

## Research



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# Oviposition drives hatching order and developmental disparities with brood mates

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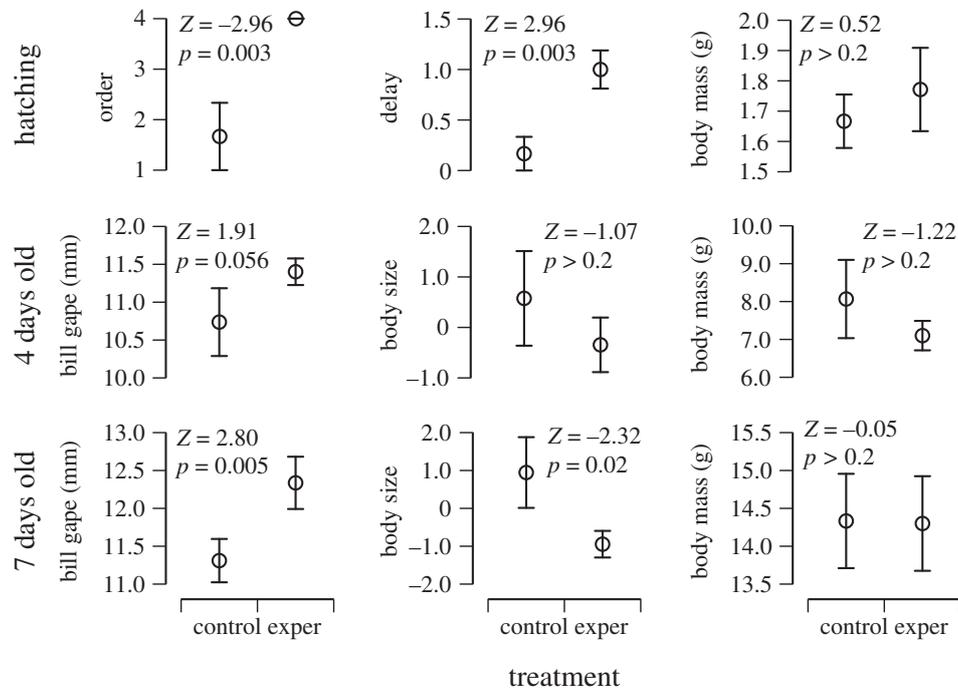
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Birth or hatching order can affect fitness. It has long been assumed that the order in which a bird's egg is laid (its oviposition) and first exposed to incubation relative to other eggs in the nest determines the order in which it hatches and the subsequent effects on development and survival. To my knowledge, this cause of hatching order has not been tested while controlling for laying-order effects on egg composition. Like those of many species, the last-laid eggs in clutches of the Lincoln's sparrow (*Melospiza lincolni*) hatch last and produce nestlings with reduced growth and survival rates. Last-hatched Lincoln's sparrows also develop wider bills that are associated with reduced attractiveness of adult courtship songs. Using wild, free-ranging Lincoln's sparrows, I replaced the first or fourth freshly laid egg in four-egg host nests with a freshly laid first egg from a donor nest. I discovered that the experimental elevation of oviposition—which controlled for egg composition—delayed hatching, inhibited growth and widened bill gape, thus confirming the mechanism for hatching order and revealing a potential role for oviposition in reproductive success. Similar effects of postnatal family position could affect fitness in other taxa, as well.

## 1. Introduction

The asynchronous hatching of eggs within a bird's clutch leads to a hierarchy among brood mates in their competition for parentally provided resources, in their development and in their survival [1]. For at least 70 years [2], it has been assumed that delayed hatching is the product of the egg's laying order (its oviposition) and therefore its delay in incubation exposure relative to other eggs in the nest [3]. However, factors other than serial oviposition could cause eggs to hatch in the order they are laid and thus lead to the sibling hierarchy. These include well-known laying-order effects on egg size and contents in numerous bird species [4], which, in some cases, affect time to hatching [5,6]. This raises the alternative hypothesis that hatching asynchrony is caused by laying-order effects on egg composition. To my knowledge, the effect of oviposition has not been tested while controlling for egg composition.

In Lincoln's sparrows (*Melospiza lincolni*), hatching (and laying) order is negatively correlated with nestling growth and survival rates [7], as it is for many species [4]. Additionally, later-hatched individuals have a wider bill than earlier-hatched brood mates [8]. Bill shape (height relative to width) in adult, male Lincoln's sparrows is associated with performance of sexual song signals [9] that, in turn, affect female preferences in a mate-choice context [10]. Moreover, adult bill shape is correlated with nestling bill shape [8], suggesting that early life experience can influence not only rates of development and survival but also a trait that later in life may be associated with reproductive success. In order to determine whether oviposition is one such early life experience, I experimentally manipulated oviposition in wild,



**Figure 1.** Effects of oviposition on timing of hatching and on body mass, body size and width of bill gape (mean  $\pm$  s.e.m.) at three ages in Lincoln's sparrows (*Melospiza lincolni*). The first (control) or fourth (exper) freshly laid egg in four-egg host nests was replaced by a freshly laid first egg from a donor nest, thus controlling for laying-order effects on egg composition. Statistical results are from a Wilcoxon rank-sum test (hatching order) and general linear models (others).

free-ranging Lincoln's sparrows while controlling for egg composition and then assessed hatching times and post-hatching development.

## 2. Methods

I performed this experiment in June and July of 2007–2009, 2011 and 2015 and have previously described the population, field setting, and how assistants and I found nests and marked eggs as they were laid [7,9]. Individuals lay one egg per day by 07.30 h local time [11] until the clutch is complete (usually at four eggs [7]). I generated control treatments by exchanging the first-laid eggs of two nests on the day those eggs were laid. This required nests with a 0-day offset in their date of clutch initiation. In other nests, I generated experimental treatments by replacing the fourth-laid egg with the first-laid egg from a donor nest, again on the day both the donor and replaced eggs were laid. This required a 3-day offset between host and donor nests in their date of clutch initiation. No nest received more than one foster egg or donated more than one donor egg. Thus, nests retained their natural clutch size after manipulation. We allowed incubation to proceed naturally.

Beginning approximately 10 days following clutch completion, we checked nests two to four times daily during daylight hours in an effort to identify the nestling that hatched from the fostered egg. Nestlings were marked by clipping a toe nail; weighed 0, 4 and 7 days post-hatching; measured 4 and 7 days post-hatching for the width of the bill gape and the length of the tarsi and wing chords; and measured 7 days post-hatching for the length of the ninth primary feathers. I then calculated body size from the first-axis factor scores of principle components analyses on mean tarsi and wing chord measurements for 4-day-olds and on mean tarsi, wing chord and ninth primary measurements for 7-day-olds. All ages apply to the nestling from the fostered egg, not necessarily the brood.

I conducted nine statistical analyses (plus one described in Results), each corresponding to one of the following dependent

measures of the nestling hatching from the fostered egg: hatching order (1–4); hatching delay (nearest half-day interval since onset of hatching in the host clutch), mass 0, 4 and 7 days post-hatching; body size 4 and 7 days post-hatching; and bill gape 4 and 7 days post-hatching. In all analyses, experimental treatment was the predictor. The first analysis was a Wilcoxon rank-sum test and the others were general linear models.

## 3. Results

Of the 243 nests found over the five seasons, 56 were used for the experiment, because they were found on or before the laying of the first egg when another, appropriately offset nest had also been found on or before the laying of the first or fourth egg. Of those 56, I analysed the 10 (three control and seven experimental) for which all eggs hatched, for which I definitively knew the identity of the nestling from the fostered egg, and for which clutch size was four eggs. The remaining 46 experimental nests were not used in the analyses due to having a three- or five-egg clutch, pre-hatching predation, weather- or investigator-triggered abandonment, incomplete hatching or the simultaneous discovery of more than one hatchling that made assignment to the fostered egg ambiguous (but see below). Over the course of the nest cycle, some nestlings from experimental (but no control) eggs died, contributing to smaller sample sizes by 4 ( $N = 8$ ) and 7 ( $N = 6$ ) days post-hatching.

Despite the small sample size (suggesting a reason nobody had done this experiment before), significant effects of the experimental treatment were evident. Specifically, each of the experimental eggs was the fourth in its host nest to hatch, with a mean hatching delay of  $1.00 \pm 0.19$  (s.e.m.) days. By contrast, two of the control eggs hatched first and one hatched third in their host nests, with a mean hatching delay of  $0.17 \pm 0.17$  days (figure 1). I detected no effect of treatment on mass at 0, 4 or 7 days post-hatching

or on size at 4 days post-hatching, but nestlings from experimental eggs were smaller at 7 days post-hatching than nestlings from control eggs (figure 1). Despite their smaller size, nestlings from experimental eggs had wider bill gapes than nestlings from control eggs 7 days post-hatching (figure 1).

In order to increase sample size, I added the four nests for which I did not know the identity of the hatchling from the fostered egg but for which I did know whether or not the fostered egg hatched fourth and for which all other criteria above were fulfilled. Among this larger sample, none of the control eggs ( $n = 7$ ) and all of the experimental eggs ( $n = 7$ ) hatched fourth (Pearson's  $\chi^2 = 14.0$ ,  $p < 0.001$ ).

## 4. Discussion

Experimentally elevating oviposition while controlling for egg composition delayed hatching, inhibited growth and widened bill gape. In this experiment, all comparisons were between fostered first-laid eggs that, on the day they were laid, replaced a freshly laid host egg that was either first- or last-laid in the host clutch. To my knowledge, this is the first time this manipulation has been conducted (L. S. Forbes, M. Krist, R. D. Magrath, R. E. Ricklefs 2018, personal communication). And yet, this was the manipulation necessary in order to validate the 70-year-old assumption that oviposition drives asynchronous hatching and the resultant developmental hierarchy among brood mates [3].

Since Lack [2] first popularized the phenomenon of hatching asynchrony, investigators have conducted numerous experiments aimed at understanding it [1]. However, I was unable to find any that explicitly addressed the long-assumed cause of hatching asynchrony—differential exposure to incubation caused by oviposition. Researchers have exchanged eggs towards the end of the incubation cycle (e.g. [12]) or just after hatching (e.g. [13]), but these experiments largely addressed consequences of hatching asynchrony, not its cause. Others have experimentally retained freshly laid eggs outside of active nests to manipulate exposure (e.g. [14]), but this manipulation demonstrates not the specific role of oviposition but rather the more general role of exposure to an active nest, a role that may be confounded by differences between experimental (eggs replaced by dummy eggs for extended periods) and control (eggs held briefly, if at all) treatments that extend beyond the question of interest.

Hatching patterns often precisely follow expectations from an interaction between oviposition and the timing of incubation onset [15], and this experiment's confirmation of

this interaction, therefore, may not seem surprising. Nonetheless, there are cases in which hatching patterns cannot be attributed to this interaction [6,16], suggesting that for some species, oviposition does not necessarily drive hatching asynchrony. Moreover, at least some yolk components that vary monotonically by laying order also affect the timing of hatching relative to nest-mates [5]. Results from the present study now show that oviposition alone can cause hatching asynchrony and at least some of the developmental disparities between brood mates. These results, however, do not rule out the possibility that other factors, including egg composition, also play a causal role.

Perhaps most intriguing is the effect of oviposition on width of the bill gape. Previously, I discovered that, in adult Lincoln's sparrows, bill shape is associated in some years with male performance of sexual song signals [9] and is correlated with nestling bill shape [8], which itself is tied to hatching order [8]. This raised the hypothesis that developmental plasticity in bill shape mediates a trade-off between acquisition of parental provisions during development and acquisition of mates during adulthood. Before this study, I could not rule out the possibility that bill shape was programmed by a hypothetical effect of egg composition tied to laying order. Results here demonstrate that this trait is a plastic response to the egg's oviposition and thus its cumulative exposure to parental incubation, possibly mediated by sibling variation in competitiveness [17].

Hatching asynchrony is probably the norm among altricial birds [1] and thus the vast majority of the 10 000 species of birds, but it has implications for family planning, parental favouritism, sibling competition and epigenetic variation in fitness even in viviparous taxa. The role of oviposition shown here raises the possibility of similar, post-natal effects of family position driving important sibling variation in other, non-avian vertebrate taxa—taxa in which siblings vary significantly in their developmental stage while simultaneously depending on parental provisioning, including some marsupials [18] and, notably, humans [19,20].

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**Data accessibility.** The data have been uploaded as the electronic supplementary material.

**Competing interests.** I declare I have no competing interests.

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