

Change in offspring sex ratio over a very short season in Lincoln's Sparrows: the potential role of bill development

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ABSTRACT. The sex ratios of offspring are targets of natural selection that can affect parental energy expenditure and fitness, adult sex ratios, and population dynamics. Parents may manipulate offspring sex ratios based on sex differences in their offsprings' potential for reproductive success. In Lincoln's Sparrows (*Melospiza lincolnii*), male bill shape is associated with the quality of songs, and song quality predicts female preferences in a reproductive context. Males and females that hatch later relative to brood mates or later in the breeding season tend to develop bill shapes that are, for males, associated with low-quality song. Because females do not sing and do not experience this selection pressure, we predicted that the sex of offspring produced late relative to their brood mates or relative to the season should be biased toward females. Using a molecular technique to sex nestlings, we found no effects of hatching order or any interaction between date of clutch initiation (season) and hatching order on offspring sex. However, we found a seasonal decline in the proportion of male offspring, from approximately 0.8 at the beginning to 0.4 at the end of a clutch initiation season only 19 d in duration. To our knowledge, this is the shortest period over which the offspring sex ratio has been shown to change in a bird population. Moreover, these findings are consistent with the hypothesis that sex differences in the potential attractiveness of offspring ultimately influence offspring sex ratios.

RESUMEN. Cambios en la proporción de sexos de pichones de *Melospiza lincolnii* durante un periodo muy corto: el papel potencial que juega el desarrollo del pico

La proporción de sexos de las crías es un parámetro sobre la cual la selección natural puede actuar y cual puede afectar el gasto de energía de los padres y su adecuación biológica, la proporción de sexos de los adultos, y las dinámicas poblacionales. Los padres pueden manipular la proporción de sexos de crías en base a diferencias sexuales en la potencial de éxito reproductivo. En *Melospiza lincolnii*, la forma del pico del macho es asociado con la calidad de los cantos, y la calidad del canto predice las preferencias de la hembra en un contexto reproductivo. Los machos y hembras que eclosionan mas tarde en relación a sus hermanos, o mas tarde durante la época reproductivo tienden a desarrollar formas del pico que son, para machos, asociados con un canto de baja calidad. Por la razón que las hembras no cantan y no experimentan esta presión selectiva, predcimos que el sexo de las crías producido mas tarde en relación a sus hermanos, o mas tarde durante la época reproductiva debería ser mas sesgado hacia hembras. Usando una técnica molecular para sexar a los pichones, no encontramos efectos del orden de eclosionamiento, o una interacción entre la fecha de la iniciación de la puesta y el orden de eclosionamiento en el sexo de las crías. Sin embargo, encontramos una disminución estacional en la proporción de las crías que fueron machos, de aproximadamente 0.8 al comienzo a 0.4 al final de una temporada de iniciación de la puesta de solo 19 días de duración. Por lo que sabemos, este es el periodo mas corto en la cual se ha demostrado que la proporción de sexos ha cambiado en una población de aves. Adicionalmente, estos resultados son consistentes con la hipótesis de que las diferencias sexuales en la potencial fuerza atractiva de las crías últimamente influye en la proporción de sexos de las crías.

Key words: beak morphology, egg laying date, maternal effects, *Melospiza lincolnii*, reproductive effort, sex allocation

The sex ratios of animal offspring are targets of natural selection that can affect parental

energy expenditure and fitness, adult sex ratios, and population dynamics (Schmicki and Karsai 2010). Consequently, there is a strong interest in understanding how prevalent unbalanced offspring sex ratios are and the ultimate (Komdeur and Pen 2002) and proximate (Pike and Petrie 2003, Uller et al. 2007) mechanisms that produce them. Fisher (1930) proposed that, at equilibrium, sex ratios will be balanced when sons and daughters are equally costly to

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produce. He also argued that parents should invest equally in sons and daughters, such that, when rearing costs differ, the sex ratio should equal the inverse ratio of the cost of rearing each sex. Trivers and Willard (1973) proposed a role for a mother's condition on offspring sex ratios. If a mother's condition has an effect on the offspring's condition that persists into adulthood and if condition more strongly affects reproductive success in sons than in daughters, then mothers should produce more sons when in good condition and more daughters when in bad condition. These general ideas can be extended to other situations that affect the reproductive success of one sex more than the other (Clark 1978, Myers 1978, Charnov 1982, Emlen et al. 1986). In particular, offspring sex ratios can be biased toward the sex with more potential for sexual attractiveness and therefore reproductive success (Burley 1981, Ellegren et al. 1996). In this study, we address the potential role of male attractiveness in offspring sex ratios, specifically by asking whether offspring sex ratio is associated with factors related to the development of a particular trait in birds—bill morphology—that recent evidence suggests can be linked with male attractiveness (Podos 2001, Badyaev et al. 2008, Snowberg and Benkman 2009).

Adult birds use their bills for foraging, manipulating food, thermoregulation, defense, preening, and, in altricial species, feeding offspring; offspring use their bills for thermoregulation, defense, and acquiring food (through begging; Welty 1975). As part of the vocal tract, the bill and associated musculature also play a role in modulating sound frequency during singing in songbirds (Podos and Nowicki 2004, Huber and Podos 2006) and, in most songbird species, singing is predominantly by males. Therefore, although both sexes face strong selection pressure on bill morphology through the bill's numerous functions, males in most songbird species face additional selection pressure through the bill's role in singing (Podos 2001, Badyaev et al. 2008).

For male Lincoln's Sparrows (*Melospiza lincolnii*), song quality declines as the ratio of bill height to bill width (hereafter bill shape) decreases from an intermediate shape value (more vertically oval in cross-section) to a low shape value (more horizontally oval in cross-section; Sockman 2009), and song quality affects female preference behavior and presumably male reproductive success (Caro et al. 2010). Nestling

bill shape predicts adult bill shape in Lincoln's Sparrows, and there is a negative relationship between the bill shape of a nestling and the date on which its clutch was initiated, a relationship which persists into adulthood (Sockman, unpubl. data). There is also a negative relationship between bill shape and hatching order in Lincoln's Sparrows (Sockman, unpubl. data). Thus, males hatching later in the breeding season or relative to their nest mates tend to develop bill shapes associated with low-quality song. Although the relationships between bill shape and hatching sequence and season show similar patterns in females and female bill shapes are, on average, indistinguishable from those of males (Sockman, unpubl. data), as is sometimes the case for sexually antagonistic traits (Cox and Calsbeek 2009), female Lincoln's Sparrows do not sing and therefore do not experience this additional selective force. If this particular measure of adult bill shape affects male reproductive success more than female reproductive success, then, taken together, these findings lead to the predictions that the proportion of male Lincoln's Sparrows produced decreases with hatching order and decreases seasonally.

We tested these predictions by examining how offspring sex ratio varies seasonally and with hatching order in Lincoln's Sparrows. As a summer migrant to high-elevation or high-latitude ecosystems, Lincoln's Sparrows have a short breeding season (Ammon 1995, Sockman 2009), necessitating the rapid production, growth, and migration of offspring before conditions become inhospitable. Females in our study population lay clutches of three to five eggs (mode = four eggs; one egg laid per day). At the population level, females initiate clutches over the course of about 4 weeks, roughly corresponding to the month of June. Incubation lasts about 13 d, and young remain in the nest for an additional 8 to 12 d, being fed by both parents before fledging. Documenting a statistically reliable decline in a population's offspring sex ratio over such a short time period requires either a large sample size or an unusually strong seasonal effect that, to our knowledge, is not often observed in birds.

METHODS

Our study was conducted at a site (37.74°N, 107.69°W) near Molas Pass, Colorado, during the 2008 breeding season (June–July). The

study site is a subalpine meadow, approximately 20 ha in area and 3200 m asl. Two species of willows (*Salix glauca* and *S. wolfii*) dominated the woody vegetation and formed the primary substrate where Lincoln's Sparrows nested and foraged.

Data collection. We found nests by searching suitable habitat and observing parental behavior. For the 4 (of 35) nests found during laying, we estimated the date of clutch initiation by subtracting one less than the number of eggs (assuming one laid per day) from the discovery date. For the 24 nests found during incubation, we estimated the date of clutch initiation by subtracting the mean incubation period of nests with known incubation periods and the number of eggs from the hatching date. We found seven nests with nestlings, including two with nestlings <1-d old, two with nestlings ~2-d old, two with nestlings ~3-d old, and one with nestlings ~6-d old. For these, we estimated date of clutch initiation by subtracting the estimated nestling age, the mean incubation period of nests with known incubation periods, and the number of nestlings from the discovery date.

We visited nests twice daily when we expected hatching to occur and recorded hatching order that, for this population of Lincoln's Sparrows, almost perfectly mirrors laying order (Sockman 2008). We were able to determine hatching order based either on the presence of a single new individual or, when multiple eggs had hatched, on overt differences in nestling dampness, a procedure previously validated for this population of Lincoln's Sparrows (Sockman 2008). As eggs hatched, we clipped a unique toenail of each nestling for later identification.

We collected blood samples from Lincoln's Sparrow nestlings via venipuncture 4 d after hatching (except at the one nest found with 6-d-old nestlings, which we sampled 8 d after hatching). We collected blood drops on filter paper (Whatman International Ltd, Maidstone, UK) and froze them until analysis. In the lab, we extracted DNA from each sample using InstaGene matrix (Bio-Rad Laboratories, Hercules, CA). We then used the polymerase chain reaction procedure of Griffiths et al. (1998) to amplify parts of the CHD-Z and CHD-W genes on the Z and W sex chromosomes. We separated the products on an ethidium bromide gel and visualized them under UV light to determine offspring sex.

Analyses. Several nests in our study (10, or 29%) did not have complete hatching success due to embryonic mortality or possible infertility. Using ordinal date (day of year; e.g., 1 January = 1, 29 February = 60, and so on) of clutch initiation as a predictor, we found no evidence for a seasonal change in the probability of a nest's exhibiting hatching failure (logistic regression $\chi^2 = 0.29$, $P > 0.2$). Additionally, we were unable to determine the sex of nine nestlings (7% of all nestlings) that died before blood collection.

We analyzed the effects of hatching order, ordinal date of clutch initiation (season), and the interaction between the two (full model) on the sex of individual offspring. We also examined the effects of hatching order and date of clutch initiation alone in separate models (reduced models). Additionally, each time we included hatching order as a predictor, it was done in two ways. First, we determined if offspring sex depended on hatching order, that is, whether a nestling hatched first, second, third, or fourth (we had no data from fifth-hatched nestlings). We defined this as numerical hatching order. Second, in separate models, we determined if offspring sex depended on whether or not nestlings were first-hatched, middle-hatched, or last-hatched, and we defined this as categorical hatching order. Because brood size in Lincoln's Sparrows typically varies from three to five nestlings, numerical hatching order is not necessarily the same as categorical hatching order. For example, a third-hatched nestling is last-hatched in a three-nestling brood, but middle-hatched in a four- or five-nestling brood (see Sockman [2008] for additional explanation of the rationale for this approach). Both numerical hatching order and categorical hatching order were treated as categorical variables in our analyses (see below). For all nestlings in our data set, we estimated date of clutch initiation. However, we frequently did not know hatching order, meaning that models with hatching order as a predictor had a smaller sample size (56 individuals in 24 broods) than models without hatching order as a predictor (113 individuals in 35 broods).

Due to between-brood variation in the number of nestlings sexed, our data set was unbalanced. In addition, the data set consisted of a combination of fixed (hatching order and ordinal date of clutch initiation) and hierarchically structured random (nestlings nested within

brood) effects, each of which may differ from the others in its correlation structure. Therefore, we used a mixed-model framework (using Stata64 IC 10.1 for the Macintosh, Stata Corporation, College Station, TX) that is readily amenable to unbalanced data sets with combinations of fixed and hierarchically structured random effects (Rabe-Hesketh and Skrondal 2005). Each model used nestling sex as the response variable and ordinal date of clutch initiation, hatching order (numerical or categorical), or both date of clutch initiation and hatching order plus their interaction as predictors. Because nestling sex is a dichotomous variable, we used a mixed-effects logistic regression. In keeping with the recommendations of Schielzeth and Forstmeier (2009), we modeled brood as a random coefficient on hatching order and as a random intercept when hatching order (that varies within broods) was a predictor and as a random intercept when only ordinal date of clutch initiation (that varies between broods) was a predictor. Finally, for all models, we used an unstructured covariance matrix for the random effects. For additional information on applying this mixed, multilevel modeling approach to individuals clustered within broods, see Sockman et al. (2008).

RESULTS

Of 113 sexed nestlings in our study, 63 were male. This proportion (0.56) was not reliably different from 0.5 (constant estimate \pm SE = 0.27 ± 0.24 , $z = 1.1$, $P > 0.2$), according to a mixed-effects logistic regression with only a constant and no predictor and with brood modeled as a random intercept. With one exception, we found no statistically reliable effects of hatching order (numerical or categorical; Fig. 1), date of clutch initiation, or their interaction on the probability of a nestling's being male, regardless of whether the models were reduced or full (as defined in Analyses; Supporting Table S1). The exception was that sex ratio declined with date of clutch initiation when it was the sole predictor in the model (Supporting Table S1, Analysis 4; Fig. 2). Apparently, removing hatching order from our analyses increased the sample size and statistical power sufficiently to reveal a reliable effect. The time over which these clutches were initiated spanned 19 d and, during this time,

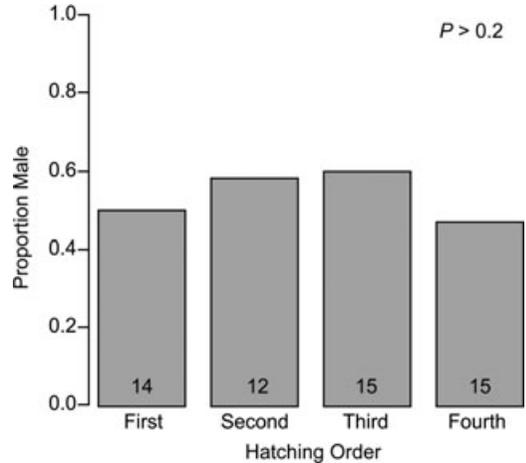


Fig. 1. Proportion of Lincoln's Sparrow nestlings that are male relative to hatching order. The number of offspring at each position is located at the base of the bars.

the predicted proportion of males declined from ~ 0.8 to ~ 0.4 (Fig. 2).

DISCUSSION

Our failure to identify a clear, reliable relationship between hatching order and sex, much less a relationship in the predicted direction, may be due to any of several factors. First, due to our sample size, we had relatively low power for this analysis. Because of this and because sex-biased hatching order is common in birds (e.g., Ankney 1982, Bednarz and Hayden 1991, Badyaev et al. 2002, 2006), it is premature to conclude that there is no relationship between hatching order and offspring sex in Lincoln's Sparrows. Nonetheless, had there been a strong, negative relationship between hatching order and the probability of being male, as we predicted, then it seems we should have detected at least the pattern even with our relatively small sample size. However, not even the pattern was detectable, perhaps because no such relationship exists.

The lack of a relationship between hatching order and offspring sex could be real, perhaps because the effect of bill shape on adult male reproductive success is not particularly strong or because it is strong, but is equally strong in females. Of course, if either of these possibilities were true, we would not have expected a

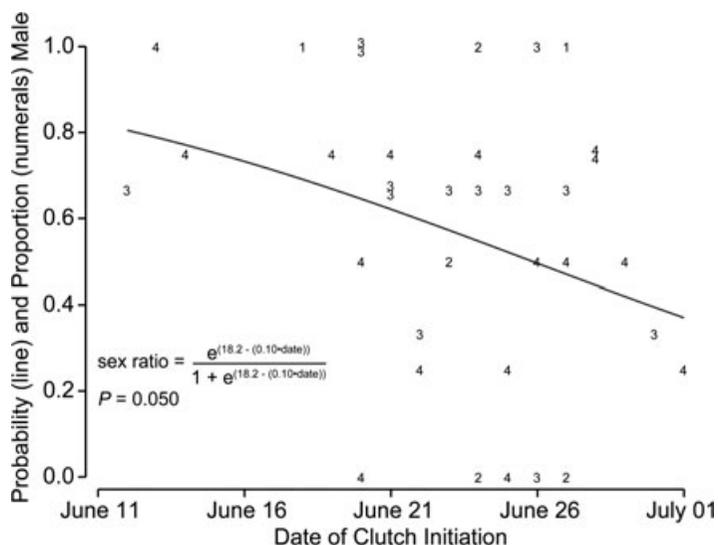


Fig. 2. Seasonal change in offspring sex ratio in Lincoln's Sparrows. The graph illustrates two different relationships. The line is a logistic curve modeling the probability of an individual nestling's being male based on the ordinal date (e.g., 12 June 2008 = 164) that an individual's clutch was initiated. Numerals correspond to individual broods and are plotted according to clutch initiation date and the proportion males in each brood. The value of the numeral indicates the number of nestlings used in that brood to estimate the sex ratio, and it varies due to variation in clutch size, hatching success, and nestling mortality prior to blood collection. Although the logistic curve was not regressed through the plotted numerals, the points through which it was regressed can be extracted from the plotted numerals. For example, the 4 plotted at 13 June and 1.0 (on the abscissa and ordinate, respectively) corresponds to a brood of four males and, therefore, the regression uses four points at 13 June and 1.0. The 3 plotted at 12 June and 0.667 corresponds to a brood of two males and one female, and therefore the regression also uses two points at 1.0 and 12 June and one point at 0 and 12 June.

relationship between date of clutch initiation and offspring sex, but we did find such a relationship. Thus, either other factors must explain the relationship between date of clutch initiation and offspring sex, which is certainly a possibility (see below), or some competing force or forces offset any sex-biased ultimate effects of hatching order on bill shape. For example, although the larger sex in strongly sexually dimorphic species is often more susceptible to variation in condition than the smaller sex (Clutton-Brock et al. 1985, Bortolotti 1986), in slightly to moderately sexually size dimorphic species, the smaller sex can be more susceptible to variation in condition (Rowland et al. 2007) and should therefore be positioned early in the hatching sequence to reduce brood reduction (cf. Howe 1977). Lincoln's Sparrows are slightly to moderately sexually size dimorphic, with males approximately 2–10% heavier than females (Ammon 1995). Thus, selection against the potentially

more susceptible females in the late-hatching position might offset selection against males in that position, giving rise to little if any sex bias in hatching order. Alternatively, there may be nonadaptive sex biases in embryonic or postembryonic mortality (Clutton-Brock et al. 1985) that could offset any female control of sex-biased laying order.

Consistent with one of our predictions, we found reasonable support for a strong seasonal decline in the proportion of offspring that were males, from ~ 0.8 for the earliest produced to ~ 0.4 for the latest, over a clutch-initiation period of only 19 d. Although seasonal changes in offspring sex ratios have been reported previously (e.g., Howe 1977, Blank and Nolan 1983, Dijkstra et al. 1990, Daan et al. 1996, Lessells et al. 1996, Krebs et al. 2002), we are not aware of other studies demonstrating a change in a bird population's offspring sex ratio over such a brief period of time. This is not to say that offspring

sex ratios do not normally change this rapidly. From a statistical perspective, if a population's sex ratio shows a linear change over 50 d (e.g., Dijkstra et al. 1990), then, theoretically, it could also change over any period within those 50 d. Whether or not this can be demonstrated statistically, however, depends on the strength of the change (i.e., the slope) and the sample size. Because our sample size (113 nestlings in 35 broods) was not extremely large, our ability to show a change over just 19 d that was reasonably reliable from a statistical perspective ($P = 0.05$) must be due to the strength of the change (from 0.8 to 0.4).

In Lincoln's Sparrows, bill shape is associated with song quality (Sockman 2009) that influences female choice (Caro et al. 2010), and males produced later in the season may, as adults, experience lower reproductive success because they are less likely to have bills suited for producing songs that attract females. The rapid seasonal decline in the proportion of male offspring in our study is consistent with the idea that parents primarily produce the sex whose reproductive success would be least negatively impacted by laying date. Nonetheless, a number of alternative explanations are also plausible. For example, in some species, females may vary offspring sex ratios in response to sex differences in several sources of offspring mortality that we did not investigate. These include mortality induced by sex differences in susceptibility to ectoparasites (Bize et al. 2005, Badyaev et al. 2006), offspring mortality induced by sex differences in immune function regulated by pre- and posthatching exposure to androgen hormones (Müller et al. 2005), or offspring mortality induced by sex differences in susceptibility to various types of ambient conditions (e.g., Kalmbach et al. 2005, Eiby et al. 2008).

Alternatively, perhaps the emphasis should be on the seasonal increase in female production, instead of a seasonal decrease in male production, if, as in some raptors (Daan et al. 1996), reproductive success of females is somehow reduced when they are produced early in the season. Still another possible explanation for the seasonal change in offspring sex ratio is that there is seasonal change in the mortality of male compared to female offspring, as has been found in mammals (Neuhaus and Pelletier 2001, Kraus et al. 2008). Young Lincoln's Sparrows produced early in the breeding season are exposed to different ambient environmental conditions than

those produced later in the season (Sockman 2008, 2009) and, if females are more susceptible to conditions early and males late in the season, a seasonal decline in the proportion of males might be expected. Additional studies will be needed to identify and distinguish between these and other possibilities.

Future studies may also contribute to understanding the reliability and generalizability of our results. For example, our study spanned only one breeding season and different results are possible if not likely in different years, especially in subalpine ecosystems where environmental conditions are highly variable from year to year (Morton 2002, Sockman 2009). Still, even if these results are specific to some, but not other years, that possibility does not diminish their potential importance. In Lincoln's Sparrows, the proportion of male offspring can decline from 0.8 to 0.4 over a period of just 19 d, if only in some years. This phenomenon is consistent with expected parental strategies to maximize the attractiveness and therefore reproductive success of offspring.

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Supporting Information

The following supporting information is available for this article online:

Table S1. Results from mixed-effects logistic regressions of offspring sex on hatching order and date of clutch initiation in Lincoln's Sparrows.

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Supporting Table S1. Results from mixed effects logistic regressions of offspring sex on hatching order and date of clutch initiation in Lincoln's Sparrows.

Analysis		Standard		
Predictor	Estimate	error	z score	P value
1. Effects of date of clutch initiation, numerical hatching order, and their interaction [56/24]				
intercept	37.47	50.00	0.75	> 0.2
date	-0.21	0.28	-0.75	> 0.2
order 1:2	58.88	86.53	0.68	> 0.2
order 1:3	-46.01	58.92	-0.78	> 0.2
order 1:4	35.23	75.22	0.47	> 0.2
date × order 1:2	-0.33	0.49	-0.68	> 0.2
date × order 1:3	0.26	0.33	0.79	> 0.2
date × order 1:4	-0.20	0.43	-0.48	> 0.2
2. Effects of date of clutch initiation and numerical hatching order [56/24]				
intercept	29.16	22.97	1.27	> 0.2
date	-0.16	0.13	-1.27	> 0.2
order 1:2	0.40	1.02	0.39	> 0.2
order 1:3	0.51	1.24	0.41	> 0.2
order 1:4	-0.42	1.40	-0.30	> 0.2
3. Effects of numerical hatching order [56/24]				
intercept	-0.02	0.88	-0.02	> 0.2
order 1:2	0.46	1.05	0.44	> 0.2
order 1:3	0.75	1.29	0.58	> 0.2
order 1:4	-0.17	1.42	-0.12	> 0.2
4. Effects of date of clutch initiation [113/35]				
intercept	18.22	9.19	1.98	0.047
date	-0.10	0.05	-1.96	0.050

5. Effects of date of clutch initiation, categorical hatching order, and their interaction [56/24]

intercept	32.70	45.83	0.71	> 0.2
date	-0.19	0.26	-0.71	> 0.2
order first:middle	14.04	53.22	0.26	> 0.2
order first:last	4.48	64.33	0.07	> 0.2
date × order first:middle	-0.08	0.30	-0.25	> 0.2
date × order first:last	-0.03	0.37	-0.07	> 0.2

6. Effects of date of clutch initiation and categorical hatching order [56/24]

intercept	39.62	33.02	1.20	> 0.2
date	-0.22	0.19	-1.20	> 0.2
order first:middle	0.65	1.19	0.55	> 0.2
order first:last	-0.28	1.44	-0.20	> 0.2

7. Effects of categorical hatching order [56/24]

intercept	0.01	0.73	0.02	> 0.2
order first:middle	0.92	1.12	0.83	> 0.2
order first:last	-0.02	1.26	-0.01	> 0.2

The hierarchical nesting structure of each model is indicated in brackets around the number of offspring, followed by the number of broods in which offspring is nested as a random effect. Offspring sex was coded 0 for female and 1 for male. Date of clutch initiation was quantified as the ordinal date of clutch initiation (i.e., January 1st = 1, February 29th = 60, etc.). Order 1:2, 1:3, 1:4, first:middle, and first:last are, respectively, the contrasts between a hatching order of first (coded 0) and second, third, fourth, middle, and last (each coded 1).
