

Experimental (Antiestrogen-Mediated) Reduction in Egg Size Negatively Affects Offspring Growth and Survival

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Accepted 12/21/2006; Electronically Published 2/13/2007

ABSTRACT

The relationship between egg size and offspring phenotype is critical to our understanding of the selective pressures acting on the key reproductive life-history traits of egg size and number. Yet there is surprisingly little empirical evidence to support a strong, positive relationship between egg size and offspring quality (i.e., offspring growth, condition, and survival) in birds, in part because of confounding effects of parental quality and the lack of experimental techniques for directly manipulating avian egg size independently of maternal condition. Previously, we showed that treatment of laying female zebra finches (*Taeniopygia guttata*) with the antiestrogen tamoxifen can decrease egg size by ca. 8% but that this reduction in egg size had few effects on offspring mass and size at fledging. Here, we extend the use of this technique to induce larger decreases in egg size (up to 50% in individual females) and show that a reduction in egg size of ca. 18% is associated with decreased embryo viability, increased hatchling mortality, and lower posthatching offspring survival. Furthermore, we show that although hatchlings from eggs reduced in size by ca. 9% can survive to fledging, these chicks show slower initial growth during the linear growth phase (5–10 d of age), fledge at lower masses than chicks from control eggs, and show postfledging compensatory growth. Our results provide empirical support for significant effects of egg size on offspring quality and further suggest that among individual females there is a minimum egg size required to maintain embryo viability and offspring quality.

Introduction

Egg size is one of the most widely studied life-history traits in evolutionary ecology, yet the selective forces that maintain this trait remain poorly understood (Bernardo 1996; Christians 2002). Life-history theory predicts that within and among species, natural selection should favor an optimal egg size that best balances conflicting demands between parental investment and offspring survival (Stearns 1992). However, there is little evidence that such a universal optimal egg size exists among avian species; in fact, there is marked intraspecific variation in egg size on both temporal and spatial levels that remains largely unexplained (reviewed in Christians 2002). Although the general physiological mechanisms involved in egg production have been well characterized (Etches 1996), not much is known regarding the mechanistic basis of interindividual variation, although it has been suggested that egg size is optimized within individuals as a result of genotypic and phenotypic constraints (Williams 1996a; Christians and Williams 2001; Salvante and Williams 2002).

What factors might determine the optimal egg size for an individual female? In terms of immediate maternal fitness (e.g., the total number of offspring that survive to reproduce per reproductive bout), maximizing fecundity by laying as many eggs as possible of a minimum viable egg size might be the most advantageous strategy. However, in terms of offspring quality (i.e., offspring growth, condition, and survival), laying a smaller clutch consisting of the largest eggs possible would seem to be the most advantageous strategy. Therefore, any optimal egg size should balance these conflicting demands between offspring quality and female fecundity, and this will, in turn, depend on the strength of the relationship between egg size and offspring quality. If this relationship is weak, females could maximize egg number and reduce egg size with relatively little impact on growth, condition, or survival of individual offspring. However, if the egg size–offspring quality relationship is strong, then selection should favor increased egg size over an increased number of eggs for a given reproductive bout.

Surprisingly, given its central importance, there is little empirical evidence supporting a strong positive relationship between egg size and offspring quality in birds (Smith et al. 1995; Hipfner and Gaston 1999; Styrsky et al. 1999; Bize et al. 2002; see Christians 2002 for review; and see “Discussion”). One reason for this might be that few studies have used experimental techniques to directly manipulate egg size independently of the laying female’s condition or environment (but see Hill 1993;

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Table 1: Comparison of reproductive traits in sham-treated and tamoxifen-treated females

Trait	Sham Treatment	Tamoxifen Treatment
Sample size	22	52
Pretreatment:		
Body mass (g)	15.64 ± .25	15.55 ± .16
Clutch size	5.5 ± .3	5.7 ± .2
Mean egg mass (g)	1.106 ± .027	1.070 ± .017
Treatment:		
Body mass (g)*	16.08 ± .25	15.18 ± .13
Laying interval (d)	6.90 ± .68	7.35 ± .44
Laying rate (eggs d ⁻¹)	.90 ± .04	.81 ± .02
Clutch size	5.3 ± .5	5.8 ± .3
Mean egg mass (g)**	1.047 ± .024	.878 ± .016
Eggs laid during treatment833 ± .014
Eggs laid posttreatment989 ± .022
Posttreatment:		
Body mass (g)	14.72 ± .22	14.73 ± .14

Note. Values are least squares means ± SEM.

* $P < 0.0025$.

** $P < 0.0001$.

Finkler et al. 1998). Although indirect methods can be used to manipulate egg size (temperature [Nager and Van Noordwijk 1992], diet quality [Williams 1996b], photostimulation [Renema et al. 2001], intraspecific interactions [Verboven et al. 2005]), such techniques are limited by a general lack of specificity in targeting the underlying mechanism of interest (Zera and Harshman 2001) and are confounded by the effects of parental condition (Bernardo 1996). Cross-fostering experiments, which can effectively control for confounding effects of parental quality (e.g., Magrath 1992; Styrsky et al. 1999; Hipfner et al. 2001; Pelayo and Clark 2003), do not eliminate the possibility that condition-dependent nutritional, hormonal, and genetic factors (i.e., maternal effects) may be transferred to offspring during egg production (Krist and Remes 2004). In order to critically evaluate resource allocation and/or trade-offs that may occur during reproduction, it is necessary to experimentally uncouple any correlation between maternal condition and egg size (sensu Sinervo et al. 1992).

Here we use the antiestrogen tamoxifen to experimentally manipulate egg size directly via disruption of the hormonal signal (estrogen action) that drives egg production. In birds, tamoxifen is considered a pure antiestrogen in most tissues (i.e., an estrogen-receptor antagonist; Capony and Williams 1981; Sutherland and Murphy 1982; but see Mathews and Arnold 1991), particularly those involved in estrogen-dependent reproductive processes, such as the hypothalamus, liver, and oviduct. The mechanism of action is not fully understood, but tamoxifen is believed to competitively bind and inhibit the nuclear estrogen receptor, preventing the activation of estrogen-responsive elements on DNA and suppressing estrogen-depen-

dent gene transcription (Sutherland and Murphy 1982; Lazier 1987). Previously, we showed that administration of tamoxifen ($10 \mu\text{g g}^{-1}$ body mass) on alternating days to egg-laying females decreased egg size by 8% but that this reduction in egg size had few effects on offspring survival, body size and mass at fledging, or reproductive performance at maturity (Williams 2000, 2001; T. D. Williams, unpublished data). In the current study, we administered the same dose of tamoxifen to egg-laying females but at a higher frequency (daily rather than on alternate days) in order to determine (a) whether this results in a further reduction in egg size and (b) whether a greater decrease in egg size negatively affects offspring quality. We predicted that an increased frequency of tamoxifen injections would result in greater reductions in egg size than previously documented (Williams 2000, 2001) while maintaining among-individual variation in egg size. Furthermore, we predicted that greater reductions in egg size would in turn reduce embryo viability and offspring quality with respect to control chicks. Our objective was to provide experimentally derived empirical data supporting a positive relationship between egg size and offspring phenotype and to determine whether there exists an intraspecific minimum egg size required for embryo viability and offspring survival in zebra finches.

Material and Methods

Study Species and Breeding Conditions

Zebra finches (*Taeniopygia guttata*) were housed under controlled environmental conditions (temperature 19°–23°C, humidity 35%–55%, constant light schedule of 14L : 10D, lights

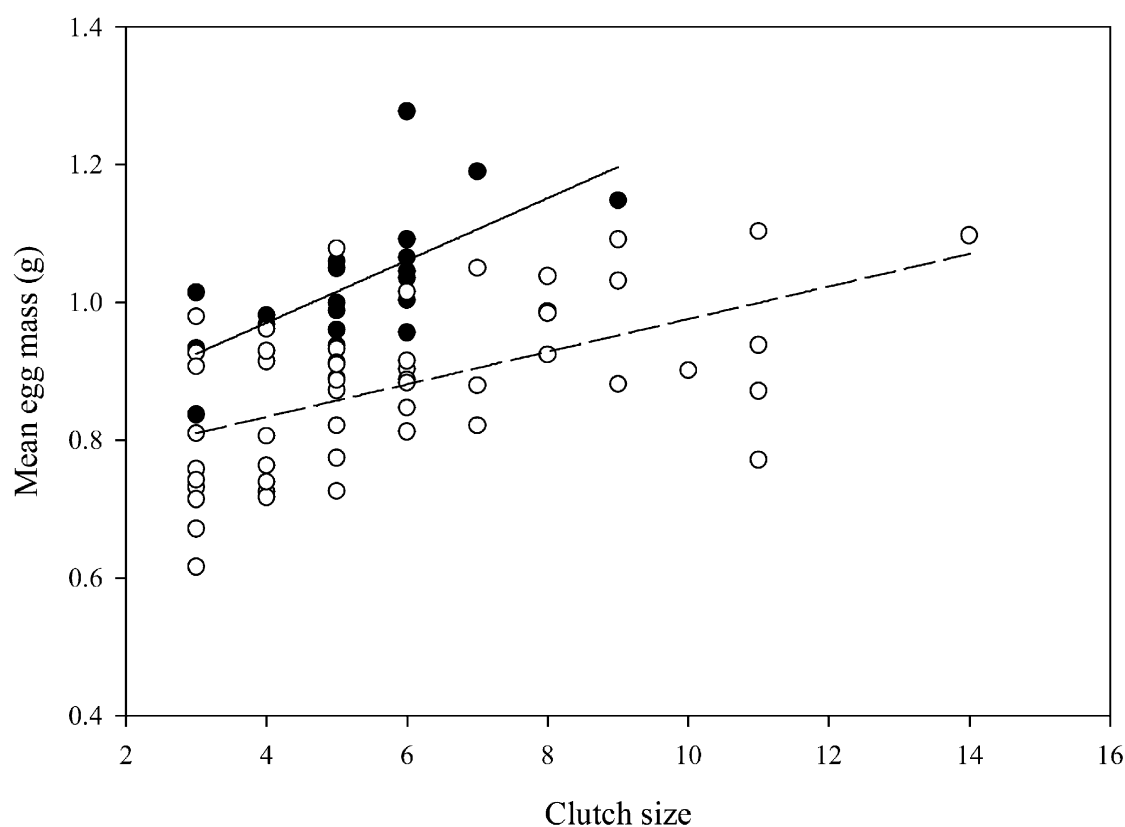


Figure 1. Relationship between mean egg mass (g) and clutch size in tamoxifen-treated females (*open circles, dashed line*) and sham-treated females (*filled circles, solid line*).

on at 0700 hours). The birds received a mixed-seed diet (panicum and white millet 1 : 1; approximately 11.7% protein, 0.6% lipid, and 84.3% carbohydrate), water, grit, and cuttlefish bone (calcium) ad lib., and a multivitamin supplement in their drinking water once per week. All experiments and animal husbandry were carried out under a Simon Fraser University Animal Care Committee permit (692B-94) following guidelines of the Canadian Committee on Animal Care.

Before the experiment, all birds were housed in same-sex cages (61 cm × 46 cm × 41 cm) but were not visually or acoustically isolated from the opposite sex. The females selected for this experiment were 6–18 mo in age and had been successfully bred once or twice, allowing for repeated-measures analyses of primary reproductive traits (i.e., pretreatment mean egg mass, clutch size, and clutch mass data were available from unmanipulated breeding trials held before the experiment). Randomly assigned breeding pairs were housed individually in cages (61 cm × 46 cm × 41 cm) equipped with an external nest box (11.5 cm × 11.5 cm × 11.5 cm). Mass (± 0.001 g), bill length (± 0.01 mm), and tarsus length (± 0.01 mm) of each bird were recorded at the time of pairing. Breeding pairs were provided with an egg-food supplement (20.3% protein, 6.6% lipid) daily from pairing to clutch completion and again during the chick-

rearing period. Nest boxes were checked daily between 0900 and 1100 hours to obtain data on laying interval, egg sequence, egg mass (± 0.001 g), and clutch size. Egg mass is highly correlated with linear and volumetric measurements of egg size in this population ($r^2 = 0.98$; T. D. Williams, unpublished data); therefore, using egg mass as an index of egg size is warranted. A clutch was considered complete when no additional eggs were produced over three consecutive days. At clutch completion, breeding pairs were weighed to obtain posttreatment mass and then left undisturbed until the hatching period, at which point nest boxes were checked daily to determine hatching success per clutch. Immediately after hatching, chicks were weighed and marked with nontoxic dye to indicate hatch order and were then individually banded at 8 d of age. The mass of each chick was recorded at 7, 14, and 21 d posthatch. At 30 d of age, final brood size for each nest was recorded, and mass, tarsus length, and bill length of each chick were measured. Chicks were then separated from the parents and placed in standard housing until development of sexually dimorphic plumage allowed them to be transferred to single-sex holding cages. Mass, tarsus length, bill length, and sex of each individual were recorded at sexual maturity (≥ 60 d of age).

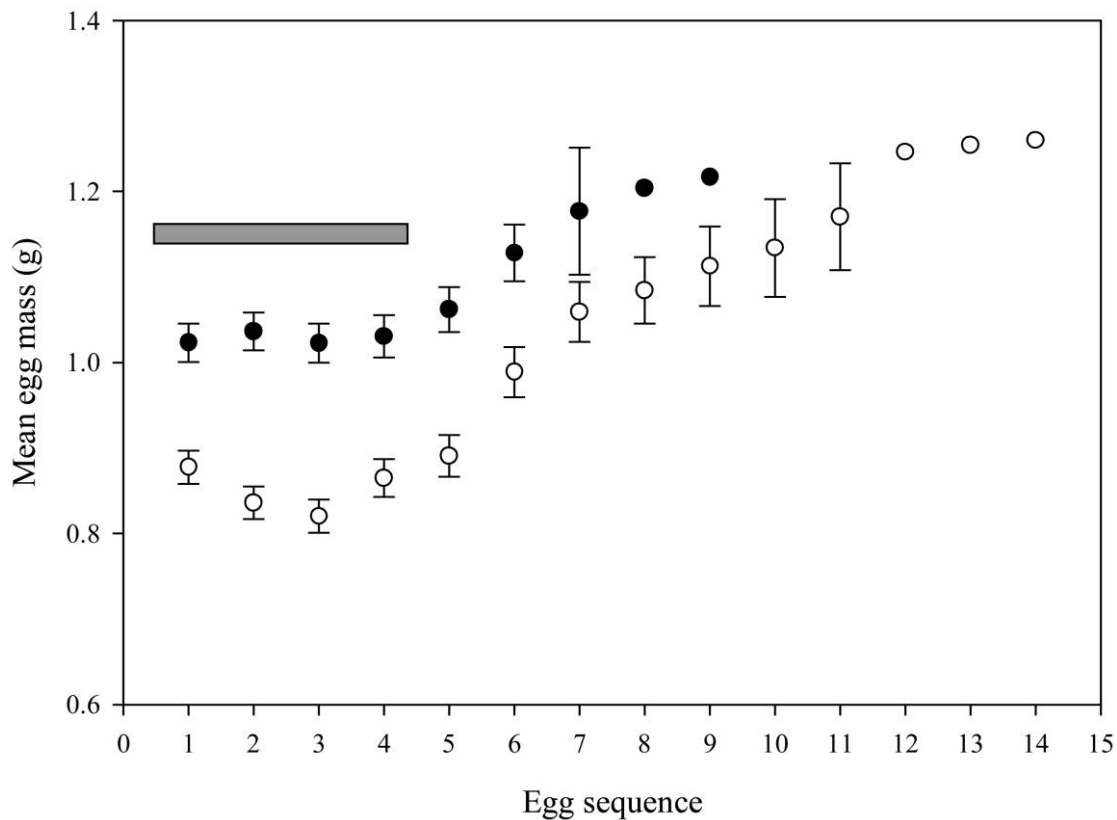


Figure 2. Variation in egg mass (g) with laying sequence in tamoxifen-treated females (*open circles*) and sham-treated females (*filled circles*); the shaded bar indicates the treatment period. Values are least squares means \pm SEM. In the sham treatment group, only one female laid nine eggs, and in the tamoxifen treatment group, only one female laid 14 eggs.

Experimental Protocol

Initially we had designed this experiment to further address the effect of short-term tamoxifen treatment on the trade-off between egg size and clutch size (Williams 2001). In the zebra finch, clutch size is believed to be determined between deposition of the third and fourth eggs (Haywood 1993), and to target this critical period we assigned females to three treatment groups, with cessation of tamoxifen injections at the two-egg ($n = 17$), three-egg ($n = 17$), and four-egg ($n = 18$) stages. However, in preliminary analysis we found no significant difference in clutch size or clutch mass between treatment groups and the control group (clutch size: $F_{4,67} = 1.07$, $P > 0.15$; clutch mass: $F_{3,71} = 1.02$, $P > 0.35$), and while tamoxifen treatment decreased egg size in all three treatment groups, there was no significant difference in egg size between the tamoxifen-treated groups ($F_{3,48} = 1.51$, $P > 0.2$). Therefore, for this article we have pooled data for the two-egg, three-egg, and four-egg treatment groups into a single tamoxifen treatment group ($n = 52$), and we focus on the relationship between egg size and offspring growth and survival. Females were thus randomly assigned to two groups. (a) Tamoxifen-treated females ($n = 52$) received

daily intramuscular (pectoral) injections of tamoxifen citrate ($10 \mu\text{g g}^{-1}$ body weight in $30 \mu\text{L}$ 1,2-propanediol) until they laid their second, third, or fourth egg (females originally assigned to the two-egg and three-egg treatments received injections of 1,2-propanediol vehicle until the fourth egg had been laid to ensure that handling was consistent between individuals). (b) Sham-treated females ($n = 22$) received daily intramuscular injections of only 1,2-propanediol until the fourth egg had been laid. All injections were given between 0830 and 1030 hours. Females that did not lay an egg on sequential days of the laying cycle continued to receive daily injections until the fourth egg had been laid. The half-life of tamoxifen in avian species is estimated at 7–14 h (Capony and Williams 1981), which is consistent with our previous findings; tamoxifen-induced changes in reproductive physiology were transient and reversible, typically lasting for 48 h after intramuscular injection (Williams 2000, 2001). Therefore, all eggs laid during the injection period or within 48 h of the last tamoxifen injection were considered eggs laid during treatment, whereas eggs laid more than 48 h after the last injection were considered eggs laid posttreatment.

Table 2: Comparison of egg fates for sham-treated and tamoxifen-treated females

Egg Fate	Sham-Treated Females	Tamoxifen-Treated Females
<i>n</i> (females)	22	52
Number of eggs where fate known	100	291
Eggs hatched	46 (46.0%)	59 (20.3%)
Infertile eggs	32 (32.0%)	99 (34.0%)
Unhatched embryo	14 (14.0%)	82 (28.2%)
Missing	8 (8.0%)	51 (17.5%)

Note. Values are absolute counts followed by percentages in parentheses.

Statistical Analysis

All statistical analyses were carried out using SAS, version 9.1 (SAS Institute 2003). Treatment effects on reproductive traits were analyzed using generalized linear models (GLM procedure) to compare tamoxifen- and sham-treated birds, with covariates (e.g., pretreatment mean egg mass, pretreatment clutch size, treatment clutch size, and treatment mean egg mass) included as terms in the model where appropriate. Paired *t*-tests were used to compare differences between pretreatment and treatment reproductive traits within individual females. Treatment effects on egg hatchability, offspring mortality, and brood sex ratio were tested with generalized linear models (GENMOD procedure) in a two-level structure with individual offspring nested within broods. Binary data (egg hatchability, offspring mortality, and brood sex ratio) were transformed by the “logit link” function and analyzed assuming a binomial error distribution. Wald statistics for type 3 contrasts were used to assess significance of fixed effects (SAS Institute 2003). Treatment effects on offspring growth were analyzed using repeated-measures mixed linear models with an unstructured covariance matrix (MIXED procedure) to compare offspring from tamoxifen-treated versus sham-treated females, with individual offspring nested within broods included as a random effect in the model (SAS Institute 2003). To analyze the effects of tamoxifen-induced changes in egg mass and clutch size on reproductive outcome (defined as the fate of eggs and/or chicks within a clutch), tamoxifen-treated females were assigned to one of three groups: (1) laid a nonviable clutch; that is, none of the eggs laid by the female hatched; (2) produced offspring that all died before fledging; or (3) produced at least one offspring that survived to fledge. Generalized linear models (GLM procedure) were then used to test for effects of mean egg mass, clutch size, and relative change in these parameters on reproductive outcome among tamoxifen-treated females, including covariates where appropriate. Values presented are least squares means \pm SEM unless otherwise stated.

Results

There were no differences in pretreatment body mass, egg mass, clutch size, clutch mass, or laying interval for birds subsequently assigned to the tamoxifen-treated and control groups ($P > 0.1$ in all cases; Table 1); in other words, birds were randomly assigned to treatments with respect to these phenotypic traits. Furthermore, when we controlled for differences in clutch size, tamoxifen treatment had no effect on laying interval ($F_{2,70} = 0.17$, $P = 0.85$), laying rate ($F_{2,70} = 2.73$, $P = 0.07$), or post-treatment body mass ($F_{1,73} = 0$, $P = 0.97$; Table 1) in comparison with sham-treated females.

Effects of Tamoxifen on Egg Size and Number

Controlling for differences in pretreatment egg mass, eggs laid by tamoxifen-treated females were significantly smaller than eggs laid by sham-treated females ($F_{2,69} = 24.65$, $P < 0.0001$). Among tamoxifen-treated females, mean egg mass was 17.8% lower (95% confidence interval [CI]: 14.7%–21.0% decrease) than pretreatment values (paired $t_{51} = -11.26$, $P < 0.0001$). In contrast, mean egg mass in sham-treated females was not significantly different from pretreatment values (mean: -2.0% , 95% CI: -6.0% to $+2.1\%$; paired $t_{20} = -1.24$, $P > 0.2$). Among tamoxifen-treated females, mean egg mass was independent of the number of tamoxifen injections received before onset of egg-laying ($F_{40,11} = 0.83$, $P = 0.6$). Thirty of the 52 tamoxifen-treated females continued laying eggs after the treatment period (i.e., 48 h after the last tamoxifen injection). For these birds, eggs laid during tamoxifen treatment (0.833 ± 0.014 g) were significantly smaller than eggs laid posttreatment (0.989 ± 0.022 g; paired $t_{29} = 6.55$, $P < 0.001$). However, both eggs laid during treatment (paired $t_{51} = -14.98$, $P < 0.0001$) and eggs laid posttreatment (paired $t_{29} = -3.32$, $P = 0.0025$) were significantly smaller than the average pretreatment egg of these individual females (1.070 ± 0.012 g; Table 1).

When we controlled for pretreatment clutch size and laying interval, tamoxifen treatment had no effect on the number of

Table 3: Hatch mass and hatching and fledging success for offspring of sham-treated and tamoxifen-treated females

Trait	Sham Treatment	Tamoxifen Treatment
<i>n</i> (clutches)	12	21
Total number of chicks where fate known	46	59
Mean hatch mass (g)**	.848 ± .026 (46)	.738 ± .021 (59)
Hatchlings that survived to fledging*	.952 ± .037 (30)	.821 ± .030 (24)
Hatchlings that did not survive to fledging	.744 ± .042 (16)	.655 ± .025 (35)
Mean brood size:		
At hatch***	4.3 ± .4	2.7 ± .3
At fledging**	2.7 ± .5	1.0 ± .3

Note. Values are least squares means ± SEM, followed by sample size in parentheses.

* $P < 0.05$.

** $P < 0.0075$.

*** $P < 0.0001$.

eggs laid by tamoxifen-treated versus sham-treated females ($F_{2,69} = 0.68$, $P > 0.5$). Among tamoxifen-treated females, there was a significant positive correlation between mean egg mass and clutch size ($r_{52} = 0.56$, $P < 0.0001$): females that laid larger eggs tended to lay larger clutches (Fig. 1). A similar positive relationship between mean egg mass and clutch size was observed among sham-treated females (Fig. 1), although the correlation was not statistically significant ($r_{22} = 0.39$, $P = 0.08$). In both experimental groups, egg mass increased with laying order (tamoxifen: $F_{13,290} = 11.42$, $P < 0.0001$; sham: $F_{8,104} = 2.13$, $P < 0.04$; Fig. 2).

Effects of Tamoxifen-Induced Reduction in Egg Size on Hatchability, Chick Growth, and Survival

In comparison to sham treatment, tamoxifen treatment resulted in a significant decrease in the proportion of eggs that hatched (Wald $\chi^2 = 6.99$, $P = 0.008$), and among tamoxifen-affected clutches, eggs laid during the treatment period were less likely to hatch than eggs laid posttreatment within a clutch (Wald $\chi^2 = 6.55$, $P < 0.01$). A greater percentage of eggs laid by tamoxifen-treated females were infertile, contained inviable embryos, or went missing (i.e., were ejected from or buried in nest or broken or eaten by the birds) before hatch than eggs laid by sham-treated females (Table 2). Furthermore, mean brood size at hatch ($F_{2,48} = 6.72$, $P < 0.003$) and fledging ($F_{2,48} = 3.81$, $P < 0.03$) was lower in tamoxifen-treated females than sham-treated females (Table 3).

For both tamoxifen- and sham-treated females, hatching mass had a significant effect on survival to fledging (Table 3; sham-treated: $F_{1,37} = 13.53$, $P < 0.0007$; tamoxifen-treated: $F_{1,57} = 18.13$, $P < 0.0001$). However, in comparison to sham treatment, tamoxifen treatment significantly reduced the proportion of hatchlings that survived to fledging (Wald $\chi^2 = 4.41$, $P = 0.035$; Fig. 3). Within tamoxifen-treated clutches, there was no significant difference in mortality between off-

spring hatched from eggs laid during tamoxifen treatment and those from eggs laid posttreatment (Wald $\chi^2 = 0.01$, $P = 0.94$). When we controlled for differences in brood size, tamoxifen treatment had a significant effect on growth rates of offspring in comparison to sham treatment ($F_{4,49.2} = 10.5$, $P < 0.0001$; Fig. 4): offspring of tamoxifen-treated females had significantly lower body masses than offspring of sham-treated females at hatch ($P < 0.0003$; Table 4) and at days 14, 21, and 30 posthatch ($P < 0.0001$ in all cases; Fig. 4). At day 30, offspring of tamoxifen-treated females had shorter bills than offspring of sham-treated females ($F_{1,15.5} = 15.99$, $P < 0.0011$; Table 4), but the difference in tarsus length was not significant between groups ($F_{1,11.2} = 3.65$, $P = 0.08$; Table 4). Among offspring of tamoxifen-treated females, there was no significant difference in growth between chicks that had hatched from eggs laid during treatment and those from eggs laid posttreatment ($F_{4,22.3} = 1.56$, $P = 0.22$). At sexual maturity (≥ 60 d posthatch), there was no significant difference in sex ratio (Wald $\chi^2 = 0$, $P = 0.96$), body mass ($F_{1,42} = 0.13$, $P = 0.7$), tarsus length ($F_{1,42} = 0.34$, $P = 0.7$), or bill length ($F_{1,12.7} = 0.80$, $P = 0.4$) between offspring of sham-treated and of tamoxifen-treated females (Table 4). Offspring sex did not influence body mass or size at any stage of development ($P > 0.25$ in all cases).

Among individual tamoxifen-treated females, reproductive outcome (i.e., the fate of the eggs or offspring in the clutch) was dependent on both absolute egg mass ($F_{2,49} = 3.44$, $P < 0.04$; Table 5) and change in egg mass (i.e., the difference between experimental and pretreatment egg mass values; $F_{2,49} = 5.04$, $P < 0.015$; Table 5). Furthermore, there was a systematic relationship between a female's reproductive outcome and the magnitude of the tamoxifen-induced change in egg and clutch size ($F_{3,49} = 15.27$, $P < 0.0001$; Fig. 5). Females that laid nonviable clutches (i.e., no eggs hatched; $n = 30$; Table 5) showed decreases in both egg mass (-15.6%) and clutch size (-1 egg). In contrast, females that showed decreased egg mass (-23.1%) but a larger relative clutch size ($+1$ egg) produced

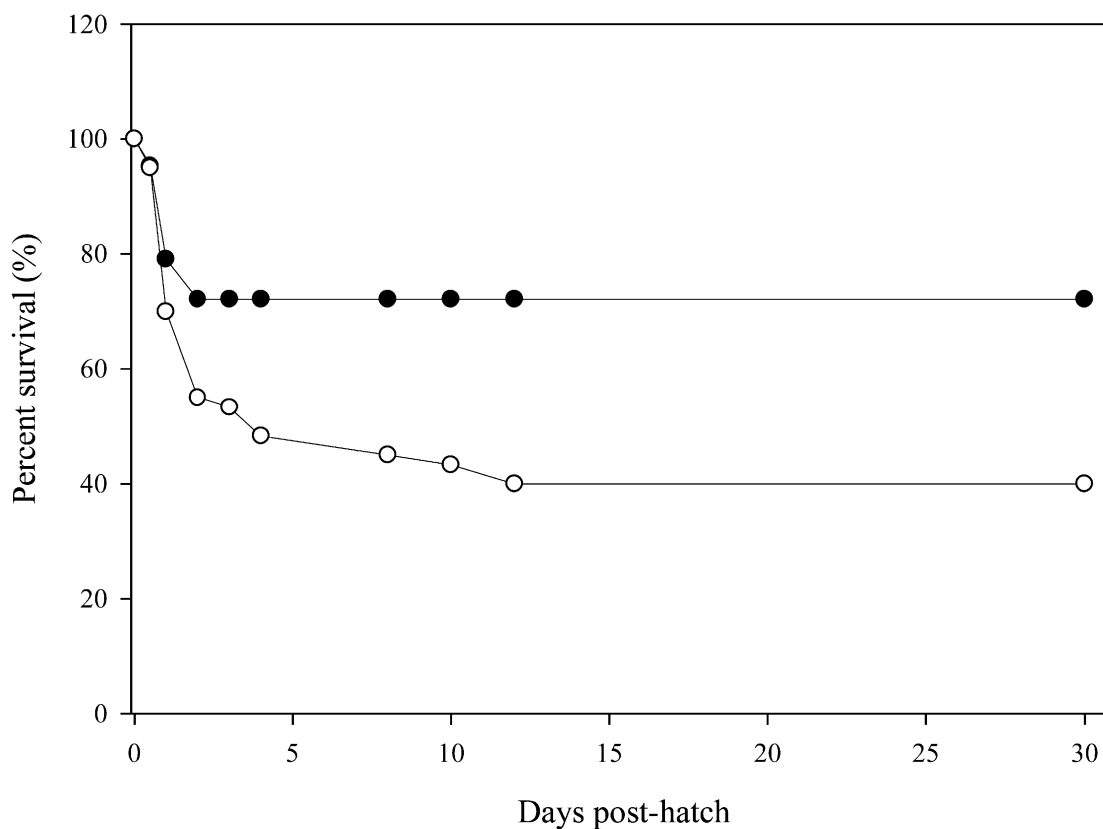


Figure 3. Comparison of survival rates of offspring from tamoxifen-treated females (*open circles*) and sham-treated females (*filled circles*) from hatching to independence (30 d posthatch).

offspring that hatched but did not survive to fledging ($n = 13$; Table 5). Finally, females that showed the smallest relative decrease in egg mass (-8.8%) and laid a larger clutch ($+2$ eggs) produced at least one offspring that survived to fledging ($n = 9$; Table 5). Among sham-treated females, there was no relationship between mean egg size or clutch size and the reproductive outcome of a clutch ($P > 0.5$ in both cases).

Discussion

Tamoxifen is considered to be a pure antiestrogen in birds (e.g., Wilson and Cunningham 1981; Jaccoby et al. 1995), and the effects on egg size that we report here are probably mediated by tamoxifen binding to estrogen receptors in the liver and suppressing yolk precursor synthesis, consequently reducing circulating yolk precursor levels available for yolk formation (Williams 2000). Hepatic production of vitellogenin is primarily an estrogenic process (Capony and Williams 1981); however, the production of albumen proteins in the oviduct can also be secondarily stimulated by receptor-mediated binding of progesterones, androgens, and glucocorticosteroids in addition to estrogens (McKnight and Palmiter 1979), and the mechanisms of these hormones are unaffected (and in some cases are potentiated) by

tamoxifen treatment (Catelli et al. 1980; Lebouc et al. 1985). Calcification of the egg shell is also not a singularly estrogenic process (Qin and Klandorf 1995), and tamoxifen does not appear to affect activity or concentration of the calcium-binding protein (calbindin) in the avian intestine or eggshell gland in laying hens (Bar et al. 1996). The specific effect of tamoxifen on egg size via the process of yolk formation is also confirmed by Williams's (2000) observation that tamoxifen treatment reduced relative yolk mass and yolk protein content but did not alter the relative proportion of yolk lipid, albumen, or shell. Our results are therefore entirely consistent with tamoxifen having specific effects on early stages of rapid yolk development via suppression of hepatic vitellogenin production; tamoxifen decreased egg size but had no effect on the timing components of egg production (laying interval or laying rate), the pattern of within-clutch variation in egg size, or, in this study, the number of eggs laid. It is notable how robust the intraclutch pattern of egg size variation is despite this tamoxifen-induced perturbation of egg formation, although the mechanisms that control this fine-scale variation in egg size remain unknown.

Increasing the frequency of tamoxifen treatment in this study (using daily injections; cf. Williams 2000, 2001) resulted in a

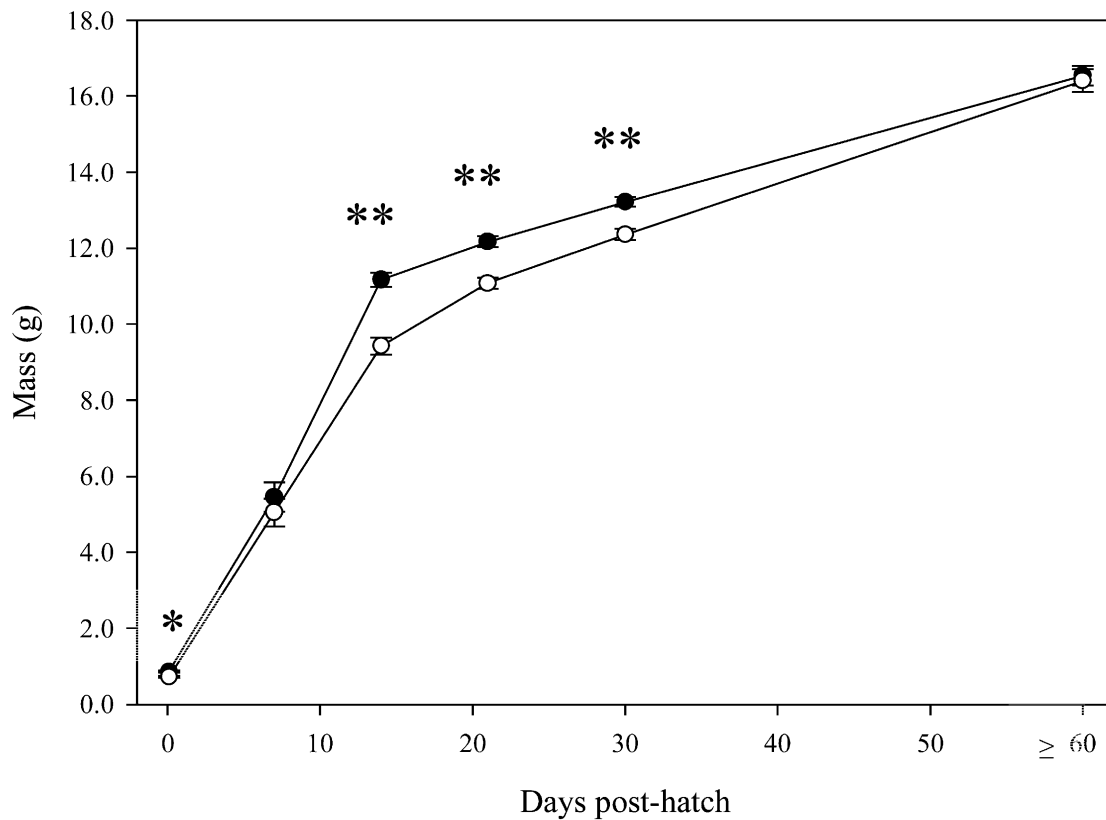


Figure 4. Comparison of growth rates of offspring from tamoxifen-treated females (*open circles*) and sham-treated females (*filled circles*) from hatching to maturity (≥ 60 d posthatch). Values are least squares means \pm SEM; one asterisk indicates $P < 0.0005$; two asterisks indicate $P < 0.0001$.

greater reduction in egg size than was observed in previous studies. On average, egg size of individual tamoxifen-treated females was 18% lower than that of control females in our study, compared with an average decrease in egg size of 8% reported by Williams (2000, 2001). Our results clearly show that this greater reduction in individual egg size has marked effects on offspring development, with a significant decrease in embryo viability and increased hatchling mortality. Although some hatchlings from tamoxifen-treated eggs did survive to fledging (those from eggs ca. 9% smaller than pretreatment values on average), these chicks still showed slower initial growth during the linear growth phase (5–10 d of age) and fledged at lower masses than chicks from eggs of sham-treated females. At sexual maturity (≥ 60 d of age), these treatment effects had disappeared, but this indicates that these birds went through a period of compensatory growth postfledging (*sensu* Metcalfe and Monaghan 2001).

We believe that the effects of tamoxifen treatment on offspring quality are due to reductions in egg size rather than direct effects of tamoxifen on maternal or offspring health for several reasons. We found no difference in posttreatment body mass between tamoxifen- and sham-treated females in this

study, and we have evidence that tamoxifen treatment does not adversely affect hematological parameters or endogenous estradiol levels (E. Wagner, unpublished data). In addition, previous studies have found that tamoxifen treatment does not alter body mass, bone density, fat stores, plasma protein and calcium levels, food consumption, or activity level in reproductively mature female birds (Jaccoby et al. 1995; Wilson and Thorp 1998; Lupu 2000; Sechman et al. 2004). It is also unlikely that tamoxifen had any direct effects on parental behavior and chick provisioning, particularly because treatment did not extend beyond initial stages of egg production and the effects of tamoxifen are transient and reversible (Williams 2000, 2001). In terms of direct effects of tamoxifen on offspring health, previous studies have found that *in ovo* administration of tamoxifen to chicken eggs ($200 \mu\text{g egg}^{-1}$) does not have negative effects on offspring growth and survival, although tamoxifen did feminize development of secondary sexual characteristics in some but not all genotypic males (Coco et al. 1992). However, all female offspring from our current experiment have been bred successfully (E. Wagner, unpublished data), confirming that tamoxifen had no effect on subsequent female reproductive development.

Table 4: Body mass and size measurements at fledging and maturity and sex ratio for adult offspring of sham-treated and tamoxifen-treated females

Trait	Sham Offspring	Tamoxifen Offspring
Fledging mass (g)**	13.22 ± .13 (30)	12.52 ± .18 (24)
Fledging tarsus length (mm)	16.69 ± .11 (29)	16.31 ± .13 (24)
Fledging bill length (mm)*	9.59 ± .09 (29)	8.88 ± .095 (24)
Adult mass (g)	16.54 ± .26 (29)	16.41 ± .30 (24)
Adult tarsus length (mm)	16.35 ± .16 (29)	16.28 ± .16 (24)
Adult bill length (mm)	10.16 ± .12 (29)	10.03 ± .13 (24)
Sex ratio at maturity	19 female : 11 male	15 female : 9 male

Note. Values are least squares means ± SEM, followed by sample size in parentheses.

* $P < 0.001$.

** $P < 0.0001$.

The negative effects of the antiestrogen tamoxifen on egg mass are clearly very robust and repeatable (this study; Williams 2000, 2001; T. D. Williams, unpublished data). However, in this study, we found no effect of tamoxifen treatment on clutch size, in contrast to the compensatory increase in clutch size in tamoxifen-treated females reported by Williams (2001). We suggest two reasons for this. First, the change in clutch size in response to tamoxifen (comparing experimental and pretreatment values) is highly variable among individuals (± 6 eggs difference; see Fig. 5), even though clutch size is in general a repeatable trait in nonmanipulated captive zebra finches (T. D. Williams, unpublished data). This might indicate that the direct physiological effect of tamoxifen on ovarian follicle development varies among individuals or that individual females make different investment decisions in terms of egg and clutch size in response to lower circulating levels of yolk precursors. Second, the different results in terms of clutch size could simply reflect differences in the experimental protocols used in dif-

ferent studies, for example, tamoxifen injections given daily until the second, third, or fourth egg was laid (this study) versus injections given every other day until the fourth egg was laid (Williams 2000, 2001). This highlights the potential complexity of using physiological or hormonal manipulations in these types of “phenotypic engineering” studies and suggests that caution is required in making conclusions from such studies about possible mechanisms linking different phenotypic traits (such as egg and clutch size). In contrast to Williams (2001), we actually found a positive phenotypic correlation between egg and clutch size and a strong positive relationship between the change in egg and clutch size in response to tamoxifen treatment: females with greatly reduced egg mass ($>20\%$) also laid fewer eggs. We suggest that the more marked inhibition of egg formation associated with more frequent tamoxifen treatment uncoupled the compensatory relationship between egg size and number that has been reported previously for birds (Williams

Table 5: Comparison of mean egg mass, clutch size, and change in mean egg mass and clutch size (compared to pretreatment values) in relation to reproductive outcome (fate of eggs and/or chicks within a clutch) for tamoxifen-treated females

Trait	Nonviable Clutch (No Eggs Hatched)	All Offspring Died before Fledging	One or More Offspring Survived to Fledging
<i>n</i> (clutches)	9	3	9
Pretreatment mean egg mass (g)	1.076 ± .088	1.071 ± .082	1.043 ± .062
Treatment mean egg mass (g)*	.876 ± .121	.824 ± .094	.952 ± .106
Percentage change in mean egg mass (compared to pretreatment egg mass)**	-18.26	-23.09	-8.81
Pretreatment clutch size	5.7 ± 1.3	5.5 ± 1.3	5.7 ± 1.7
Treatment clutch size**	5.2 ± 2.7	6.0 ± 2.3	7.9 ± 2.4
Difference in clutch size (compared to pretreatment clutch size)*	-1	+1	+2

Note. Values for mean egg mass and clutch size are least squares means ± SEM.

* $P < 0.05$.

** $P < 0.01$.

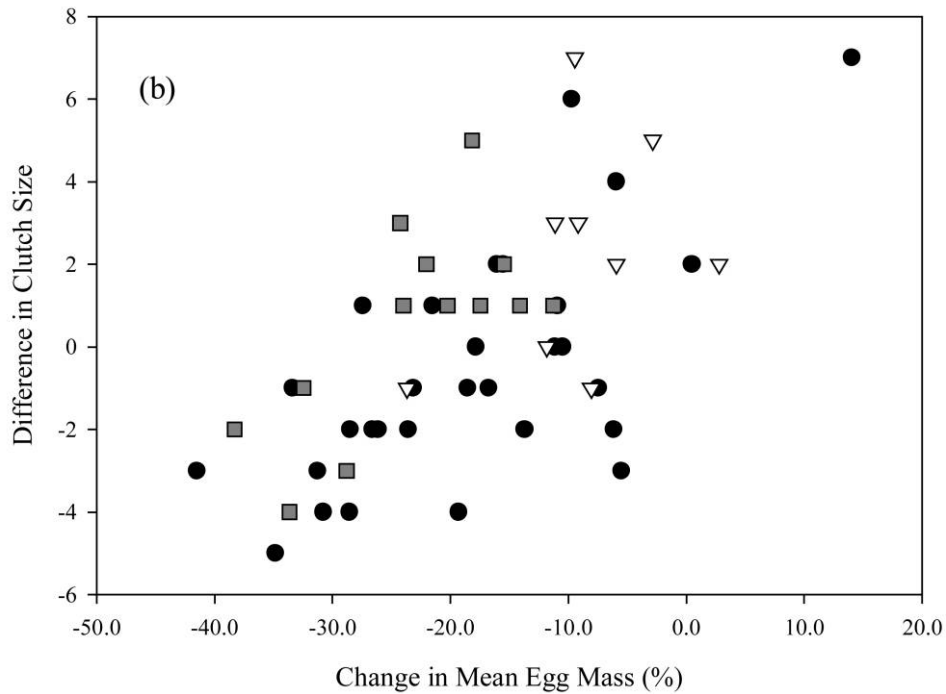
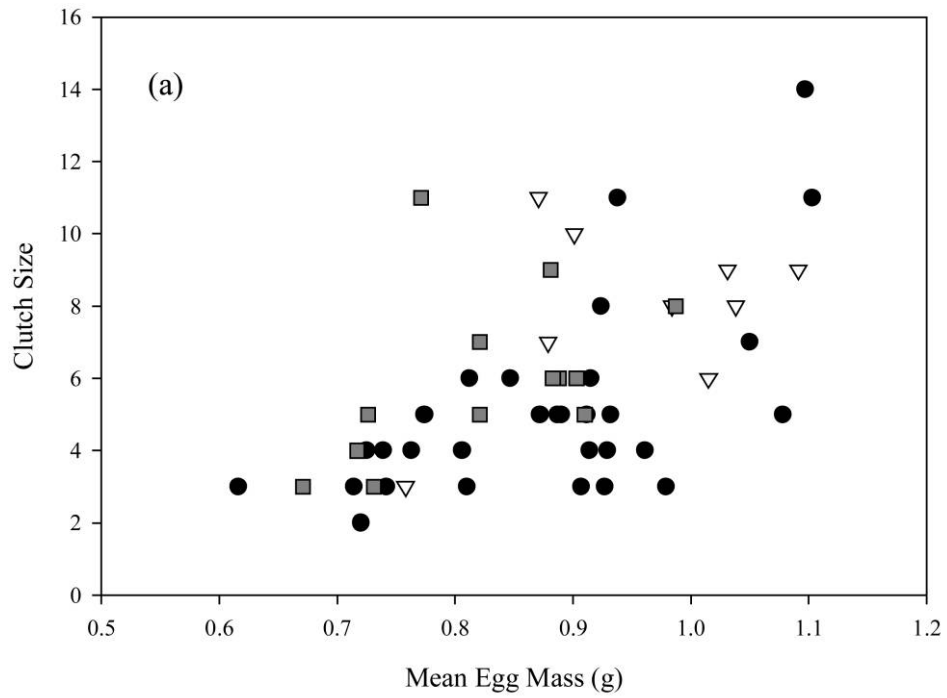


Figure 5. Relationship between reproductive outcome (i.e., the fate of eggs and/or offspring within a clutch) and (a) absolute clutch size and mean egg mass or (b) difference in clutch size and mean egg mass (relative to pretreatment values) for tamoxifen-treated females. Relative to pretreatment values, females that laid nonviable clutches (circles) showed decreases in both egg mass and clutch size, females that produced offspring that hatched but did not survive to maturity (squares) showed decreased egg mass but a larger relative clutch size, and females that produced at least one offspring that survived to fledging (triangles) showed the smallest relative decrease in egg mass and laid a larger clutch.

2001) and the lizard *Uta stansburiana* (Sinervo and Licht 1991; Sinervo 1999).

Despite the existence of a very high level of interindividual variability in egg size in most avian populations (Christians 2002), there is still surprisingly little empirical evidence for a strong, positive relationship between egg size and offspring fitness in birds (Williams 1994; Bernardo 1996; Christians 2002). For example, some studies have reported effects of egg size on hatchability (largely due to small eggs not hatching; e.g., Magrath 1992; Serrano et al. 2005), while other studies have failed to find an effect of among-clutch variability in egg mass on hatchability (Reid and Boersma 1990; Smith et al. 1995; Sanchez-Lafuente 2004). Factors affecting hatchability remain poorly understood, and few, if any, field studies have documented the cause of hatching failure, that is, whether it arises as a result of egg infertility, embryo inviability, or mortality during the process of hatching itself. Similarly, surprisingly few studies have reported consistent, positive effects of egg size on posthatching growth and survival of offspring. Hatching mass is strongly correlated with egg mass in all studies (reviewed in Williams 1994), and egg size is often correlated with offspring mass and size within the first week after hatching (Smith et al. 1995; Amundsen et al. 1996; Styrsky et al. 1999; Bize et al. 2002). However, far fewer studies have found a significant effect of egg size on offspring mass or body size at fledging (e.g., Erikstad et al. 1998; Hipfner and Gaston 1999), and this mainly occurs, as would be predicted, where selection on offspring development is likely to be stronger, for example, in low-quality environments (Smith et al. 1995) or with late-season broods when food is more scarce (Styrsky et al. 1999; but see Styrsky et al. 2000).

This general lack of support for strong positive effects of egg size on offspring phenotype in birds has led some authors to suggest that egg size is a neutral trait that is under no selection pressure (Jager et al. 2000; Van de Pol et al. 2006). However, we believe that this conclusion is premature and probably incorrect for the following reasons. First, as we have demonstrated by direct experimental manipulation (cf. indirect manipulation via changes in environmental cues, female condition, nutritional state, etc.), egg size can have substantial effects on embryo and chick viability and chick growth, even under relatively benign conditions (e.g., in captivity with a constant environment and ad lib. food). Second, our results suggest that the change in egg size for each individual female might have greater effects on offspring development than absolute egg mass. Data from our colony show that eggs of mass ≥ 0.827 g can produce female offspring that survive and breed at 3 mo of age (lower 95% CI: 0.912 g, $n = 44$; T. D. Williams, unpublished data). In this study, 64% of all eggs laid by tamoxifen-treated females that failed to produce viable embryos or offspring were larger than this minimum viable egg size, and 56% of all tamoxifen-treated females had a mean egg mass >0.827 g. One possible explanation for this is that individual females produce eggs of an

optimum size for the growth of their chick(s). For example, embryo growth and metabolism are genetically determined by the maternal/parental genotype, and each egg contains the precise amount of nutrients required to maintain expression of this phenotype in the chick. In this case, it is only when egg size is experimentally manipulated such that it deviates significantly from each individual female's optimum that marked negative effects on offspring development are observed. This could explain why, even with robust cross-fostering experiments, egg size appears to have little effect on offspring growth or survival: offspring development is not uncoupled from female-specific maternal effects; for example, egg size is still the optimum size for the growth of that particular chick, regardless of the foster environment. It could also explain why studies more commonly find effects of within-clutch variation in egg size (i.e., variability around some individual-specific optimum) on offspring development (e.g., Slagsvold et al. 1984; Potti and Merino 1996) even though the magnitude of variation at this level is much smaller (Christians 2002): deviations from a female's mean egg size are more significant than absolute variation in egg size among females. Finally, in this study, chicks that hatched from smaller, tamoxifen-affected eggs but survived to reproductive maturity showed compensatory growth, possibly mediated through increased provisioning from parents. Given the relative lack of parental care in precocial species, effects of reduced egg size might be even more marked because chicks would have to rely on self-feeding to compensate. In other words, final size at maturity is not a good indicator of effects of egg size on offspring development, and the lack of effects of egg size on fledging mass or size reported in many field studies could mask very different growth trajectories in chicks hatching from different-sized eggs. Because compensatory growth itself can incur long-term costs (Metcalf and Monaghan 2001; Mangel and Munch 2005), this suggests that egg size could have similar long-term effects on offspring survival and/or future fecundity that have simply gone undetected in studies to date.

Acknowledgments

We thank Katrina G. Salvante and the Simon Fraser University Animal Care Facility for their assistance with this project. Two anonymous reviewers provided helpful comments and criticisms on an earlier draft of this manuscript. This work was supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada operating grant to T.D.W. and an NSERC Undergraduate Student Research Award to E.C.W.

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