

Manipulating developmental stress reveals sex-specific effects of egg size on offspring phenotype

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Abstract

The general lack of experimental evidence for strong, positive effects of egg size on offspring phenotype has led to suggestions that avian egg size is a neutral trait. To better understand the functional significance of intra-specific variation in egg size as a determinant of offspring fitness within a life-history (sex-specific life-history strategies) and an environmental (poor rearing conditions) context, we experimentally increased developmental stress (via maternal feather-clipping) in the sexually size-dimorphic European starling (*Sturnus vulgaris*) and measured phenotypic traits in offspring across multiple biological scales. As predicted by life-history theory, sons and daughters had different responses when faced with developmental stress and variation in egg size. In response to developmental stress, small egg size in normally faster-growing sons was associated with catch-up growth prior to attaining larger adult size, resulting in a reduction in developmental stability. Daughters apparently avoided this developmental instability by reducing growth rate and eventual adult body mass and size. Interestingly, large egg size provided offspring with greater developmental flexibility under poor growth conditions. Large-egg sons and daughters avoided the reduction in developmental stability, and daughters also showed enhanced escape performance during flight trials. Furthermore, large egg size resulted in elevated immune responses for both sexes under developmental stress. These findings show that there can be significant, but complex, context-specific effects of egg size on offspring phenotype at least up to fledging, but these can only be demonstrated by appreciating variation in the quality of the offspring environment and life histories. Results are therefore consistent with egg size playing a significant role in shaping the phenotypic outcome of offspring in species that show even greater intra-specific variation in egg size than starlings.

Introduction

The functional significance of intra-specific variation in avian egg size as a determinant of offspring and maternal fitness remains surprisingly unresolved (Christians, 2002; Williams, 2005; see Krist, 2011 for a recent review). Indeed, the fact that there is still little experimental evidence for strong, positive effects of egg size on offspring phenotype has led some authors to suggest

that egg size is a neutral trait, i.e. under no selection pressure (Jager *et al.*, 2000; Van de Pol *et al.*, 2006; although see Krist, 2011). Early studies concluded that a strong positive relationship between egg size and offspring fitness existed (Clutton-Brock, 1991); however, the effects of parental quality confounded many of these correlational studies (Williams, 1994). Earlier studies used cross-fostering experiments (reciprocal exchanges between 'large- and small-egg' females; e.g. Meathrel *et al.*, 1993) as a means to experimentally control for parental quality, e.g. examining the assumed proximate and ultimate costs of raising large offspring in low-quality rearing environments (i.e. small-egg mothers). However, although often assumed, large offspring may not

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necessarily be at a consistent disadvantage in low-quality environments because they may have a greater competitive capability coupled with an increased capacity to reduce energetic demands on parents during harsh periods (Williams, 1994). More recently, studies have begun to investigate the evolution of egg size within more biologically relevant contexts (Bize *et al.*, 2002; Krist, 2009; Pryke & Griffith, 2009). In particular, it may be quite important to consider the role of environmental and life-history context on the fitness of small egg offspring, because they are expected to have less ability to buffer poor conditions given that they maybe are closer to a energetic/survival threshold compared with large-egg offspring (Råberg *et al.*, 2005; Rowland *et al.*, 2007; Love & Williams, 2008).

Support for the idea that small-egg offspring face greater fitness costs than those from large eggs comes from (i) the disappearance of early developmental costs for large-egg offspring raised by small-egg mothers by fledging (Smith *et al.*, 1995; Smith & Bruun, 1998; Styrsky *et al.*, 1999; Risch & Rohwer, 2000), (ii) recent direct manipulations of egg and yolk size suggesting a significant fitness cost for small-egg offspring (Wagner & Williams, 2007; Saino *et al.*, 2010) and (iii) recent work indicating sex-specific effects of hatching size on offspring phenotype and survival (Love *et al.*, 2005; Love & Williams, 2008). As such, we may need a better appreciation of the effects of egg size (and indeed even other phenotypic traits, e.g. Roulin, 2009), within the appropriate ecological and developmental context to properly understand the potential role of egg size as a maternal effect (Krist, 2011).

A recent meta-analysis indicated that we still do not fully understand how egg size affects offspring quality in birds (Krist, 2011). We still lack studies that (i) manipulate the quality of the offspring environment, (ii) appreciate the context of variation in life histories, (iii) examine traits directly related to survival and (iv) examine relationships between egg size and offspring behavioural and physiological traits (Krist, 2011). Although some studies have incorporated a greater life-history and environmental context (see Smith & Bruun, 1998; Styrsky *et al.*, 1999; Christians, 2002), most recent research has shifted away from egg size *per se* to consider other putative aspects of egg quality (i.e. yolk hormones, carotenoids and maternal antibodies (Blount *et al.*, 2000; Grindstaff *et al.*, 2003; Groothuis *et al.*, 2005). However, the current lack of interest in egg size as an important life-history trait might be premature and even misleading for a number of important reasons (Wagner & Williams, 2007; Reed *et al.*, 2009). First, ignoring life-history and environmental context makes it very difficult to predict how a given maternal effect will influence offspring fitness (Pfennig & Murphy, 2000; Heath *et al.*, 2003; Marshall & Uller, 2007; Love *et al.*, 2009). Second, many early egg size studies focused on offspring morphology and survival as fitness-related endpoints, whereas the

numerous, multi-level phenotypic mechanistic responses (i.e. molecular/physiological/behavioural) that many evolutionary ecologists currently study may also be important drivers of variation in fitness (Love & Williams, 2008; Travers *et al.*, 2010). Consequently, earlier studies of egg size did not have the opportunity to evolve to the state of integration within which other maternal effects are currently scrutinized (Wagner & Williams, 2007). Ironically, we would argue that if a novel trait with as much intra- and inter-individual variability as egg size were to arise (Christians, 2002; Kontiainen *et al.*, 2008), researchers would predict that maternal effects have a very good chance of influencing offspring fitness, especially within the context of (i) low-quality mothers or environments, (ii) sex-specific differences in energetic and evolutionary demands/goals (i.e. in sexually dimorphic species) and (iii) having effects on traits that are sensitive to developmental stress (see Saino *et al.*, 2010).

Here, we use an experimental approach to examine how small egg size affects offspring phenotype across relevant biological scales (body mass, structural size, fluctuating asymmetry, immune function and flight performance) within a life-history context (a species with sex-specific differences in reproductive variance) and an environmental context (post-natal developmental stress). We examine our questions in free-living European starlings (*Sturnus vulgaris*) because they show moderate sexual size dimorphism (7–8% in juvenile and adult mass/structural size; see Rowland *et al.*, 2007) and because male size is important for nest-site competition (Flux & Flux, 1992; Cabe, 1993). Theory predicts that larger males needing to compete for access to mates will have higher relative reproductive variance than females (Clutton-Brock, 1988), and these males will only have high fitness if they can successfully compete for mating opportunities (Shuster, 2009). We therefore predicted that male starlings forced to develop under low-quality conditions (small eggs, developmental stress) would shift investment towards traits that maximize eventual adult reproductive potential (i.e. body mass and size) over traits related to immediate offspring survival (i.e. immune function, escape performance). Essentially, male starlings under developmental stress are predicted to have a higher ratio of reproductive-to-survival-trait investment than females because a small male that survives to adulthood gains nothing if it cannot successfully compete against larger males for access to females (Clutton-Brock, 1988).

Developmental stress is a biologically relevant stressor faced by many nest-bound avian species (Love *et al.*, 2005; McNamara & Buchanan, 2005) and can be experimentally induced in the starling system by reducing maternal offspring-rearing capacity (via primary feather-clipping; Rowland *et al.*, 2007). We examined variation in body mass and size because they are consistently regarded as meaningful indicators of developmental stress (Råberg *et al.*, 2005); fluctuating asymmetry in

structural size as a measure of developmental instability (Van Dongen, 2006) because it can vary in a sex-specific manner in response to environmental stress (Söderman *et al.*, 2007) and has been related to juvenile recruitment (Lemberget & McCormick, 2009); cell-mediated immune response as it has been shown to be sensitive to poor developmental conditions (Fargallo *et al.*, 2002) and can respond in a sex-specific manner under these conditions (Tschirren *et al.*, 2003; Chin *et al.*, 2005; Love *et al.*, 2008); and flight performance as a quantifiable survival-related trait influenced in a sex-specific manner by the quality of the rearing environment via variation in body mass, size and flight musculature (i.e. wing-loading, size and capacity of flight muscle; Verspoor *et al.*, 2007; Chin *et al.*, 2009). Using this experimental design, we can apply biological context to examine two important but largely untested aspects of egg size on offspring fitness. Firstly, by experimentally manipulating 'ecological context' (developmental stress: analogous to good vs. poor year/habitat), we can test the prediction that egg size is most important for offspring fitness in poor conditions (see Smith & Bruun, 1998; Styrsky *et al.*, 1999). Second, because male and female starlings appear to have different life-history strategies, they respond differently to experimental manipulations of hatching size (i.e. egg size; Love & Williams, 2008), and we can therefore explore the 'life-history context' (i.e. sex-specific effects) of egg size variation on offspring fitness.

Methods

Research occurred from April to July 2005 in Langley, BC, Canada under a Simon Fraser University Animal Care permit (657B-96) and Canadian Council on Animal Care guidelines. Daily nest-box checks determined clutch initiation dates and laying sequence. As outlined in Love & Williams (2008), individual females were caught just prior to nestling hatching and randomly assigned (matched for laying date) into either a feather-clipping treatment ($N = 16$) designed to reduce maternal provisioning rates (Winkler & Allen, 1995; Hill, 2003) or an unmanipulated treatment (control: captured and handled only: $N = 16$). Females were measured, weighed, banded and released; individuals were recaptured at day 7–8 of nestling rearing to determine post-treatment changes in body mass. We ensured that all individual nestlings were matched to respective eggs by removing all clutches within 0.5 days of hatching and placing them in an incubator for approximately 6–10 h until hatching – artificial eggs were used to maintain maternal incubation behaviour. Parental rearing ability was assessed using 30-min behavioural observations of each nest over two consecutive days when nestlings were 6–8 days of age (linear phase of growth), calculated as the number of feeding visits per chick per hour for each parent (Rowland *et al.*, 2007). Nestling hatching order, weight and size were tracked from hatching through 5, 10, 15

and 17 days of age (see Love & Williams, 2008). Developmental instability was calculated as the difference between the left and right tarsus (Swaddle *et al.*, 1994), with high within-individual repeatability in both left and right measurements ($r^2 = 0.91$ and 0.92 , respectively), and in the difference between the left and right leg ($r^2 = 0.93$). Nestlings underwent a phytohemagglutinin test at 17–18 days evaluate cell-mediated immunity (Martin *et al.*, 2006) and previously described in more detail for this study group (Rowland *et al.*, 2007). Repeatability of both initial ($r^2 = 0.85$, $P = 0.0001$) and final ($r^2 = 0.86$, $P = 0.0001$) measurements was high. Nestling sex was determined by PCR amplification (as in Love *et al.*, 2005) using small blood samples collected at hatching. A detailed description of the flight performance methodology is outlined in Verspoor *et al.* (2007). Briefly, fledglings were collected from nest-boxes on the brood mean 21st day of age, and two flight trials (to avoid habituation to the flight apparatus) in a horizontal performance chamber were filmed with a high-speed video camera; trials were analysed frame by frame on a monitor. Average mechanical energy [calculated as the per unit mass (Energy, in joules per kilogram) for each individual according to Williams & Swaddle, 2003] describes both the height and velocity gain components of flight performance in a single variable, referred to hereafter as 'flight performance'.

We used general linear mixed models to analyse maternal treatment by egg size interactions on nestling morphology (body mass, tarsus length, wing cord), structural stability (fluctuating asymmetry of tarsus length), immune function and flight performance within each sex, with maternal identity as a random factor to control for the nonindependence of siblings. We analysed the sexes separately given that male and female starlings are expected to have different life-history strategies (with respect to relative investment in reproductive-compared with survival-related traits), because strong male–male competition is expected to produce greater relative reproductive variance in males compared to females (see Introduction; Clutton-Brock, 1988; Love & Williams, 2008). We used repeated-measures ANOVA to examine treatment-induced changes in maternal body mass.

Results

Egg size across individual females ranged from 5.93 to 8.39 g (1.41-fold variation), but there were no significant differences in laying date, mean egg mass, clutch size, structural size or body mass for females assigned to the two maternal treatments (Love & Williams, 2008); we also found no differences in egg size for eggs that hatched sons or daughters ($F_{1,94} = 1.65$, $P = 0.23$). Furthermore, the feather-clipping treatment had no effect on whether females did or did not return to clutches following the clipping treatment compared to controls ($\chi^2: 1.21$, $P = 0.27$). Maternal and total parental provisioning

rates (number of feeds per nestling per hour) were significantly lower in the feather-clipped group [maternal: $F_{1,29} = 5.67$, $P = 0.02$; CLIP: 2.56 ± 1.26 , CTL: 5.77 ± 1.10 (mean \pm SEM); parental: $F_{1,29} = 4.87$, $P = 0.03$; CLIP: 6.51 ± 1.15 , CTL: 9.49 ± 0.98], and feather-clipped females lost more body mass during provisioning than control females (repeated-measures ANOVA: time effect: $F_{1,29} = 8.54$, $P = 0.007$), CLIP: 13.28 ± 1.52 g, CTL: 9.27 ± 1.16 g; see also Love & Williams, 2008).

Smaller egg size negatively impacted growth (body mass, tarsus and wing cord) in developmentally stressed daughters, but not sons, compared to control individuals (Table 1; Fig. 1a, b, f, g). Smaller egg size increased developmental instability in developmentally stressed sons, but not daughters compared with control individuals (Table 1; Fig. 1c, h). Larger egg size had a positive effect on immune responses in both developmentally stressed sons and daughters compared with controls (Table 1; Fig. 1d, i). Likewise, larger egg size had a positive effect on flight performance in daughters, but not sons, under developmental stress compared with controls (Table 1; Fig. 1e, j).

Discussion

In this study, we experimentally confirmed that egg size (as measured by egg mass) can have significant effects on offspring phenotype up to fledging but that the effect of egg size can be sex specific and dependent on ecological context or developmental rearing conditions. By feather-clipping female parents, we reduced parental quality and significantly reduced total provisioning rates in experimental broods, an important manipulation that has been largely lacking when examining the effects of egg size on offspring fitness (Krist, 2011). Under these conditions, there were strong, clear effects of egg size on several components of offspring phenotype, whereas in control broods, there were no effects of egg size. Furthermore, as

we predicted, there were sex-specific effects of egg size on offspring phenotype, e.g. body mass and tarsus at fledging were strongly positively correlated with egg mass in female offspring but not in males, whereas there were opposite sex-specific effects of egg size on developmental stability.

In daughters reared by feather-clipped (low-quality) mothers (i.e. under developmental stress), egg size had a strong impact on mass and size at fledging. For example, body mass at fledging averaged 57.4 ± 0.3 g in small-egg (6.0–6.5 g) offspring compared with 77.8 ± 0.4 g in large-egg (7.75–8.5 g) offspring, i.e. a difference of almost 45%. Similarly, tarsus length was 9.3% smaller at fledging in small- vs. large-egg daughters, and similar results were found for wing cord (15.8% smaller). In addition, in daughters, there was evidence that large egg size negated this reduction in body mass and size and increased escape flight performance. In marked contrast to daughters, there was no relationship between egg size and body mass or tarsus at fledging in sons regardless of rearing conditions. Indeed, body mass of small-egg sons averaged 78.7 ± 0.4 g and was identical to that of large-egg sons (79.1 ± 0.2 g) and of large-egg daughters. Also in contrast to daughters, we found no positive effects of larger egg size on flight performance in stressed sons, although large egg size did prevent the negative effects on developmental stability. Interestingly, both sexes appeared to somehow 'invest' in cell-mediated immune responses to the same degree regardless of egg size; however, large-egg offspring of both sexes showed higher immune responses under developmental stress. Although highly speculative, it is possible that offspring raised by feather-clipped mothers interpret poor developmental conditions as a physiological threat to immediate survival (see Martin, 2009) and divert resources towards the immune system, but only when they are given the flexibility (see Love *et al.*, 2008) of hatching from a large egg. Given that we did not see this same response in control offspring, we would predict that this

Table 1 Sex-specific interactive effects of developmental stress (treatment) and egg size on fledgling phenotype in free-living European starlings (from a GLMM).

Sex	Trait	Treatment		Egg size		TRT \times ES		N
		F	P	F	P	F	P	
Male	Body mass	0.00	0.99	0.06	0.80	0.006	0.94	47
	Tarsus	1.63	0.21	0.37	0.54	0.06	0.81	47
	Tarsus asymmetry	19.22	<0.0001	10.6	0.0023	11.5	0.0016*	47
	Immune response	1.89	0.18	4.16	0.047	3.21	0.03*	47
	Flight performance	0.55	0.46	0.94	0.34	0.24	0.63	34
Female	Body mass	6.03	0.02	10.1	0.003	4.50	0.04*	46
	Tarsus	7.24	0.01	12.4	0.001	9.57	0.004*	46
	Tarsus asymmetry	3.77	0.06	0.0007	0.98	0.72	0.40	46
	Immune response	1.99	0.16	3.89	0.055	3.03	0.04*	46
	Flight performance	7.06	0.01	2.89	0.10	10.9	0.003*	29

*A significant interaction between maternal treatment and egg size of $P < 0.05$.

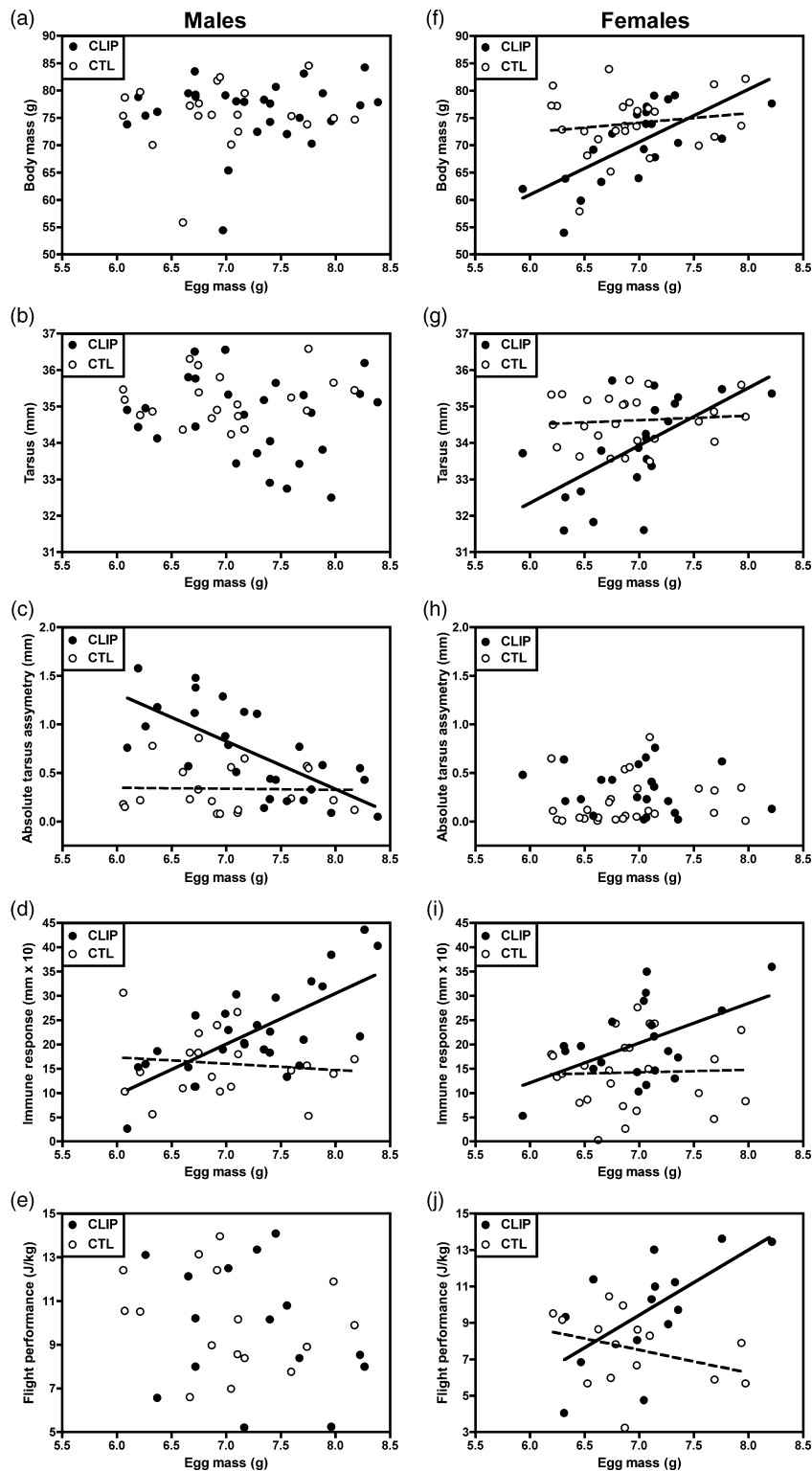


Fig. 1 Treatment-specific developmental responses of European starling fledglings in relation to egg size (male, female): body mass (a, f), tarsus length (b, g), developmental instability (c, h), immune response (d, i) and escape performance (e, j) for. Solid lines represent a significant relationship between the trait and egg size for individuals raised by feather-clipped mothers (i.e. significant treatment \times egg size interaction; see Results); nonsignificant relationships for control treatment (dashed lines) are shown only for comparison sake for significant interactions.

potentially short-term investment may result in future costs for other systems in stressed offspring.

We predicted that under developmental stress, sons from small eggs would invest in future reproductive potential (mass and size) over immediate survival, whereas daughters hatching from small eggs would trade-off growth to adult size for survival-related traits (see Introduction). Our data suggest that small-egg sons had higher initial growth rates to achieve the same fledging mass/size as large-egg sons (i.e. they exhibited catch-up growth – Metcalfe & Monaghan, 2001), but this appeared to come at a cost of increased developmental instability (i.e. developmental errors). Interestingly, large-egg males raised under poor developmental conditions could not increase escape performance ability like their sisters, potentially because of the higher wing-loading that would result from maintaining body mass. However, stressed large-egg males did exhibit elevated immune responses. Developmentally stressed daughters from small-eggs showed the predicted reduced investment in body mass and size without an apparent cost to developmental instability. Moreover, large-egg daughters raised under poor developmental conditions appeared able to increase escape performance and immune responses. It seems likely that females may pay possible future costs for these investments, although we are not able to examine these currently. It is well known that catch-up growth can induce a wide range of costs to phenotypic and life-history traits and even fitness (e.g. Álvarez & Metcalfe, 2007; Inness & Metcalfe, 2008; Kolss *et al.*, 2009), something that small-egg males under developmental stress appear to have faced. Indeed, the ‘developmental stress hypothesis’ has been suggested to explain the evolution of complex traits as indicators of male quality and why males and females often differ in their developmental responses to neonatal stress (Buchanan *et al.*, 2003; Arnold *et al.*, 2007; Walling *et al.*, 2007).

Our results appear consistent with egg size playing a significant and complex sex-specific role in shaping the phenotypic outcome in starlings, especially under stressful developmental conditions. Furthermore, although egg size had sizeable effects on fitness-related traits in starling offspring, egg size variation in this study was relatively low (i.e. varied 1.41-fold) compared with the degree of intra-specific variation reported in other species (i.e. up to two-fold variation, Christians, 2002). As such, egg size may be an even more important life-history trait in species and populations that show greater variance. It is of course important to note that there may be a number of factors that change with variation in egg size (i.e. finer scale components), and as such it is indeed possible that some of the effects of egg size on offspring phenotype may have been indirectly caused by natural variation in egg size. Nonetheless, our results indicate that egg size is likely an important maternal effect that deserves more careful consideration within the context of environmental and life-history variation.

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