

Laying order, hatching asynchrony and nestling body mass in Tree Swallows *Tachycineta bicolor*

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Clotfelter, E. D., Whittingham, L. A. and Dunn, P. O. 2000. Laying order, hatching asynchrony and nestling body mass in Tree Swallows *Tachycineta bicolor*. J. Avian Biol. 31: 329–334.

We studied the reproductive biology of a box-nesting population of Tree Swallows *Tachycineta bicolor* in southeastern Wisconsin, USA. We were interested particularly in the relationship between laying order and hatching order and the extent to which each was a predictor of nestling body mass. We found that laying order was a significant predictor of hatching order. Laying and hatching order were related to nestling mass at 4 days of age and to a lesser extent at 12 days of age. In addition, we investigated the effects of natural variation in hatching asynchrony. Hatching asynchrony was positively related to the range of nestling body masses within a brood at days 4 and 12. The probability that brood reduction occurred was also positively related to the degree of hatching asynchrony, though this effect was significant only at day 4. Our results suggest that laying order and hatching order have their greatest effects on nestling Tree Swallows early in the nestling period.

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In most birds, females lay eggs at approximately 24-hour intervals. The order in which the eggs hatch depends on when incubation begins (Ricklefs 1993). If incubation is delayed until the ultimate egg, embryonic development is synchronized and the eggs hatch synchronously. If incubation begins before clutch completion, however, embryonic development is staggered and the eggs hatch asynchronously over a period of one or more days. The adaptive significance of asynchronous hatching was first addressed by Lack (1954). Since then, approximately 20 hypotheses have been proposed to explain this phenomenon, but none explains hatching asynchrony in all species (Magrath 1990, Slagsvold et al. 1995, Stoleson and Beissinger 1995, Stenning 1996). An assumption implicit in many hypotheses is that hatching order reflects laying order. This assumption is rarely tested, however (Stoleson and Beissinger 1995, Wiebe et al. 1998). The relationship between laying and hatching order is relevant to other areas of inquiry, including the evolution of sibli-

cide (Mock 1984), pre-hatching communication among nestlings (Davies and Cooke 1983), the allocation of maternal testosterone to eggs (Schwabl 1993) and the relationship between parentage and parental investment (Riley et al. 1995).

In the current study, we examined several aspects of the reproductive biology of Tree Swallows *Tachycineta bicolor* in southeastern Wisconsin, USA for the period 1997–1998. The data we present are part of a larger, ongoing study of the relationship between parentage and parental investment in this species. The objectives of this study were to determine: (1) the extent to which laying order predicts hatching order, (2) the effects of these variables on nestling body mass and (3) the influence of natural variation in hatching asynchrony on nestling survival and body mass. Tree Swallows are a good species in which to address these questions because they have large clutches (typically 4–8 eggs) with considerable intraspecific variation in clutch size and hatching asynchrony (Robertson et al. 1992).

Methods

We studied Tree Swallows at the University of Wisconsin-Milwaukee Field Station near Saukville, WI (43° 23'N, 88° 01'W) beginning in 1997. That year, we placed a total of 58 nestboxes with aluminum predator guards on two meadows. In 1998, we added an additional 40 nestboxes. We set up grids in which nestboxes were 40 m apart along a row and 28 m on the diagonal between rows. The density of nestboxes in these grids is similar to natural Tree Swallow densities and to densities in other box-nesting populations (Robertson and Rendell 1990).

Once nest construction began in early May, we checked nestboxes daily for eggs. We numbered eggs with pencil when they were laid. Final clutch size was recorded when no additional eggs were laid for two consecutive days. The average incubation period for Tree Swallows is 14 days (Robertson et al. 1992); after 12 days we checked nests for signs of hatching every 2 hours between 0600 and 2000 CDT (approximately dawn and dusk, respectively). We continued these visits every 2 hours until all eggs in the clutch hatched. Newly hatched nestlings were marked on the flanks with a unique color combination using indelible markers. If more than one egg hatched in the same 2-hour interval, the eggs were assigned the same hatching order and the nestlings were given the same marking. These marks were applied twice per day or as necessary. Hatching asynchrony data are reported for 1998 only because nest checks were made more regularly this year than in 1997. There were no cases of nest abandonment as a result of frequent nest checks.

Hatching date was recorded as the date on which the majority of eggs in the clutch hatched. If four eggs in a six-egg clutch hatched on the first day, for example, that day was recorded as the hatching date. A small number of clutches, however, hatched in a different pattern: one egg on the first day, several on the second day, and one on the third day. In these cases, the hatching date was the second day. Four days after hatching (day 4), we weighed all nestlings to the nearest 0.1 g with an electronic balance. On day 4, we also fitted nestlings with plastic colored leg bands, which we checked daily and replaced if necessary. On the twelfth day after hatching (day 12), nestlings were weighed again and given an aluminum USFWS leg band. We measured body mass on days 4 and 12 because day 4 is the earliest age at which all nestlings in a brood can be measured (i.e. all eggs have hatched by this time; Zach 1982) and day 12 is when most nestlings reach their asymptotic mass (Zach and Mayoh 1982, Quinney et al. 1986). Adult female swallows were recorded as second-year or after second-year on the basis of plumage coloration (Husell 1983).

Multiple regressions and ANOVAs were conducted using JMP version 3.0 (SAS Institute 1997). In ANOVAs, we included clutch size, brood size, hatching date and year in all models to account for differences among nests and years. We were concerned that brood reduction might confound the relationships among laying order, hatching order and nestling body mass. In addition, there were some cases in which multiple eggs hatched between successive nest checks, making it impossible for us to determine exact hatching order. For these reasons, we conducted our analyses using three data sets: (1) the complete set of all nests, (2) only nests in which no brood reduction occurred before day 4 and (3) only nests in which no brood reduction occurred before day 4 and for which we had complete information on hatching order. We defined brood reduction as any partial brood loss before the day in question. These three data sets gave similar results, except for the analysis of nestling mass on day 12 (see Results). The text and figures report only analyses of nests with no brood reduction, because this data set provided the best compromise between large sample sizes and few confounding effects. A summary of all analyses is reported in Table 1. Means are presented \pm SE. All tests are two-tailed and differences were considered significant at $P < 0.05$.

Results

We obtained laying and hatching orders for 287 eggs from 58 clutches. Laying order was a highly significant predictor of hatching order (partial correlation coefficient $R = 0.77$, $F_{1,175} = 264$, $P < 0.001$; Fig. 1) in an ANOVA that also included clutch size ($P = 0.87$), hatching date ($P = 0.005$) and year ($P < 0.0001$) as predictors (model $R^2 = 0.62$; Table 1, data set with no brood reduction). The effect of hatching date indicated that eggs in a given position in the laying sequence hatched later when the date of hatching was later in the season. For example, late in the season the third laid egg was more likely to hatch fourth or later in sequence, whereas early in the season it would be more likely to hatch third or earlier. Similarly, eggs hatched later than would be expected based on their laying order in 1998 as opposed to 1997. This was the only significant year effect in our study.

Day 4 mass was related negatively to both laying order (partial correlation coefficient $R = -0.53$, $F_{1,168} = 65$, $P < 0.001$; Fig. 2a) and hatching order ($R = -0.57$, $F_{1,169} = 83$, $P < 0.001$; Fig. 2b) in ANOVAs that also included brood size, hatching date and year (P values > 0.22 for these last three variables). On day 4, the difference in mass between first

Table 1. Relationships between laying order, hatching order and nestling body mass for broods of Tree Swallows. Sample sizes represent numbers of broods.

	All nests (n = 58) ¹	Nests with no brood reduction (n = 33) ²	Nests with complete hatching information and no brood reduction (n = 10) ³
Laying order versus:			
Hatching order	F _{1,281} = 312 P < 0.0001	F _{1,175} = 264 P < 0.0001	F _{1,44} = 41 P < 0.0001
Mass at day 4	F _{1,261} = 66 P < 0.0001	F _{1,168} = 65 P < 0.0001	F _{1,44} = 3.3 P = 0.013
Mass at day 12	F _{1,253} = 4.5 P = 0.03	F _{1,169} = 5.8 P = 0.02	F _{1,46} = 1.9 P = 0.18
Hatching order versus:			
Mass at day 4	F _{1,262} = 133 P < 0.0001	F _{1,169} = 83 P < 0.0001	F _{1,48} = 21.3 P < 0.0001
Mass at day 12	F _{1,254} = 3.9 P = 0.049	F _{1,170} = 6.1 P = 0.02	F _{1,46} = 1.4 P = 0.24

¹ Results are from ANOVAs with year, hatching date, clutch size and brood size as additional predictors. ² Results are from ANOVAs with year, hatching date and brood size as additional predictors (clutch size = brood size for these nests). ³ Results are from ANOVAs with hatching date and brood size as additional predictors (all nests were from 1998).

and last hatched young averaged 3.0 g or 37.5% of the mass of the first hatched young. The difference in mass between young from first and last laid eggs was 3.5 g or 44.2% of the day 4 mass of the nestling from the first laid egg. All three data sets gave similar results (Table 1).

In contrast, on day 12 the relationships between mass and both laying and hatching order varied depending on the data set we analysed. When we examined the complete data set or the data set with no brood reduction, nestling mass on day 12 was related negatively to laying order and hatching order (Table 1, Fig. 3a, b). However, there was no significant relationship between mass on day 12 and laying order or hatching order for the data set with complete hatching order information and no brood reduction (Table 1). In all these analyses the difference in mass on day 12 between first and last hatched young or young from first and last laid eggs was 1.2 g or 6% of the day 12 mass of the first hatched young. In summary, the relationship between laying or hatching order and nestling body mass was much stronger on day 4 than on day 12.

We observed considerable variation in hatching asynchrony among nests in 1998 (range = 2–70 h), with a mean hatching spread of 28.4 ± 2.0 h (n = 47 nests) between the first- and last-hatched eggs. More than 70% of all eggs hatched during the period for which we checked nests (0600–2000 hours), meaning that our estimates of hatching asynchrony were good approximations of true hatching spreads. Spread in hatching was not related to clutch size, nest initiation date or female age in a simultaneous multiple regression (overall $R^2 = 0.077$, $F_{3,37} = 1.03$, $P = 0.39$, n = 41 nests). On day 4, the degree of hatching asynchrony was related positively to the range of nestling body mass within a brood ($R^2 = 0.52$, $P < 0.001$, n = 35 nests). Similarly, on day 12 the degree of hatching asynchrony was

related positively to the range of nestling body mass, although the relationship was not as strong as on day 4 ($R^2 = 0.18$, $P = 0.01$, n = 35 nests). As might be expected, brood reduction was more likely in nests with a greater spread in hatching. Broods in which nestlings died or disappeared for any reason prior to day 12 had a mean hatching spread of 31.3 ± 4.0 h (n = 17 nests) versus a mean hatching spread of 26.7 ± 2.2 h (n = 30 nests) for broods with no mortality. In a logistic regression, brood reduction by day 4 (coded as: 1 = one or more nestlings died, 0 = no nestlings died) was more likely if there was a larger spread in hatching ($\chi^2 = 4.6$, $P = 0.03$, n = 47 nests). By day 12 there was no such

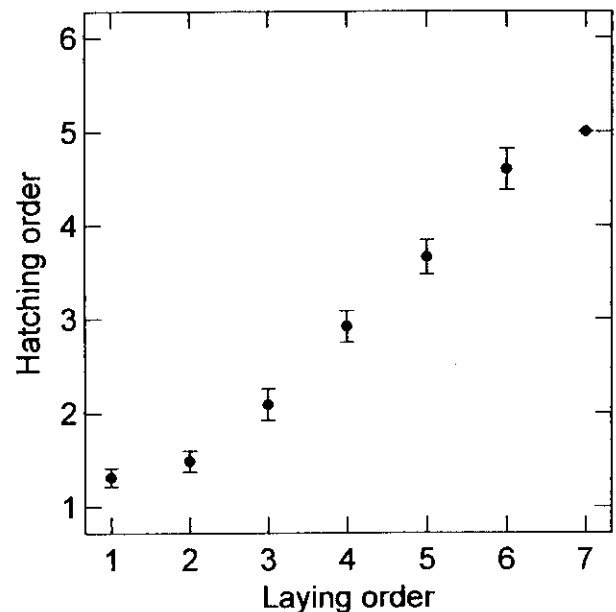


Fig. 1. Relationship between laying order and hatching order for 179 eggs from 33 clutches of Tree Swallow eggs (1997–1998).

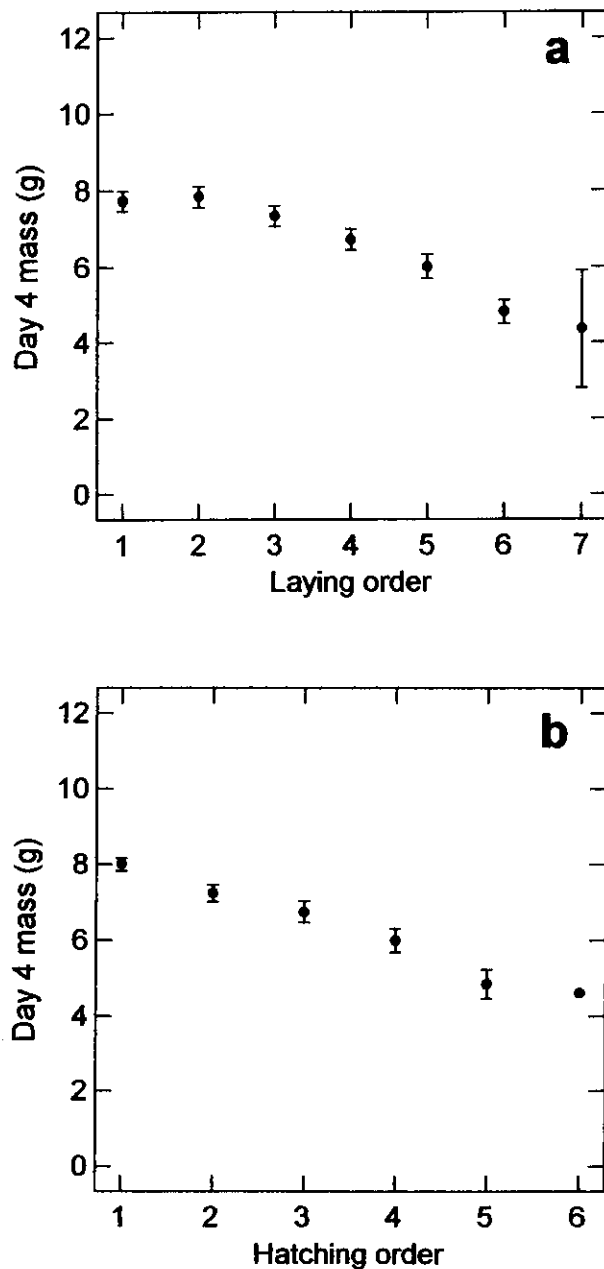


Fig. 2. (a) Relationship between laying order and day 4 mass for 172 nestlings from 33 broods of Tree Swallows (1997–1998). (b) Relationship between hatching order and day 4 mass for 173 nestlings from 33 broods of Tree Swallows (1997–1998).

relationship between brood reduction and hatching spread ($\chi^2 = 2.3$, $P = 0.13$, $n = 47$ nests).

Discussion

For most bird species, it is assumed that eggs within a clutch hatch in the order in which they were laid. This assumption, however, has been tested in relatively few species (Cargill and Cooke 1981, Stokland and Amundsen 1988, Magrath 1990, Beissinger and Waltman 1991,

Wiebe et al. 1998). Deviations from expected hatching order may result from unequal heat distribution (Bortolotti and Wiebe 1993), differences in incubation time between first- and last-laid eggs (Viñuela 1991) and accelerated or delayed hatching as a result of communication among developing embryos (Davies and Cooke 1983, Schwagmeyer et al. 1991). Given that the majority of hypotheses for the evolution of hatching asynchrony are based on the assumption that females can control the order in which eggs hatch (Stoleson and

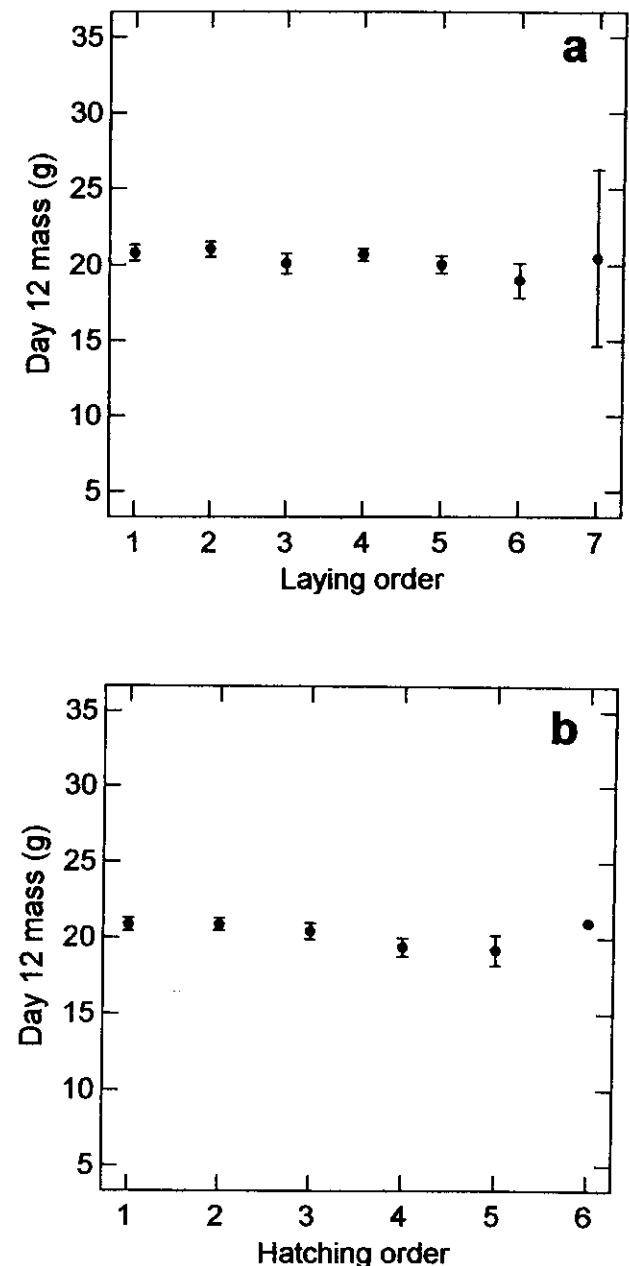


Fig. 3. (a) Relationship between laying order and day 12 mass for 173 nestlings from 33 broods of Tree Swallows (1997–1998). (b) Relationship between hatching order and day 12 mass for 174 nestlings from 33 broods of Tree Swallows (1997–1998).

Beissinger 1995), the presumed correlation between laying and hatching order merits further attention. We found that laying order was a highly significant predictor of hatching order in Tree Swallow clutches.

As predicted by the brood reduction hypothesis (Lack 1954, Ricklefs 1965), we found that laying order and hatching order had strong influences on body mass of Tree Swallow nestlings at day 4. Early-hatched nestlings were larger than later-hatched nestlings. Similar results have been reported from a Tree Swallow population in Manitoba, Canada (Zach 1982). Zach (1982) also reported that egg size increased with laying order, but that increased egg size did not compensate for the disparity in nestling body masses. By the time nestlings reached day 12, the evidence was not as strong for a relationship between laying order or hatching order and nestling mass. Similarly, Blancher and McNicol (1988) reported that nestling mass at hatching was correlated with mass at days 4 and 8, but not at day 12 or 16.

Incubation by female Tree Swallows usually begins following the penultimate egg (Clark and Wilson 1981, Robertson et al. 1992). This incubation pattern suggests that most eggs within a clutch should hatch relatively synchronously (Ricklefs 1993, Stoleson and Beissinger 1995, Wiebe et al. 1998). The degree of hatching asynchrony we observed is consistent with such a pattern; the mean hatching spread between first and last eggs within the clutch was approximately one day. However, there was considerable variation around the mean, including several nests with hatching spreads of two or more days. These results are similar to those obtained from other Tree Swallow populations (Zach 1982, Quinney et al. 1986). Intraspecific variation in hatching asynchrony is common, even in theoretically synchronously-hatching species where incubation begins after the penultimate egg (Clark and Wilson 1981). Several factors may contribute to this variation, including clutch size (Smith 1988, Briskie and Sealy 1989, Stouffer and Power 1990), timing of onset of incubation (Haftorn 1981, Harper et al. 1994, Clotfelter and Yasukawa 1999) and hatching date (Hussell 1972, Slagsvold 1986, Skagen 1987). Hatching may be a stochastic process over which incubating females have little control (Clark and Wilson 1981, Bortolotti and Wiebe 1993, but see Wiebe et al. 1998). Interestingly, we observed no effect of clutch size or hatching date on hatching asynchrony in our study. We also found no evidence that older (after second-year) females differed from younger (second-year) females in the degree of hatching asynchrony, as might be expected if incubation was affected by maternal condition (e.g. Slagsvold 1986, Slagsvold and Liffeld 1989).

Another prediction of Lack's (1954) hypothesis is that hatching asynchrony produces size hierarchies among nestlings that allow for efficient brood reduction in times of food scarcity. We found that Tree Swallow

clutches that hatched asynchronously resulted in broods with greater size ranges than synchronously hatching clutches. Similar results have been reported in a variety of species (Ricklefs 1965, Parsons 1975, Zach 1982, Hussell 1985, Stokland and Amundsen 1988). As predicted, we found that brood reduction was more likely to occur in nests prior to day 4 when there was greater hatching asynchrony; however, there was no such relationship when we examined brood reduction up to day 12.

In summary, laying order was a significant predictor of hatching order in our population of Tree Swallows. These variables were related significantly to nestling body mass on day 4, and to a lesser extent to mass on day 12. Clutches differed considerably in the synchrony with which they hatched, and asynchronous hatching resulted in greater mass ranges among nestlings. Apparently as a consequence, hatching asynchrony affected nestling survival to day 4. Our results suggest that factors such as laying order and hatching order have an effect on offspring growth and survival that is most pronounced early in the nestling period.

Acknowledgements We thank the staff of the UWM Field Station for facilitating this research, particularly Lou Nelson for constructing the nestboxes. Kevin Thusius and Lisa Belli assisted in the field. Comments by Ellen Ketterson, Martyn Stenning and an anonymous reviewer greatly improved an earlier version of this paper. This research was funded in part by a UWM Graduate School Research Award.

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(Received 8 March 1999, revised 6 September 1999, accepted 5 October 1999.)