on the growth of male and female offspring.

Acknowledgements We thank Wendy Lammers for assistance in data collection and NIMH (R01 49877 to H.S.), SICB (Grant-in-Aid of Research to K.W.S.), and the WSU Department of Zoology (James R. King Fellowship to K.W.S.) for financial support. This study complies with the current laws of the USA, where it was performed.

References

Aggrey SE (2002) Comparison of three nonlinear and spline regression models for describing chicken growth curves. Poultry Sci 81:1782–1788

Anderson DJ, Reeve J, Gomez JEM, Weathers WW, Hutson S, Cunningham HV, Bird DM (1993) Sexual size dimorphism and food requirements of nestling birds. Can J Zool 71:2541–2545

Badyaev AV, Hill GE, Beck ML, Dervan AA, Duckworth RA, McGraw KJ, Nolan PM, Whittingham LA (2002a) Sex-biased hatching order and adaptive population divergence in a passerine bird. Science 295:316–318

Badyaev AV, Hill GE, Whittingham LA (2002b) Population consequences of maternal effects: sex-bias in egg-laying order facilitates divergence in sexual dimorphism between bird populations. J Evol Biol 15:997–1003

Balgooyen TG (1976) Behavior and ecology of the American kestrel (*Falco sparverius* L.) in the Sierra Nevada of California. Univ Calif Publ Zool 103:1–87

Ball GF, Riters LV, Balthazart J (2002) Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones. Front Neuroendocrin 23:137–178

Balthazart J, Absil P, Foidart A, Houbart M, Harada N, Ball GF (1996) Distribution of aromatase-immunoreactive cells in the forebrain of zebra finches (*Taeniopygia guttata*): implications for the neural action of steroids and nuclear definition in the avian hypothalamus. J Neurobiol 31:129–148

Bernardo J (1996) Maternal effects in animal ecology. Am Zool 36:83–105

Bird DM (1988) American kestrel. In: Palmer RS (ed) Handbook of North American birds, vol 5. Yale University Press, New Haven, Connecticut, pp 253–290

Burnham HP, Anderson DR (2002) Model selection and multimodel inference. Springer, Berlin Heidelberg New York

Clutton-Brock TH (1986) Sex ratio variation in birds. Ibis 128:317–329

Cox RM, John-Alder HB (2005) Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.) with opposite patterns of sexual size dimorphism. J Exper Biol 208:4679–4687

Crews D, Diamond MA, Whittier J, Mason R (1985) Small male body size in garter snake depends on testes. Am J Physiol Regul Integr Comp Physiol 249:62–66

de Freitas AR (2005) Growth curves in animal production. Braz J Anim Sci 34:786–795

Eising CM, Eikenaar C, Schwabl H, Groothuis TGG (2001) Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. Proc R Soc Lond B 268:839–846

Godsave SF, Lohmann R, Vloet RPM, Gahr M (2002) Androgen receptors in the embryonic zebra finch hindbrain suggest a function for maternal androgens in perihatching survival. J Comp Neurol 453:57–70

Herbst KL, Bhasin S (2004) Testosterone action on skeletal muscle. Curr Opin Clin Nutr Metab Care 7:271–277

Kahn NW, St. John J, Quinn TW (1998) Chromosome-specific intron size differences in the avian CHD gene provide an efficient method for sex identification in birds. Auk 115:1074–1078

Krackow S, Tkadlec E (2001) Analysis of brood sex ratios: implications of offspring clustering. Behav Ecol Sociobiol 50:293–301

Müller W, Groothuis TGG, Eising CM, Dijkstra C (2005) An experimental study on the causes of sex-biased mortality in the black-headed gull—the possible role of testosterone. J Anim Ecol 74:735–741

Nahashon SN, Aggrey SE, Adefope NA, Amenyenu A, Wright D (2006) Growth characteristics of pearl gray Guinea fowl as predicted by the Richards, Gompertz, and logistic models. Poultry Sci 85:359–363

Pilz KM, Smith HG, Sandell MI, Schwabl H (2003) Interfemale variation in egg yolk androgen allocation in the European starling: do high-quality females invest more? Anim Behav 65:841–850

Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. Springer, Berlin Heidelberg New York

R Development Core Team (2004) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ricklefs RE (1968) Patterns of growth in birds. Ibis 110:419–451

Ricklefs RE (1973) Patterns of growth in birds. II. Growth rate and mode of development. Ibis 115:177–205

Royle NJ, Surai PF, Hartley IR (2001) Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? Behav Ecol 12:381–385

Rubolini D, Romano M, Martinelli R, Saino N (2006) Effects of elevated yolk testosterone levels on survival, growth and immunity of male and female yellow-legged gull chicks. Behav Ecol Sociobiol 59:344–352

Saino N, Bertacche V, Ferrari RP, Martinelli R, Møller AP, Stradi R (2002) Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. Proc R Soc Lond B 269:1729–1733

Saino N, Ferrari RP, Romano M, Martinelli R, Lacroix A, Gil D, Møller AP (2006) Maternal allocation of androgens and antagonistic effects of yolk androgens on sons and daughters. Behav Ecol 17:172–181

SAS Institute Inc (2004) SAS/STAT User's Guide, Version 9.1. SAS Institute, Carey, North Carolina

Schwabl H (1993) Yolk is a source of maternal testosterone for developing birds. Proc Natl Acad Sci USA 90:11446–11450

Schwabl H (1996) Maternal testosterone in the avian egg enhances postnatal growth. Comp Biochem Phys A 114:271–276

Schwabl H, Mock DW, Gieg JA (1997) A hormonal mechanism for parental favouritism. Nature 386:231

Sengül T, Kiraz S (2005) Non-linear models for growth curves in large white turkeys. Turk J Vet Anim Sci 29:331–337

Smallwood PD, Smallwood JA (1998) Seasonal shifts in sex ratios of fledgling American kestrels (*Falco sparverius paulus*): the early bird hypothesis. Evol Ecol 12:839–853

Sockman KW, Schwabl H (1998) Hypothermic tolerance in an embryonic American kestrel (*Falco sparverius*). Can J Zool 76:1399–1402

Sockman KW, Schwabl H (2000) Yolk androgens reduce offspring survival. Proc R Soc Lond B 267:1451–1456

Sockman KW, Schwabl H (2001a) Covariation of clutch size, laying date, and incubation tendency in the American kestrel. Condor 103:570–578

Sockman KW, Schwabl H (2001b) Plasma corticosterone in nestling American kestrels: effects of age, handling stress, yolk androgens, and body condition. Gen Comp Endocrinol 122:205–212

Sockman KW, Schwabl H, Sharp PJ (2001) Regulation of yolk-androgen concentrations by plasma prolactin in the American kestrel. Horm Behav 40:462–471

Sockman KW, Sharp PJ, Schwabl H (2006) Orchestration of avian reproductive effort: an integration of the ultimate and proximate bases for flexibility in clutch size, incubation behaviour, and yolk-androgen deposition. Biol Rev 81:629–666

Trivers RL, Willard DE (1973) Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90–92

Tschirren B, Richner H, Schwabl H (2004) Ectoparasite-modulated deposition of maternal androgens in great tit eggs. Proc R Soc Lond B 271:1371–1375

von Engelhardt N, Carere C, Dijkstra C, Groothuis TGG (2006) Sex-specific effects of yolk testosterone on survival, begging and growth of zebra finches. Proc R Soc Lond B 273: 65–70

Wang Z, Zuidhof MJ (2004) Estimation of growth parameters using a nonlinear mixed Gompertz model. Poultry Sci 83:847–852

Westneat DF (1990) Genetic parentage in the indigo bunting: a study using DNA fingerprinting. Behav Ecol Sociobiol 27:67–76

Williamson KA, Surai PF, Graves JA (2006) Yolk antioxidants and mate attractiveness in the zebra finch. Funct Ecol 20:354–359