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Source: The Condor, 111(4) : 752-755

Published By: American Ornithological Society

URL: <https://doi.org/10.1525/cond.2009.090061>

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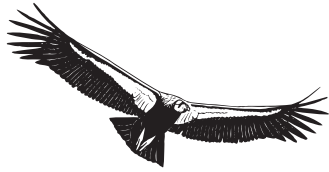
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## SHORT COMMUNICATIONS

*The Condor* 111(4):752–755  
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### EXTENDED LAYING INTERVAL OF ULTIMATE EGGS OF THE EASTERN BLUEBIRD

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**Abstract.** Proximately, clutch size is determined by the termination of the sequential pattern of egg formation and laying. Egg laying is difficult to study, and documentation of detailed patterns is scarce. We used archived video recordings of the Eastern Bluebird (*Sialia sialis*) to contrast the times of day of laying with variability in egg-laying intervals. Ultimate eggs were laid over a significantly longer interval than previous eggs. The extended interval over which the ultimate egg is laid could be a symptom of physiological constraints in the formation of the ultimate egg, extra resources provided to compensate the ultimate egg, and/or a late egg may become the ultimate egg because being late prevents further ovulation.

**Key words:** clutch size, Eastern Bluebird, egg formation, egg-laying interval, ovulation, photoperiod, *Sialia sialis*.

#### Intervalo Extendido de Puesta del Último Huevo de *Sialia sialis*

**Resumen.** En primera instancia, el tamaño de la nidada está determinado por la finalización del patrón secuencial de formación y puesta del huevo. La puesta de huevos es difícil de estudiar, y la documentación detallada de los patrones es escasa. Usamos grabaciones de archivo de *Sialia sialis* para contrastar las horas del día de la puesta con la variabilidad de los intervalos entre la puesta de los huevos. Los últimos huevos fueron puestos con intervalos significativamente más largos que los de los primeros huevos. La mayor duración del último intervalo antes de la puesta del último huevo podría ser un síntoma de (i) limitaciones fisiológicas en la formación del último huevo, (ii) la provisión de recursos extra para compensar la producción del último huevo y/o (iii) que un huevo tardío puede tornarse el último huevo porque al demorarse impediría que la ovulación continúe.

Most birds form eggs one at a time, and the proximate regulation of clutch size involves factors that control the termination of laying. The sequential pattern of egg formation and laying can result in variation in parental allocations among embryos (Lipar et al. 1999), size differences among offspring (Clotfelter et al. 2000),

differences in sex ratios of offspring (Badyaev et al. 2002, Krebs et al. 2002), and fitness consequences for offspring (Schubert and Cooke 1993, Wiebe and Martin 1995). While many studies have examined the potential fitness consequences of laying order, few have addressed factors associated with order such as the time of day of laying. Yet the details of laying patterns may provide clues to the proximate mechanism determining clutch size (Aparicio 1994; [http://scienceblogs.com/clock/2006/08/does\\_circadian\\_clock\\_regulate.php](http://scienceblogs.com/clock/2006/08/does_circadian_clock_regulate.php)).

In wild birds, patterns of laying are difficult to study because identifying the timing of laying during the day requires a high frequency of observations during a period when most birds are sensitive to disturbance. Consequently, patterns of laying in wild birds have rarely been described systematically and in detail, so the extent of intra- and interspecific variation is largely unknown (reviewed in Skutch 1952, Astheimer 1985, Wiebe and Martin 1995). Additionally, casual observations suggest that taxa differ in the typical time of day of laying. For example, many Passeriformes generally lay in the early morning, while some Galliformes (e.g., quail) lay in the afternoon (Wiebe and Martin 1995, Zivkovic et al. 2000). Yet, on closer examination, interspecific variation in laying intervals and the time of day of laying have been evident (Watson et al. 1993, Wiebe and Martin 1995), and, in intraspecific studies, it is possible to manipulate laying intervals and subsequent clutch size, as through food supplementation (Aparicio 1994) and changes in photoperiod in the laboratory (Zivkovic et al. 2000).

Examinations of patterns of laying in wild birds typically have involved experiments in which eggs are either removed as they are laid or added during laying (reviewed in Kennedy 1991). The response of birds to removal/addition experiments has been described as some species laying to achieve a particular clutch size (indeterminate layer) and others laying a particular number of eggs (determinate layer). Although phylogenetic constraints play a role in the determination of clutch size (Yom-Tov 1987, Postma and van Noordwijk 2005), the termination of laying has been attributed to (1) extrinsic cues that trigger the cessation of laying (e.g., the tactile stimulation of the brood patch; Haywood 1993a, b), (2) nutrient limitations on egg production (Houston et al. 2007), and (3) hormonal and circadian regulation of clutch size (Haywood 1993a, b, Zivkovic et al. 2000). If any of these mechanisms play proximate roles in

Manuscript received 6 April 2009; accepted 16 September 2009.

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*The Condor*, Vol. 111, Number 4, pages 752–755. ISSN 0010-5422, electronic ISSN 1938-5422. © 2009 by The Cooper Ornithological Society. All rights reserved. Please direct all requests for permission to photocopy or reproduce article content through the University of California Press's Rights and Permissions website, <http://www.ucpressjournals.com/reprintInfo.asp>. DOI: 10.1525/cond.2009.090061

determining clutch size in wild birds, detailed patterns of laying may complement insights gained from egg-removal and addition experiments. For example, discerning the potential role of the permissive zone (Opel 1966, Zivkovic et al. 2000) in the termination of egg laying requires, among other things, specifics on the time of day of egg laying.

We used video cameras to examine the time of laying during the day and intervals between eggs in the Eastern Bluebird (*Sialia sialis*). We review potential implications of these patterns with regard to the proximate regulation of clutch size.

## METHODS

We used images archived at the Cornell Lab of Ornithology that had been extracted at intervals of 0.5 to 2 min from video footage of nesting birds, video that covers almost 70 nest attempts from 17 species from 1999 to 2008. Even within these archives, sufficient imagery of the laying period is rare, and we found suitable imagery from 12 nests of the Eastern Bluebird in three nest boxes, two in Kentucky and one in Maryland. One clutch contained three eggs, six contained four eggs, four clutches contained five eggs, and one contained six eggs. During laying, bluebirds did not attend the nest at night and attended it infrequently during the day, so we could view nest contents often. Females spent on average 33 min ( $\pm 13$ , range from <1 to 84 min) on the nest while laying each egg. While the female was on the nest, the nest contents were not visible, and so we could not record the exact time of egg laying. Instead, we recorded the “laying window,” that is, the interval from the last minute when the nest contents were visible in one state ( $n$  eggs) to the first minute when nest contents were visible in another state ( $n + 1$  eggs).

## STATISTICAL ANALYSES

We used the “laying window” to estimate the time of day of laying under three assumptions: (1) start—that laying occurred at the start of the laying window, (2) middle—that laying occurred at that middle of the laying window, and (3) end—that laying occurred at the end of the laying window. We computed these estimates for each egg in four categories of reverse sequence (ultimate, penultimate, antepenultimate, earlier) rather than in forward sequence in order to standardize across the range of clutch sizes (four to six eggs) by clutch completion rather than by clutch initiation. We defined a category of “earlier” eggs as the first, second, and third eggs of six-egg clutches and first and second eggs of five-egg clutches.

Results regarding the time of day of laying were consistent across the three assumptions (see Results), so we used the middle laying window for the remaining analyses. We computed the estimated mean laying interval between eggs. Because nest attempts were at different locations and times of year, we also computed the mean time of laying during the day relative to sunrise (minutes since sunrise). We obtained sunrise data from the U.S. Navy’s website <[aa.usno.navy.mil/data/](http://aa.usno.navy.mil/data/)> for the dates and locations of the nest cameras. All times were in their local daylight time.

We modeled all response variables as a function of laying order as categorical (earlier, antepenultimate, penultimate, ultimate) using linear mixed models, with maximum likelihood variance component estimates in SAS (Littell et al. 1996), and nest attempt as a random effect to control for the repeated measures on each nest. We report least-squares means with standard deviations and significance tests using Bonferroni correction for multiple comparisons.

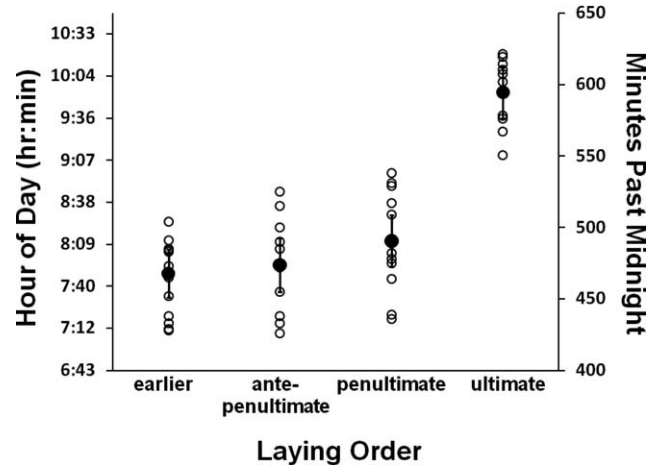


FIGURE 1. Least-squared means and 95% confidence intervals from mixed models of the time of egg laying (raw data are open circles; local time), which increases with laying order (data for Eastern Bluebird eggs,  $n = 12$  nests).

## RESULTS

Earlier and penultimate eggs differed in the duration of the middle laying window from which we estimated the time of day of laying ( $F_{3,29} = 3.7$ , adjusted  $P = 0.02$ ). The middle laying window was largest for penultimate eggs (39 min, 95% CI 32–41 min), so the estimate of the time of day of laying was least precise for this egg; the mean middle laying window was 34 min (CI 26–41 min) for ultimate eggs, 31 min (CI 23–39 min) for antepenultimate eggs, and 25 min (CI 17–33 min) for earlier eggs.

Bluebirds laid their eggs in the morning between 7:06 and 10:20 (mean 8:25  $\pm$  60), which corresponded to about 154 ( $\pm$  67; range 31–277) min after sunrise. Time of day of laying differed with order of laying, on the basis of the start, middle, and end of the laying window, respectively ( $F_{3,31} = 14.9$ ,  $P < 0.001$ ,  $F_{3,31} = 51.8$ ,  $P < 0.001$ ,  $F_{3,35} = 12.9$ ,  $P < 0.001$ ). The time of day of laying relative to sunrise (Fig 1;  $F_{3,31} = 35.5$ ,  $P < 0.001$ ) differed with laying order. Bluebirds laid nonultimate eggs at approximately the same times of day each morning and relative to sunrise but laid ultimate eggs at a later time of day and later relative to sunrise (Table 1). The interval between eggs varied with laying order (Fig 2;  $F_{3,20} = 18.7$ ,  $P < 0.001$ ) in a similar way, with no differences among nonultimate eggs and marked increase for ultimate eggs (Table 1).

## DISCUSSION

Nonultimate and ultimate eggs differed in the time of day of they were laid and the length of the interval from laying of the preceding egg. We observed a substantially later laying of ultimate eggs, generated by a substantial increase in the interval following laying of the preceding egg. Last-laid eggs have been noted for differing from earlier eggs in size (Aparicio 1999) and hatchability (e.g., Sockman 2008, Beissinger and Waltman 1991). Last-laid eggs can also differ in eggshell qualities (Gosler et al. 2005), yolk and egg size (Ardia et al. 2006), and hormone concentrations (e.g., Schmaltz et al. 2008). Some studies have found differences in sex ratios, hatchling quality, and paternity (e.g., Barber and Robertson 2007) based on laying order. Here we report one previously overlooked distinguishing characteristic of last-laid

TABLE 1. Bonferroni-adjusted *P*-values for multiple comparisons among laying intervals and time of laying of Eastern Bluebird eggs. Ultimate eggs were laid later in the day and later relative to sunrise than were previous eggs.

Pairwise comparison		Time of day	Time relative to sunrise	Inter-egg interval
Ultimate	Earlier	<0.0001	<0.0001	<0.0004
Ultimate	Antepenultimate	<0.0001	<0.0001	<0.0001
Ultimate	Penultimate	<0.0001	<0.0001	<0.0001
Earlier	Antepenultimate	NS	NS	NS
Antepenultimate	Penultimate	NS	NS	NS
Penultimate	Earlier	NS	NS	NS

eggs: they are laid later in the day and after a longer interval than eggs laid earlier in the sequence.

What is the significance of the ultimate egg's being laid after an extended interval? Although the extended interval for the ultimate egg may reflect a difficulty of making the last egg, other explanations are worth consideration. Watson et al. (1993) noted the longer laying interval of ultimate eggs in the Common Eider (*Somateria mollissima*) and attributed the pattern to the onset of incubation causing hormonal changes that impede formation of the last egg. Specifically, rising prolactin levels may reach a point of causing the ovary to start regressing even as the last egg is traveling through the oviduct. The longer laying interval could be adaptive, reflecting compensations given to last egg to offset its inherent disadvantages (Howe 1976). Through supplemental feeding of European Kestrels (*Falco tinnunculus*), Aparicio (1994) experimentally demonstrated an effect of food supply on clutch size through laying interval. That is, supplemented females laid larger clutches with shorter intervals between the eggs so that these clutches took less time to complete than the smaller clutches of nonsupplemented females. This result suggests that photoperiod may also be an important proximate mechanism, which could work in combination with nutrient constraints by the following scenario: females have an open period or permissive zone during which ovulation can occur, and the duration of

the period is determined proximately by photoperiod (Opel 1966, Phillips et al. 1985, Zivkovic et al. 2000) and perhaps ultimately by diel patterns of food availability, predation, or weather (Wiebe and Martin 1995). If females have trouble acquiring resources for an egg, then the egg takes longer to travel down the oviduct, and if that delay pushes its laying out of the open period then ovulation for a subsequent egg will not be stimulated.

Unfortunately, the patterns of laying intervals do not permit us to distinguish whether the ultimate egg is laid late because of nutrient constraints or whether the ultimate egg becomes last because it arrives outside the permissive zone. Nevertheless, patterns of time of day of egg laying should be incorporated into frameworks for testing the proximate mechanisms behind maternal effects related to laying order.

The extended interval preceding laying of ultimate eggs could be useful for field experiments. If field biologists can track the time of laying each day, then researchers can use the interval between eggs to identify ultimate eggs (i.e., an egg laid >25 hr after the preceding egg; Fig. 2) or researchers could, with a relatively high degree of certainty, distinguish the ultimate from preceding eggs on the basis of the time of day it is laid (though this is likely species-specific, e.g., after 9:00 for the Eastern Bluebird; Fig. 1). If a last-laid egg is experimentally removed, it is possible that a female will not be able to lay another the next day because she will not have ovulated, may have moved out of the permissive zone, and/or her oviduct may already be regressing (Williams and Ames 2004). If she does replace the egg, it might be after a delay, extending the laying period (Zivkovic et al. 2000). Therefore, identifying a clutch as complete on a basis other than hindsight could facilitate field experiments aimed at reducing clutch size, extending the laying period, or generating gaps in laying (i.e., attempting to generate embryos/young of different ages).

We are grateful to T. Phillips for coordinating the NestCam program at the Cornell Lab of Ornithology and thankful for the foresight of T. Phillips and P. Allen for establishing the NestCam program and archiving the images. We greatly appreciate the dedicated volunteers who manage the nest cameras for all to view.

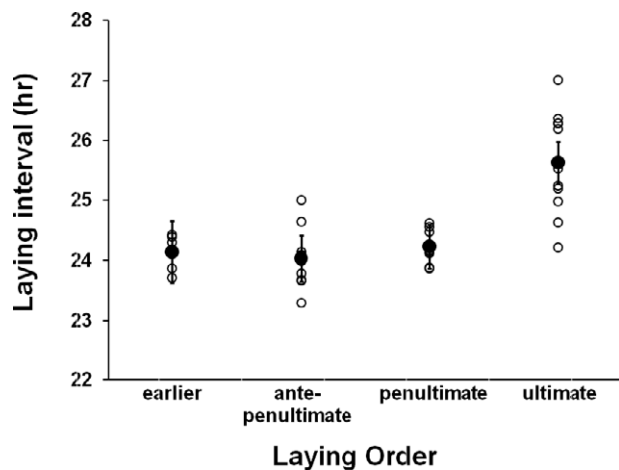


FIGURE 2. Least-squared means and 95% confidence intervals from mixed models illustrating that the inter-egg laying interval (raw data are open circles) is longest for ultimate eggs (data for Eastern Bluebird eggs,  $n = 12$  nests).

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