Interindividual variation in yolk mass and the rate of growth of ovarian follicles in the zebra finch (*Taeniopygia guttata*)

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Abstract The amount of resources invested in an individual egg yolk must be determined by its rate of growth and/or the duration of growth. We examined interindividual variation in the growth rate of yolks by injecting radiolabeled amino acid into breeding female zebra finches and measuring the activity associated with protein in the yolks of eggs laid subsequently. We predicted that (1) there would be a positive correlation between yolk mass and the rate of uptake of activity into the yolk; and (2) there would be a negative correlation between clutch size and the amount of activity taken up by each of the follicles due to competition between follicles for circulating yolk precursors. The rate of uptake of activity by the yolks was positively related to yolk mass ($r^2 = 0.24, 0.35$ and $0.50$ for the yolks of the third-, fourth- and fifth-laid eggs, respectively), suggesting that interindividual variation in yolk mass is due, at least in part, to variation in the rate of follicle growth. However, we found no evidence of a trade-off between yolk size and number. The uptake of activity was generally repeatable between breeding attempts (repeatability $= 0.23–0.44$), as was mean yolk mass (repeatability $= 0.35$), suggesting that these traits are characteristics of individual females.

Key words Intraspecific variation · Egg size · Rapid yolk development · Repeatability · Vitellogenin

Abbreviations $dpm$ disintegrations per minute · $RYD$ rapid yolk development · $VLDL$ very-low-density lipoprotein

Introduction

Natural selection acts on variation within species, and thus studying intraspecific variation in physiology and life history parameters is necessary to understand the evolution of such traits (Bennett 1987; Bradley and Zamer 1999). Little is known about the sources of interindividual variation in avian egg size (Christians and Williams 2001) despite extensive knowledge of the physiological mechanisms involved in egg production in domesticated species (Etches 1996). To determine the mechanistic basis of egg size variation, it is useful to examine the various components of the egg separately since the yolk develops in the ovary whereas the albumen and shell are formed in the oviduct (Etches 1996). In birds, the vast majority of the mass of the yolk is deposited in a relatively short period of time compared to other oviparous vertebrates; in some passerines rapid yolk development ($RYD$) may last only 3 days per yolk (Ricklefs 1974). During $RYD$ the liver produces two main yolk precursors, vitellogenin and yolk-targeted very-low-density lipoprotein ($VLDL$), which are secreted into the circulation (Wallace 1985). Vitellogenin and $VLDL$ are taken up from blood by the growing follicles of the ovary via receptor-mediated endocytosis (Griffin and Hermier 1988; Shen et al. 1993), where they provide the primary sources of yolk protein and lipid, respectively. The growth of the ovarian follicles occurs in a hierarchy (Fig. 1), and at most one follicle reaches maturity and ovulates each day.

Clearly, variation in the mass of ovarian follicles at ovulation (and hence in yolk mass) must result from variation in the rate of growth of the follicles and/or the
duration of their growth. Although the relationship between the duration of RYD and yolk mass has been studied within domesticated species (Bacon and Koontz 1971; Imai 1983), little work has been done on other species (but see Birkhead and Del Nevo 1987; Hatchwell and Pellatt 1990). Furthermore, the importance of interindividual variation in follicle growth rate in determining yolk mass has yet to be examined.

The goal of this study was to investigate whether large yolks are formed more rapidly than small yolks (versus taking longer to form), using protein deposition as a measure of growth rate. We injected radiolabeled amino acid into breeding female zebra finches (Taeniopygia guttata), and examined the activity associated with protein in the yolks of eggs laid subsequently. We predicted that, if larger yolks were the result of more rapid rates of protein deposition, there would be a positive relationship between the rate of uptake of activity into the yolk and yolk mass. Measuring the rate of protein deposition also allowed us to examine the potential trade-off between the number of growing yolks and yolk size. Thus, our second prediction was that, if there were a trade-off between yolk number and size due to competition between follicles for circulating yolk precursors, the rate of uptake of activity per yolk would decrease with increasing clutch size. Finally, because reproductive parameters such as egg size and clutch size are generally repeatable within individual females (Boag and Noordwijk 1987), we predicted that the rate of protein deposition would be repeatable between breeding attempts.

**Materials and methods**

**General**

A captive-breeding population of zebra finches was maintained in controlled environmental conditions (temperature 24–28°C; humidity 35–55%; constant light schedule, 14L:10D, lights on at 0700 h), with birds kept in single-sex cages prior to experiments. All birds were provided with mixed seed (white and paniced millet; 11.7% protein, 0.6% lipid, and 84.3% carbohydrate), water, grit, and cuttlefish bone ad libitum and received a multi-vitamin supplement in the drinking water once per week. During breeding, birds also received a daily egg-food supplement (20.3% protein, 6.6% lipid) and were housed in pairs in cages (51 cm×39 cm×43 cm), each with an external nest box (14 cm×14 cm×20 cm). Females were introduced to the breeding cages in the morning and a single male partner was added within 3 h. Nest boxes were checked daily for eggs. Eggs are generally laid at daily intervals, but females occasionally “skip” a day, i.e. lay two eggs ca. 48 h apart (Williams 1996). Clutch size was therefore defined as the number of eggs laid, allowing interruptions of 1 day.

**Injection of radiolabeled amino acid**

Thirty-one females were paired with males. On the day a female laid her first egg (i.e. one-egg stage), she was weighed (±0.1 g), a blood sample was taken for measurement of plasma vitellogenin levels (see below), and the female was injected intraperitoneally with 1 μCi L-[14C]serine (151 mCi mmol⁻¹; Amersham Pharmacia Biotech) in 100 μl phosphate-buffered saline. Figure 1 shows the timing of isotope injection relative to the stage of growth of the various ovarian follicles. In chickens, radiolabeled amino acids are incorporated rapidly into newly synthesized vitellogenin (~15 min; Jost et al. 1978) and the apoprotein components of VLDL (<2.5 h; Evans and Burley 1987). We used radiolabeled serine because vitellogenin, the primary source of yolk protein, has an unusually high serine content and therefore serine would selectively (but not exclusively) label this protein (Wang and Williams 1982). Since [14C]serine would also label all other newly synthesized protein, we refer to “protein uptake” by the ovary. All [14C] injections were performed between 5 h and 7.5 h after lights-on to ensure that isotope was administered after that day’s ovulation (i.e. the ovulation of the yolk of the second egg). 91% of eggs are laid within 1–3 h of lights-on (Williams 1996) and ovulation occurs 30–45 min after oviposition of the preceding egg (Etchles 1996).

We repeated the experiment a second time using the same females as in the first experiment so that the repeatability of reproductive and physiological parameters could be assessed. The interval between isotope injection in the first and second experiments was approximately 9 weeks. In the second experiment the time of oviposition (±0.5 h) was recorded as an estimate of the time of ovulation (Hammond et al. 1980; Calvo and Bahr 1983; Etchles 1996).

**Measurement of activity in the yolks of eggs laid after isotope injection**

The first five eggs laid by each female were removed on the day they were laid, weighed (±0.001 g), and replaced with “dummy” eggs (i.e. eggs from other, non-manipulated females). Within 48 h of laying, yolks were separated from eggs, weighed fresh, and placed in a 1.5-ml micro-tube. To measure only protein-associated activity (i.e. incorporated into ovarian follicles via receptor-mediated processes), rather than activity associated with free serine, we precipitated protein from the yolks by mixing each yolk with 500 μl of 7% trichloroacetic acid solution, leaving the suspension on ice for 10 min, and centrifuging at 13000 rpm for 10 min. The resulting supernatant was discarded and the precipitate was rinsed three times with water, resuspended in 1 ml water and centrifuged again at 13,000 rpm for 10 min. The resulting precipitate was rinsed once and then frozen.

Yolk protein was later thawed and resuspended in 15 ml scintillation cocktail (Amersham biodegradable counting scintillant). Samples were left for 3 days (first experiment) or 6 days (second experiment) to improve the suspension of yolk protein in the cocktail, and were placed in the dark at least 24 h prior to counting to reduce interference due to chemiluminescence. All samples were counted on a Beckman LS6500 scintillation counter for 5 min.