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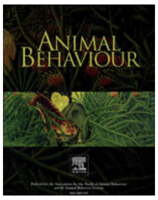

Variation in the onset of incubation and its influence on avian hatching success and asynchrony

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Variation among species in the onset of incubation has been attributed to differences in life history traits, and variation within species has been related to individual and environmental factors. We quantified within- and among-species variation in the onset of incubation in five cavity-nesting passerines, using a continuous record of diurnal and nocturnal incubation from clutch initiation through completion. We documented 11 potential patterns for the onset of incubation and showed that onset patterns were significantly related to hatching success. The onset of diurnal partial incubation and nocturnal full incubation generally occurred before diurnal full incubation, which started around clutch completion. Increases in precipitation or wind speed significantly delayed most types of incubation onset, supporting predictions of the energy constraints hypothesis. Ancillary predictions of rain and wind disproportionately delaying incubation for aerial foragers, and for species with male feeding during incubation, were not upheld. Larger clutch size accelerated the timing of full incubation onset in diurnal and nocturnal full incubation, supporting predictions of the egg viability hypothesis. Predictions of both hypotheses for the effects of minimum temperature, proportion of time above 24 °C, and seasonality on incubation onset were not supported. We observed egg neglect for up to 4 days or nights; neglect was more common at night, and consecutive nights of neglect occurred during nocturnal full incubation. Egg neglect did not significantly affect hatching success or incubation period. In conclusion, the timing of incubation onset was strongly affected by environmental and individual factors, and patterns of incubation onset affected hatching success.

Parental care has important consequences for individual fitness and life history trade-offs, but factors that influence variation in the onset of parental care are poorly understood (Clutton-Brock 1991). In birds, parental care begins with incubation and its onset potentially affects the clutch size that females lay, hatching asynchrony and success of eggs, subsequent brood reduction of young, and risk of predation to offspring and adults (Stoleson & Beissinger 1995, 1999; Cooper et al. 2005). The onset of incubation is highly variable, occurring from the first egg to after the last egg of a clutch has been laid (Clark & Wilson 1981; Stoleson & Beissinger 1995). Among-species variation in the onset of incubation has been attributed to many factors, such as differences in developmental mode, nest type, uniparental versus biparental incubation, nest failure and environmental effects on egg viability (Stoleson & Beissinger 1995; Hebert 2002; Cook et al. 2005b). Individual variation in the onset of incubation is a hallmark of the relatively few studies that have analysed intraspecific patterns. Within species, differences in the onset of incubation have been related to individual factors such as age, experience and body condition (Bortolotti & Wiebe 1993; Hanssen et al. 2002; Ardia & Clotfelter 2007), and to environmental factors such as temperature, precipitation and food supply during egg laying (Nilsson 1993; Nilsson & Svensson 1993; Wiebe & Bortolotti 1994).

Standardized criteria for describing the patterns of incubation onset that can accommodate interspecific and intraspecific variation are sorely needed. The onset of incubation has been commonly interpreted as the commencement of a steady incubation rhythm, or full incubation, but partial incubation (i.e. attentiveness during laying) appears to be common (Haftorn 1981; Loos & Rohwer 2004). Wiebe et al. (1998) qualitatively categorized trajectories of individual attentiveness during laying in the American kestrel, *Falco sparverius*, into three main types of incubation onset patterns that were related to female body condition and nestling survival: rising, steady and pulsed incubation. Grenier & Beissinger (1999) decomposed the rising pattern into slowly rising and rapidly rising for the green-rumped parrotlet, *Forpus passerinus*. More variation in patterns of onset undoubtedly occurs. Differences may exist between diurnal and nocturnal patterns of onset, in the timing of

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incubation onset during laying (e.g. the egg in the laying sequence with which full incubation begins) and in incubation fidelity.

Here we develop a classification system that includes all likely patterns of the onset of incubation in birds, and provide a method to distinguish among the patterns. We apply it by quantifying within- and among-species variation in the onset of incubation in five cavity-nesting passerines, based on a continuous record of diurnal and nocturnal incubation from clutch initiation through clutch completion. The study species are single-sex intermittent incubators that nest in similar or identical habitat, overlap in breeding season and clutch size, and experience similar environmental conditions (Table 1). They differ in nest construction, foraging mode and whether the male feeds the female during incubation (Table 1).

Predictions for intra- and interspecific differences in the onset of incubation reflect the multiple, complex biological phenomena that can create the wide variety of avian hatching patterns (Stoleson & Beissinger 1995). We predicted that the timing of incubation onset might be: (1) accelerated by ambient temperatures above physiological zero if earlier-laid eggs need protection from fluctuating ambient temperatures (egg viability hypothesis: Arnold et al. 1987; Stoleson & Beissinger 1999; Beissinger et al. 2005) or if warm ambient temperatures lead to increased food availability (energy constraints hypothesis: Nilsson & Svensson 1993); (2) delayed by cold weather because eggs can remain in stasis below physiological zero (egg viability: Fasenko 2007) or because of increased energetic costs of self-maintenance (energy constraints: Nilsson & Svensson 1993); and (3) delayed for eggs laid earlier in the season, which are less likely to experience temperatures above physiological zero (egg viability) and are more likely to experience cold weather (energy constraints). In predictions unique to one hypothesis, the egg viability hypothesis predicts (4) earlier onset of incubation for individuals or species laying larger clutches if they need to protect earlier-laid eggs from mortality as a result of fluctuating ambient temperatures (Arnold et al. 1987), while the energy constraints hypothesis predicts (5) delayed onset of incubation during rain for insectivorous species and (6) delayed onset of incubation during windy conditions for aerial foragers.

Finally, we relate patterns of incubation onset, timing of incubation onset and egg neglect to fitness outcomes through effects on hatching success, hatching asynchrony (spreads) and incubation (i.e. development) periods. We hypothesize that monotonically rising patterns of onset, which previously have been linked to better adult condition, result in higher hatching success (Wiebe et al. 1998), that earlier onset of incubation results in larger hatching spreads (Nilsson 1993; Nilsson & Svensson 1993; Wiebe et al. 1998; Clotfelter & Yasukawa 1999) and that egg neglect results in decreased hatching success (Sockman & Schwabl 1998) and extended incubation periods (Boersma & Wheelwright 1979; Gaston & Powell 1989).

Table 1
Life history characteristics of the five study species

Life history trait	TRES	VGSW	OATI	WEBL	ATFL
Incubating sex	F	F	F	F	F
Male feeds incubating female	No	No	Yes	No	Yes
Foraging style	Aerial	Aerial	Gleaning	Perch-forage	Low sallying
Clutch initiation date ($\bar{x}\pm$SD)	133±14.7	154±8.21	91.3±17.0	121±21.7	147±14.9
Nest material	Grass	Grass	Moss	Grass	Fur
Nest-lining material	Feathers	Feathers	Wool	Grass	Fur
No. of first clutches	169	121	50	305	32
Clutch size ($\bar{x}\pm$SD)	5.1±0.93	4.3±0.76	6.9±0.97	5.1±0.82	4.0±0.86
Clutch size range	2–7	3–6	4–8	2–7	3–5
No. of hatched first clutches	100	75	34	175	25
Hatching spread (days; $\bar{x}\pm$SD)	0.74±0.72	0.87±0.72	0.79±0.54	0.76±0.73	0.52±0.51

TRES: tree swallow, *Tachycineta bicolor*; VGSW: violet-green swallow, *Tachycineta thalassina*; OATI: oak titmouse, *Baeolophus inornatus*; WEBL: western bluebird, *Sialia mexicana*; ATFL: ash-throated flycatcher, *Myiarchus cinerascens*. Clutch initiation dates are reported in Julian days. Information on incubating sex (F = female), male feeding, foraging style and diet was obtained from Birds of North America species accounts (Brown et al. 1992; Robertson et al. 1992; Cicero 2000; Guinan et al. 2000; Cardiff & Dittmann 2002).

METHODS

Study Site and Data Collection

The study was performed from 2003 to 2007 at the Hopland Research and Extension Center in Mendocino County, California (39°00′ N, 123°04′ W) using 159–216 nestboxes in seven study plots that were located in sheep pastures and oak woodland along an elevation gradient from 150 to 915 m. The top- or front-opening wooden boxes were mounted on wooden or metal fence posts, metal poles, or trees.

Nestboxes were checked between 0800 and 1300 hours. Checks occurred every 3 days until the nest cup was formed, and then daily until the clutch size remained the same for 3 consecutive days. Eggs were marked and weighed on the day of laying. Checks for hatching were done daily, starting with the 10th day after clutch completion and ending when every egg's fate had been determined as hatched, disappeared, depredated, or failed to hatch. Hatching spreads were calculated for each nest as the number of days between hatching of the first and last young. Hatching spread was denoted as 0 if all eggs in a clutch hatched within 24 h, 1 if hatching lasted 24–48 h, and 2 if hatching lasted more than 48 h.

Incubation was monitored using temperature loggers in western bluebird, *Sialia mexicana* (abbreviated WEBL, $N = 26$), tree swallow, *Tachycineta bicolor* (TRES, $N = 17$), violet-green swallow, *Tachycineta thalassina* (VGSW, $N = 10$), oak titmouse, *Baeolophus inornatus* (OATI, $N = 12$), and ash-throated flycatcher, *Myiarchus cinerascens* (ATFL, $N = 8$), nests from the laying of the first egg until 4–6 days after clutch completion (~11.5 days total). Thermistors were secured into the bottom of nest cups and connected to temperature data loggers (Stowaway XTI, Onset Corporation, Bourne, MA, U.S.A.) installed below the nestbox. Nest-cup temperature was recorded to the nearest 0.2 °C every 30 s. Because first days with <4 h of data were removed from the analysis, data for seven nests started on the day after clutch initiation; analysis of these nests proceeded under the assumption of no diurnal incubation on the day of clutch initiation.

Environmental conditions were monitored locally. Ambient temperature and relative humidity were recorded every 5 min using data loggers (HOBO H8 Pro, Onset Corporation, Bourne, MA, U.S.A.) installed in each plot at a shaded location at nestbox height. Daily precipitation and wind speed were recorded at two California Irrigation Management Information Stations (http://www.cimis.water.ca.gov/cimis/infoStnSensorSpec.jsp) permanently located on the Hopland Research and Extension Center (no. 85, Hopland FS, and no. 106, Sanel Valley) at 354 and 160 m above sea level, respectively. Wind speed was measured by an anemometer every 60 s (accurate to 1.5% or 0.11 m/s) and reported as daily averages. Daily precipitation was measured by a tipping-bucket rain gauge with 0.1 mm resolution.

Inferring Incubation Bouts and the Onset of Partial Incubation

The software programs BoxCar Pro (version 4.3, Onset Corporation, Bourne, MA, U.S.A.), Raven version 1.2 and Rhythm (Cornell

Laboratory of Ornithology, Ithaca, NY, U.S.A.) (Cooper & Mills 2005) were used to mark incubation on- and off-bouts from nest-cup temperatures collected by loggers. The criteria for the beginning or end of on-bouts was a change of at least 2 °C within an interval of 0.5–10 min. Bout selections were also visually examined for substantial differences from concurrently recorded ambient temperature. In a few nocturnal records, thermistor temperature fell gradually with declining ambient temperature, then flattened at ≥8 °C above ambient. Such intervals were considered on-bouts, following Figure 1 in Clotfelter & Yasukawa (1999).

Bouts were assigned to day or night occurrence according to the civil sunrise and civil twilight for each date at the study site's latitude and longitude (U.S. Naval Observatory, http://aa.usno.navy.mil/faq/docs/RST_defs.html), and the proportion of each day or night incubated was calculated. The onset of partial incubation was defined as the first day or night in which nest attendance totalled at least 1 h. One hour is a conservative threshold that accommodates the observation that nest attendance during egg laying often exceeds 30 min (Wang & Weathers 2009).

We calculated two measures of the onset of incubation relative to laying stage. The first measure was in relation to the day of clutch completion, which was assigned to 0, so that negative numbers indicate the number of days before clutch completion and positive numbers indicate the number of days after clutch completion (Loos & Rohwer 2004). This measure allowed comparison of the onset of incubation across different clutch sizes. Clutch completion could have occurred on one of two consecutive days in six nests; in these cases the date of clutch completion was assigned to the mean of the two serial dates, resulting in day and night intervals ending in 0.5. The second measure was the number of days elapsing between clutch initiation and the onset of incubation, which indicates the maximum length of exposure to ambient conditions that the first-laid egg would have experienced, and is related to clutch size.

Verification of Inferred Incubation

Over 117 h of externally videotaped observation at 33 nests ($\bar{x}$ = 3.5 h per nest) with temperature loggers were used to test the accuracy of inferring nest attendance from temperature measurements. During diurnal full incubation, the mean ± SD percentage of time that females spent incubating as calculated from the temperature loggers was 46.1 ± 17.6%, which was slightly but significantly lower than the percentage of time that females spent incubating as determined from cameras, 51.8 ± 15.9% (Wilcoxon signed-ranks test: $Z = 3.28$, $N = 33$, $P < 0.001$). Cameras recorded an average of 2.4 ± 5.2 more on-bouts (paired t test: $t_{32} = 2.70$, $P = 0.011$) and 2.4 ± 5.0 more off-bouts (paired t test: $t_{32} = 2.73$, $P = 0.010$) than reported from the temperature data. Differences between the two methods may have occurred because a bird inside a nestbox may not necessarily have been incubating, which would result in overestimation of incubation by the cameras. Temperature data are more conservative for inferring incubation than external video cameras, and the small differences between methods of inferring incubation did not appear to alter the rest of our analyses.

Determining the Onset of Full Incubation

We assigned the onset of full incubation for each individual by comparing its daily, arcsine-transformed proportion of time spent incubating to a nest-specific lower-limit criterion of incubation after clutch completion. Correlations among daily or nightly attendance at each nest were controlled in a random-coefficient model using the MIXED procedure in SAS (version 9.1.3, Cary, NC, U.S.A.) with species as a fixed effect, the nest identifier as a random effect, and a pre-specified error-covariance structure across days or nights within the same nests (Loos & Rohwer 2004). This method provides more flexibility than repeated measures ANOVA by allowing the error-covariance structure to vary (Diggle et al. 2004; Fitzmaurice et al. 2004). Random-coefficient models with an autoregressive error-covariance structure better described the diurnal and nocturnal incubation data than either random effect autoregressive models (ΔAICc = 9.7 and 5.2 for diurnal and nocturnal, respectively) or random effect compound symmetry models (ΔAICc = 48.8 and 21.0 for diurnal and nocturnal, respectively) (Burnham & Anderson 2002). Information criteria are recommended for determining the best-fitting covariance structures among repeated measures mixed models. This step is necessary before making inferences from such models (Littell et al. 2006).

We obtained empirical Bayes estimates of each nest's lower 90% confidence interval of the mean proportion of the day or night incubated after clutch completion. We defined the onset of full incubation as the first day or night that the proportion of incubation reached or exceeded each nest's 90% lower confidence interval. Within- and among-species variation in the onset of incubation was investigated with random effects models having the day or night of onset as the dependent variable and the nest identifier as a random effect nested within species. The intraclass correlation coefficient, or the proportion of total variation accounted for by within-species variation, was calculated for each of the four measures of incubation.

Modelling Variation in the Timing of Incubation Onset

We evaluated whether variation in the timing of incubation differed between species, clutch size, clutch initiation date and Julian date of clutch initiation. Clutch size and clutch initiation date were standardized into normal deviates from species-specific yearly means. Species interactions with clutch size and Julian date of clutch initiation were also included to test whether species responded differently to changes in standardized clutch size or seasonality.

Four weather variables were chosen to address the egg viability and energy constraints hypotheses for the onset of incubation. The proportion of time at or above 24 °C before the onset of incubation indicates how long the first-laid egg is exposed to temperatures above physiological zero (Beissinger et al. 2005). The minimum temperature signifies the lowest nocturnal temperature to which the first-laid egg is exposed. Minimum temperature was included for its possible effects on egg viability at the lower extreme of temperatures, even though it was significantly correlated with the proportion of time that temperatures were at or above 24 °C (Pearson product–moment correlations: diurnal partial: $r = 0.701$; diurnal full: $r = 0.720$; nocturnal partial: $r = 0.621$; nocturnal full: $r = 0.656$; all $P < 0.05$). Total precipitation and maximum daily average wind speed were also included because they relate to the adversity of foraging conditions. Species interactions with precipitation and wind speed were included to test predictions for species differences in foraging style and diet.

Two life history variables were also chosen to directly test predictions of the energy constraints hypothesis: whether species were aerial foragers and whether males feed females on the nest during incubation (Table 1). Precipitation and wind were predicted to disproportionately delay incubation for aerial foragers, and male feeding was predicted to reduce the effect of precipitation and wind on incubation delays.

Weather data were summarized for each nest attempt in 24 h intervals starting with the morning of clutch initiation. For diurnal full incubation, weather data ended with the 24 h interval prior to the onset of full incubation. For diurnal partial incubation, weather data ended on the day of the onset of partial incubation, because partial incubation sometimes started on the day of clutch initiation

(9.6% of nests). Likewise, for nocturnal partial and full incubation, weather data ended with the 24 h interval in which incubation began, because nocturnal partial and full incubation commonly started on the night of clutch initiation. Three nests had incomplete or unavailable weather data on the relevant dates and were assigned weather data from the nearest study plot, 1.5–2 km away.

The onset of full incubation was measured relative to the day or night of clutch completion, and the onset of any incubation was measured relative to the day or night of clutch initiation. ANCOVA with stepwise variable selection was performed for the onset of partial and full incubation with individual and environmental variables using the GLMSELECT procedure in SAS, with an effect entry and stay significance threshold of 0.15. All models included a species effect and used GLM coding for classification variables, with western bluebird as the reference species.

We recognize that phylogenetic relatedness is a source of nonindependence among the five species that we examined. In behavioural traits, which are thought to be more labile than morphological traits, nonindependence may also occur indirectly if more closely related species are more likely to share a similar habitat or niche (Freckleton et al. 2002). However, our sample size of five species was too small to apply phylogenetically independent contrasts and is unlikely to have a detectable phylogenetic signal because of the low power of comparative methods (Freckleton et al. 2002). High intraspecific variation in the timing of incubation onset would also result in extremely low power to detect a phylogenetic signal (Harmon & Losos 2005). Our data set of individual trait values allows partitioning of total variance into across-species and within-species components; the within-species component is comparable to Lynch's (1991) nonphylogenetic component (including nonadditive genetic effects, environmental effects and measurement error).

Modelling the Hatching Outcomes of Incubation Onset Patterns and Egg Neglect

The relationships between species, incubation onset patterns and groups of incubation onset patterns were examined using chi-square with exact *P* values (SAS version 9.2 User's Guide, March 2008). To assess differences in hatching success across grouped incubation onset patterns, species and laying order, we used the generalized estimating equations (GEE) approach to logistic regression to model per-egg hatching success with nest as a cluster variable and an exchangeable correlation structure within nests, using SAS PROC GENMOD. This approach provides parameter estimates, group means and standard errors that are robust to within-nest associations of hatching success (Fitzmaurice et al. 2004). The nonparametric correlation between hatching spread and the timing of onset of incubation was assessed with Kendall's tau-b for all nests with hatching spreads known to the nearest day ($N = 42$).

For each egg's date of laying until clutch completion, we derived measures of neglect, exposure and ambient temperature. We defined egg neglect as any day or night after the onset of partial incubation where less than 1 h of incubation was performed and summed the number of days and nights that incubation was neglected. We calculated the average proportion of day and night incubated and the average of daily average temperatures. We also counted the number of days between the date of laying and the onset of full diurnal incubation, roughly approximating the length of exposure to ambient conditions. Laying order was created as a class variable with the following values: first, second, middle, penultimate, and last. The second egg in three-egg clutches was assigned to the penultimate laying order, following the common practice in the literature of labelling incubation onset with the second of three eggs as occurring with the penultimate egg. In four-egg clutches the second and third eggs were assigned to the second and penultimate laying orders, respectively. In clutches of five or more eggs, any eggs in between the second and penultimate eggs were assigned to the middle laying order.

To determine whether egg neglect affected hatching success, we used GEE logistic regression and a within-nest exchangeable correlation structure to model each egg's hatching success ($N = 55$ nests, 278 eggs). In addition to egg neglect, days exposed before full incubation, average daytime temperature and laying order, we tested the following variables: species, clutch size, standardized clutch size, Julian date of clutch initiation and standardized date of clutch initiation. We proceeded with forward stepwise model selection with an enter-and-stay significance threshold of 0.15. We used GLM coding for the species effect, with western bluebird as the reference species.

To determine whether egg neglect affected incubation periods, we used mixed model ANCOVA in the MIXED procedure with nest as a random effect and a within-nest autoregressive error structure ($N = 34$ nests, 123 eggs). We calculated incubation periods by summing the proportions of 24 h periods incubated from laying through clutch completion and adding them to the number of days between hatching and clutch completion, not including the day of clutch completion. We tested the same factors as for hatching success using forward stepwise selection and an enter-and-stay significance threshold of 0.15.

RESULTS

Patterns of the Onset of Incubation

Potential patterns of the onset of incubation are more diverse (Fig. 1) than previously characterized. Onset patterns can be classified first by whether or not they rise monotonically after initiation, and second by whether or not they showed flat portions with a constant level of incubation. Finally, patterns can be differentiated by the speed and direction of change.

These characteristics lead us to identify 11 potential patterns for the onset of incubation and all except one were observed in our study (Fig. 1). In addition to the 'Rapid Rise', 'Slow Rise', 'Pulsed' and 'Irregular' patterns described previously (Wiebe et al. 1998; Grenier & Beissinger 1999), there were patterns with multiple irregularities, which we labelled 'W-shape', and patterns with a gradual decrease in incubation followed by a gradual increase, which we named 'U-shape'. We also observed steady, nonzero incubation at a level less than that attained during full incubation, which was classified as 'Step', 'Flat-rise', or 'Flat-fall' depending on the direction and timing of change. Finally, nests could show constant incubation immediately upon laying of the first egg, which was classified as 'Flat', and, conceivably, nests could show declining incubation throughout laying, which was classified as 'Falling'.

Diurnal patterns of onset varied significantly among species ($\chi^2_{24} = 48.2$, $P = 0.002$), but species' differences in nocturnal patterns were less apparent ($\chi^2_{36} = 47.7$, $P = 0.092$). The most common patterns of diurnal incubation onset were Step for the tree swallow, Slow Rise for the violet-green swallow and ash-throated flycatcher, and Flat-rise for the oak titmouse and western bluebird (Fig. 1). There were no nests with diurnal patterns of W-shape, Flat-fall, Falling, or Flat. The most common nocturnal patterns, regardless of species, were Rapid Rise, Irregular and U-shape (Fig. 1).

The patterns of full incubation onset (Fig. 1) were arranged into four groups that encompassed the general characteristics of uninterrupted rising (Rising), interrupted rising (Irregular Rising), high followed by low levels of incubation (Not Rising), and a consistently high level of incubation (Flat). Diurnal incubation onset groups

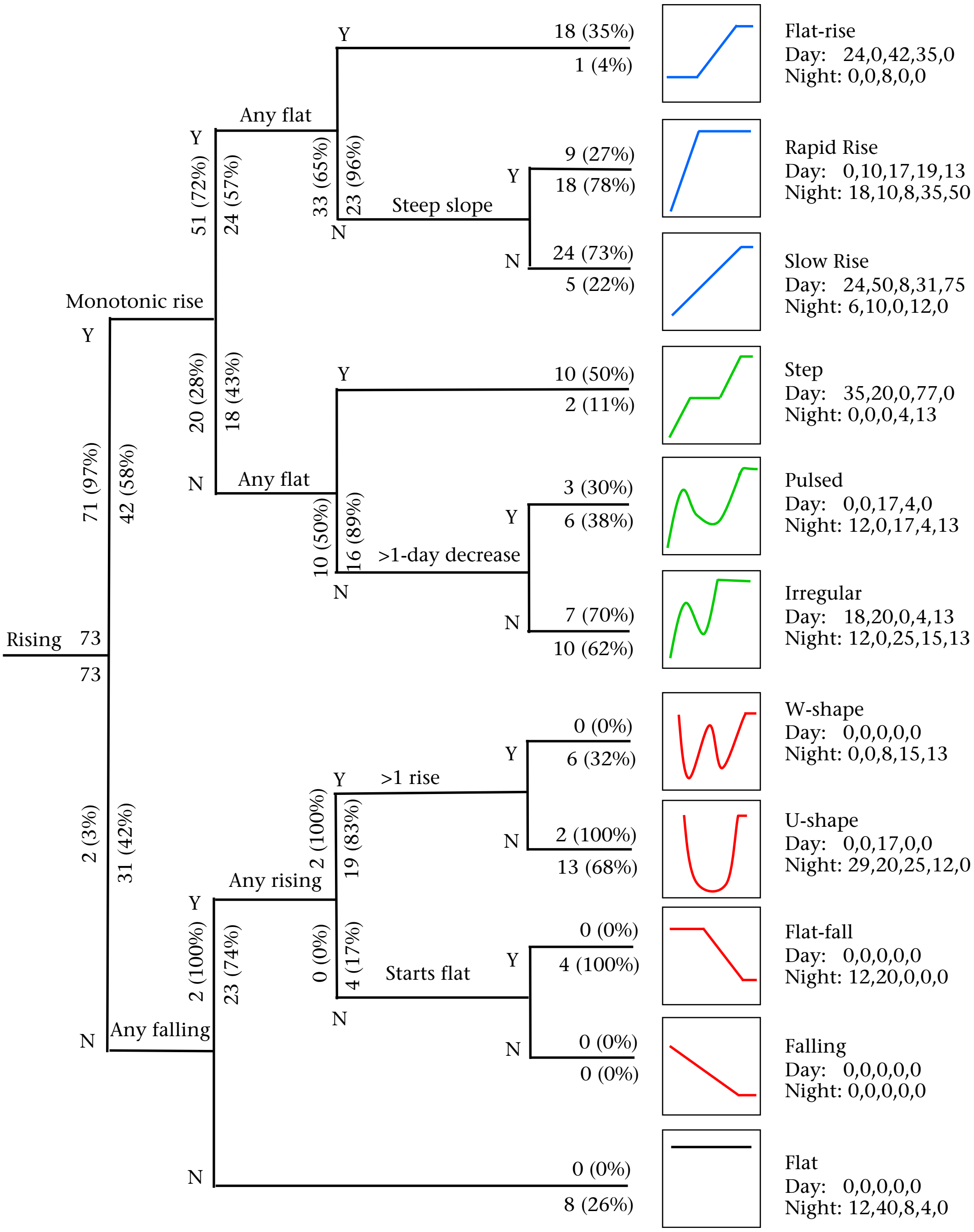

Figure 1. General patterns for characterizing the onset of full incubation based on whether or not incubation rises monotonically, whether the rise occurs continuously, and the speed and duration of rises. For all patterns, the *X* axis is days or nights up to the completion of laying or on the second consecutive day or night after full incubation (whichever came later) and the *Y* axis is the proportion of the day or night incubated. The numbers above and below each fork are the diurnal and nocturnal sample size and percentage of nests, respectively. The numbers to the right of each pattern are the diurnal and nocturnal percentages of nests for each species, in taxonomic order: tree swallow, violet-green swallow, oak titmouse, western bluebird and ash-throated flycatcher. The patterns were categorized into four groups based on similarity: Rising (blue), Irregular Rising (green), Not Rising (red) and Flat (black). The data examined for each nest started with the last day or night having 1 h or less of incubation (all flat sections shown are nonzero proportions). The Irregular and Pulsed patterns could have one or more decreases in incubation.

differed significantly between species ($\chi^2_8 = 19.9$, $P \leq 0.011$). The majority of western bluebird and ash-throated flycatcher diurnal patterns fell in the Rising group, with the remainder in the Irregular Rising group (Fig. 2). Diurnal patterns of the two swallow species were split between the Rising and Irregular Rising groups. The oak titmouse diurnal patterns were distributed among the Rising, Irregular Rising and Not Rising groups (Fig. 2).

Nocturnal onset groups differed marginally between species ($\chi^2_{12} = 20.2$, $P \leq 0.064$). The most common groups were Rising and Not Rising, although Irregular Rising and Flat were also well represented (Fig. 2). Within species, the most frequent nocturnal onset groups were Not Rising for the tree swallow, Irregular Rising for the oak titmouse, Not Rising and Flat for the violet-green swallow, and Rising for the western bluebird and ash-throated flycatcher (Fig. 2).

We tested whether the diurnal or nocturnal onset groups displayed by each nest depended on weather during the laying period, including the day and night of clutch completion. Multinomial logit models with three diurnal outcomes (Rising, Irregular Rising, Not

Rising) and four nocturnal outcomes (Rising, Irregular Rising, Not Rising, Flat) were used to model the effects of species, minimum temperature, total precipitation, maximum daily average wind speed and proportion of time ≥24 °C. None of the factors significantly affected the diurnal or nocturnal onset groups (all $P > 0.19$).

Thresholds for the Onset of Incubation

Example nests from each species show the thresholds for the onset of partial and full diurnal and nocturnal incubation (Fig. 3). The onset of diurnal partial incubation generally occurred before diurnal full incubation, but nocturnal partial incubation often started concurrently with nocturnal full incubation in individuals that began nocturnal incubation above the threshold of full incubation (Fig. 4). This reflects a trend for nocturnal incubation to be initiated at a relatively high level that exceeded the nest-specific threshold for full incubation (e.g. VGSW and ATFL panels in Fig. 3). While the range of diurnal full incubation onset values straddled the day of clutch completion, the vast majority of nocturnal full incubation began before the night of clutch completion (Fig. 4).

Among-species variation in incubation onset was overshadowed by within-species variation. Species-specific lower 90% confidence intervals of incubation after clutch completion (Table 2) did not consistently predict the onset of full incubation at all nests. For all four measures of the onset of incubation, within-species variation accounted for the majority of within- and among-species variation (Fig. 4) (intraclass correlation coefficient: diurnal partial, 0.712; diurnal full, 0.735; nocturnal partial, 0.654; nocturnal full, 0.832).

Egg Neglect

Days or nights where less than 1 h of incubation was performed after the onset of partial or full incubation were surprisingly common (Fig. 5). During the day all species except the violet-green swallow neglected eggs after partial incubation, and the oak titmouse and western bluebird also neglected eggs in the daytime after full incubation was achieved. Diurnal neglect lasted for 1 day in most nests (77%), but one oak titmouse nest was neglected for 4 days during partial incubation and two oak titmouse nests were neglected for 4 and 5 days, respectively, during full incubation. Nocturnal neglect was seen in the tree swallow, oak titmouse and western bluebird during partial incubation, and in all species during full incubation. Multiple nights of neglect were common, occurring in 14–25% of nests (Fig. 5). Incubation was neglected for consecutive days and nights at one tree swallow nest and four oak titmouse nests (mean = 1.6, range 1–3 24 h periods). All consecutive days and nights of neglect started before clutch completion, and four started at night. Three of the nests with consecutively neglected days and nights had multiple bouts of neglect, two of them occurring after clutch completion.

Table 2
Species-specific least-squares means for the proportion of time incubated after clutch completion

Period	Species	N	LS mean	Lower 90% CI
Diurnal	Tree swallow	17	0.556	0.502
	Violet-green swallow	10	0.518	0.453
	Oak titmouse	12	0.611	0.545
	Western bluebird	26	0.512	0.468
	Ash-throated flycatcher	8	0.588	0.512
Nocturnal	Tree swallow	17	0.775	0.676
	Violet-green swallow	10	0.961	0.893
	Oak titmouse	12	0.945	0.862
	Western bluebird	26	0.949	0.902
	Ash-throated flycatcher	8	0.878	0.759

Least-squares means and lower 90% confidence intervals were backtransformed from arcsine square-root estimates from a random-coefficient model with an autoregressive error-covariance structure.

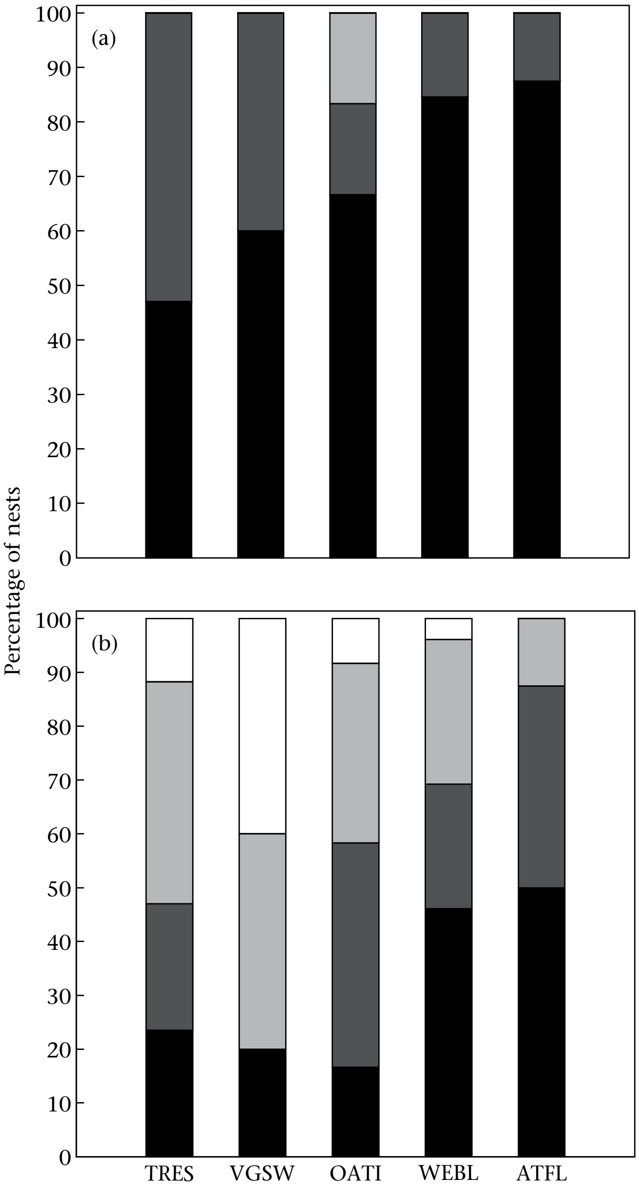

Figure 2. Distribution of the percentage of nests in (a) diurnal and (b) nocturnal full incubation onset groups that showed Flat (□), Not Rising (▒), Irregular Rising (▓) and Rising (■) incubation patterns. Species are presented in taxonomic order: TRES: tree swallow; VGSW: violet-green swallow; OATI: oak titmouse; WEBL: western bluebird; ATFL: ash-throated flycatcher.

Do Environmental and Individual Factors Explain Variation in Timing of the Onset of Incubation?

The onset of both partial and full incubation was influenced by weather (Table 3). Increases in wind speed or precipitation significantly delayed the onset of all four measures of incubation. Each 1 m/s increase in wind delayed partial incubation by 0.79 days and 0.82 nights and delayed full incubation by 0.59 days and 1.54 nights. The effect of wind was significant for all measures of incubation except diurnal full ($P = 0.076$). Similarly, each 1 mm increase in total precipitation delayed partial incubation by 0.03 days and 0.05 nights, and full incubation by 0.01 days and 0.04 nights. Precipitation delayed the onset of nocturnal partial incubation

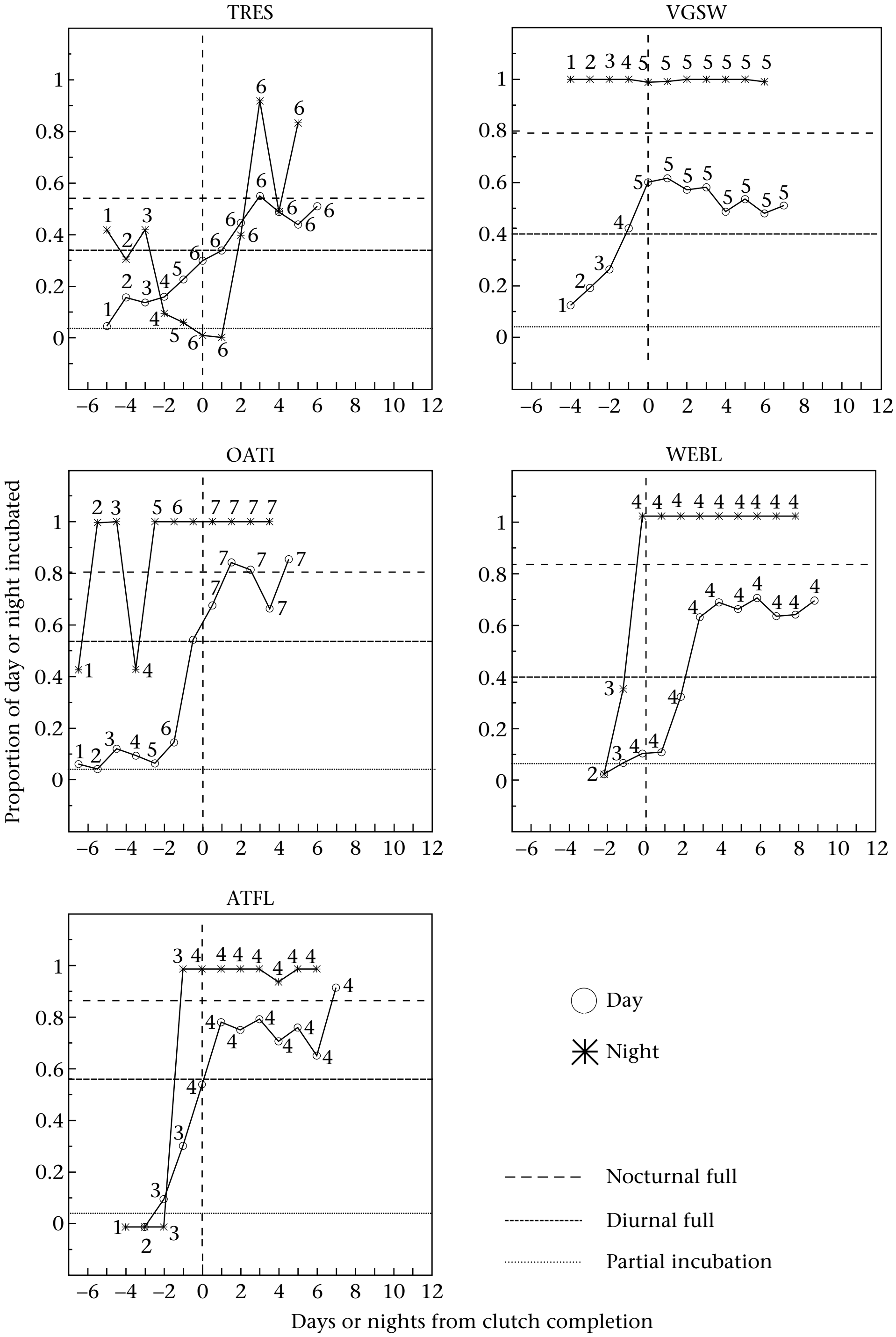

Figure 3. Example incubation plots of the study species. Numeric labels next to observed values show the number of eggs in the nest on each day or night; clutch size is the maximum label value. The dashed vertical lines denote the day or night of clutch completion. The lower, middle, and upper dashed horizontal lines denote the 1 h threshold for the onset of partial incubation and the nest-specific thresholds for the onset of diurnal and nocturnal full incubation, respectively. Species are labelled as follows: tree swallow: TRES; violet-green swallow: VGSW; oak titmouse: OATI; western bluebird: WEBL; ash-throated flycatcher: ATFL. The diurnal and nocturnal full incubation onset patterns represent common patterns shown by each species. In order from diurnal to nocturnal, they are Step and U-shape (TRES), Slow Rise and Flat (VGSW), Flat-rise and Irregular (OATI), Flat-rise and Rapid Rise (WEBL), and Slow Rise and Rapid Rise (ATFL). Monitoring at the WEBL nest started on the day the second egg was laid. The day of clutch completion for the OATI nest was assigned to the midpoint of days 7 and 8.

significantly more for the tree swallow and oak titmouse than for the western bluebird (Table 3). An average 24 h period of rain during the study period (mean ± SD 8.8 ± 10.1 mm, range 1–60 mm) would delay the onset of incubation by 0.12–0.41 days or nights. An increase in the proportion of time $\geq$24 °C significantly delayed the onset of nocturnal partial and full incubation. Every 10% increase in the proportion of time $\geq$24 °C delayed partial incubation by 0.19 nights and full incubation by 0.36 nights. Of the nest-level effects, an increase in clutch size significantly accelerated the onset of full incubation but not partial incubation. For each standard deviation above a species' average yearly clutch size, the onset of full incubation was earlier by 0.41 days and 0.58 nights.

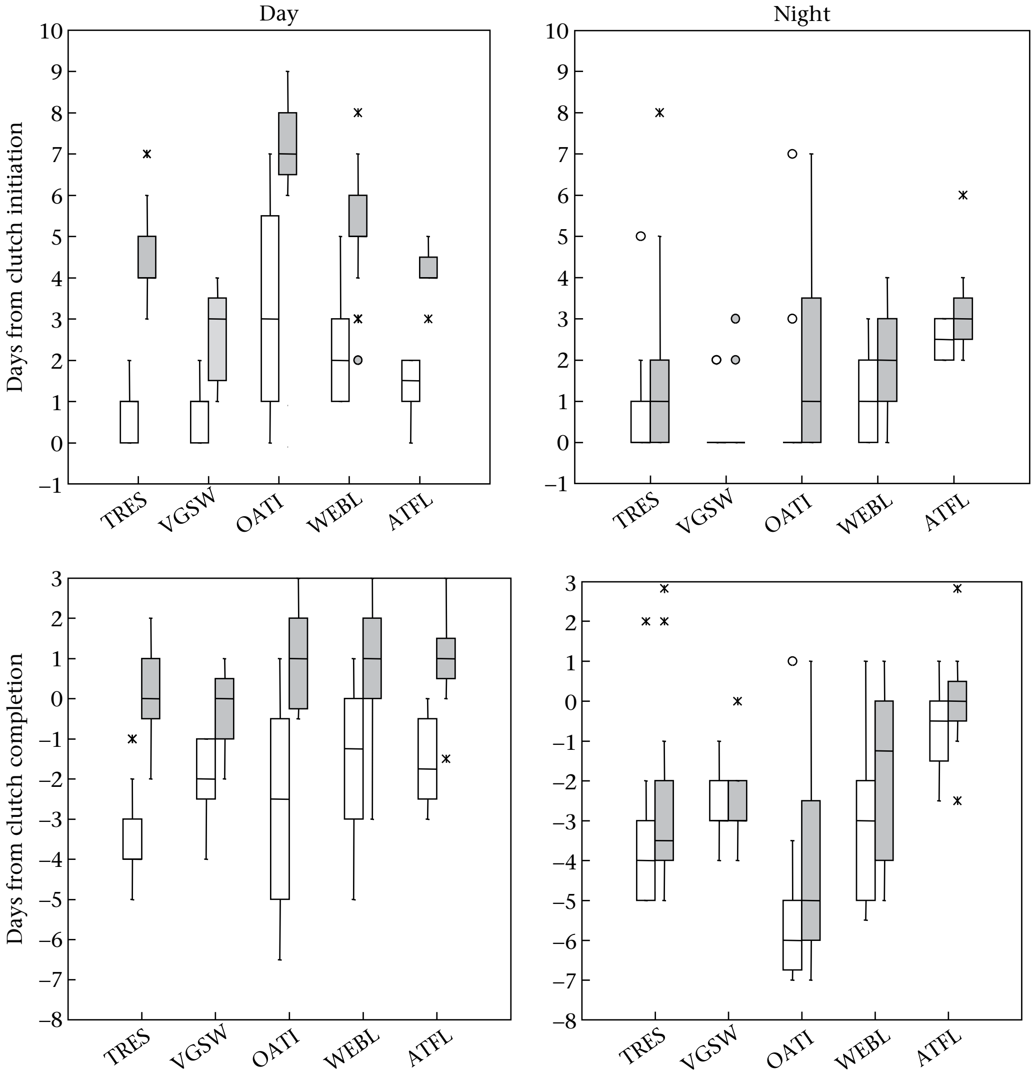

Figure 4. The onset of partial and full incubation grouped by species for day and night, relative to clutch initiation or clutch completion. Species are labelled as follows: tree swallow: TRES ($N = 17$); violet-green swallow: VGSW ($N = 10$); oak titmouse: OATI ($N = 12$); western bluebird: WEBL ($N = 26$); ash-throated flycatcher: ATFL ($N = 8$). Open boxes refer to the onset of partial incubation; shaded boxes refer to the onset of full incubation. Horizontal lines within boxes indicate the median. Top and bottom edges of the boxes indicate the first and third quartiles, respectively. Whiskers encompass observed values falling within 1.5 times the interquartile range from the nearest box edge. Asterisks and open circles are observed values falling between 1.5 and 3 times the interquartile range and beyond 3 times the interquartile range, respectively.

Minimum temperature, clutch initiation date, aerial foraging, male feeding, and all other species interactions did not influence any measure of the onset of incubation.

Does Variation in the Onset of Incubation and Egg Neglect Affect Hatching Success or Hatching Asynchrony?

Both diurnal and nocturnal groups of full incubation onset (Fig. 1) affected hatching success (GEE type 3 score statistic: diurnal group $\chi^2_1 = 5.55$, $P = 0.019$; nocturnal group $\chi^2_1 = 8.00$, $P = 0.046$). Uninterrupted patterns of diurnal incubation had higher hatching success than interrupted patterns; in the subset of clutches that were incubated to term ($N = 55$), mean hatching success was lower for the diurnal group Irregular Rising than for Rising (GEE pairwise differences of least-squares means: $\chi^2_1 = 6.53$, $P = 0.011$), after controlling for differences between species (Fig. 6). Oak titmouse eggs had significantly higher hatching success than most other species after step-down Bonferroni adjustment (GEE pairwise differences of least-squares means: tree swallow: $\chi^2_1 = 7.83$, $P = 0.041$; violet-green swallow: $\chi^2_1 = 14.37$, $P = 0.002$; western bluebird: $\chi^2_1 = 12.09$, $P = 0.005$; ash-throated flycatcher: $\chi^2_1 = 4.99$, $P = 0.179$); only one of 63 oak titmouse eggs in nine clutches failed to hatch. Least-squares means of hatching success among nocturnal incubation groups did not significantly differ after Bonferroni adjustment (Fig. 6). Laying order within the clutch did not affect hatching success (GEE type 3 score statistic: $\chi^2_1 = 5.08$, $P = 0.280$).

The timing of incubation onset did not significantly affect hatching spread. In clutches where hatching spread was known to the nearest 24 h, hatching spread was not related to the onset of diurnal or nocturnal partial incubation (Kendall's tau-b $= -0.078$, $N = 42$, $P = 0.569$ and tau-b $= -0.104$, $N = 42$, $P = 0.464$, respectively), or with the onset of diurnal or nocturnal full incubation (Kendall's tau-b $= 0.033$, $N = 42$, $P = 0.808$, and tau-b $= -0.135$, $N = 42$, $P = 0.306$, respectively). Hatching spread did not significantly differ between species ($\chi^2_8 = 4.92$, $P = 0.766$).

Egg neglect did not significantly affect egg-specific hatching success, after controlling for species (GEE type 3 score statistic: neglect: $\chi^2_1 = 2.13$, $P = 0.145$; species: $\chi^2_4 = 11.88$, $P = 0.018$; Table 4). Neither days of exposure before full incubation nor average daytime temperature significantly affected the probability

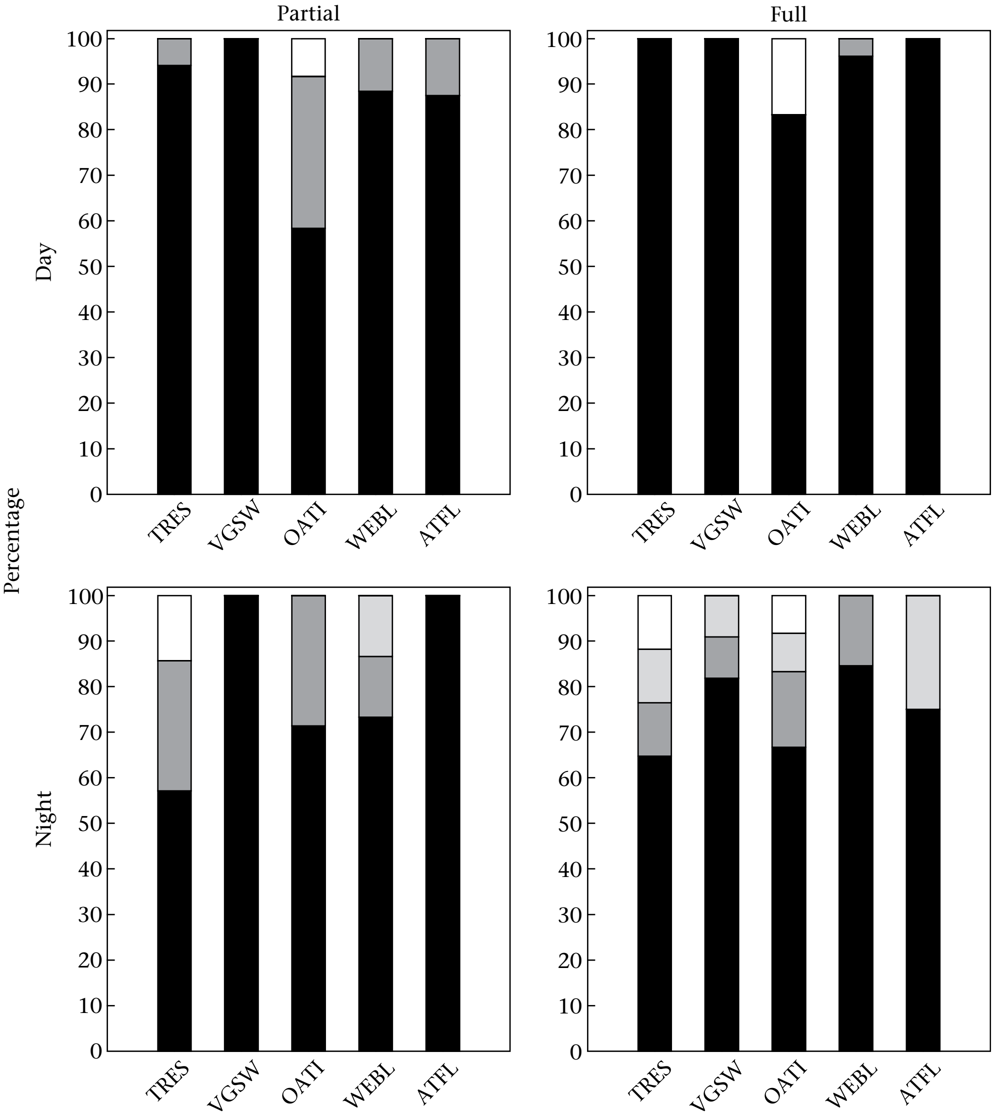

Figure 5. Percentage of nests per species where incubation was skipped (1 h or less) after the onset of partial or full incubation. The duration of skipping was categorized as 0 days (■), 1 day (▩), 2–3 days (▨) and 4–7 days (□).

of hatching, and these parameters were not included in the final model. Laying order, clutch size and date of clutch initiation were also not included in the final model for hatching success.

Incubation period was significantly negatively correlated with the Julian date of clutch initiation when laying order was controlled for (type 3 tests: Julian date: $F_{1,33} = 18.74$, $P < 0.001$; laying order: $F_{4,75} = 2.55$, $P = 0.046$; Table 5). Each day's delay in clutch initiation led to a 0.8 h decrease in incubation period. Incubation periods were shorter for penultimate eggs compared to first and second eggs (Tukey–Kramer test: $P = 0.037$, 0.042, respectively) (Table 5). Neglect, days of exposure, and all other factors did not significantly affect incubation periods and were not included in the final model.

DISCUSSION

In vertebrates with internal fertilization and development, parental care begins immediately upon fertilization and the subsequent birth of offspring occurs synchronously within a relatively short period of time compared to the time required for development (e.g. most mammals, and some fishes, lizards and snakes; Stoleson & Beissinger 1995; but see While & Wapstra 2008). Synchronous reproduction also occurs in animals with external fertilization, where all zygotes are subject to similar environmental conditions during development (e.g. many insects, anurans and fishes). In contrast, avian eggs are laid externally and typically need to be warmed by a parent to develop. Thus, birds have the potential to control the hatching (birthing) intervals through the onset of incubation, and hatching patterns range from highly asynchronous, if incubation begins upon the laying of the first egg, to synchronous, when incubation starts on or after the last egg (Clark & Wilson 1981; Stoleson & Beissinger 1995). It is not clear, however, to what extent variation in the onset of incubation reflects benefits to offspring and costs to parents, or constraints imposed by environmental limitations, endogenous factors and phylogenetic history (Stoleson & Beissinger 1995). Understanding has been slowed by the lack of a classification system that includes all likely patterns of the onset of incubation, and because few studies have quantified incubation from laying of the first to the last egg. We have attempted to address both shortcomings by presenting a general framework for classifying incubation onset patterns (Figs 1–4), by testing whether these patterns were related to environmental or individual conditions (Table 3), and by determining whether fitness

Table 3
Stepwise regression results of partial and full incubation onset with individual and environmental factors ($N = 73$ nests)

Onset	Parameter	Diurnal estimate	$P\leq$	Nocturnal estimate	$P\leq$
Partial	Intercept	−0.031	0.969	−1.287	0.013
	TRES	−0.913	0.020	−0.455	0.091
	VGSW	−1.110	0.014	−0.737	0.024
	OATI	1.064	0.015	−0.152	0.613
	ATFL	−0.587	0.216	1.557	0.001
	WEBL	0	—	0	—
	Proportion≥24°C	—	—	1.881	0.006
	Precipitation	0.030	0.001	0.047	0.001
	Wind speed	0.785	0.014	0.815	0.001
	Precipitation*TRES	—	—	0.080	0.009
	Precipitation*VGSW	—	—	0.126	0.406
	Precipitation*OATI	—	—	0.068	0.001
	Precipitation*ATFL	—	—	−0.070	0.087
	Precipitation*WEBL	—	—	0	—
Full	Intercept	−0.987	0.269	−6.631	0.001
	TRES	−0.367	0.314	−0.292	0.599
	VGSW	−0.830	0.059	−0.411	0.515
	OATI	0.291	0.486	−0.994	0.102
	ATFL	0.156	0.748	1.681	0.015
	WEBL	0	—	0	—
	Clutch size	−0.413	0.005	−0.583	0.006
	Proportion≥24°C	—	—	3.590	0.015
	Precipitation	0.014	0.032	0.043	0.004
	Wind speed	0.589	0.076	1.544	0.001

TRES: tree swallow, *Tachycineta bicolor*; VGSW: violet-green swallow, *Tachycineta thalassina*; OATI: oak titmouse, *Baeolophus inornatus*; WEBL: western bluebird, *Sialia mexicana*; ATFL: ash-throated flycatcher, *Myiarchus cinerascens*.

Table 4
Forward stepwise model selection results of GEE logistic regression for the effect of egg neglect on hatching success ($N = 55$ nests, 278 eggs)

Parameter	Estimate	$P\leq$	Odds ratio	Probability[*]
Intercept	1.482	0.001		
Total neglected days/nights	0.413	0.126	1.511	0.057
TRES	−0.755	0.179	0.470	−0.104
VGSW	−0.884	0.130	0.413	−0.122
OATI	2.379	0.026	10.79	0.329
ATFL	1.619	0.113	5.048	0.224
WEBL	0		1	
Alpha[†]	1.063	0.005		

TRES: tree swallow, *Tachycineta bicolor*; VGSW: violet-green swallow, *Tachycineta thalassina*; OATI: oak titmouse, *Baeolophus inornatus*; WEBL: western bluebird, *Sialia mexicana*; ATFL: ash-throated flycatcher, *Myiarchus cinerascens*.

[*] Probabilities were calculated using the overall probability of hatching, 0.8345, and the equation for the slope of the logistic curve: $\partial p/\partial x_i = \beta \times 0.8345\,(1 - 0.8345)$. For the neglect variable, this represents the change in probability of hatching with a 1-day or 1-night increase in neglect. For the species variables, it represents the change in probability of hatching when compared to the western bluebird.

[†] The shape parameter for the binomial distribution.

outcomes were related to patterns of incubation onset, timing of incubation onset and egg neglect (Fig. 6, Tables 4, 5).

Variation in the Onset of Incubation

We present a general framework for characterizing variation in the onset of incubation both within and among species (Fig. 1). Standardized criteria for describing 11 a priori patterns of incubation onset are based on whether or not incubation rises monotonically, whether periods of plateau or decrease occur, and the rate of change in incubation. Nests were categorized into these patterns by comparing incubation throughout the laying period to the criteria outlined in Fig. 1. Classification could also be achieved by fitting and comparing a set of competing linear and nonlinear functions to the incubation onset data using methods of model selection (Burnham & Anderson 2002) if sufficient data were available. To our surprise, 10 of the 11 potential patterns were shown in our study. Moreover, we combined patterns into useful onset groups that reflected general trends (Rising, Irregular, Not Rising and Flat) and affected fitness outcomes (Fig. 6). We believe this to be a more insightful approach to describing the onset of incubation than simply reporting the egg in the laying sequence with which diurnal incubation in a clutch is first detected, or whether eggs hatch synchronously or asynchronously.

The onset of incubation was highly variable both within and among the five passerines we studied, yet broad trends emerged when comparing the patterns of diurnal and nocturnal onset. Nocturnal onset was much more likely to start earlier than diurnal incubation and at a higher level of incubation, with subsequent periods of decreasing incubation (e.g. Flat-fall, U-shape, W-shape in Fig. 1). Partial incubation, in which the amount of incubation during the laying period is less than that during full incubation, was more likely to occur during the day. Different diurnal and nocturnal patterns also occurred within the onset groups. Within the Rising group, Rapid Rise was more common at night, while Slow Rise and Flat-rise were more common during the day (Fig. 1). Within the Irregular Rising group, Step was more common in the daytime, and Pulsed and Irregular were more common at night.

Contrary to life history accounts listing species-specific days for the onset of incubation, we found a large degree of intraspecific variation (Fig. 4). Diurnal partial incubation had not been documented in the violet-green swallow, oak titmouse, western bluebird, or ash-throated flycatcher, although it has been seen in the few passerines that have been continuously monitored during

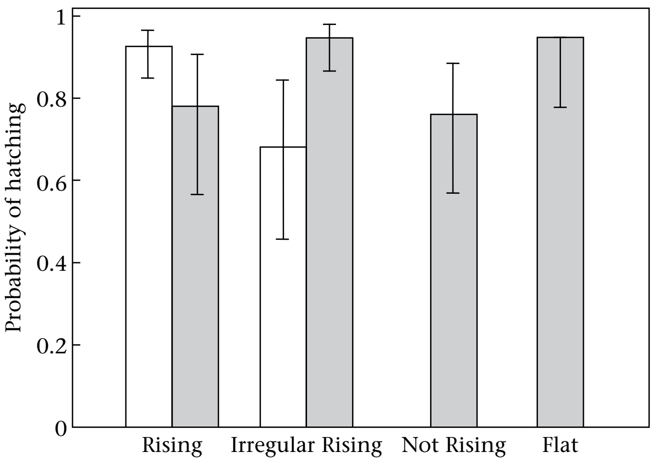

Figure 6. Hatching success across onset groups of diurnal (□) and nocturnal (▨) full incubation, as the probability of an egg hatching (least-squares means and 95% confidence intervals were backtransformed from logit values). Values were obtained after controlling for interspecific differences and within-nest associations in hatching success.

Table 5
Forward stepwise model selection results of mixed model ANCOVA for effects on incubation period ($N = 35$ nests, 126 eggs)

Parameter	*df*	Estimate	$P\leq$
Intercept	33	19.21	0.001
Julian date of clutch initiation	33	−0.034	0.001
Laying order: first	75	0.383	0.046
Laying order: second	75	0.301	0.074
Laying order: middle	75	0.079	0.630
Laying order: penultimate	75	−0.094	0.455
Laying order: last		0	

Least-squares means ± SE by laying order (in days): first: 15.61 ± 0.26; second: 15.53 ± 0.25; middle: 15.30 ± 0.26; penultimate: 15.13 ± 0.26; last: 15.23 ± 0.26.

laying (Holcomb 1974; Zerba & Morton 1983; Morton & Pereyra 1985; Anderson 1997).

We also found large variation in nocturnal incubation during the laying period, in contrast to other studies (Morton & Pereyra 1985; Anderson 1997; Grenier & Beissinger 1999). Part of this variation may be due to egg neglect during some evenings and from incomplete nest attendance at night. The latter may occur if the adult stands over the eggs during nocturnal rest, which was observed in a nearby population of western bluebirds (Wang & Weather 2009) and has been documented during laying in other cavity-nesting species (Haftorn 1978, 1979, 1981). Standing over eggs rather than incubating them would decrease hatching asynchrony. Alternatively, birds could roost in the nest cup without incubating to reduce their own thermoregulatory costs (Pendlebury & Bryant 2005). Others have reported periods of nest temperatures elevated above ambient but below incubation temperature at night during egg laying, with varied interpretations (Wiebe et al. 1998; Clotfelter & Yasukawa 1999).

Causes of Variation in the Timing of Incubation Onset

Intra- and interspecific differences in the onset of incubation reflect the interaction of multiple, complex biological phenomena that create the wide variety of avian hatching patterns. At least 17 hypotheses for the evolution of avian hatching patterns have been identified, including factors that affect the survival of eggs, nestlings and parents (Stoleson & Beissinger 1995). We concentrated on examining environmental constraints affecting parental behaviour and egg viability that might account for variation in the onset of incubation in five passerines.

We found direct evidence that inclement weather delays incubation onset. Previous tests of the energy constraints hypothesis used supplemental feeding to show that food limitation delays onset (Nilsson 1993; Nilsson & Svensson 1993), while the mechanism of inclement weather causing food shortages was often assumed. Extreme weather events can result in neglect during full incubation (Morton & Pereyra 1985), but evidence that poor weather delays incubation onset separately from other energetic constraints, such as wing-clipping (Slagsvold & Lifjeld 1989), has been lacking. The ancillary predictions of rain and wind disproportionately delaying incubation for aerial foragers and for species with male feeding during incubation were not supported. Inclement weather may universally increase the costs of self-maintenance relative to better weather conditions.

Predictions of the egg viability and energy constraints hypotheses for the effects of cold temperature, warm temperature and seasonality on the onset of incubation were not supported. Diurnal incubation onset was not affected by warm temperatures, while nocturnal onset was delayed by warmer temperatures, in direct contrast to predictions of the egg viability hypothesis (Ardia et al. 2006). It is possible that once partial incubation has begun, eggs are largely resistant to fluctuations in ambient temperature. Egg neglect in Cassin's auklets, *Ptychoramphus aleuticus*, does not affect hatching success when restricted to the first third of the incubation period, when embryonic metabolic rates are lower (Astheimer 1991), and neglect is restricted to the early incubation period in other alcids (Murray et al. 1983; Gaston & Powell 1989). Detailed examination of passerine embryonic developmental stages achieved during partial incubation, and their hardiness to temperature fluctuations, would provide additional tests of this hypothesis. Regular nocturnal nest desertion of waterbird colonies during predation may not strikingly affecting hatching success (Nisbet 1975; Chardine & Morris 1983).

The onset of incubation begins earlier in larger clutches of many species (Lessells & Avery 1989; Magrath 1992). We found that increases in clutch size significantly accelerated the onset of full incubation relative to clutch completion but did not affect partial incubation prior to clutch completion. Clutch size may be a proxy for individual quality or condition, where better-quality individuals are able to facultatively start incubation earlier, while the onset of partial incubation may be more tightly regulated by hormonal mechanisms (Mead & Morton 1985; Hebert & Sealy 1992). However, clutch size had no effect on incubation onset in another study of the tree swallow (Ardia & Clotfelter 2007). In summary, there seems to be more support for constraints on adult behaviour than on egg viability for the onset of incubation in our study species in the dry, moderate temperatures of northern California.

Fitness Outcomes of Early Incubation and Egg Neglect

Implicit in studies examining the evolution of hatching asynchrony is the expectation that variation in the amount and the onset of incubation will affect fitness in the form of hatching success, and provides parental control of development times and hatching spreads (Stoleson & Beissinger 1995). Yet, hatching success and development times can be unrelated to variation in the onset of incubation (Bortolotti & Wiebe 1993).

Patterns of incubation onset affected hatching success in the five passerines we studied. Nests in which the daily increase in incubation proportion was interrupted had decreased hatching success (Fig. 6). Hence, nests in the Rising group may be viewed as having more optimal onset patterns than nests in the Irregular Rising group. Wiebe et al. (1998) found that females with 'pulsed' patterns of incubation onset had significantly poorer body condition than females with 'rising' or 'steady' patterns. An interruption of the rising pattern could be due to intrinsic (adult condition) or extrinsic (environmental) factors, but is probably the result of interplay between both. Although hatching success in our study was significantly related to nocturnal nest attendance, we found no differences between nocturnal onset groups (Fig. 6).

An early onset of incubation did not contribute strongly to embryonic development in our study species. Hatching spreads were not significantly related to the onset of diurnal, nocturnal, partial, or full incubation. Partial incubation or early nocturnal incubation may act to maintain egg viability (Fasenko 2007). In poultry, incubation of chicken eggs for 6 h and of turkey eggs for 12 h before a 14-day storage period significantly lessens the reduction in hatchability (Fasenko et al. 2001a,b). Pre-storage incubation achieved complete hypoblast formation in embryos of both species, suggesting this stage of development may be more able to withstand developmental arrest (Fasenko 2007). On the other hand, partial incubation may be the external manifestation of a gradual hormonal build-up of prolactin in the incubating adult (Sockman & Schwabl 2001), or may serve to catalyse antibiotic defences in avian eggs (Cook et al. 2005a,b; Shawkey et al. 2008). It is not clear whether partial incubation is an evolutionarily selected behaviour or a corollary of necessary physiological change (Hebert & Sealy 1992).

The most extreme instance of day-to-day variability in incubation was the phenomenon of egg neglect prior to and after clutch completion (Fig. 5). Usually defined as the interruption of incubation for 1 day or more, egg neglect regularly occurs in procellariiforms and alcids (Astheimer 1991) and has been infrequently documented in Passeriformes (Morton & Pereyra 1985), Falconiformes (Sockman & Schwabl 1998) and Podicipediformes (Nuechterlein & Buitron 2002). In species where it commonly occurs, egg neglect has resulted in lengthened incubation periods but does not decrease hatching success (Boersma & Wheelwright 1979; Boersma et al. 1980; Gaston & Powell 1989), suggesting that embryos of these species are resistant to extended cooling periods during incubation. Although neglect usually occurs at ambient temperatures well below physiological zero (Boersma & Wheelwright 1979; Lill 1979; Gaston & Powell 1989), egg neglect increased incubation periods in the Xantus' murrelet, *Synthliboramphus* (*Endomychura*) *hypoleucus*, but decreased the number of days that eggs were incubated (Murray et al. 1983), suggesting that ambient temperatures at that site supported continued embryonic development.

Egg neglect did not significantly affect hatching success or incubation periods in our study species (Tables 4, 5). These results support the idea that wild eggs may require fluctuations in temperature to develop (Robinson et al. 2008). Our finding that advances in laying date are correlated with shorter incubation periods has also been demonstrated in temperate-nesting species (Cooper et al. 2005), although the correlation between laying date and incubation period may differ between species (Feldheim 1997) or interact with egg size (Arnold 1993). Our failure to find the same effect on incubation periods for increases in daily temperature suggests that seasonality affects incubation periods independently of ambient temperature, contrary to other studies (e.g. Ardia et al. 2006).

Incubation periods of first and second eggs were longer than those of penultimate eggs (Table 5). Including laying-stage incubation in measures of incubation period is often done when the data are available (Nicolai et al. 2004; Hepp et al. 2006) and more closely approximates exact incubation periods from artificially incubated eggs (MacCluskie et al. 1997; Wells-Berlin et al. 2005; Robinson et al. 2008). Thus, nest-level measures of incubation period that do not take into account embryonic development during the laying period (e.g. Martin et al. 2007; Schwabl et al. 2007; Martin & Schwabl 2008) may be misleading estimates, even for synchronously hatching species. Incubation periods of last-laid eggs are often shorter than those of earlier-laid eggs when the onset of natural incubation is experimentally controlled in precocial (Nicolai et al. 2004) and semialtricial species (Vinuela 1997).

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