

VARIATION IN INCUBATION PERIOD WITHIN A POPULATION OF THE EUROPEAN STARLING

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ABSTRACT.—We switched eggs among nests of European Starlings soon after the eggs were laid, and we measured incubation periods to within 2 h in most cases. Data from two years were analyzed to determine how much the natural parent of the egg and the incubating parent contributed to variation in the length of the incubation period. In one year, the relative size and composition of the yolk of one egg from each clutch were measured in order to assess the additional influence of egg composition on the length of the incubation period. In both sets of experiments, nest (incubating parent) had a significant effect, whereas clutch (natural parent) had either no effect or a marginally significant effect on the length of the incubation period. The standard deviation of incubation periods within nests was 2.6 and 3.5 h in the two experiments. Incubation periods increased significantly both with the percentage of yolk in eggs laid by the natural parent and with the percentage of yolk in eggs laid by the incubating parent.

Our results suggest that the length of the incubation period is determined primarily by the incubating behavior of the adult, thermal properties of the nest, or both. Genetic differences in the growth rates of embryos and maternal effects expressed through egg composition were small and generally insignificant. Incubation period was, however, weakly related to egg composition of both the natural parent and the incubating parent, suggesting a phenotypic correlation between egg formation and incubation behavior. *Received 20 December 1982, accepted 18 May 1983.*

THE natural incubation periods of birds vary considerably within populations (e.g. Kendeigh 1952). Although much of this variation can be related to environmental temperature and differences in the thermal properties of nests (e.g. Kendeigh 1940, Skutch 1976), the incubation periods of eggs hatched under uniform conditions in incubators also vary. Experiments with domestic fowl have demonstrated both the heritability and response to selection of hatching time (Crittenden and Bohren 1961, Smith and Bohren 1974). Natural populations have not, however, been subjected to an experimental investigation of the influence of genetic and environmental factors on incubation period. This paper reports two sets of experiments designed to determine the relative contributions of egg quality (genetic parentage of the embryo and maternal effects) and the combination of incubation behavior and nest microclimate on the length of the incubation period in the European Starling (*Sturnus vulgaris*) in southeastern Pennsylvania.

METHODS

Experiments were conducted in 1976 at a colony of starlings at the Stroud Water Research Center near

Avondale, Pennsylvania, approximately 48 km east of Philadelphia (see Ricklefs and Peters 1979), and in 1982 at a similar colony located at the Waterloo Mills Field Station of the University of Pennsylvania near Wayne, Pennsylvania. The experiments were conducted with first clutches initiated during late April and early May. In 1976, eggs were switched at random among 13 nests. Each of the complete clutches contained 4 or 5 eggs. Nests were checked each day during the laying period; new eggs were removed from the nests in the early afternoon and replaced with eggs from nests not used in the experiment. Experimental eggs were held at room temperature. After laying had been completed in all 13 nests, which were highly synchronized, the eggs were replaced in nests at random and the dummy eggs removed. The switching was completed between 1200 and 1300 on one day. As all dummy clutches were warm prior to switching, all eggs were assumed to have commenced incubation at that time.

In 1982, eggs were removed from nests according to the following schedule: on day 1, the first egg was left in the nest; on day 2, the first egg was removed and a plaster egg was added; on day 3, the second egg was removed and a second plaster egg was added; on day 4, the third and fourth eggs were removed and a third plaster egg was added; on day 5, the eggs were switched among nests and the plaster eggs removed, generally in the early afternoon; all the clutches were warm at the time of switching. Fifth

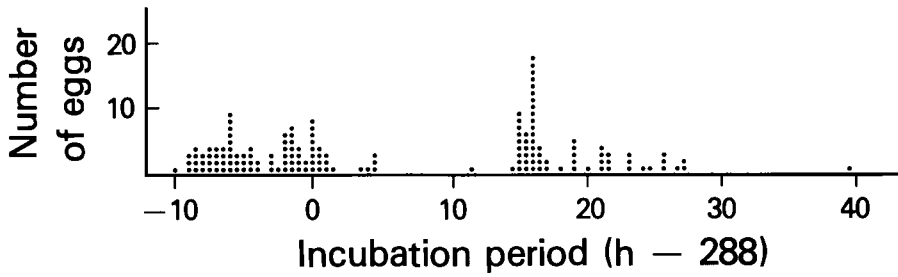


Fig. 1. Distribution of hatching times, expressed as hours deviation from 12 days (288 h), of 154 eggs of the European Starling in 1982.

and sixth eggs, if laid, were removed for analysis of composition. Eggs were individually marked with felt-tip pens.

During 1982, 2 of the first 4 eggs in the laying sequence were switched among pairs of nests. Within any pair, the same eggs in the laying sequence were switched, but the switched eggs were varied at random among pairs of nests. That is, among one pair of nests eggs number 1 and 3 might have been switched while among another pair eggs 2 and 4 were switched, and so on. All eight eggs hatched, and we were able to match all neonates to eggs with certainty, in seven pairs of switched nests ($n = 56$ eggs). Predation, hatching failure, and inability to match eggs and hatchlings caused incomplete results for many switched pairs, for which incubation periods were determined for an additional 98 eggs.

Chemical composition was determined as follows. We separated eggs into shell, albumen, and yolk components. The yolks were rolled on aluminum pans to remove adhering albumen before being placed in tared weighing pans. Yolks and albumen were air dried at 60°C. Yolks were soaked in 2 baths of a 5:1 mixture of petroleum ether and chloroform to remove lipids. We estimated the fresh mass of the egg from its length and breadth according to the equation, $M = 0.035 + 0.530 L B^2$; in a sample of eggs whose fresh masses and dimensions were known precisely, the standard deviation of mass about this regression line was 0.12 g (Ricklefs 1983). We calculated the percentage of water in the albumen, the yolk fraction (yolk mass/total mass), and the percentage of lipid of the dry matter in the yolk. Based on a principal components analysis of egg composition, Ricklefs (1983) determined that these were the major components of variation among eggs of European Starlings.

In both years, the incubation period was determined by periodic visits to the nest on the day of hatching. The natural incubation period of the starling is approximately 12 days (Kessel 1957). In 1976, eggs were set between 1200 and 1300; in 1982, most of the experiments were started in mid-afternoon (1500–1700). On the 12th day after the initiation of

incubation, nests were checked approximately every 2 h from 0600 until the eggs hatched. Nests were not checked at night, roughly between 2000 and 0600. Incubation periods were recorded as number of hours deviation from 12 days (288 h) at the time the chick was discovered hatched. For example, an egg set at 1400 and hatched at 1000 on the 12th day was recorded as -4 h. An egg discovered hatched at 0600 could have hatched during the previous 10 h. Similarly, eggs hatched after 2000 and before 0600 of the following day were recorded as having hatched at 0600. Hence, we systematically overestimated the incubation periods for such eggs.

Data were analyzed according to procedures in Sokal and Rohlf (1969) with Statistical Analysis System (SAS) procedures (Helwig and Council 1979). Statistical designs are discussed in the appropriate part of the Results section.

RESULTS

Incubation period.—In 1976, the average of the incubation periods of 45 eggs was -2.31 h (± 3.70 SD, range -6 to $+6$). In 1982, the average of 154 eggs was $+5.87$ h (± 11.63 SD, range -10 h to $+39.5$ h). In 1976, all eggs hatched during the 12th day of incubation. The distribution of hatch times in 1982 is illustrated in Fig. 1. The peak of hatching at $+15$ to $+16$ h represents eggs that were found hatched on the first visit of the second day, hence eggs that may have hatched at any time during the previous night (2000–0600). The single value of $+39.5$ h represents an egg that was found hatched at the first visit on the third day and thus could have hatched any time between about $+29$ and $+39$ h. Assignment of nighttime hatching to the first morning visit in 1982 resulted in our overestimating the average incubation period, probably by 1–2 h.

Laying order.—In 1976, eggs were removed from nests and placed at room temperature

TABLE 1. Analysis of variance in length of incubation period in European Starlings.^a

Source	df	SS	MS	F	P
Experiment	6	6,909	1,151	5.7 ^b	<0.005
Clutches (within experiments)	7	184	26	2.2	0.059
Incubation nests (within experiments)	7	2,640	377	31.4	<0.0001
Error	35	420	12	—	—
Total	55	10,152	185	—	—

^a Seven experiments in which 4 eggs were switched among pairs of nests and 4 were left unswitched. Analyzed as a two-way ANOVA (clutch \times incubation nest) nested within experiments. Clutch \times incubation nest interaction assumed negligible and subsumed in error MS. df = degrees of freedom, SS = sums of squares, MS = mean squares, F = effect MS/error MS, P = probability that F does not exceed 1.

^b The denominator of the F-ratio for the effect of experiments is the Clutches (expt) MS + Nests (expt) MS, or $(184 + 2,640)/14 = 202$; degrees of freedom are 6 and 14.

within a few hours of laying. The length of the incubation period was independent of the order in which the eggs were laid, hence also of the time between laying and incubation [$F(4,40) = 1.29$, $P = 0.29$]. In 1982, eggs 1 through 3 were left in the nest for approximately 24 h before they were removed and placed at room temperature, and incubation period then was significantly related to order of laying [$F(4,149) = 2.79$, $P = 0.029$, $R^2 = 0.07$]. The incubation periods for each of the laying-order classes were 1 = +9.94 h, 2 = +5.35 h, 3 = +5.50 h, and 4 = +3.94 h. A Duncan's Multiple Range test revealed that eggs 1 and 4 differed significantly, but, because laying order accounted for so little of the total variance in incubation period and because switched eggs were assigned at random with respect to laying order, it was not entered as an effect in subsequent analyses.

Most eggs were held at room temperature between 0 and 4 days before they were replaced in nests to begin incubation. In 1982, the length of this period had no effect on the length of the incubation period [$F(5,148) = 1.26$, $P = 0.28$].

Natural- and foster-parent effects.—For the 1976 data, the effects of natural parent (clutch) and foster parent (nest) on the length of the incubation period were determined separately in one-way analyses of variance. Clutch had no effect [$F(12,32) = 1.24$, $P = 0.30$]. Nest, however, did significantly influence the length of the incubation period [$F(10,34) = 5.49$, $P < 0.0001$, $R^2 = 0.62$]. The standard deviation of incubation periods within nests was 2.6 h. The number of nests was smaller than the number of clutches, because two nests were lost during the course of the experiment.

In 1982, because eggs were switched among pairs of nests, we could apply a two-way analysis of variance to the length of the incubation period. From each clutch of the pair, 2 eggs were incubated in the nest of laying and 2 were incubated in the transfer nest. Each experiment involved 8 eggs; degrees of freedom were distributed with 1 for the nest in which the egg was laid (clutch), 1 for the nest in which the egg was incubated (nest), and 5 for error. Seven such experiments were successfully completed; hence, the sample comprised 14 nests and 56 eggs (Table 1). The average incubation period was +7.1 h (± 13.6 SD, range -10 to +39.5, $n = 56$). Within experiments, nest was a large, significant effect, explaining approximately 81% of the variance in incubation period. Clutch was a minor effect of marginal significance [$F(7,35) = 2.20$, $P = 0.059$], explaining only 6% of the variance. The error sum of squares, which accounted for almost 13% of the variance within experiments, represents variation among eggs that were laid in the same clutch and incubated in the same nest. The standard deviation among these eggs was 3.46 h. Most of the variation in this study was among the seven experiments and presumably represented differences among pairs of experimental nests in the quality of parents as incubators or in the microclimate of the nest boxes. Because experiments were a significant source of variation, pairs of switched nests evidently were not chosen at random with respect to factors that influence incubation period.

The total variance in incubation period within the 1982 study was 184.69 h² (13.59 h SD). The variances attributable to differences between clutches and nests were calculated by the expressions

TABLE 2. Size and composition of eggs analyzed in the 1982 study.^a

Variable	<i>n</i> ^b	Mean	SD	CV ^c	1976 (<i>n</i> = 88)	
					Mean	CV
Length (mm)	51	30.04	1.46	4.8	30.08	3.7
Breadth (mm)	52	21.06	0.67	3.2	21.17	2.8
Mass (g)	51	7.11	0.56	7.9	7.19	7.5
Yolk (% egg mass)	48	15.87 ^c	1.76	11.1	17.7	8.5
Lipid (% yolk dry mass)	50	58.02 ^d	2.00	3.5	67.3	—

^a *n* = sample size, SD = standard deviation, CV = coefficient of variation (100 × SD/mean).

^b Total sample = 54 eggs; numbers reduced for each component owing to various processing accidents.

^c Significantly less than 1976 sample, probably due to change in analysis procedure.

^d Significantly less than 1976 sample.

* Values do not differ significantly from 1976 values.

$$V_C = \frac{MS_C - \text{error } MS}{nb}$$

and

$$V_N = \frac{MS_N - \text{error } MS}{na}$$

where *V* is the variance; *MS* the mean square; subscripts *C* and *N* refer to components among clutches and among nests, respectively; *a* is the number of clutches per experiment (2); *b* the number of nests per experiment (2); and *n* the number of replicates per treatment (2). The variance among clutches was 3.59 h²; this is equivalent to a standard deviation of 1.89 h, which, when divided by the average incubation period, gives a coefficient of variation of 0.64%. The variance in incubation period among nests was 91.27 h² (SD = 9.55 h). Hence, the standard deviation attributable to variation among clutches is about one-half the standard deviation within treatments (error), whereas that attributable to variation among nests is almost three times that within treatments.

When the effects of clutch and nest were analyzed in two-way analyses of variance within each experiment separately, clutch was not a significant effect in any of the experiments, whereas nest was significant in 4 out of the 7 experiments. Of these, eggs hatched on the same day in 2 of the experiments and on different days in each of the nests in the other 2 experiments.

Egg composition.—Fifth or sixth eggs were analyzed from 54 switched clutches in 1983. Ricklefs (1983) determined that egg composition can be described by three variables with low intercorrelation: total mass, yolk fraction of the egg, and lipid fraction of the dry matter in the yolk. The values of these variables in the

present study are similar to those reported for another sample of eggs collected in 1976 (Table 2). The yolk fraction was somewhat lower in the present sample than that reported by Ricklefs (1983), primarily because we removed albumen adhering to the surface of the yolk. In addition, the lipid fraction of the dry matter in the yolk was lower in this study than in the earlier sample.

In order to determine the relationship between egg composition and incubation period, we first calculated the average incubation period of each pair of eggs switched to a foster nest, whether the switching experiment was a complete success or not. These averages were entered as the dependent variable in a stepwise regression in which the independent variables were the egg components. This analysis showed that the length of the incubation period was significantly related to the yolk fraction of the egg [$F(1,27) = 7.65$, $P = 0.01$, $R^2 = 0.22$] but to no other variable. The slope of the regression relating incubation period to yolk fraction was 2.72 (± 0.98 SE) h/%. The standard deviation of the incubation period related to yolk fraction was 5.18 h. We performed a similar analysis in which the average incubation period of the pair of eggs transferred to a nest was related to the composition of eggs laid by the incubating parent. In this analysis, the yolk fraction, but no other variable, exerted a significant effect on the length of the incubation period [$F(1,34) = 4.17$, $P = 0.049$, $R^2 = 0.11$]. The slope of the relationship was 2.06 (± 1.01 SE) h/%.

DISCUSSION

In our 1982 sample of 154 eggs, the average incubation period was 12 days 6 h, with a standard deviation of 11.6 h. For a population in

southwest Germany, Hund and Prinzing (1981) reported the average of accurately determined incubation periods of 72 last eggs in unmanipulated clutches to be 12 days 5 h with a standard deviation of 14 h and a range of 11.8–13.3 days. Although the German data do not provide a control for the effects of manipulation in our study, they provide some reassurance that our experimental procedures did not produce uninterpretable results.

Experiments conducted in 1976 indicated that variation in the length of the incubation period was due primarily to characteristics associated with the incubation nest. In that study, the genetic parents of the egg bore no significant relationship to the incubation period. In 1982, with a different experimental design, 81% of the sum of squares within each switching experiment could be attributed to the incubation nest. Of the remainder, 6% was related to the genetic parents (clutch) and 13% to variation within clutch and nest (error). The pattern of variation within switching experiments suggests that most of the variation among experiments was due to differences in incubation behavior among the pairs of parents in each experiment. We did not, however, gather data on egg temperatures or incubation behavior. Furthermore, although nest boxes were of standardized design and construction, their location and orientation may have produced variation in nest microclimate, which we did not measure.

In a larger sample of nests ($n = 29$), incubation period varied in direct relation to the relative size of the yolk but was unrelated to overall egg size. The variation in incubation period related to egg composition (SD = 5.2 h) was on the same order as the variation due to clutch within the switching experiments (3.5 h). This suggests that most of the clutch effect identified in the switching experiments may have resulted from maternal effects associated with the composition of the egg rather than from genetic variation for embryonic growth rate among embryos. In addition, we found that the length of the incubation period was related significantly to the composition of eggs laid by the incubating parent. This variation, which had a standard deviation of 3.8 h, suggests that whatever general qualities of the parent affect incubation also influence the composition of the egg. Although females assume the greater role, both male and female starlings incubate,

and so the male contribution to incubation pattern probably obscures somewhat the relationship between egg formation and incubation behavior of the female.

In the domestic fowl, Crittenden and Bohren (1961) estimated a phenotypic variation in hatching time of 8.22 h with a genetic heritability of 16%. Hence, the standard deviation of hatching time due to genetic factors in the fowl is approximately 3.3 h, a coefficient of variation of approximately 0.65%. The among-clutches standard deviation in our 1982 experiments was 1.9 h (0.64% CV) and therefore of the same magnitude as genetic variation for hatching time in chickens. The relationship of incubation period to yolk fraction observed in our study, however, suggests that maternal effects rather than genetic factors were primarily responsible for the among-clutches variation. We were not able to factor egg composition out of the analysis of the switching experiments, because composition data were not available for several of the clutches used in the experiments. Further resolution of these effects will require additional, larger experiments.

ACKNOWLEDGMENTS

We thank P. Greenwood and L. Kiff for comments on the manuscript. This study was supported by National Science Foundation grants GB42661 and DEB80-21732.

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