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Correlated evolution of maternally derived yolk testosterone and early developmental traits in passerine birds

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Recent studies on hormone-mediated maternal effects in birds have highlighted the influence of variable maternal yolk androgen concentration on offspring phenotype, particularly in terms of early development. If genetic differences between laying females regulate variation in yolk hormone concentration, then this physiological maternal effect is an indirect genetic effect which can provide a basis for the co-evolution of maternal and offspring phenotypes. Thus, we investigated the evolutionary associations between maternally derived yolk testosterone (T) and early developmental traits in passerine birds via a comparative, phylogenetic analysis. Our results from species-correlation and independent contrasts analyses provide convergent evidence for the correlated evolution of maternal yolk T concentration and length of the prenatal developmental period in passerines. Here, we show these traits are significantly negatively associated (species-correlation: $p<0.001$, $r^2=0.85$; independent contrasts: $p=0.005$). Our results highlight the need for more studies investigating the role of yolk hormones in evolutionary processes concerning maternal effects.

Keywords: maternal effects; yolk hormones; testosterone; early development; independent contrasts

1. INTRODUCTION

Recent work on maternally derived yolk hormones in avian systems has made an important contribution to our understanding of the physiological mechanisms by which mothers influence their offspring's phenotype (i.e. maternal effects; Schwabl 1993, 1996*b*; Lipar & Ketterson 2000; Sockman & Schwabl 2000). During the rapid yolk development phase of oogenesis, steroid hormones of maternal origin are transferred from females to the yolk of their developing ovarian follicles (Schwabl 1993, 1997*a*; Adkins-Regan *et al.* 1995; Hackl *et al.* 2003). This hormonal milieu provided by adult females may modulate offspring phenotype as exposure to hormones during early ontogeny can have considerable organizational effects in terms of development and behaviour (Clark & Galef 1995; Lindström 1999; Dufty *et al.* 2002).

Most avian yolk androgen studies have considered the ecological determinants, or the developmental and behavioural effects, of variation in maternal yolk hormone concentration. Many studies have reported marked intraspecific differences in yolk androgen concentration over the egg laying sequence of individuals (Schwabl *et al.* 1997; Lipar *et al.* 1999*b*; Lipar & Ketterson 2000; Groothuis & Schwabl 2002), as well as differences between conspecifics (Schwabl 1997*b*; Gil *et al.* 1999; Müller *et al.* 2002; Pilz & Smith 2004). This variation in maternal yolk androgen concentration influences early developmental and behavioural phenotypes of offspring (Schwabl 1996*b*; Lipar & Ketterson 2000; Eising *et al.* 2001; Eising & Groothuis 2003), potentially affecting offspring survival or reproductive success (e.g. Sockman & Schwabl 2000; Eising & Groothuis 2003; Pilz *et al.* 2004). Therefore, such physiological maternal effects can have significant evolutionary consequences. However, the current literature has neglected linking yolk androgens to evolutionary processes concerning maternal effects.

Theoretically, if genetic differences between laying females regulate variation in maternal yolk hormone concentration, then this physiological maternal effect is an indirect genetic effect (IGE; Wolf *et al.* 1998) which can provide a basis for the co-evolution of maternal and offspring phenotypes (Wade 1998; Wolf & Brodie 1998; Wolf 2000). Thus, we conducted a comparative, phylogenetic analysis using species-correlation and independent contrasts analyses to test for evolutionary associations between maternally derived yolk testosterone (T) concentration and early developmental traits of offspring including length of the prenatal and postnatal developmental periods, as well as postnatal developmental growth rate in the Passeriformes. We predicted that higher yolk T concentrations should be associated with shorter developmental periods and higher growth rates (*sensu* Eising *et al.* 2001; Eising & Groothuis 2003).

2. MATERIAL AND METHODS

(a) *Data collection*

We compiled data from the published literature on species clutch mean yolk T concentration for all available passerine species (figure 1). Our analyses were restricted to the Passeriformes in order to address the statistical concerns of taxon sampling in comparative studies (Ackerly 2000). We focused on yolk T in this study as concentration data were available for 11 passerine species. Concentration data for other yolk androgens such as androstenedione (A4) and 5α-dihydrotestosterone (DHT) were available for only a small number of passerines (i.e. 4 and 5 species, respectively), thus these data were excluded from our analyses. Species yolk T values were calculated by either using the reported clutch mean concentration, or calculating the clutch mean concentration from reported data over the egg laying sequence. When possible, multiple clutch mean values for a single species were used to calculate an overall value for that species (figure 1). Data from experimental studies were always obtained from control samples. Data on developmental traits including length of the prenatal (i.e. number of days of incubation) and postnatal (i.e. number of days between hatch and fledge) developmental periods, as well as postnatal developmental growth rate (i.e. grams of mass gained per day, controlling for hatch mass) were obtained from general references (Cramp 1983; Poole *et al.* 1992–2002; Zann 1996). Yolk T concentration data, as well as length of the prenatal and postnatal developmental period data, were log transformed for normality (Shapiro–Wilk test).

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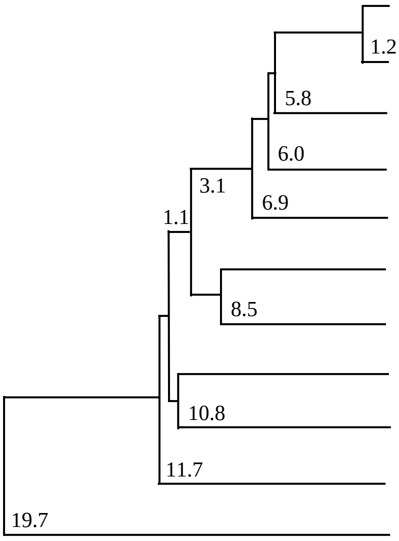

species	yolk T (pg mg^{-1})	data source
red-winged blackbird (*Agelaius phoeniceus*)	37.26	Lipar *et al.*(1999*a*), Lipar *et al.*(1999*b*), Lipar & Ketterson (2000)
brown-headed cowbird (*Molothrus ater*)	14.81	Schwabl (1997*a*), Hauber & Pilz (2003)
yellow warbler (*Dendroica petechia*)	54.00	Hauber & Pilz (2003)
dark-eyed junco (*Junco hyemalis*)	29.67	Lipar *et al.*(1999*b*)
canary (*Serinus canaria*)	55.03	Schwabl (1993), Schwabl (1996*a*)
zebra finch (*Taeniopygia guttata*)	4.18	Schwabl (1993), Gil *et al.*(1999)
house sparrow (*Passer domesticus*)	27.52	Schwabl (1997*a*), Schwabl (1997*b*), Mazuc *et al.* (2003)
tree swallow (*Tachycinta bicolor*)	1.20	Whittingham & Schwabl (2002)
house wren (*Troglodytes aedon*)	4.78	Schwabl (1997*a*)
European starling (*Sturnus vulgaris*)	1.33	Schwabl (1997*a*), Lipar (2001), Pilz *et al.* (2003)
eastern pheobe (*Sayornis saya*)	0.50	Hauber & Pilz (2003)

Figure 1. DNA–DNA hybridization phylogeny for all 11 passerine species including clutch mean yolk T concentration and associated data sources. Branch lengths after Sibley & Ahlquist (1990). Phylogenetic tree produced using TREEVIEW (Page 1996).

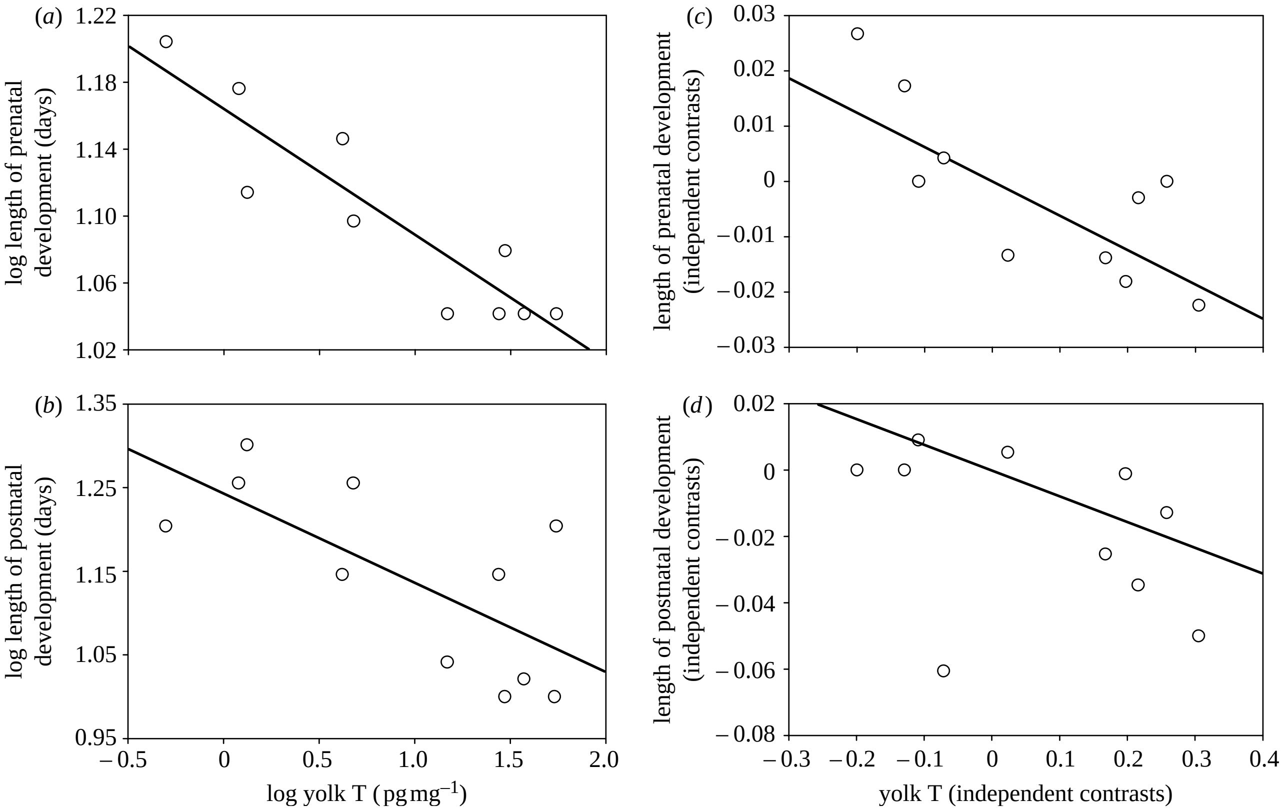

Figure 2. Species-correlations between log yolk T concentration and log (*a*) length of the prenatal and (*b*) postnatal developmental periods, and independent contrasts relationships for yolk T concentration and (*c*) length of the prenatal and (*d*) postnatal developmental periods.

(b) *Data analysis*

We used species-correlation and independent contrasts analyses to test for evolutionary associations between maternally derived yolk T concentration and offspring early developmental traits. Species-correlation analyses assume inter-specific data are phylogenetically independent (Martins 2000) and describe current associations between extant taxa (Price 1997). Independent contrasts analyses incorporate phylogenetic information into comparative data to control for the statistical problems associated with species-relatedness (Felsenstein 1985; Martins 2000), and are therefore more conservative. We report the results of both analyses as similarity in results is suggestive of robust relationships, while discrepancies may be biologically relevant (Ricklefs & Stark 1996; Price 1997).

We derived phylogenetic relationships from the DNA–DNA hybridization phylogeny reported by Sibley & Ahlquist (1990; figure 1) for use in independent contrasts analyses. COMPARE software 4.5 (Martins 2003) was used to produce independent contrasts of maternal yolk T and offspring early developmental trait data. Adequate standardization of independent contrasts was tested by regression of the absolute value of the contrasts by their standard deviation (Garland *et al.* 1992). All contrasts were adequately standardized for all analyses ($p > 0.05$). Species-correlation and independent contrasts analyses were analysed using linear regression models in JMP 4.0 (2001). Independent contrasts analyses forced the regression line through the origin (Garland *et al.* 1992).

3. RESULTS

(a) *Species-correlation analyses*

Linear regression analyses of species yolk T concentration and length of the prenatal developmental period indicated these traits were significantly negatively related ($p<0.001$; $r^2=0.85$; figure 2*a*). Furthermore, species yolk T concentration was significantly negatively associated with length of the postnatal developmental period ($p=0.02$; $r^2=0.49$; figure 2*b*). There was no relationship between species yolk T concentration and postnatal developmental growth rate ($p=0.45$; $r^2=0.08$).

(b) *Independent contrasts analyses*

Linear regression analyses of independent contrasts for species yolk T concentration and length of the prenatal developmental period resulted in a significant negative relationship ($p=0.005$; figure 2*c*). However, length of the postnatal developmental period (figure 2*d*) and postnatal developmental growth rate were independent of species yolk T concentration ($p>0.10$ in both cases).

4. DISCUSSION

We have demonstrated a significant evolutionary association between maternally derived yolk T concentration and length of the prenatal developmental period in passerine birds, corroborated by species-correlation and phylogenetically independent contrasts analyses. Species-correlation analysis also indicated an evolutionary association between yolk T concentration and length of the postnatal developmental period, although this relationship was not supported by the more conservative result from independent contrasts analysis.

In our comparative study, higher yolk T concentration was associated with shorter prenatal development. Lipar & Ketterson (2000) showed that treatment of egg yolk with T in red-winged blackbirds (*Agelaius phoeniceus*) resulted in an increase in the relative mass of the young's hatching muscle, which might be associated with more rapid hatching (see also Lipar 2001). Black-headed gull (*Larus ridibundus*) embryos hatching from eggs with experimentally elevated yolk androgen concentration showed a decrease in the length of prenatal development; young hatched half a day earlier in experimental eggs compared with control eggs (Eising *et al.* 2001; Eising & Groothuis 2003). Thus, our results corroborate that which has been shown in studies examining the intraspecific effects of variation in yolk T concentration on offspring development, and further demonstrate the correlated evolution of these maternal and offspring traits. Genetic differences in hormonal traits, such as maternal yolk hormone concentration and offspring developmental response to yolk hormones (*sensu* Ketterson & Nolan 1999), can provide a basis for the co-evolution of maternal and offspring phenotypes via interactions between these genomes such as genotype × genotype epistasis (Wade 1998; Wolf & Brodie 1998; Wolf 2000). Other yolk androgens of maternal origin, such as A4 and DHT, may also show correlated evolution with avian developmental phenotypes. These relationships should be examined as more interspecific yolk hormone data become available.

Our results highlight the need for further investigations of the role of maternal yolk hormones in evolutionary processes concerning maternal effects. In theory, yolk hormones as IGEs can have important implications for the direction and rate of short-term trait evolution (*sensu* Wolf *et al.* 1998) as the sign of the covariance between direct and maternal additive genetic effects drives the response to selection (Kirkpatrick & Lande 1989; Wolf *et al.* 1998). An appreciation for the evolutionary dynamics of maternal effects systems is important for better understanding the transgenerational relationships between maternal physiology and offspring phenotype.

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