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Experimental manipulation of female reproduction reveals an intraspecific egg size–clutch size trade-off

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A negative relationship, or trade-off, between egg size and clutch size is a central and long-standing component of life-history theory, yet there is little empirical evidence for such a trade-off, especially at the intraspecific level. Here, I show that female zebra finches (*Taeniopygia guttata*) treated chronically during egg formation with the anti-oestrogen tamoxifen lay smaller eggs (by 8%) but produce larger clutches (on average two eggs more) than controls. Decreased egg mass in tamoxifen-treated females was associated with a 50% decrease in plasma levels of the two yolk precursors, vitellogenin and very-low-density lipoprotein. Although tamoxifen-treated females laid more, smaller eggs (and had a higher total expenditure in their clutch), they did not differ from controls in the number of chicks fledged, the mass or size of these chicks at fledging, or the chicks' egg-production performance at three months of age. However, tamoxifen-treated females had lower relative hatching success: they laid more eggs but hatched the same number of chicks. Among individual tamoxifen-treated females, birds that laid the smallest eggs early in their laying sequence laid the largest number of additional eggs, that is, there was a negative correlation, or trade-off, between egg size and clutch size.

Keywords: egg size; clutch size; trade-off; physiological manipulation

1. INTRODUCTION

The existence of a trade-off between size and number of propagules (or offspring) is a central and long-standing (Smith & Fretwell 1974) component of life-history theory (e.g. Clutton-Brock 1991; Stearns 1992). Such a trade-off should exist both within and between species but direct evidence to support intraspecific trade-offs between egg size and clutch size in oviparous vertebrates is limited (Bernardo 1996). This is particularly true for birds, where intraspecific analyses have commonly failed to find evidence for a trade-off (e.g. Lessells *et al*. 1989; Roff 1992; see §4). As Van Noordwijk & de Jong (1986) pointed out, the most likely explanation for this is that phenotypic correlations of these two traits will tend to be positive if individuals vary in the absolute amount of resources they have to allocate to reproduction. Experimental manipulations are therefore more likely to reveal trade-offs than will phenotypic correlations (Reznick 1985; Lessells 1991), in part because they will decouple any correlation between the mother's condition and egg size or clutch size (Roff 1992).

Recent studies have highlighted the importance of processes involved in the actual production of young, i.e. egg formation (cf. post-hatch rearing of young), to our understanding of the evolution of clutch size and the fitness costs of reproduction in birds (Heaney & Monaghan 1995; Monaghan *et al*. 1998; see also Partridge & Harvey 1985). However, the ecological and evolutionary consequences of egg-size variation, in particular, remain poorly understood (Bernardo 1996), as do the mechanism(s) underlying a trade-off between egg size and clutch size. One reason for this is that we currently lack a technique for direct experimental manipulation of egg size in birds (cf. indirect manipulation via changes in environmental cues, female condition or nutritional state, which are subject to the same problems of interpretation as phenotypic correlations (Lessells *et al*. 1989)). Here, I show, first, that chronic treatment with tamoxifen significantly decreases the mean egg size of individual birds while maintaining among-individual variation; second, that the decrease in egg size is mediated by a decrease in the supply (plasma pool) of the yolk precursors vitellogenin (VTG) and very-low-density lipoprotein (VLDL) available for uptake into the yolk; and third, that within individuals the change in egg size is negatively correlated with a change in clutch size, i.e. that a trade-off exists between egg size and clutch size.

2. METHODS

(a) *Animals, husbandry and breeding*

Zebra finches were maintained in controlled environmental conditions (temperature 19–23 °C; humidity 35–55%; constant light schedule 14 L:10 D, lights on at 07.00). All birds were provided with a mixed-seed diet (panicum and white millet 1:3; 11.7% protein, 0.6% lipid and 84.3% carbohydrate by dry mass), water, grit and cuttlefish bone (calcium) *ad libitum* and received a multi-vitamin supplement in the drinking water once per week (Williams 1996*a*,*b*). Breeding pairs were also provided with an egg food supplement (20.3% protein, 6.6% lipid) daily between pairing and clutch completion, and again during the chick-rearing period. Birds were assigned to experimental groups and pairs at random. All birds used in this experiment had bred (laid eggs) either once or twice previously and were 6–18 months old. Experiments and animal husbandry were carried out under a Simon Fraser University Animal Care Committee permit (number 399B), in accordance with guidelines from the Canadian Committee on Animal Care.

Breeding pairs were housed individually in cages (61 cm × 46 cm × 41 cm), each with an external nest-box

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(11.5 cm × 11.5 cm × 11.5 cm). Females were weighed (±0.1 g, initial mass) at the time of pairing and again at clutch completion. Nest-boxes were checked daily between 09.00 and 11.00 and all new eggs were weighed (±0.001 g) and numbered to obtain data on egg size, clutch size and laying interval (the time between pairing and laying of the first egg). All females were blood sampled between 10.30 and 11.30 on the day of first laying (one-egg stage) for measurement of plasma yolk-precursor levels, VTG and VLDL (see Williams & Christians 1997; Williams 1999*a*). If no new eggs were laid over two days the clutch was considered to be complete and the birds were left undisturbed until hatching. For all birds data were available on their previous reproductive history (egg size, clutch size, laying interval, mass loss) for at least one previous breeding attempt. Just prior to hatching, nest-boxes were again checked daily to determine hatching success per clutch (brood size at hatching). Unhatched eggs were opened to determine the stage of embryo development (some eggs were buried, broken or eaten by the birds, so stage of embryo development could not be determined for all eggs). All chicks were individually banded at eight days of age. Chicks generally 'fledged', i.e. they left the nest-box, at around 18 days of age, and all chicks were weighed and measured (tarsus length, ±0.01 mm) at 21 days of age; brood size at fledging was recorded. Chicks were then separated from the adult pair until they could be sexed based on development of sexually dimorphic plumage at around two months of age.

(b) *Tamoxifen treatment*

Tamoxifen-treated breeding females ($n = 21$) were given intramuscular injections (pectoralis muscle) of tamoxifen (10 μg g^{-1} tamoxifen citrate in 30 μl 1,2-propanediol) on the third and fourth days after pairing and then on alternate days until the third or fourth egg was laid. In all previous experiments, modal clutch size on this diet has been six eggs (Williams 1996*a*, 1999*a*) and thus I predicted that these were the latest daily injections that could affect developing eggs. Egg formation takes four days (three days for rapid yolk development, one day for albumen and shell formation) and laying starts on average seven days after pairing (Williams 1999*a*), so with this treatment protocol all birds should have been exposed to tamoxifen during the formation of all eggs. However, actual clutch size could not be predicted and tamoxifen-treated birds laid more eggs than controls (see §3(a)). Discontinuing injections at the three- or four-egg stage meant that eggs 7–11 probably developed without significant exposure to tamoxifen (see §§3 and 4). Control females ($n = 17$) were vehicle-injected only on the same schedule as experimental birds. All injections were given between 10.30 and 11.30.

All statistical analyses were carried out using SAS (SAS Institute 1990). Values are presented as means of least-squares means ± s.e.m. unless otherwise stated.

3. RESULTS

(a) *Egg size and clutch size*

There were no differences in pre-treatment egg mass, clutch size or clutch mass for birds subsequently assigned to the tamoxifen-treated and control groups ($p > 0.80$ in all cases (table 1) using mean values where data for two previous breeding attempts were available). Also, there was no difference in either laying interval ($p > 0.50$, table 1) or rate of laying (number of days over which eggs were laid, controlling for clutch size, $p > 0.15$) between tamoxifen-treated and control females, i.e. tamoxifen treatment had no effect on onset or pattern of egg laying. Body mass decreased over the laying cycle in both control and tamoxifen-treated females (1.88 ± 0.39 g, $t_{17} = 4.71$, $p < 0.001$; and 1.81 ± 0.28 g, $t_{20} = 6.38$, $p < 0.001$, respectively) but was typical of that reported for non-manipulated birds (e.g. Williams 1996*a*) and was independent of treatment ($p > 0.40$).

Table 1. *Comparison of reproductive traits for tamoxifen-treated females* ($n = 21$) *and control* (*vehicle-injected*) *females* ($n = 17$)

trait	control females	tamoxifen-treated females
pre-treatment		
egg mass (g)	1.119 ± 0.030	1.116 ± 0.027
clutch size	5.72 ± 0.26	5.79 ± 0.24
clutch mass (g)	6.45 ± 0.38	6.49 ± 0.34
treatment (all eggs)		
laying interval (days)	5.65 ± 0.34	5.95 ± 0.30
egg mass (g)*	1.105 ± 0.030	1.020 ± 0.027
clutch size**	5.67 ± 0.36	7.51 ± 0.32
clutch mass (g)*	6.28 ± 0.45	7.65 ± 0.40
treatment (eggs 1–6)		
egg mass (g)**	1.105 ± 0.031	0.985 ± 0.028

*$p < 0.05$ and **$p < 0.01$.

Tamoxifen-treated females laid smaller eggs ($F_{1,37} = 4.46$, $p < 0.05$), larger clutches ($F_{1,37} = 14.32$, $p < 0.001$) and had a larger total clutch mass ($F_{1,37} = 5.10$, $p < 0.05$) than controls (including all eggs in the analysis, table 1). Restricting analysis to the first six eggs, i.e. those exposed to tamoxifen during development, revealed that eggs of tamoxifen-treated females were, on average, 11% smaller than those of controls ($F_{1,37} = 8.11$, $p < 0.01$; table 1). Later-laid eggs from tamoxifen-treated females (eggs 7–11) were larger than eggs 1–6 within clutches (1.121 ± 0.033 g versus 0.937 ± 0.035 g; paired t-test, $t_{14} = 8.56$, $p < 0.001$) but were not significantly different from eggs 1–6 laid by control females (1.107 g, $t_{29} = 0.31$, $p > 0.70$; figure 1). This confirms that the effects of tamoxifen were restricted to eggs laid early in the laying sequence (eggs 1–6). Plasma yolk-precursor levels, measured at the one-egg stage, were significantly lower in tamoxifen-treated females than in control females (VTG: 1.03 ± 0.13 μg ml^{-1} versus 2.05 ± 0.15 μg ml^{-1}, $F_{1,31} = 25.6$, $p < 0.001$; VLDL: 10.4 ± 1.6 mg ml^{-1} versus 18.1 ± 1.9 mg ml^{-1}, $F_{1,32} = 9.83$, $p < 0.01$).

There were no significant differences in body mass, egg mass, clutch size or clutch mass in individual control females between pre-treatment and experimental clutches (paired t-test, $p > 0.10$ in all cases). However, in individual tamoxifen-treated females, clutch size (paired $t_{21} = 3.90$, $p < 0.001$) and clutch mass (paired $t_{21} = 2.41$, $p < 0.05$) were larger in the experimental clutch than in the pre-treatment clutch. Egg masses were smaller in the experimental clutches of these females (paired $t_{21} = 6.25$, $p < 0.001$) even though females were, on average, 7% heavier than for their pre-treatment clutch (paired $t_{21} = 3.34$, $p < 0.01$), i.e. the change in egg mass was independent of body mass (the mass of control females did not differ between clutches, $p > 0.70$).

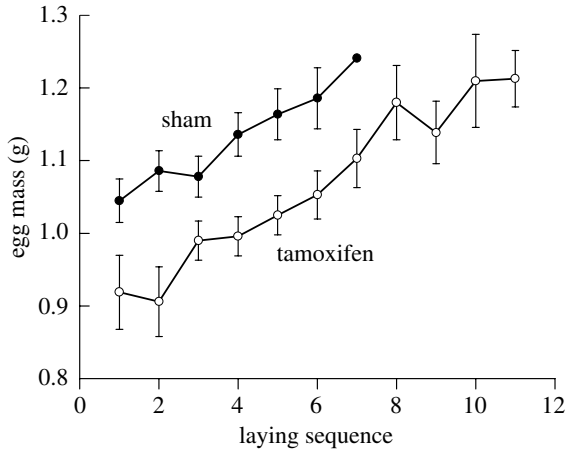

Figure 1. Relationship between egg mass (g) and laying sequence for tamoxifen-treated females (open circles) and control females (filled circles). Values are means ± s.e.m. In the control group only one female laid seven eggs.

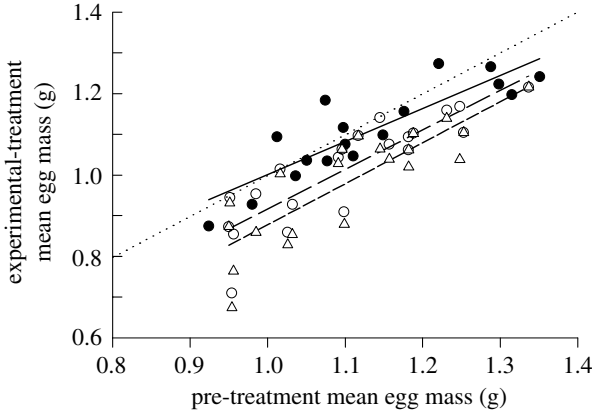

Figure 2. Relationship between pre-treatment egg mass and experimental egg mass in individual female zebra finches (control group: closed circles, solid line; tamoxifen-treated group, all eggs: open circles, short-dashed line; tamoxifen-treated group, eggs 1–6 only: triangles, long-dashed line; dotted line indicates slope with regression coefficient $b = 1$).

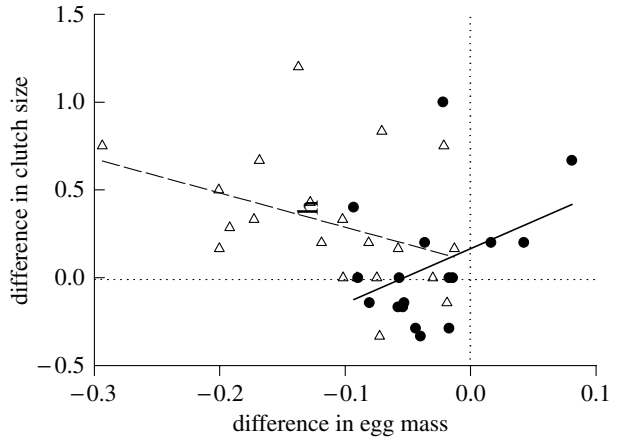

Figure 3. Relationship between change in clutch size and change in egg mass for early-laid eggs (eggs 1–6) of tamoxifen-treated females (triangles) and control females (filled circles). Data plotted are relative changes calculated as (pre-treatment clutch-experimental clutch)/pre-treatment clutch).

Table 2. *Hatching and fledging success, egg fate and sex ratio of chicks for tamoxifen-treated females* ($n = 21$) *and control (vehicle-injected) females* ($n = 17$)

(Values are means ± s.e.m. unless otherwise stated. Figures in parentheses refer to data for eggs 1–6 only.)

trait	control females	tamoxifen-treated females
brood size at hatching	4.00 ± 0.34	3.77 ± 0.32
total number of eggs where fate known	62 (62)	105 (85)
eggs hatched (%)	64.5 (64.5)	46.7 (44.7)
infertile eggs (%)	24.2 (24.2)	22.9 (23.5)
pre-term embryo (%)	11.3 (11.3)	10.1 (17.6)
lost pre-incubation (%)	0 (0)	11.4 (14.1)
brood size at fledging	3.60 ± 0.40	3.54 ± 0.30
sex ratio at fledging	16 male: 20 female	20 male: 24 female

Within individuals, the mean egg mass of experimental clutches was dependent on both pre-treatment egg mass ($F_{1,37} = 105.7$, $p < 0.001$) and treatment ($F_{1,37} = 10.3$, $p < 0.01$; figure 2). Mean egg masses in pre-treatment and experimental clutches were highly correlated in both control females ($r_{17} = 0.874$, $p < 0.001$) and tamoxifen-treated females ($r_{17} = 0.867$, $p < 0.001$; figure 2) and the slopes did not differ (interaction term, $F_{1,37} = 0.84$, $p > 0.30$). None of the regression coefficients of the relationship of experimental egg mass to pre-treatment egg mass differed significantly from $b = 1$ (control, tamoxifen, tamoxifen eggs 1–6; $p > 0.10$ in all cases; figure 2). In other words, tamoxifen treatment did not reduce the level of individual variation in mean egg size but it did decrease the mean absolute value.

In control females, the change in egg mass between experimental and pre-treatment clutches (expressed as a proportion of the pre-treatment value) was positively correlated with the difference in clutch size ($r_{17} = 0.55$, $p < 0.025$; figure 3), i.e. individual control females that laid larger eggs in their experimental clutch also laid larger clutches. In tamoxifen-treated females there was no similar significant relationship when the data for all eggs were considered (eggs 1–11; $p > 0.70$). However, when considering the mean egg mass for eggs 1–6 only, the change in egg mass was negatively correlated with change in clutch size in tamoxifen-treated females ($r_{21} = -0.44$, $p < 0.05$), i.e. females that laid the smallest eggs relative to their pre-treatment egg size had the largest increase in clutch size (figure 3).

(b) *Breeding success and chick growth*

There was no effect of treatment on brood size at hatching ($t_{21} = 0.38$, $p > 0.70$) or fledging ($t_{21} = 0.10$, $p > 0.90$) for pairs that hatched at least one chick (table 2). However, tamoxifen-treated females laid more eggs (see § 3(a)) and, consequently, hatching success was lower in these females ($\chi^2 = 4.99$, $p < 0.05$; table 2). Where egg fate was identified, the only major effect of

treatment was that 14% ($n = 85$) of eggs laid by tamoxifen-treated females were lost before the onset of incubation, compared with none of those laid by control females. These eggs were all laid early in the laying sequence (eggs 1–6) and were significantly smaller than all other eggs (0.852 ± 0.042 g versus 1.077 ± 0.011 g, $F_{1,166} = 27.2$, $p < 0.001$); there were no differences in egg size for hatching, infertile and pre-term embryo eggs ($p > 0.25$). For eggs 1–6, the masses of eggs that hatched varied from 0.904 g to 1.328 g (5th–95th percentile) in control females and from 0.825 g to 1.262 g in tamoxifen-treated females. There were no differences in sex ratio (table 2) or mass or tarsus length of chicks that fledged from control or tamoxifen-treated broods (data not shown). Furthermore, there were no differences in egg-production performance of daughters of control and tamoxifen-treated females when they themselves were bred at three months of age (clutch size, mean egg mass, laying interval, plasma levels of VTG and VLDL at the one-egg stage; $p > 0.15$ in all cases).

4. DISCUSSION

Female zebra finches treated chronically during egg formation with the anti-oestrogen tamoxifen laid smaller eggs but produced larger clutches, compared with both controls and with each individual's pre-treatment egg size and clutch size. The absolute change in egg size was smaller (8% decrease) than the change in clutch size (two eggs more), and the change in egg size was repeatable across tamoxifen-treated females, i.e. the decrease in egg mass was similar in all females relative to their initial (pre-treatment) egg mass. This is consistent with the idea that egg size is the less plastic of these two traits and is an inherent characteristic of individual females (Williams 1999*b*). Decreased egg mass was associated with a 50% decrease in plasma levels of the two yolk precursors, VTG and VLDL. This was probably due to tamoxifen competitively binding to and inhibiting oestrogen receptors in the liver, suppressing VTG production and, in turn, decreasing the plasma pool of yolk precursors below the level required to maintain receptor-mediated yolk uptake at V_{max} (see also Williams 2000).

Among individual tamoxifen-treated females, birds that laid the smallest eggs early in the laying sequence also laid the largest number of additional eggs, that is, there was a negative correlation, or trade-off, between egg size and clutch size. To my knowledge, this is the first study to demonstrate experimentally the existence of an intraspecific egg size–clutch size trade-off in birds. The only other research showing similar results in an oviparous vertebrate is that of Sinervo and colleagues (e.g. Sinervo 1999). In the lizard *Uta stanburiana*, Sinervo & Licht (1991) were able to demonstrate an egg size–clutch size trade-off using 'yolkectomy' to reduce clutch size and hormone treatment (follicle-stimulating hormone) to increase clutch size. These two treatments resulted in a compensatory increase and decrease in egg size, respectively.

Tamoxifen can have many, highly variable species- and tissue-specific effects, acting as a potent oestrogen agonist, a partial agonist or a potent oestrogen antagonist (Jordan & Robinson 1987; Jordan 1995). However, in birds it appears to act as a 'pure' anti-oestrogen in most tissues, particularly with regard to oestrogen-dependent reproductive function (e.g. Wilson & Cunningham 1981; Delville & Balthazart 1987; Jaccoby *et al*. 1995). Tamoxifen treatment completely suppresses egg laying and female sexual behaviour in hens (Jaccoby *et al*. 1995, 1996) and quail (*Coturnix c. japonicus*; Delville & Balthazart 1987) at doses similar to those used in the present study (typically 10 mg kg^{-1}). However, on a metabolic body-mass basis, the zebra finches in this study received a much lower dose of tamoxifen (approximately 0.1×), which may explain why tamoxifen modulated, rather than suppressed, reproductive function. Several results suggest that the effects on egg size and clutch size in this study were not pharmacological. First, tamoxifen treatment had no effect on the timing or pattern of egg laying. Second, all females lost mass during laying (as reported in previous studies, e.g. Williams 1996*b*) but mass loss was not affected by treatment. Third, although tamoxifen-treated females showed decreased egg size and plasma yolk-precursor levels, both were within the range observed in non-manipulated females (e.g. Williams 1996*a*,*b*; Williams & Christians 1997).

A negative correlation between egg size and clutch size was seen in this study only if mean egg mass was calculated for eggs laid early in the laying sequence (eggs 1–6). This was due to the fact that later-laid eggs were probably not affected by tamoxifen, since injections were stopped after the third or fourth egg was laid (see §2(b)). The egg mass of late-laid eggs in tamoxifen-treated females was the same as in control females. Williams (2000) reported a transient decrease in egg mass in response to short-term tamoxifen treatment, suggesting that tamoxifen only affected yolk development for 24–48 h after injection (consistent with an estimated half-life for the initial elimination phase of 7–14 h (Hardman & Limbard 1996)). In this previous study (Williams 2000), tamoxifen treatment had no effect on clutch size and tamoxifen-treated birds laid larger eggs late in the laying sequence, so there was no effect on individual mean egg mass. Clutch size is determined sometime between the third and fourth day of egg production in zebra finches (Haywood 1993), which is consistent with the negative correlation between the size of early-laid eggs and clutch size in the current study. In other words, tamoxifen-treated females appear to have based their clutch size 'decision' on some aspect of the magnitude or rate of development of eggs during this critical period (e.g. egg size *per se*, yolk size, etc.).

The importance of an experimental approach in decoupling the correlation between the mother's condition and egg size or clutch size (Roff 1992) was demonstrated in this study by the positive correlation between egg size and clutch size in the control females. This is probably due to differences in the absolute amount of resources available to different females for reproduction (*sensu* Van Noordwijk & de Jong 1986); most studies in birds have reported either a positive correlation or no correlation between egg size and clutch size. In a recent review of 63 avian studies only five (8%) reported a negative relationship between egg size and clutch size, 15 (24%) reported a positive relationship and 43 (68%) found no relationship (J. K. Christians, personal communication).

Similarly, in zebra finches there is a positive correlation between egg size and clutch size in non-manipulated females over the range of most common clutch sizes (three to six eggs, $n = 99$; T. D. Williams, unpublished data). However, there is also substantial overlap in total clutch mass between clutch sizes in zebra finches suggesting the existence of trade-offs among individuals: females with the same total expenditure either produce a clutch of n large eggs or a clutch of $n + 1$ smaller eggs (see also Flint & Sedinger 1992; Flint & Grand 1999).

The simplest explanation for trade-offs is that individuals have finite resources, which they can divide amongst many small eggs or a few large eggs, that is, the trade-off is resource-based (Stearns 1992). This would predict that, for females with the same resources, the total expenditure in the clutch should be the same regardless of any egg size–clutch size trade-off. In contrast, in this study tamoxifen-treated females had a higher total clutch mass than controls (cf. lizards; Sinervo 1999), which is inconsistent with a simple resource-based trade-off model. Females that obtain resources for egg formation primarily from daily dietary intake (cf. stored nutrients), such as zebra finches (Williams 1996*b*), can increase clutch size by forming eggs for more days without increasing peak daily expenditure. Since total clutch mass is determined more by the number of eggs laid than by the size of those eggs, this could potentially uncouple any effect of finite resource availability on female expenditure in egg formation (Bernardo 1996; Williams 1996*b*). An alternative explanation for the trade-off observed in this study is that females 'ignored' their small, early-laid, tamoxifen-affected eggs and continued laying replacement eggs in order to obtain a 'normal' egg-size clutch (a 'behavioural adaptive decision'; M. Lambrechts, personal communication). In support of this, relative hatching success was lower in tamoxifen-treated females because small, early-laid eggs were less likely to be retained and incubated, so these eggs contributed less to brood size at fledging (although this effect was not absolute: some tamoxifen-treated females hatched all of their smaller, earlier-laid eggs). In this case, by laying additional (larger) eggs, individual females effectively restored or maintained expression of their phenotypic trait value for egg size. This is consistent with the lack of individual flexibility in egg size in this, and other, species: egg size is highly repeatable even when resource availability or quality changes (e.g. Williams 1996*b*). There does not appear to have been selection for a flexible strategy of variable egg size in female birds, which could be traded off against clutch size depending, for example, on environmental circumstances, even though variability can be generated through experimental physiological manipulation.

Clutch size can be easily manipulated after laying by addition or removal of eggs or chicks from nests, and egg quality can also be manipulated after laying to investigate the effects on offspring fitness (e.g. Vander Werf 1992; Hill 1993; Finkler *et al*. 1998). However, these post-laying manipulations do not incorporate the physiological costs of producing more eggs (Monaghan & Nager 1997). Egg production can be manipulated through diet (e.g. Selman & Houston 1996; Williams 1996*a*) or photoperiod (e.g. Shanawany *et al*. 1993), but in both cases the female's resources and/or environment are changed and this can confound interpretation of these studies (Lessells *et al*. 1989). For example, with dietary manipulation, females on higher-quality diets lay larger eggs and more eggs (Selman & Houston 1996). However, experimental (physiological) manipulations, such as tamoxifen treatment (this study) or other hormonal or physical manipulations of egg formation (e.g. Sinervo 1999), can be used to modify egg size and/or clutch size in females with constant resource availability under constant environmental conditions. This should facilitate progress in our understanding of the life-history consequences of these traits (in particular egg size, see Bernardo 1996) as well as the physiological mechanisms underlying them and their trade-offs.

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