

Egg ejection risk and hatching asynchrony predict egg mass in a communally breeding cuckoo, the Greater Ani (*Crotophaga major*)

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The Greater Ani (*Crotophaga major*) is a neotropical cuckoo in which several females lay eggs in a single nest. Group members synchronize egg laying and compete for reproduction by ejecting early-laid eggs from the communal nest. Eggs are large (~17% of female body mass) and vary greatly in size. I assessed the effects of egg size, hatching asynchrony, and position in the laying order on the survival and growth of nestlings to test the hypothesis that females invest more resources in eggs that are likely to give rise to surviving offspring. The egg's position in the female's laying sequence was a significant predictor of egg mass, with first-laid eggs and last-laid eggs consistently smaller than those in the middle of the clutch. Females that initiated laying in the communal nest almost always lost their first-laid eggs, and these females also exhibited the most extreme variation in egg mass. Nestlings from last-laid eggs were more likely to hatch asynchronously and starve before fledging, and the mass of last-laid eggs decreased as the degree of hatching asynchrony increased. Ejection and starvation probabilities were not affected by egg mass; therefore, low survivorship of first-laid and last-laid eggs was due solely to their position in the laying order, not to their smaller size. These data suggest that individual females allocate more resources to eggs that are likely to survive to fledging and that reproductive competition among communally breeding females explains much of the variation in egg size in this species. *Key words:* allocation, *Crotophaga major*, Crotophaginae, maternal effects, maternal investment. [*Behav Ecol*]

The life-history strategies of all animals are shaped by the trade-off between the number of offspring that an individual can produce and the size or quality of each offspring (Clutton-Brock 1991; Roff 1992). Large, well-provisioned offspring are often more likely to survive and reproduce than their smaller siblings (Williams 1994); however, selection for increased offspring size is constrained by selection on the lifetime reproductive fitness of the parent (Williams 1966). The interaction between these 2 opposing pressures is thought to give rise to variation in offspring size within a species (Parker and Begon 1986). In birds, for example, increases in egg size are correlated with higher hatching success, size at hatching, growth rate, size at fledging, and survival (Ricklefs et al. 1978; Blomqvist et al. 1997; D'Alba and Torres 2007). Conversely, calcium, protein, yolk lipids and carotenoids, and other nutrients are energetically costly to females (Graveland et al. 1994; Blount et al. 2004, reviewed in Williams 2005), and increased investment in a clutch can lower maternal condition as well as investment in future clutches (Nager et al. 2001; Visser and Lessells 2001).

Egg mass tends to vary considerably within most bird species, with the largest egg in the population averaging about 50% larger than the smallest. However, as much as 70% of this variation is explained by differences among females, not within individual females' clutches (reviewed in Christians 2002). In fact, egg mass appears to be fairly constant within individuals because it is heritable (Noordwijk 1987; Budden and Beissenger 2005) and highly repeatable within and across breeding attempts (Lessells et al. 1989; Valkama et al. 2002).

Though clutch size, ambient temperature, and maternal size, age, food availability, and nutritional condition may also influence egg mass, these factors typically explain only a small amount of its variation (reviewed in Christians 2002).

Because intra-female egg mass appears to be relatively inflexible in birds, the species that do show variation within clutches have long been of interest to evolutionary biologists (Warham 1974; Koenig 1980; Slagsvold et al. 1984; Williams 1990). Life-history theory posits that intraclutch variation is most likely to be adaptive to females when offspring within a brood predictably differ in their likelihood of survival or future reproductive success (O'Connor 1979). In many birds, for example, incubation begins before all the eggs in the clutch are laid, leading to asynchronous hatching. Early-laid eggs hatch before their later-laid counterparts, creating a size hierarchy that favors older nestlings. Depending on the degree of asynchrony and the severity of competition among nest mates, mothers are predicted to skew their investment in members of a clutch either by increasing the size of late-laid eggs in order to compensate for the effects of hatching asynchrony (the "brood survival" hypothesis) or by decreasing the size of late-laid eggs in order to accentuate the effects of hatching asynchrony (the "brood reduction" hypothesis; Slagsvold et al. 1984).

In practice, however, it is often difficult to determine whether variation in egg size reflects an adaptive response to externally imposed differences in nestling survivorship or whether it simply results from proximate physiological limitations and leads to differences in nestling survivorship as a secondary consequence (Mousseau and Fox 1998; Marshall and Uller 2007). Without considering the effects of position on the laying order on nestling mortality, correlations between egg size and nestling survivorship cannot be used to infer the selective pressures underlying variation in these traits. Distinguishing between adaptive and nonadaptive

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explanations for variation in egg mass, therefore, requires analyzing the effects of egg size, maternal identity, and position in the laying order on nestling survivorship (Mueller 1990; Maddox and Weatherhead 2008). Because few studies have included the experimental or statistical controls necessary for such analyses, it is often unclear whether intra-female variation in egg size reflects individual plasticity or proximate constraints.

In this paper, I present data on the variation in the mass of 333 Greater Ani (*Crotophaga major*) eggs laid by 70 females over 4 years at Gatún Lake, Panama (2006–2009). This large neotropical cuckoo is obligately communal, nesting in groups of 2 to 5 socially monogamous pairs. Adult group members are generally unrelated to one another, and neither pairs nor groups are stable from year to year (Riehl C, unpublished data). As in the other 3 species of crotophagine cuckoos (*C. ani*, *C. sulcirostris*, and *Guira guira*), all of the females in a group lay their eggs in a single nest; incubation, nest defense, and chick rearing are shared among group members (Davis 1941, 1942).

Females in each group compete for reproduction and synchronize laying by ejecting each others' eggs from the nest. Experimental cross-fostering of eggs during the laying period suggests that female Greater Anis are incapable of recognizing their own eggs (Riehl C, in preparation). As a result, only early-laid eggs are ejected: each female ceases ejection once she enters the laying sequence, presumably to avoid ejecting her own eggs. The first female to begin laying, therefore, always loses her first egg to ejection, whereas the last female to enter the laying sequence loses none (Vehrencamp 1977). Each female typically lays 3 to 5 eggs at 2-day intervals. Males perform the majority of incubation, which lasts only 11 days and usually begins with the penultimate egg, leading to asynchronous hatching of the last-laid egg. Hatching asynchrony produces substantial differences in size between late-hatching nestlings and their older nest mates, which are exacerbated by rapid nestling development: Because nestlings typically double in mass between their first and second day of life, a nestling that hatches just 1 day late must compete with nest mates twice its size. This leads to a significantly increased risk of starvation for nestlings from last-laid eggs (Riehl and Jara 2009).

Several features of the life history of crotophagine cuckoos suggest that egg mass may be under strong selection. First, their communal breeding systems lead to predictable differences in egg survivorship both among females in a group and across the laying sequence, with first-laid eggs virtually always ejected from the nest and last-laid eggs at increased risk of hatching asynchronously (Riehl and Jara 2009). Second, eggs vary greatly in size in all the crotophagines (Payne 2005): In this study population of Greater Anis, the largest egg in the population is nearly twice as heavy as the smallest (range = 19–37 g). Finally, eggs are unusually large relative to female body mass and are energetically costly for females to produce (mean egg mass = 17% of female body mass; Riehl and Jara 2009). Because an individual female's position in the laying order of the group may change within a season or across years, variation in egg investment may result from individual plasticity rather than from effects related to age, experience, or body condition (Riehl C, unpublished data).

In this study, I tested the hypothesis that female Greater Anis adjust investment in their eggs based on the egg's probability of surviving to fledging. The main goals of the study were to identify factors predicting variation in: 1) egg mass among and within females, 2) egg and nestling survivorship over the laying sequence, and 3) nestling mass at hatching and fledging. Biased investment should be most pronounced in the first and last eggs laid in the communal clutch because

these eggs have demonstrably reduced survivorship due to egg ejection and hatching asynchrony, respectively. Therefore, I predicted that egg mass should be significantly influenced by the egg's position in the female's laying sequence and by the female's position in the laying order of the group. Predictions for the direction of investment bias for first- and last-laid eggs differ as follows:

- *First-laid eggs.* I predicted that females should allocate fewer resources to first-laid eggs because ejection risk depends on the egg's position in the laying sequence rather than on its mass. Furthermore, reduced investment should be most pronounced for the female that initiates laying in each group because the first egg to be laid in the communal clutch is nearly always ejected.
- *Last-laid eggs.* I predicted that the direction of investment bias in last-laid eggs should depend on whether an egg's size can compensate for hatching asynchrony. If increased egg mass can mitigate the effects of hatching asynchrony, I predicted that females should allocate more resources to last-laid eggs than to eggs in the middle of the clutch (the "brood survival" hypothesis). Alternatively, if hatching asynchrony leads to lowered survivorship independent of egg mass, I predicted that females should invest less in last-laid eggs (the "brood reduction" hypothesis). Because any female in the group may lay the last egg in the clutch, I predicted that patterns of investment in last-laid eggs should not differ among females.

METHODS

Study species and general methods

The Greater Ani is a 150–200 g cuckoo that inhabits forested lake and river edges from Panama to Northern Argentina (Payne 2005). I studied a color-banded population of Greater Anis during June to October 2006 to 2009 at Gatún Lake, Panama, an area including the 1500-ha Barro Colorado Island as well as 4 adjacent mainland peninsulas within the 5400-ha Barro Colorado Nature Monument. The habitat is tropical moist forest (Holdridge et al. 1971). Annual rainfall averages 265 cm with a marked dry season lasting from mid-December through mid-April (Rand AS and Rand WM 1982).

Greater Anis in this study population are obligately communal and nest in groups of 2–5 socially monogamous pairs. Groups of 2 and 3 pairs are most common (ca. 70% and 30% of groups in the population, respectively); groups containing more than 3 pairs are rare and their nests are usually abandoned during the laying period (Riehl and Jara 2009). For this paper, I restricted analyses to 2-pair groups (the majority of groups in the study population) to simplify identification of egg maternity and to avoid confounding correlations between group size and ejection rates. Each group, therefore, consisted of 1 female that initiated laying (female "A") and 1 female that began laying after the clutch had already been initiated (female "B").

Nests are large, open-cup structures of small twigs, usually placed in emergent shoreline vegetation 0.3–2 m above the surface of the water. Nests were located and monitored by boat. Group size was determined by counting all adults present at each visit to the nest (validated in Riehl and Jara 2009). Nests were checked daily prior to laying and throughout the laying period. Each egg was weighed on an electronic balance (± 0.1 g) and numbered with a permanent felt-tip marker to identify its position in the laying sequence of the communal clutch. Greater Ani groups occasionally renest if the first clutch of eggs is depredated; however, for this paper, I analyzed data

from first clutches only because mean avian egg mass often changes over subsequent nesting attempts (Magrath 1991; Valkama et al. 2002). Nests were checked every 2–3 days during incubation, then several times per day beginning on the expected day of hatching of the first egg. Nestlings were banded, genetically sexed, and weighed both at hatching and at 5 days of age (White and Densmore 1992; Griffiths et al. 1998). I recorded the fate of each egg (ejected, depredated, unhatched, and hatched) and nestling (starved, depredated, and fledged). Details on DNA collection, nestling measurements, and nestling fate are given in the Supplementary Materials.

To determine maternity of each egg, I used a recently developed technique to isolate maternal DNA from blood stains and shed cells on the external surface of the eggshell (Schmaltz et al. 2006) and genotyped the samples using a set of 12 polymorphic microsatellite markers developed for the Greater Ani (Riehl and Bogdanowicz 2009). I assigned genetic egg maternity to females in communal clutches using the “identity check” function in CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al. 2007), a maximum likelihood-based program for parentage analysis that can also be used to identify repeat samples from the same individual. The accuracy of maternity assignment was cross-checked and validated by genotyping maternal DNA from 2 other sources: whole blood samples from breeding females captured at a majority of the nests in the study and extraembryonic membranes collected from ejected eggs (Strausberger and Ashley 2001). After genotyping, I assigned eggs in each communal clutch to either female A (the female that initiated laying) or female B and assigned each egg to its correct position in the laying order in the individual female’s clutch. Details of genotyping, DNA collection, and validation of maternity assignment are given in the Supplementary Materials.

Sample sizes and statistical analysis

Overall, the study included 35 two-female groups (333 eggs laid by 70 females) for which I had complete information on egg mass, maternity, laying order, and fate (ejected vs. incubated). This is the data set that I used to analyze factors influencing egg mass and ejection probability. Of these, 22 groups successfully fledged offspring (120 nestlings). I used a subset of 97 nestlings from 18 groups, for which I could match each nestling to its egg of origin, to examine relationships between egg mass, laying order, embryo sex, hatching asynchrony, and starvation risk. Sample sizes vary among analyses because it was not possible to collect all data from each individual. Clutches that were depredated during the laying phase were not included in analyses concerning eggs, and clutches depredated during the nestling phase were not included in analyses concerning nestlings.

To identify factors predicting egg mass and the effects of egg mass on ejection, survival, and nestling growth, I constructed models in SAS (SAS Institute, Cary, NC). Response variables with continuous normal distributions (egg mass and nestling mass) were assessed with linear mixed-effects models using a restricted/residual maximum likelihood approach (PROC MIXED). Response variables with binary distributions (egg ejection and nestling survival) were assessed with generalized linear mixed models using a restricted pseudolikelihood approach, a binomial error structure, and a logit link function (PROC GLIMMIX). Initial models included all variables and their 2-way interactions; final best-subset models were chosen using the Akaike Information Criterion following Johnson (1998). Group identity and individual female identity (nested in group identity) were included as random effects in all models, thereby controlling for nonindependence of eggs and young from the same female or the same nesting group.

In the first set of models, I investigated whether egg size varied in relation to year, laying date, embryo sex, clutch size, group identity, female identity, the female’s position in the laying order of the group (first laying, “A,” or second laying, “B”), and the egg’s position in the female’s laying order (nested within female identity). Because I was interested in variation both within and among females, I ran parallel models using absolute egg mass and relative egg mass (the difference between an egg and its intra-female clutch mean) as response variables. (By definition, female identity was not included in the model predicting relative egg mass.) The same analyses included clutches with different numbers of eggs, so I coded position in each female’s laying order as a 3-level factor (first-, middle-, and last-laid egg). In a 4-egg clutch, for example, the second and third eggs laid were both coded as “middle” eggs. This was necessary because variables related to position in the laying order, such as the probability of hatching asynchronously, depend on the position of the egg in the laying sequence relative to the rest of the clutch rather than the absolute number of eggs laid in the clutch. The main goal of these analyses was to identify factors predicting absolute egg mass across females and patterns of investment within females.

Next, I used mixed-effects logistic regression models to investigate the relative effects of egg mass versus position in the laying order on egg survival (i.e., the probability of ejection) and nestling survival (i.e., the probability of starvation). The 2-way interactions between mass and position were included as fixed-effects covariates, and group identity and female identity were retained as random effects in both models. Nestling sex was also included as a fixed effect in the model concerning nestling survival; however, I was not able to include embryo sex in the model concerning egg ejection because I could not determine the sex of ejected eggs. Because position in the laying order is already known to affect egg ejection and nestling starvation (through hatching asynchrony), the main purpose of these analyses was to determine whether increased egg size could compensate for a disadvantageous position in the laying order.

Finally, I constructed a third set of models to investigate whether nestling mass and at day 0 (hatching) and day 5 varied in relation to female identity, clutch size, egg mass, nestling sex, and degree of hatching asynchrony. Because the maximum recorded degree of hatching asynchrony was 2 days, this was coded as a 3-way factor (0, 1, and 2, indicating that the nestling hatched on the same day as the first-hatched nestling, the day after, or 2 days after, respectively). The main purpose of these analyses was to determine whether egg size influenced nestling size at hatching, whether the effect (if present) persisted until fledging, and whether increased egg mass could compensate for asynchronous hatching.

RESULTS

Variation in egg mass within and among females

Egg mass was not significantly repeatable within individual females (estimate based on 3–7 eggs from each of 70 females; repeatability \pm standard error [SE] = 0.006 ± 0.174 , $F_{69,140} = 1.24$, $P = 0.39$). In fact, the greatest range of variation observed within a single female’s clutch was 20.3–29.6 g, with the largest egg in the clutch approximately 46% larger than the smallest. Nevertheless, linear mixed-effects models showed that female identity was a significant predictor of absolute egg mass (Table 1), indicating that average egg size does differ among females despite high intra-female variation. Position in the laying order was also a significant predictor of egg mass, whether egg mass was measured on an absolute scale or

Table 1

Final linear mixed-effects models testing the effects of female identity, the female's position in the laying order of the group (A vs. B), and the egg's position in the female's clutch (first, middle, or last laid) on absolute egg mass and on relative egg mass (the difference between an egg's mass and its clutch mean). Other model terms and interactions were not significant (see "METHODS" for details of full model)

Variable	Absolute egg mass			Relative egg mass		
	df	Z/F ^a	P	df	Z/F ^a	P
Female ID	—	1.7	0.04	—	—	—
Female position	1, 329	0.97	0.33	1,329	0.32	0.57
Egg position	2, 329	7.6	<0.0001	2,329	8.4	<0.001
Female position × egg position	2, 329	1.3	0.27	2,329	5.5	<0.005

df = degrees of freedom; ID = identification.

^a A Z value is given for "female ID" (a random effect); all other test statistics are F values.

relative to other eggs laid by the same female (Table 1). Post hoc tests showed that eggs laid in the middle of a female's clutch were relatively larger than her first-laid egg ($F_{1,259} = 31.4$, $P < 0.001$) or her last-laid egg ($F_{1,261} = 10.6$, $P < 0.005$; Figure 1). This was true for all females, regardless of whether they initiated laying in the communal nest ("A" females) or entered the laying sequence afterward ("B" females; Figure 1).

However, within-clutch differences in egg mass were most extreme for "A" females (laying order × female position interaction, $F = 5.5$, $P < 0.005$; Table 1 and Figure 1). Post hoc tests showed that, relative to their clutch means, the first-laid eggs of "A" females were smaller than those of "B" females ($F_{1,65} = 4.9$, $P = 0.03$) and middle-laid eggs of "A" females were larger than those of "B" females ($F_{1,164} = 5.1$, $P = 0.02$; Figure 1). The relative masses of last-laid eggs did not differ between "A" and "B" females ($F_{1,67} = 0.9$, $P = 0.4$). In terms of mean absolute egg mass, "A" females laid slightly larger eggs than did "B" females, but this difference was not statistically significant (mean egg mass ± SE for "A" females = 30.4 ± 3.4 g and for "B" females = 28.9 ± 2.7 g; Table 1). Year, laying date,

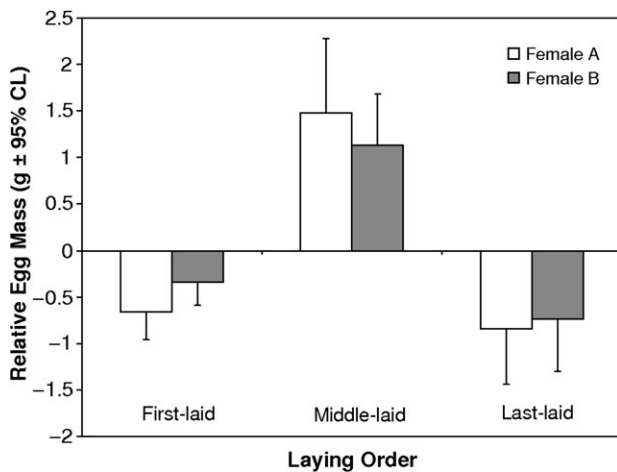


Figure 1

Mean relative egg mass ($g \pm 95\%$ confidence limits) in relation to laying order for first-laying (A) and second-laying (B) females in communal groups of Greater Anis.

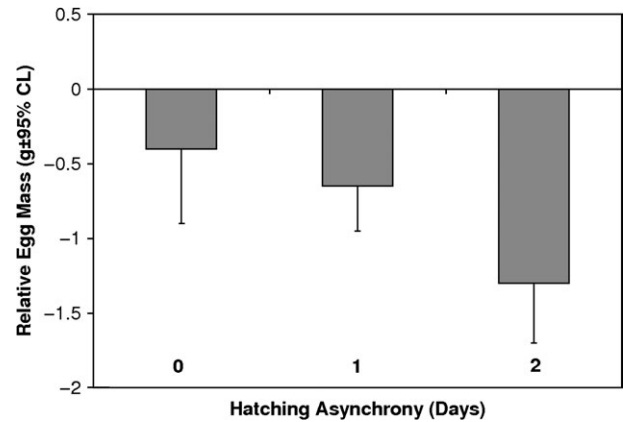


Figure 2

Mean relative egg mass ($g \pm 95\%$ confidence limits) in relation to the degree of hatching asynchrony for last-laid eggs.

embryo sex, clutch size, and group identity were not significant predictors of egg mass. Finally, the mass of last-laid eggs decreased significantly with increasing hatching asynchrony (pooled for both "A" and "B" females; Spearman rank correlation $r_s = 0.48$, $n = 70$, $P < 0.01$; Figure 2).

Variation in egg and nestling survivorship over the laying sequence

The first egg laid in the communal clutch (i.e., the first egg laid by female "A") was ejected at all but one of 34 nests (Figure 3), regardless of the egg's mass (Table 2). As a result, the probability of an egg being ejected was predicted by the mother's position in the laying order of the group and by the egg's position within the laying order of each female's clutch (Table 2). The probability of nestling starvation was predicted by the egg's position within the laying order of each female's clutch and by the degree of hatching asynchrony (Table 2), with post hoc tests showing that nestlings from last-laid eggs were more likely to starve than those from first-laid eggs ($F_{1,65} = 6.7$, $P = 0.01$) or middle-laid eggs ($F_{1,81} = 5.4$, $P = 0.02$; Figure 3). The interaction between egg mass and position in the female's laying order was also a significant

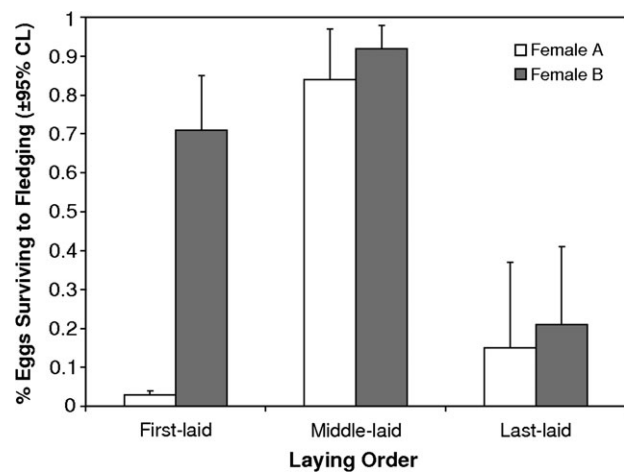


Figure 3

Proportion of eggs that survive to fledging ($\pm 95\%$ confidence limits) in relation to laying order for first-laying (A) and second-laying (B) females in communal groups of Greater Anis.

Table 2

Final mixed-effects logistic regression models testing the effects of egg mass, the female's position in the laying order of the group (A vs. B), and the egg's position in the female's clutch (first, middle, or last laid) on the probability of ejection and starvation. The degree of hatching asynchrony (0, 1, or 2 days) was included as a predictor variable in the model concerning starvation only. Other model terms and interactions were not significant (see "METHODS" for details of full model)

Variable	Probability of ejection			Probability of starvation		
	df	χ^2	<i>P</i>	df	χ^2	<i>P</i>
Egg mass	329	345	0.26	96	106	0.23
Female position	329	420	<0.001	96	71	0.97
Egg position	329	411	0.001	96	130	0.01
Hatching asynchrony	—	—	—	96	158	<0.0001
Egg mass × egg position	329	284	0.97	96	123	0.03

df, degrees of freedom.

predictor of starvation risk, indicating that starvation risk was greatest for last-laid eggs that were also small; however, the effect of egg mass alone was not significant (Table 2). Female identity, group identity, and nestling sex did not affect starvation probabilities. Overall survival probabilities for first-, middle-, and last-laid eggs of "A" and "B" females (including mortality from ejection and starvation, but excluding predation) are shown in Figure 3.

Variation in nestling mass at hatching and fledging

Egg mass was the only significant predictor of nestling mass at hatching ($F_{1,96} = 5.2$, $P = 0.02$; Table 3). By the time nestlings reached 5 days of age, however, the effect of egg mass on nestling mass was no longer significant ($F_{1,74} = 0.06$, $P = 0.81$). Rather, nestling mass at day 5 was predicted by hatching asynchrony, clutch size, and group identity (Table 3). Male nestlings were slightly heavier than females at 5 days of age (mean male mass ± standard deviation [SD] = 75.3 ± 4.6 g; mean female mass ± SD = 71.1 ± 6.4 g), but this difference was not significant ($F_{1,74} = 1.9$, $P = 0.17$; Table 3).

DISCUSSION

In this 4-year study, I used genetic identification of egg maternity to show that the remarkable variation in egg size in the Greater Ani is due mainly to variation within individual

Table 3

Final linear mixed-effects models testing the effects of egg mass, clutch size, nestling sex, and degree of hatching asynchrony (0, 1, or 2 days) on nestling mass at day 0 (hatching) and day 5 (fledging). Other model terms and interactions were not significant

Variable	Nestling mass at day 0			Nestling mass at day 5		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Egg mass	1, 96	5.2	0.02	1, 74	0.06	0.81
Clutch size	1, 96	2.3	0.13	1, 74	5.5	0.02
Nestling sex	1, 96	0.81	0.45	1, 74	1.9	0.17
Hatching asynchrony	2, 96	1.7	0.19	2, 74	13.7	<0.0001

df, degrees of freedom.

females. The egg's position in the female's laying sequence was a significant predictor of egg mass, with first-laid eggs and last-laid eggs consistently smaller than those in the middle of the clutch. Females that initiated laying in the communal nest ("A" females) almost always had their first-laid eggs ejected from the nest, and these females also exhibited the most extreme variation in egg mass. The first-laid eggs of "A" females were significantly smaller than those of "B" females, and the middle-laid eggs of "A" females were significantly larger than those of "B" females. Because incubation began before clutch completion, the last-laid eggs of both females were at increased risk of hatching asynchrony and starvation, and the mass of last-laid eggs decreased as the degree of hatching asynchrony increased. Ejection and starvation probabilities were not affected by egg mass; therefore, the lowered survivorship of first-laid and last-laid eggs was due solely to their position in the laying order, not to their smaller size. Taken together, these results support the hypothesis that first-laying females allocate fewer resources to eggs that are likely to be ejected from the communal nest.

Plasticity in resource allocation across the laying sequence

The first-laid eggs of both "A" and "B" females were significantly smaller than their clutch means, even though the first-laid eggs of "B" females were rarely ejected. There are several possible explanations for this pattern. First, although I restricted this study to groups composed of 2 pairs (2 breeding females), approximately 30% of nesting groups in the study population are composed of 3 or more pairs. Because each female in the nesting group will eject the eggs of other females until she has laid her first egg, the number of eggs that are ejected increases with group size (Riehl and Jara 2009). In groups containing more than 2 laying females, the "B" female's first egg is frequently ejected by other group members that have not yet begun to lay ("C" females and so on). Because late-laying females also risk losing their early eggs to ejection in larger groups, reducing investment in the first egg may be a general strategy employed by all females. Second, Greater Ani frequently renest if the first clutch is depredated, and "A" and "B" females have been observed to switch positions in the laying order in subsequent nesting attempts (Riehl C, unpublished data). Thus, the roles of "first-laying female" and "second-laying female" may change over relatively short time periods, and females may not be able to predict their position in the laying order of the group. Given that a female's role in the nesting group apparently changes several times over her lifetime, the first-laid eggs of all females will, on average, experience lower survivorship than subsequently laid eggs in a nesting attempt. In either case, selection should favor the evolution of small first eggs regardless of a female's role in a given nesting attempt. However, I found that the first-laid eggs of "A" females were significantly smaller than those of "B" females on both absolute and relative scales, suggesting some degree of plasticity in allocation depending on the egg's immediate probability of ejection.

Correlations between egg mass and position in the laying order may be partially due to nonadaptive physiological factors that I was not able to measure in this study. In many bird species with large clutches, absolute egg mass follows a bell-shaped curve with early and late eggs being smaller than those in the middle of the clutch, even when these eggs are not at increased risk of mortality (Arnold 1991; Budden and Beissenger 2005). This may be a proximate consequence of changing levels of hormones in circulation and in the ovary during egg production because follicles mature and are ovulated in a sequential hierarchy (Challenger et al. 2001). Plasma estradiol, a gonadal steroid that simulates the production of yolk precursors, shows

a similar bell-shaped pattern (levels increase with the onset of yolk development and decrease linearly over the later stages of follicle development; Christians and Williams 1999; Williams et al. 2004). Decreases in egg mass toward the end of the laying sequence have also been attributed to declines in the endogenous lipid and protein reserves of the female (Selman and Houston 1996; Reynolds et al. 2003), though few experimental studies have successfully linked body condition and egg mass (reviewed in Williams 2005).

Particularly for last-laid eggs, physiological and adaptive bases for differences in egg mass are not mutually exclusive and may even reinforce one another. However, if declines in maternal nutrient reserves or body condition were wholly responsible for decreases in egg mass, such decreases should be most pronounced in females that lay large clutches. I found that clutch size had no effect on absolute egg mass and relative mass of the last-laid egg decreased sharply regardless of clutch size. In addition, one would expect “A” females to lay smaller last eggs than “B” females because “A” females lay significantly larger clutches to compensate for the ejection of their first eggs (Riehl and Jara 2009). By contrast, I found no differences in the mean absolute or relative mass of last-laid eggs between “A” and “B” females. Finally, the mean mass of the last-laid egg decreased as the degree of hatching asynchrony increased, consistent with adaptive explanations for differential investment.

Consequences of egg mass variation on maternal and nestling fitness

Many factors other than egg size have been shown to influence nestling size, including hatching asynchrony, nestling sex, yolk androgen concentrations, and the relative amounts of yolk and albumen deposited in the egg (Williams 1994; Schwabl et al. 1997; Badyaev et al. 2005). Substantial recent evidence suggests that, in the context of these other variables, egg mass explains relatively little variation in nestling fitness (Bitton et al. 2006; Maddox and Weatherhead 2008). Consistent with these results, I found that in Greater Anis, the effect of egg size cannot compensate for that of hatching asynchrony and that differences in egg mass do not persist until fledging. Studies that fail to find correlations between egg mass and nestling growth or fitness have frequently been interpreted to mean that variation in egg mass is of limited adaptive consequence (cf. Krist et al. 2004; Fernández and Reboreda 2008). This view is misleading, for it considers only selection on offspring fitness and not on maternal fitness (Trivers 1974; Marshall and Uller 2007). Particularly in species that lay relatively large eggs, mothers could reduce the physiological costs of reproduction by allocating fewer resources to eggs that are unlikely to survive. In addition, egg size might have more pronounced effects on nestling fitness when environmental conditions are poor, as in years of food scarcity during the chick-rearing period.

The costs of egg laying are substantial in the crotophagine cuckoos: among female groove-billed Anis (*C. sulcirostris*), increases in egg mass correlated with decreased wing feather growth, indicating an energetic trade-off (Vehrencamp et al. 1986). In the Greater Ani, egg size variation is so extreme that it is likely to have biologically relevant effects on maternal body condition. On average, total clutch mass for “A” females was 131 g. By contrast, if a hypothetical “A” female was to lay an average-sized clutch in which all eggs were the size of the mean middle-laid egg, her total clutch mass would be 140 g—an increase of almost 7% of total investment, or the rough equivalent of laying an extra one third of an egg. An “A” female does not risk compromising offspring fitness by reducing the size of her first-laid egg because this egg is

virtually always ejected regardless of its size. These results suggest that if the same female was to lay in the “B” position the following year, she might not reduce the size of her first egg to the same extent. However, in order to test the adaptive value of differential resource allocation to eggs, future studies are needed to determine the effects of egg mass on lifetime female survival and reproductive success.

Patterns of egg size variation in other crotophagine cuckoos

The other 3 species in the subfamily Crotophaginae (the groove-billed Ani, *C. sulcirostris*; the smooth-billed Ani, *C. ani*; and the Guira Cuckoo, *Guira guira*) share many characteristics of their breeding biology with the Greater Ani and might be expected to demonstrate similar patterns of reproductive investment across the laying order. All 4 species may breed in communal groups; because group members begin laying asynchronously, early eggs are ejected from the nest or buried in the nest lining (Vehrencamp and Quinn 2004). All lay eggs that are unusually large relative to female body mass and vary greatly in size population wide (Payne 2005). Nestling mass at hatching may be a more important determinant of competitive ability for crotophagine nestlings than for other altricial species because ani nestlings grow extremely rapidly and leave the nest after an unusually short period of time. Rapid development, in turn, has probably been favored by the communal breeding system of crotophagine cuckoos because nestlings in communal clutches must compete with both related and unrelated nest mates. Within-group infanticide, which has been extensively documented in Guira Cuckoos, may also select for rapid nestling development (Macedo et al. 2001). Intra-female variation in egg mass has not been extensively studied in the other crotophagines, but the available data are consistent with the patterns presented here.

In groove-billed Anis, variation in egg mass appears to be greater among females than within females, such that Vehrencamp (1977, 1978) was able to use characteristics of egg size and shape to assign eggs in communal clutches to their respective mothers. Within clutches, however, first-laid eggs tended to be smaller than subsequently laid eggs (though this difference was not quantified) and first-laying “A” females laid larger eggs than did “B” females. As in the Greater Ani, “A” females appear to allocate more resources to synchronously laid eggs in order to increase nestling survival, thereby compensating for the reproductive costs imposed by egg ejection (Vehrencamp et al. 1986). In Guira Cuckoos, identification of egg maternity through yolk protein electrophoresis demonstrated that egg size, shape, and shell patterns vary as much within a female’s clutch as among females (Cariello et al. 2002, 2004). In terms of absolute mass, ejected eggs were not significantly smaller than eggs that were incubated. However, early-laid eggs had relatively smaller yolks (Macedo et al. 2004) and lower concentrations of androstenedione (a yolk androgen; Cariello et al. 2006), indicating lower maternal investment in eggs that were likely to be ejected. Similarly, concentrations of yolk testosterone increased with position in the laying order in communal clutches of smooth-billed Ani eggs (Schmaltz et al. 2008), though intra-female variation was not quantified.

Exactly how female anis are able to alter egg mass so dramatically between the first and second egg and between the penultimate and last egg remains unclear. Very little is known about the physiological mechanisms through which immediate environmental and social cues influence egg formation in birds (reviewed in Williams 2005). The physiological mechanisms responsible for the costs of egg production are equally obscure; however, the ability to vary egg mass is not itself thought to be costly (Christians 2002). As Williams (1998)

pointed out, the physiological systems involved in egg formation exhibit great plasticity, including the secretion of yolk precursors from the liver, their uptake by developing follicles in the ovary, and the structures of the ovary and oviduct. Future research, therefore, should focus on the physiological mechanisms controlling intra-individual plasticity in egg mass. Without a better understanding of the factors that constrain egg mass variation in individual females, it is difficult to determine why such variation is relatively rare in birds.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>.

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REFERENCES

- Arnold TW. 1991. Intraclutch variation in egg size of American coots. *Condor*. 93:19–27.
- Badyaev AV, Schwabl H, Young RL, Duckworth RA, Navara KJ, Parlow AF. 2005. Adaptive sex differences in growth of pre-ovulation oocytes in a passerine bird. *Proc R Soc B Biol Sci*. 272:2165–2172.
- Bitton P-P, Dawson RD, O'Brien EL. 2006. Influence of intraclutch egg-mass variation and hatching asynchrony on relative offspring performance within broods of an altricial bird. *Can J Zool*. 84:1721–1726.
- Blomqvist D, Johansson OC, Götmark F. 1997. Parental quality and egg size affect chick survival in a precocial bird, the lapwing *Vanellus vanellus*. *Oecologia*. 110:18–24.
- Blount JD, Houston DC, Surai PF, Møller AP. 2004. Egg-laying capacity is limited by carotenoid pigment availability in wild gulls *Larus fuscus*. *Proc R Soc Lond B Biol Sci*. 271(Suppl 3):S79–S81.
- Budden AE, Beissenger SR. 2005. Egg mass in an asynchronously hatching parrot: does variation offset constraints imposed by laying order? *Oecologia*. 144:318–326.
- Cariello MO, Schwabl H, Lee R, Macedo RH. 2002. Individual female clutch identification through yolk protein electrophoresis in the communally-breeding guira cuckoo (*Guira guira*). *Mol Ecol*. 11:2417–2424.
- Cariello MO, Lima MR, Schwabl HG, Macedo RH. 2004. Egg characteristics are unreliable in determining maternity in communal clutches of guira cuckoos *Guira guira*. *J Avian Biol*. 35:117–124.
- Cariello MO, Macedo RHF, Schwabl HG. 2006. Maternal androgens in eggs of communally breeding guira cuckoos (*Guira guira*). *Horm Behav*. 49:654–662.
- Challenger WO, Williams TD, Christians JK, Vézina F. 2001. Follicular development and plasma yolk precursor dynamics through the laying cycle in the European starling (*Sturnus vulgaris*). *Physiol Biochem Zool*. 74:356–365.
- Christians JK. 2002. Avian egg size: variation within species and inflexibility within individuals. *Biol Rev*. 77:1–26.
- Christians JK, Williams TD. 1999. Effects of exogenous 17- β -estradiol on the reproductive physiology and reproductive performance of European starlings (*Sturnus vulgaris*). *J Exp Biol*. 202:2679–2685.
- Clutton-Brock TH. 1991. The evolution of parental care. Princeton, NJ: Princeton University Press.
- D'Alba L, Torres R. 2007. Seasonal egg-mass variation and laying sequence in a bird with facultative brood reduction. *Auk*. 124:643–652.
- Davis DE. 1941. Social nesting habits of *Crotophaga major*. *Auk*. 58:179–183.
- Davis DE. 1942. The phylogeny of social nesting habits in the Crotophaginae. *Q Rev Biol*. 17:115–134.
- Fernández GJ, Rebores J. 2008. Between and within clutch variation of egg size in greater rheas. *Wilson J Ornithol*. 120:674–682.
- Graveland J, van der Wal R, van Balen JH, van Noordwijk AJ. 1994. Poor reproduction in forest passerines from decline of snail abundance on acidified soils. *Nature*. 368:446–448.
- Griffiths R, Double MC, Orr K, Dawson RJG. 1998. A DNA test to sex most birds. *Mol Ecol*. 7:1071–1075.
- Holdridge W, Grenke C, Hatheway WH, Liang T, Tosi JA Jr. 1971. Forest environments in tropical life zones: a pilot study. New York: Pergamon Press.
- Johnson DE. 1998. Applied multivariate methods for data analysts. Belmont, CA: Duxbury Press.
- Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol*. 16:1099–11006.
- Koenig W. 1980. The incidence of runt eggs in woodpeckers. *Wilson Bull*. 92:169–176.
- Krist M, Remeš V, Uvířová L, Nádvořník P, Bureš S. 2004. Egg size and offspring performance in the collared flycatcher (*Ficedula albicollis*): a within-clutch approach. *Oecologia*. 140:52–60.
- Lessells CM, Cooke F, Rockwell RF. 1989. Is there a trade-off between egg weight and clutch size in wild lesser snow geese (*Anser c. caerulescens*). *J Evol Biol*. 2:457–472.
- Macedo RHF, Cariello MO, Muniz L. 2001. Context and frequency of infanticide in communally breeding guira cuckoos. *Condor*. 103:170–175.
- Macedo RHF, Cariello MO, Pacheco AM, Schwabl HG. 2004. Significance of social parameters on differential nutrient investment in guira cuckoo, *Guira guira*, eggs. *Anim Behav*. 68:685–694.
- Maddox JD, Weatherhead PJ. 2008. Egg size variation in birds with asynchronous hatching: is bigger really better? *Am Nat*. 171:358–365.
- Magrath R. 1991. Seasonal changes in egg-mass within and among clutches of birds: general explanations and a field study of the blackbird *Turdus merula*. *Ibis*. 134:171–179.
- Marshall DJ, Uller T. 2007. When is a maternal effect adaptive? *Oikos*. 116:1957–1963.
- Marshall TC, Slate J, Kruuk L, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol*. 7:639–655.
- Mousseau TA, Fox CW. 1998. The adaptive significance of maternal effects. *Trends Ecol Evol*. 13:403–407.
- Mueller HC. 1990. The evolution of reversed sexual dimorphism in size in monogamous species of birds. *Biol Rev*. 65:553–585.
- Nager RG, Monaghan P, Houston DC. 2001. The cost of egg production: increased egg production reduces future fitness in gulls. *J Avian Biol*. 32:159–166.
- Noordwijk AJ. 1987. Quantitative ecological genetics of great tits. In: Cooke F, Buckley PF, editors. Avian genetics: a population and ecological approach. London: Academic Press. p. 363–380.
- O'Connor RJ. 1979. Egg weights and brood reduction in the European swift (*Apus apus*). *Condor*. 81:133–145.
- Parker GA, Begon M. 1986. Optimal egg size and clutch size: effects of environment and maternal phenotype. *Am Nat*. 128:573–592.
- Payne RB. 2005. The cuckoos. London: Oxford University Press.
- Rand AS, Rand WM. 1982. Variation in rainfall on Barro Colorado Island. In: Rand AS, Windsor DM, Leigh EG, editors. The ecology of a tropical forest: seasonal rhythms and long-term changes. Washington (DC): Smithsonian Institution Press. p. 47–60.
- Reynolds SJ, Schoech SJ, Bowman R. 2003. Nutritional quality of pre-breeding diet influences breeding performance of the Florida scrub-jay. *Oecologia*. 134:308–316.

- Ricklefs RE, Hahn DC, Montevecchi WA. 1978. The relationship between egg size and chick size in the laughing gull and Japanese quail. *Auk*. 95:135–144.
- Riehl C, Bogdanowicz SM. 2009. Isolation and characterization of microsatellite markers from the greater ani *Crotophaga major* (Aves: cuculidae) [Internet]. *Mol Ecol Res*. Available from: <http://tomato.biol.trinity.edu/manuscripts/9-6/mer-09-0270.pdf>.
- Riehl C, Jara L. 2009. Natural history and reproductive biology of the communally breeding greater ani (*Crotophaga major*) in Gatún Lake, Panama. *Wilson J Ornithol*. 121:679–687.
- Roff DA. 1992. The evolution of life-histories: theory and analysis. New York: Chapman and Hall.
- Schmaltz G, Quinn JS, Schoech SJ. 2008. Do group size and laying order influence maternal deposition of testosterone in smooth-billed ani eggs? *Horm Behav*. 53:82–89.
- Schmaltz G, Somers CM, Sharma P, Quinn JS. 2006. Non-destructive sampling of maternal DNA from the external shell of bird eggs. *Conserv Genet*. 7:543–549.
- Schwabl H, Mock DW, Gieg JA. 1997. A hormonal mechanism for parental favouritism. *Nature*. 386:231.
- Selman RG, Houston DC. 1996. The effect of prebreeding diet on reproductive output in zebra finches. *Proc R Soc Lond B Biol Sci*. 263:1585–1588.
- Slagsvold T, Sandvik J, Rofstad G, Lorentsen O, Husby M. 1984. On the adaptive value of intraclutch egg-size variation in birds. *Auk*. 101:685–697.
- Strausberger BM, Ashley MV. 2001. Eggs yield nuclear DNA from egg-laying female cowbirds, their embryos and offspring. *Conserv Genet*. 2:385–390.
- Trivers RL. 1974. Parent-offspring conflict. *Am Zool*. 14:249–264.
- Valkama J, Korpimäki E, Wiehn J, Pakkanen T. 2002. Inter-clutch egg size variation in kestrels *Falco tinnunculus*: seasonal decline under fluctuating food conditions. *J Avian Biol*. 33:426–432.
- Vehrencamp SL. 1977. Relative fecundity and parental effort in communally nesting anis, *Crotophaga sulcirostris*. *Science*. 197:403–405.
- Vehrencamp SL. 1978. The adaptive significance of communal nesting in groove-billed anis (*Crotophaga sulcirostris*). *Behav Ecol Sociobiol*. 4:1–33.
- Vehrencamp SL, Bowen BS, Koford RR. 1986. Breeding roles and pairing patterns within communal groups of groove-billed anis. *Anim Behav*. 34:347–366.
- Vehrencamp SL, Quinn JS. 2004. Avian joint laying systems. In: Koenig WD, Dickinson J, editors. *Ecology and evolution of cooperative breeding in birds*. Cambridge (MA): Cambridge University Press. p. 177–196.
- Visser ME, Lessells CM. 2001. The costs of egg production and incubation in great tits (*Parus major*). *Proc R Soc Lond B Biol Sci*. 268:1271–1277.
- Warham J. 1974. The Fiordland crested penguin *Eudyptes pachyrhynchus*. *Ibis*. 116:1–27.
- White PS, Densmore LD. 1992. Mitochondrial DNA isolation. In: Hoezel AR, editor. *Molecular genetic analysis of populations: a practical approach*. New York: Oxford University Press. p. 29–58.
- Williams GC. 1966. Natural selection costs of reproduction and a refinement of Lack's principle. *Am Nat*. 100:687–690.
- Williams TD. 1990. Growth and survival in macaroni penguin, *Eudyptes chrysolophus*, A- and B-chicks: do females maximise investment in the large B-egg. *Oikos*. 59:349–354.
- Williams TD. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. *Biol Rev*. 68:35–59.
- Williams TD. 1998. Avian reproduction, overview. In: Knobil E, Neill JD, editors. *Encyclopedia of reproduction*, volume 1. San Diego (CA): Academic Press. p. 325–336.
- Williams TD. 2005. Mechanisms underlying the costs of egg production. *Bioscience*. 55:39–48.
- Williams TD, Kitaysky AS, Vezina F. 2004. Individual variation in plasma estradiol-17 β and androgen levels during egg formation in the European starling *Sturnus vulgaris*: implications for regulation of yolk steroids. *Gen Comp Endocrinol*. 136:346–352.