sources of yolk protein and lipid, respectively (Deeley et al. 1975; Wallace 1985; Walzem et al. 1999). Vitellogenin and VLDLy particles are secreted into the circulation and taken up by developing oocytes of the ovary via receptor-mediated endocytosis (Wallace 1985; Barber et al. 1991). Vitellogenin production alone comprises approximately 50% of the daily hepatic protein synthesis of the laying hen (*Gallus gallus domesticus*) and may triple the amount of protein secreted into the blood (Gruber 1972). VLDLy production also involves changes in the physical structure and biochemical properties of hepatically produced VLDL (Walzem 1996). Thus, there are likely to be energetic, metabolic, and possibly osmoregulatory costs associated with yolk precursor production, which would predict a close matching between the amount of yolk precursors produced by the liver (i.e., supply) and the amount required for ovarian follicular growth (i.e., demand).

In this article, we first present data on the pattern of follicle development in a free-living passerine, the European starling (*Sturnus vulgaris*), including information on the timing of follicular atresia during follicle development. We then compare variation in follicle mass (as an index of demand) with changes in plasma yolk precursor concentrations at three levels: (1) daily variation throughout the laying cycle, (2) variation among in-

curred 12–15 and 0–2 h after ovulation, respectively. To control for any effect of handling or confinement of females in nest boxes, a second group of females ($n = 18$) were blood sampled in the morning only (10:00 A.M.–12:00 P.M.) on the day their second egg was laid. Between-clutch repeatability of yolk precursor concentrations was determined in females that were blood sampled during their first clutch and then again during their replacement clutch laid after desertion or in response to removal of the first clutch (see Christians and Williams 1999*a*).

Statistical Analysis

All statistical analyses were carried out using SAS (SAS Institute 1990). Preliminary analyses showed that total yolky follicle mass (i.e., F_1–F_4), plasma VTG, and plasma VLDL concentrations were independent of nonreproductive body mass ($P > 0.10$ in all cases). Similarly, time between capture and blood sampling had no effect on plasma VTG and VLDL concentrations ($P > 0.10$ in both cases). Therefore, statistically controlling for these factors was not necessary. Variation in the mass of the yolky follicles and in the plasma concentrations of VTG and VLDL was assessed using general linear models (GLM procedure; SAS Institute 1990), and pairwise comparisons between groups were performed using contrasts (Sokal and Rohlf 1995). When assessing changes in follicle mass and the plasma concentrations of VTG and VLDL throughout the laying cycle, only four pairwise comparisons were made (described below) to reduce the number of contrasts; a Bonferroni-adjusted level of significance was used for each comparison (Sokal and Rohlf 1995), that is, 0.05/4 comparisons = 0.0125, to maintain an experimentwise error rate of 0.05. When examining the effects of diurnal variation on the plasma concentrations of the yolk precursors, comparisons between all three groups were made; thus, = 0.05/3 = 0.017. Repeatability of yolk precursors was calculated following Lessells and Boag (1987).

Results

Variation in Follicular Growth and Plasma Yolk Precursor Concentrations throughout the Laying Cycle

As expected, total yolky follicle mass varied markedly throughout the laying cycle, as follicles were recruited into the hierarchy and initiated rapid yolk development ($F_{11,79} = 268.9$, $P < 0.001$; Fig. 1). Total yolky follicle mass increased from 0.067 ± 0.012 g ($n = 6$) when only the first yolky follicle was present to 1.970 ± 0.046 g ($n = 26$) for birds with a complete F_1–F_4 hierarchy and then decreased to 0.940 ± 0.043 g ($n = 9$) when only a single large yolky follicle was present. Plasma VTG and VLDL concentrations varied significantly with stage of follicle development (VTG, $F_{10,77} = 22.41$, $P < 0.001$; VLDL, $F_{10,77} = 13.52$, $P < 0.001$), and both yolk precursors matched changes in follicle mass fairly closely on the broad scale (Fig. 1). Plasma VTG and VLDL were strongly positively correlated

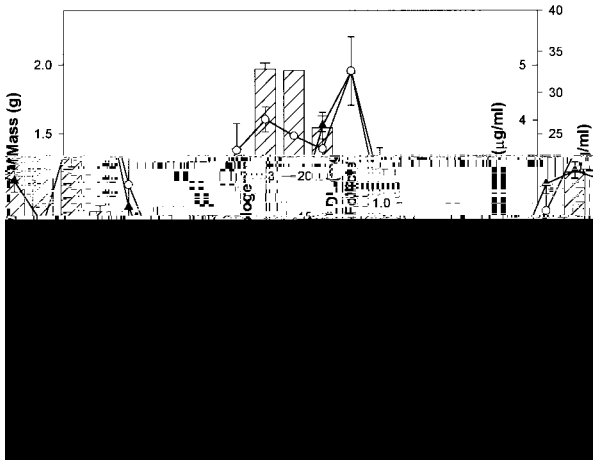

Figure 1. Variation in plasma concentrations of vitellogenin (*open circles*) and very low density lipoprotein (*closed triangles*) in relation to total yolky follicle mass during the laying cycle (*hatched bars*) and pattern of follicle development (*bottom panel*) in female starlings. *NBR* = nonbreeders; *PB* = prebreeders; *PEN* = day of penultimate egg; *CC* = clutch completion; *CC* + 2 = 2 d after clutch completion. Values are means ± 1 SE.

with each other ($F_{1,76} = 78.41$, $P < 0.001$, $r^2 = 0.84$; controlling for stage of follicle development). Both yolk precursors were basal in nonbreeding females (VTG, 0.02 ± 0.02 g/mL; VLDL, 3.5 ± 0.4 mg/mL) and then increased rapidly to 1.66 ± 0.33 g/mL and 10.7 ± 2.4 mg/mL, respectively, as soon as a single, small yolky follicle was present ($P < 0.001$ in both cases). When the full follicle hierarchy (F_1–F_4) was established, mean plasma concentrations of VTG and VLDL were 4.01 ± 0.23 g/mL and 19.4 ± 1.4 mg/mL, respectively (pairwise comparisons with females with single yolky follicle, $P < 0.001$) but varied fourfold between individuals (1.67–6.67 g/mL and 9.2–39.8 mg/mL respectively). Then, even though total follicle mass declined to the point where the ovary contained only a single F_1 follicle, plasma yolk precursor concentrations remained high and actually continued to increase (VTG, $P = 0.04$; VLDL, $P < 0.001$; pairwise comparison with birds with full hierarchy) reaching a peak on the last day of follicle development (VTG, 4.90 ± 0.62 g/mL; VLDL, 26.0 ± 1.6 mg/mL; VLDL). Only after follicle development had ceased did the precursor concentrations decrease, returning to basal concentrations 2 dcon(peak)-326.eeiD0

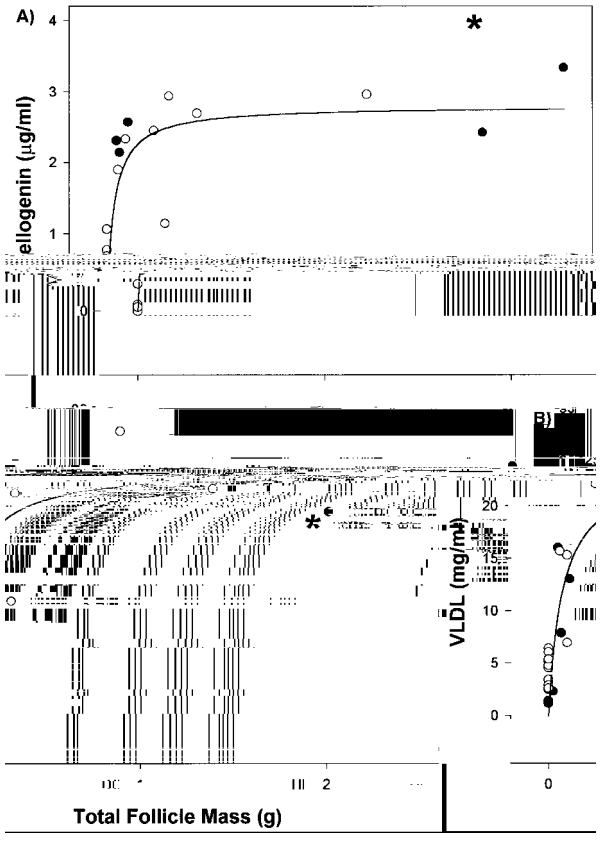

Figure 2. Relationship between total yolky follicle mass and (*A*) plasma vitellogenin and (*B*) plasma very low density lipoprotein (VLDL) concentrations in female starlings before the laying of their first egg. Precursor concentrations remain low until follicles start to develop. *Open circles*, 1997; *filled circles*, 2000. The asterisk indicates mean follicle mass and yolk precursor concentration for birds with a follicle hierarchy (F_1–F_4).

However, once the full follicle hierarchy had been established and during subsequent follicle development (when follicle recruitment had ceased), the relationship between yolk precursor concentrations and yolky follicle mass was reversed, becoming significantly negative (linear regression; VTG, F = ,

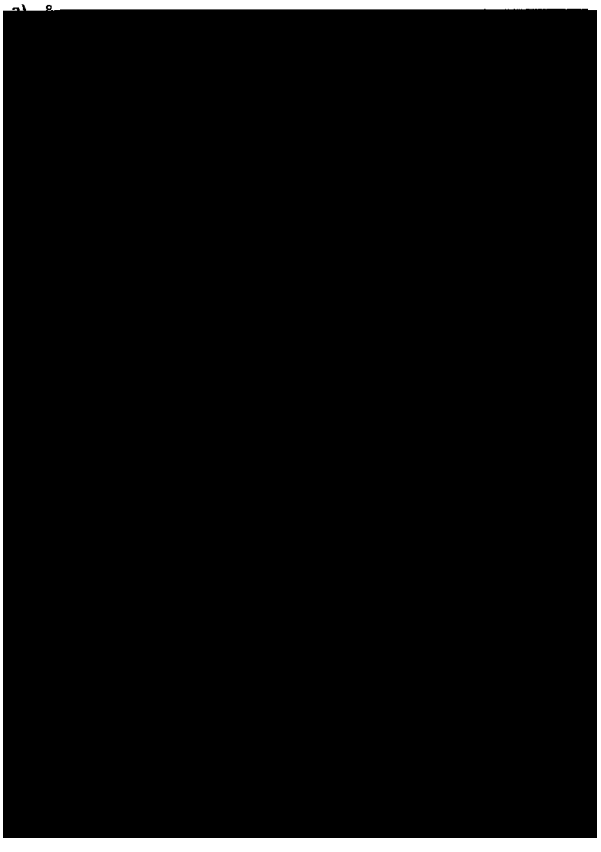

Figure 3. Relationship between total yolky follicle mass and (*a*) plasma vitellogenin and (*b*) plasma very low density lipoprotein (VLDL) concentrations for female starlings with either a full follicle hierarchy or subsequent to this stage (i.e., during the period with no new follicle recruitment).

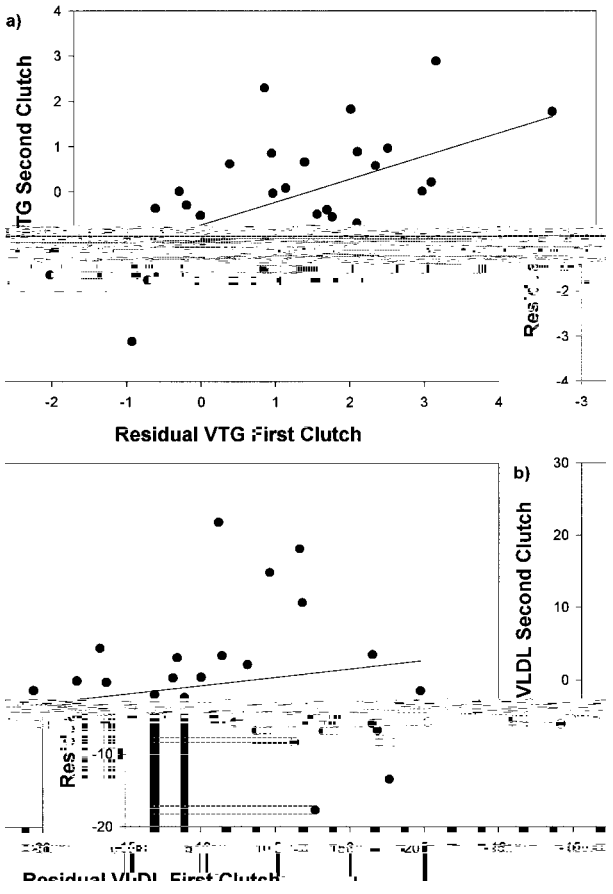

Figure 5. Repeatability of (*a*) plasma vitellogenin (VTG) and (*b*) plasma very low density lipoprotein (VLDL) concentrations (mg/mL) in female starlings sampled during their first and replacement clutches. Values are residuals controlling for variation in stage of oviposition.

stage) always having low VTG values by restricting the analysis to birds sampled at the two- and three-egg stage. First-clutch VTG concentrations were still a significant predictor of second-clutch VTG concentrations ($F_{1,7} = 6.49$, $P < 0.05$), with individuals accounting for 72.5% of the total variation ($F_{8,10} = 6.27$, $P < 0.001$).

Discussion

As expected, there was very large variation in total follicle mass over the 9-d period of yolk formation in the European starling. Although the structure of the ovarian follicular hierarchy has been studied empirically in only a few nondomesticated birds (Etches and Petitte 1990), the pattern of variation in starlings was identical to that previously described for models of egg development based on egg composition data (e.g., Ojanen 1983; Williams and Ternan 1999). At the broad scale, plasma yolk precursor concentrations (i.e., the pool of precursors available to the developing follicles) matched this variation in total follicle mass (see Fig. 1). However, there was evidence for mismatching between supply and demand at two levels. (1) Once the full follicle hierarchy (F_1–F_4) was established, yolk precursor concentrations continued to increase even though follicle mass decreased (with no new follicles being recruited), and yolk precursors concentrations only declined once follicle development was completed. (2) In birds with a full follicle hierarchy, there was a negative relationship between plasma yolk precursor concentrations and total follicle mass.

Yolk Precursor Supply and Follicular Demand

Yolk precursor concentrations were only elevated during the phase of RYD, so European starlings do not start to produce yolk precursors in advance of RYD in readiness for the onset of egg formation, as occurs with some other components of the reproductive system and with male gonads (Williams 1999)

yolk formation and that required for the female's own metabolic needs. This is supported by the fact that diurnal variation

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