

INFLUENCE OF HATCH ORDER ON BEGGING AND PLUMAGE COLORATION OF NESTLING EASTERN BLUEBIRDS

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ABSTRACT.—Hatching asynchrony in altricial songbirds can influence the morphology and behavior of nestling birds. We compared the position of nestling Eastern Bluebirds (*Sialia sialis*) in the hatching hierarchy to their (1) position in the egg-laying order, (2) rate of nestling begging, (3) circulating corticosterone, (4) size, and (5) plumage coloration. Most nestlings hatched within 36 hrs of each other, and nestlings hatched in the order in which eggs were laid. Early-hatched nestlings were heavier than late-hatched nestlings for the duration of the growth period and begged less intensely than their late-hatched siblings. There was little evidence of severe effects of hatch order. Hatch order did not influence nestling corticosterone levels nor did we find effects of hatch order on ornamental plumage coloration. Our data suggest no long-term effect of hatching asynchrony on the development of sexually selected plumage coloration. Received 7 January 2011. Accepted 28 June 2011.

Hatching asynchrony is common in many species of birds and results from incubation prior to clutch completion. Early-hatched nestlings are generally older and larger than their later-hatched siblings which creates a size hierarchy within the nest with early-hatched nestlings assuming a dominant role as later-hatched nestlings often grow slower (Clotfelter et al. 2000, Nilsson and Gardmark 2001, Saino et al. 2001, Magrath et al. 2003). Size hierarchies within broods often create differences in need for food among siblings, and offspring can use behavioral strategies to alter the rate at which they are provisioned. Nestling begging has been found to be an honest indicator of need (Price and Ydenburg 1995, Lotem 1998, Saino et al. 2000), although this is not definitive across species (Wright et al. 2010). Successful begging behavior usually results in acquisition of more food and translates into an increase in mass (Kilner 1995).

Hormones may be the proximate mechanism underlying variation in begging behavior. The nutritional state of nestlings is negatively correlated with activity of the hypothalamus–pituitary–adrenal axis in several species of seabirds (Nuñez-de la Mora et al. 1996; Kitaysky et al. 1999,

2001a). Experimental elevation of corticosterone has been shown to cause increased begging rates of nestling Black-legged Kittiwakes (*Rissa tridactyla*) (Kitaysky et al. 2001b). Late-hatched nestlings are assumed to experience nutritional stress and are expected to increase corticosterone secretion, which in turn, should increase begging behavior (Marra and Holberton 1998, Kitaysky et al. 1999).

Nestling condition also can be honestly signaled by plumage coloration (Fitze et al. 2003, Tschirren et al. 2003, Jacot and Kempenaers 2007, Siefferman and Hill 2007). Juvenal plumage coloration might function in parent-offspring communication and mediate parental favoritism either at the nest (Tschirren et al. 2003, Galvan et al. 2008, Griggio et al. 2009) or later during the post-fledgling dependence period (Tanner and Richner 2008, Ligon and Hill 2010). Nestling condition can have long-term effects by influencing adult plumage used in sexual signaling, if nestlings retain portions of the juvenal plumage into adulthood. Parental strategies for maximizing fitness may include adjusting the relative investment in juvenile males and females (Trivers and Willard 1973) by manipulating their positions in the laying order (Badyaev et al. 2002).

Eastern Bluebirds (*Sialia sialis*) are sexually dichromatic songbirds that, on average, lay 4–5 egg clutches in Alabama. Males display bright UV-blue structural coloration on the plumage of their back, head, wings and tail while females display similar coloration but are duller overall. Brighter individuals gain higher reproductive success as adults (Siefferman and Hill 2003, 2005). Bluebirds in Alabama begin incubation on

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the day the female lays the penultimate egg (M. Liu, unpubl. data). Incubation lasts ~15 days, and all nestlings in the brood hatch over a period of between 6 and 36 hrs (Gowaty and Plissner 1998).

The rectrices and remiges of nestling bluebirds begin to emerge within 11 days after hatching and exhibit UV-blue ornamental coloration. The color of remiges can be quantified by 14 days after hatching (Siefferman and Hill 2007). Young bluebirds retain these juvenile wing and tail feathers as part of their first nuptial plumage (Gowaty and Plissner 1998). Experimental manipulations of food availability to nestlings demonstrate that male nestling bluebirds reared in poor natal conditions grow more slowly and display duller wing color compared to those reared in better natal environments (Siefferman and Hill 2007). Parents exhibit preferences for the more-colorful fledglings (Ligon and Hill 2010).

Our objectives were to: (1) test the assumption that laying order reflects hatching order, (2) investigate whether sex ratio varies between early- and late-hatched nestlings, (3) ascertain whether late-hatched chicks remain smaller than early-hatched siblings throughout the nestling period, (4) test whether late-hatched nestlings have higher circulating corticosterone and beg more vigorously than their early-hatched siblings, and (5) investigate whether late-hatched nestlings have duller plumage coloration than their early-hatched siblings.

METHODS

Study Area and Field Procedures.—We studied a population of Eastern Bluebirds breeding in nest boxes in Lee County, Alabama, USA (32° 35' 52" N, 85° 28' 51" W; elevation 216 m) in 2007. The study site includes pasture and edge habitat. We monitored first nests of Eastern Bluebirds every other day during the nest building stage. We visited nests each day during the laying period and marked each new egg with a Sharpie® marker to establish laying order. We identified two groups of eggs in each brood in relation to laying order, defined as early- and late-laid eggs. Eggs laid in the first half of the clutch were early-laid in clutches with even number of eggs and those laid in the last half were late-laid eggs. The middle egg was considered a late-laid egg in nests with an odd number of eggs.

We ascertained which chick hatched from each egg during the hatching period by visiting each nest every 3 hrs (0600–1900 hrs) during daylight

until all eggs hatched. We identified individual nestlings by marking their tarsi with a unique color of Sharpie® marker. Nestlings were assigned the same hatching order if more than one egg hatched in the same 3-hr interval but were given different markings.

Nestlings that hatched in the first 3-hr interval during which hatching occurred were 'early-hatched nestlings'. These were nestlings with early spots in the hierarchy of hatching positions. Similarly, nestlings that hatched after the first 3-hr hatching interval were 'late-hatched nestlings'. These were nestlings with late spots in the hierarchy of hatching positions. We monitored >200 clutches but were only able to assign egg laying and nestling hatch order to 31 broods because most eggs hatched during the night.

We defined the age of the brood by the hatching date of the first-hatched nestling (day 1 = hatch day). We measured mass of nestlings to the nearest 0.1 g on day 2, 5, 8, 11, and 14 post hatch. We banded nestlings at 8 days of age and collected a 150- μ l blood sample within 3 min of first handling each nestling. We spun the blood sample in a centrifuge, separated the sera and plasma, and froze the samples. We measured the right tarsi and wing to the nearest 0.1 mm at day 14 post hatch. Nestlings increase rapidly in mass from hatching until they are about 11 days of age, but by 13 days of age, the mass of nestlings begins to reach the asymptote (Pinkowski 1975). Thus, mass at 14 days is an accurate estimate of fledging mass. Nestlings generally fledge between 15 and 18 days post hatch.

Nestlings at 8 days of age have feather sheaths. Feathers begin to emerge from the feather sheaths at 11 days of age and 2 cm of the feathers have emerged from the sheaths at 14 days of age. We cut the distal 2 cm of both primary 5 feathers of nestlings for spectrophotometric plumage analysis on day 14 post hatch. We stored the feathers in envelopes in a climate-controlled environment until spectrophotometric analyses were conducted. Juvenile Eastern Bluebirds are sexually dichromatic. We classified male and female nestlings using sexually dichromatic plumage coloration. Previous experience with plumage coloration and molecular classification showed that 95% of young could be properly classified using plumage coloration (L. Siefferman, pers. obs.).

Nestling Begging Behavior.—We stimulated nestlings to beg and video recorded nestling begging behavior in the morning when the oldest

nestlings were 5 days of age. Nest holes were first blocked for 30 min prior to recording behavior to ensure that no nestlings were satiated. Nestlings were removed from the nest box and placed in a cup with nesting material. A researcher stimulated the nestlings every 30 sec for 5 sec over the duration of 3.5 min by gently rattling the cup and whistling.

One researcher (NS) quantified begging behavior of individual nestlings through measurements of begging intensity. We scored the maximum posture to measure intensity during each trial as 0 = not begging; 1 = mouth open; 2 = mouth open, head back; 3 = mouth open, head back, neck stretched; 4 = mouth open, head back, neck stretched, back vertical. We summed the intensity rankings for each nestling to assess the overall begging intensity during the trial; thus, begging intensity includes both frequency and degree of begging.

Reflectance Spectrometry.—One researcher (LS) recorded spectral data with an Ocean Optics S2000 spectrometer (range = 250–880 nm: Dunedin, FL, USA) and a light source illuminated with both a deuterium bulb (UV light source) and a tungsten-halogen bulb (visible light source). We generated reflectance data relative to a WS-2 white standard (Labsphere Inc., North Sutton, NH, USA) and placed a micron fiber-optic probe at a 90-degree angle 1 mm from the feather surface. Reflectance data were summarized by calculating two standard descriptors: brightness and UV-chroma. Brightness was calculated as the mean of the summed reflectance from 300 to 700 nm and UV-chroma as the proportion of the total reflectance that is in the ultraviolet range ($[300-400]/[300-700]$).

Hormone Analyses.—Serum corticosterone and testosterone were extracted and separated using celite column chromatography following methods modified from Schwabl (1993). Briefly, 20 μ l of serum was mixed with 3 ml diethyl ether, vortexed, and allowed to settle for 20 min. Samples were snap frozen and the liquid portion containing the steroid hormones was reserved and dried using a N₂ stream. Samples were resuspended in 1 ml of 10% ethyl acetate in isoctane (VanWatersRogers, Suwanee, GA, USA) after which steroid hormones were eluted through the columns in the following fractions of ethyl acetate in isoctane: dihydrotestosterone –10%, testosterone –20%, and corticosterone –30%. Samples were further dried using a N₂ stream, and

testosterone and corticosterone were quantified using a competitive binding radioimmunoassay following Wingfield and Farner (1975). Testosterone recoveries averaged 60%; however, testosterone was below detectable levels in all samples, and testosterone concentrations were not used in analyses. Corticosterone recoveries averaged 70%, and intra-assay variation was 5.19.

Statistical Analyses.—We tested whether laying order predicted hatching order and whether the sex ratio within broods differed between early- and late-hatched nestlings using Generalized Linear Models with binomial error distribution and logit link. We performed Linear Mixed Effect Models to analyze the effects of early- versus late-hatching on begging behavior, corticosterone level, structural size, and plumage coloration. All models include gender of the nestlings and early-versus late-hatching as fixed factors, brood size as a covariate, and nest as the random factor. We performed a mixed-effect model to analyze the effects of the early- versus late-hatching on juvenile body mass (measured at ages 2, 5, 8, 11, and 14 days). The model included gender of nestlings and early- versus late-hatching as fixed factors, age and brood size as covariates, and nest as the random factor. We used a stepwise backward procedure for simplification of the mixed models and tested two-way interactions between the covariate and the fixed factors. Data were normally distributed. None of the interaction terms was significant ($P > 0.05$) and interactions were removed from models. SPSS (2006: Version 15.0) software was used to analyze data and all tests were two-tailed.

RESULTS

Brood Size and Incidence of Brood Reduction.—Seven of 31 clutches had a total of two nestlings each, nine had three nestlings, nine had four nestlings, and six had five nestlings. Seventeen percent of the 208 Eastern Bluebird nests we monitored experienced total nest failure. Eight percent of the 172 successful nests (at least 1 nestling fledged) had one or more nestlings die from apparent starvation. We were able to identify whether the dead was an early- or late-hatched nestling in nine nests; 44% were early-hatched nestlings and 56% were late-hatched nestlings.

Laying Order, Hatching Order, Hatching Span, and Nestling Gender.—Laying order predicted hatching order (Wald $X^2 = 14.46$, $n = 31$, $P < 0.001$); 90% of late-laid eggs resulted in late-

TABLE 1. Effects (estimates \pm SE) of hatch order and gender on body size, begging behavior, corticosterone concentration, and plumage coloration traits of nestling Eastern Bluebirds.

Trait	Age (day)	Factor	Estimate \pm SE	df	F	P
Mass (g)		Hatch order ^a	1.24 \pm 0.24	1, 525	26.4	<0.001
		Gender ^b	-0.18 \pm 0.24	1, 525	0.58	0.45
		Age	2.09 \pm 0.03	1, 525	5,734.90	<0.001
		Brood size	-0.34 \pm 0.11	1, 525	9.37	0.002
Tarsus length (mm)	14	Hatch order ^a	0.46 \pm 0.52	1, 85.0	0.77	0.38
		Gender ^b	0.43 \pm 0.51	1, 85.0	0.72	0.40
		Brood size	0.30 \pm 0.26	1, 85.0	1.27	0.26
Wing length (mm)	14	Hatch order ^a	1.01 \pm 0.42	1, 65.4	5.79	0.02
		Gender ^b	-0.65 \pm 0.44	1, 67.8	2.23	0.14
		Brood size	0.20 \pm 0.56	1, 26.7	0.14	0.72
Begging intensity	5	Hatch order ^a	-2.90 \pm 1.27	1, 66.5	5.26	0.02
		Gender ^b	-2.00 \pm 1.31	1, 72.2	2.33	0.13
		Brood size	0.51 \pm 1.08	1, 27.7	0.23	0.64
Cort (ng/ml)	8	Hatch order ^a	-0.03 \pm 0.04	1, 42.7	0.42	0.52
		Gender ^b	0.04 \pm 0.04	1, 50.0	0.85	0.36
		Brood size	0.01 \pm 0.02	1, 15.9	0.07	0.80
Brightness (%)	14	Hatch order ^a	0.15 \pm 0.43	1, 58.4	0.12	0.73
		Gender ^b	-2.56 \pm 0.45	1, 66.3	32.8	<0.001
		Brood size	-0.92 \pm 0.35	1, 24.1	7.03	0.01
UV chroma (%)	14	Hatch order ^a	-0.01 \pm 0.01	1, 58.8	0.02	0.89
		Gender ^b	-0.05 \pm 0.01	1, 66.9	159.7	<0.001
		Brood size	-0.01 \pm 0.01	1, 24.3	11.0	0.003

^a Estimates are relative to late-hatched nestlings.^b Estimates are relative to males.

hatched nestlings. Eggs in 14 of the 31 clutches measured hatched within 24 hrs and 17 clutches exceeded a 24-hr hatching span. We found no effect of laying order on nestling gender ($Wald X^2 = 0.18$, $n = 31$, $P = 0.67$); 56% of the early-laid eggs were male and 54% of late-laid eggs were female.

Effect of Hatch Order and Gender on Nestling Size, Begging Behavior, and Hormones.—Early-hatched nestlings were significantly ($P < 0.001$) heavier than late-hatched nestlings throughout the nestling period (no significant age \times hatch order interaction; $P = 0.83$), brood size negatively affected mass, and we found no effect of gender on mass (Table 1; Fig. 1). Late-hatched nestlings begged significantly more vigorously than their early-hatched siblings, but neither brood size nor gender influenced begging rates (Table 1; Fig. 2). We found no effect of hatch order, gender, or brood size on nestling corticosterone levels (Table 1). We also found no difference in length of tarsi of early- and late-hatched nestlings at 14 days of age, but early-hatched nestlings had longer wings (Table 1). Hatch order did not significantly influence either brightness or chroma

of plumage coloration. However, males were significantly more colorful than females and nestlings from smaller broods were more colorful than those from larger broods (Table 1).

DISCUSSION

We were able to identify the hatching order of all eggs in only $\sim 15\%$ of the clutches despite close monitoring for hatching, demonstrating that many nestlings hatch during the night and many clutches hatch synchronously in this population of Eastern Bluebirds. Thus our data may represent nests with the greatest hatching asynchrony. Position in laying order was a strong predictor of position in hatching order, and hatching order influenced the morphology and behavior of nestlings. Late-hatched siblings had lower mass compared to their early-hatched siblings throughout the nestling period, and late-hatched nestlings begged more vigorously than early-hatched nestlings at 5 days of age. Late-hatched nestlings were lighter in mass and had shorter wing length near the age of fledging. It is possible that late-hatched nestling bluebirds are more likely to experience post-fledging mortality compared to their older

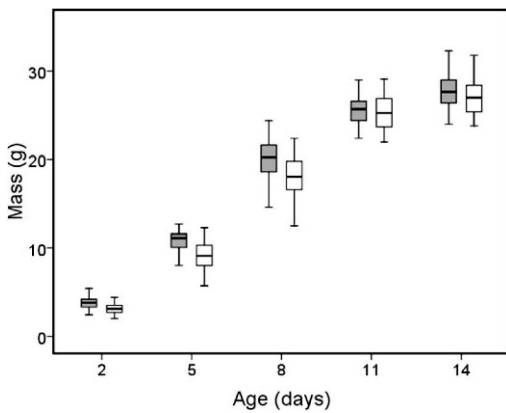


FIG. 1. Mass of nestling Eastern Bluebirds that hatched early (gray) or late (white). The line within each box represents the median mass; the upper and lower borders of each box represent the 25 and 75% percentiles; the lower and upper bars are the 10 and 90% percentiles.

siblings as larger body mass and wing length influence post-fledging survival in other species of altricial birds (Råberg et al. 2005). We failed to detect any interaction between hatch order and gender, and found no effect of hatch order on nestling corticosterone or plumage coloration. Lack of effect of hatch position on stress hormones and color suggest that late-hatched chicks may not have experienced extreme stress as a result of hatching position.

Studies of other species of birds also found laying order can be a strong predictor of hatching order (Beissinger and Waltman 1991, Clotfelter et al. 2000, Saino et al. 2001). This relationship is expected when females commence incubation prior to laying of the final egg (Magrath 1990). Laying order affects hatching order, and female bluebirds could potentially strategize their investment in eggs from different positions in the laying sequence to compensate for chick disparity caused by hatching asynchrony. Thus, although females could have differentially invested in eggs from different positions in the laying sequence, we found no evidence of a greater incidence of males hatching early. Our observations are similar to those of Lombardo (1982) for Eastern Bluebirds and Koenig and Dickinson (1996) for Western Bluebirds (*Sialia mexicana*), who also found no evidence of sex ratio bias associated with environmental conditions.

Early-hatched nestlings were significantly larger than their late-hatched siblings throughout the growth measurement period (up to day 14). The

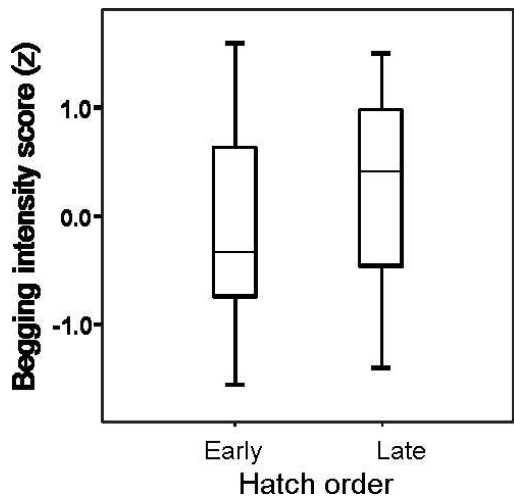


FIG. 2. Comparison of the begging intensity of nestling Eastern Bluebirds that hatched early and late, standardized by nest. The line within each box represents the median begging intensity score; the upper and lower borders of each box represent the 25 and 75% percentiles; the lower and upper bars are the 10 and 90% percentiles.

early fledgling period is a time of high mortality in passerine birds (Sullivan 1989), and all siblings in a brood tend to fledge on the same day (L. Siefferman, pers. obs.); thus, nestlings with relatively shorter wings may be less able to survive the fledgling period. We found no evidence that bluebirds in our population use a strategy of brood reduction; nestlings that appeared to die of starvation were no more likely to have hatched early or late in the clutch. Bluebirds in our study population experienced low incidence of nestling starvation compared to birds in other studies (Kendeigh 1942, Pinkowski 1977).

Hatching asynchrony created differences in begging rates among Eastern Bluebird nestlings; the late-hatched nestlings likely experienced greater need for food as they had lower mass and begged more than their early-hatched siblings. Begging has been shown to be an honest signal of need in other passerine species (Mondloch 1995, Price and Ydenburg 1995, Lotem 1998, Saino et al. 2000). Late-hatched nestlings did not have higher concentrations of circulating corticosterone at 8 days of age. Past experiments demonstrate that corticosterone promotes nestling begging (Kitaysky et al. 2001b, Loiseau et al. 2008). Corticosterone concentrations in those studies mimicked levels exhibited by nestlings during

extreme food shortages (Kitaysky et al. 2001a). Thus, late-hatched Eastern Bluebirds were not likely experiencing extreme food shortage as glucocorticoid concentrations do not suggest a high level of stress. Hatching later in the brood may not be extremely stressful for Eastern Bluebirds. Our data should be interpreted with caution because corticosterone levels and associated responses by nestlings can vary across development (Schwabl 1999, Sockman and Schwabl 2001), and we did not measure circulating corticosterone and begging on the same day.

Early-hatched chicks were larger than late-hatched nestlings, but hatch order did not influence plumage coloration. Past research suggests UV-blue structural coloration is a condition-dependent trait in nestling Eastern Bluebirds that can be negatively influenced by natal stress induced by experimental increases in brood size (Siefferman and Hill 2007). We found nestlings reared in larger broods weighed less and were duller compared to those reared in smaller broods, suggesting some effect of natal environment on nestling coloration. It may be that conditions created by hatching asynchrony are not sufficiently costly to negatively affect plumage development of late-hatched bluebird nestlings.

Our study provides a good estimation of the effects of hatching position on nestling condition. Late-hatched nestlings weighed less and had shorter wings than their siblings, and hatching asynchrony may jeopardize the first-year survival of late-hatched nestlings. We found few costs associated with late hatching. Indicators of extreme stress were not evident in late-hatched nestlings and, as a result, plumage ornamentation was not negatively influenced. Hatching asynchrony did not appear to be costly within this population, perhaps because Eastern Bluebirds do not show extreme variance in hatching asynchrony and brood reduction is rare. It is also possible that parents are able to compensate for potential detrimental effects on morphology and stress levels of late-hatched nestlings.

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