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Local variation in weather conditions influences incubation behavior and temperature in a passerine bird

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Incubation is an important component of avian parental care and slight changes in incubation temperature can affect offspring phenotype. Although many extrinsic and intrinsic factors may generate variation in incubation temperature, they remain underexplored under natural conditions. Using a robust data set encompassing 55 nests, 22 816 behavioral observations, and >1 million paired ambient and egg temperatures, we describe the relationships among abiotic factors, female incubation behavior, incubation temperature, and incubation period for tree swallows *Tachycineta bicolor*. We report a large amount of individual variation in incubation behaviors and average incubation temperatures for our study population. The average on-bout incubation temperature was 34.1°C, with daily egg temperatures ranging from 18.0–39.2°C. Females modulated the number of times they left the nest and the amount of time they stayed off the nest according to interactions between precipitation and temperature patterns. Models generated from our observations predicted that the number of female off-bouts was the lowest under warm and dry conditions while more off-bouts were taken under cold and dry or warm and wet conditions. During cold and dry conditions, females stayed off their nest ~4 times longer than under warm and dry conditions. However, this pattern was reversed under periods of rainfall; females tended to take shorter off-bouts when it was rainy and cold compared to longer off-bouts during warmer rain events. Furthermore, variation in female behavior was associated with differences in overall incubation temperature such that females that maintained greater incubation constancy produced higher incubation temperatures at a given ambient temperature than those that displayed lower incubation constancy. Our results provide perspective on the timing of breeding, as some of the advantages of breeding early may be countered by cooler, early season temperatures and precipitation that cause reproducing females to favor self-maintenance at a potential cost to optimal incubation temperatures for offspring development.

In birds, egg incubation is an important component of parental care, in which parents must maintain an optimal thermal environment while simultaneously meeting their own energetic demands. Even slight variation (<1°C) in average egg incubation temperature can influence embryonic development and affect numerous offspring traits including proximate body composition, growth, body condition, immune function, thermoregulation, locomotion, stress and thyroid endocrinology, survival, and recruitment (Hepp et al. 2006, DuRant et al. 2010, 2011, 2012a, b, 2014, Hopkins et al. 2011, Nord and Nilsson 2011, reviewed by DuRant et al. 2013). While incubation temperature clearly affects offspring phenotype, factors that generate variation in egg temperature and female incubation behavior under various environmental conditions have received comparatively little attention (but see Haftorn and Reinertsen 1985).

Many extrinsic and intrinsic factors have been suggested to influence parental incubation behavior. Along with predation pressure, food availability, clutch size, and female age and condition, variation in weather may cause incubating parents to adjust the duration of on- and off-bouts in order to maintain a relatively stable thermal environment (Yerkes 1998, Deeming 2002, Hepp and Kennamer 2011). For example, a few studies have shown that higher ambient temperatures can be associated with reduced incubation period (Ardia et al. 2006), increased off-bout duration (Conway and Martin 2000, Camfield and Martin 2009, Boulton et al. 2010), and greater on- and off-bout egg temperatures relative to cooler ambient temperatures (Ardia et al. 2009). In some species, precipitation has also been shown to play a role in determining female incubation behavior through increasing off-bout duration (Skrade and Dinsmore 2012). The effects of weather conditions on incubation behavior may be mediated in part by the costs of self-maintenance, as lower ambient temperatures may require more frequent and longer off-bouts for incubating adults to obtain adequate resources and maintain body condition. For example, Chaurand and Weimerskirch (1994) found that blue petrels *Halobaena caerulea* terminated incubation bouts when their body mass fell to a certain threshold, cueing the incubating parent to leave the nest and forage. Thus, ambient temperature and precipitation may independently influence incubation behavior indirectly through their effects on parental condition or directly through their effects on the thermal environment of the nest. However, how precipitation and ambient temperature interact to influence avian incubation behavior remains poorly understood and may be an important factor in species whose food sources are linked to such weather conditions, like aerial insectivores.

Parental decisions regarding the frequency and duration of off-bouts can have important consequences for developing embryos. In poor weather conditions, longer off-bouts may be necessary to maintain parental condition but expose embryos to greater temperature fluctuations, which can contribute to nest failure (Zicus et al. 1995) and increase the cost of rewarming eggs (Vleck 1981, Williams 1996). Likewise, long periods spent foraging can lead to decreases in egg temperature, thus slowing embryonic development and increasing incubation period (Nuechterlein and Buitron 2002, Martin et al. 2007, Carter et al. 2013). Cooler incubation temperatures could make nests more vulnerable to predation due to prolonged incubation periods (Nuechterlein and Buitron 2002, Martin et al. 2007) and decrease phenotypic quality of offspring (DuRant et al. 2013). Moreover, in most passerines, embryos do not develop below 26°C (i.e. physiological zero; Webb 1987, Haftorn 1988, Stoleson and Bessinger 1995, Cooper et al. 2005), and extended time periods at or below physiological zero can cause abnormal development and mortality (Deeming and Ferguson 1992, but see Wang and Beissinger 2009, MacDonald et al. 2013).

Despite the critical role that incubation plays in avian reproduction (Deeming 2002), few studies have examined the relationship between incubation behavior and egg temperatures during development in natural systems. Most previous studies that have examined incubation temperature have used nest temperature as a proxy for egg temperature (Camfield and Martin 2009) or have measured egg temperature during a small time frame in the incubation period (Ardia et al. 2006, 2009). However, nest cup temperatures do not fully reflect the thermal properties of an egg, which has important implications for parameters such as heating and cooling rates (Turner 1992). Additionally, egg incubation temperature may vary considerably over the course of incubation, requiring that the entire incubation period be monitored to capture the full thermal regime that embryos experience. Understanding the effects of local weather conditions (e.g. ambient temperature and rainfall) on daily variation in incubation behavior and thus egg temperatures may shed light on tradeoffs instrumental in avian reproductive ecology as well as contemporary conservation issues related to climate change.

Here, we describe the variation in incubation behavior, temperature, and period in response to local variation in weather conditions for tree swallows *Tachycineta bicolor* nesting in eastern Tennessee, USA. Tree swallows are a widely studied passerine bird, yet a detailed description of their incubation behavior in relation to egg temperatures is lacking. Based on previous studies (Conway and Martin 2000, Ardia et al. 2006, Camfield and Martin 2009, Boulton et al. 2010, Skrade and Dinsmore 2012), we predicted that ambient temperature and rainfall, individually and/or interactively, would influence female incubation behavior through differences in number of off-bouts, duration of off-bouts, and incubation constancy (total time spent incubating divided by the total time of the active period; 06:30–20:00). Furthermore, we predicted that female behavior would be associated with changes in incubation temperature such that at a given ambient temperature, increased incubation constancy would increase egg temperature and decrease egg temperature variation.

Methods

Study species

Tree swallows *Tachycineta bicolor* are aerial insectivores that readily occupy artificial nest boxes and breed throughout the central and northern USA (Robertson et al. 1992). One egg is laid per day, and partial incubation may begin 1 to 2 d after clutch initiation (Wang and Beissinger 2009). Full incubation begins after the penultimate egg is laid or at clutch completion (Robertson et al. 1992). Males do not contribute to incubation or provision incubating females, who must leave the nest periodically to forage. Incubation periods have been reported to range between 11 and 19 d, but average 14 to 15 d (Robertson et al. 1992). Ardia et al. 2010 monitored egg temperature for a 24 h period and reported average on-bout egg temperatures to be 35.8°C.

General field methods

We monitored active nest boxes ($n = 55$) in six colonies ($n = 39$–99 boxes/colony) in Roane and Loudon Counties in eastern TN, USA, from April–July 2012. Three of our colonies overlapped with an area that was recently remediated following a coal ash spill that occurred in 2008, ~3.5 yr before our study (Tennessee Valley Authority (TVA), 2011). Major remediation efforts were completed in 2010, ~2 yr prior to initiation of our study (TVA 2009, 2011). Concentrations of remaining trace elements were below those associated with adverse effects in wildlife and did not influence the stress physiology, and reproductive success (Beck et al. 2014), or nest incubation characteristics (in all cases $p \geq 0.22$) of swallows in this population. Because there was no evidence that the remediated conditions adversely influenced swallow biology, we combined the colonies for all analyses.

We placed nest boxes in each colony in late February, when tree swallows were arriving and prospecting for nest sites. All boxes were located within 70 m of the shore and were spaced 15–20 m apart. We checked nest boxes every 4 d starting in late March to monitor nesting activity so that we could accurately record clutch initiation dates. We obtained rainfall data online (<www.tva.gov/kingston/met>) from information recorded by TVA, at a small weather station located at one of the colonies. We used temperature data collected from the data loggers (see below) attached to each individual nest box to determine ambient temperatures synchronously with egg temperatures.

Incubation temperature

On the day each female laid her fifth egg (i.e. five days after a clutch was initiated), we installed a HOBO U23 Pro ver. 2

2 × external temperature data logger (Onset Corporation) to the nest box. We could not install loggers before this day as likelihood of female abandonment increased (Coe unpubl.). The HOBO U23 is a weatherproof data logger with two metal temperature probes on 6-foot cables. The main body of the logger was mounted to the back of the box and two temperature probes were placed inside the nest box through small holes in the side of the box. One of temperature probes, designated to measure ambient temperature within the nest box, was secured towards the front inside panel of the nest box, outside of the nest material and away from any direct solar or female incubation influences. To measure egg temperature, we modified the other temperature probe by removing the 2 cm metal probe and replacing it with a 10K thermistor (± 0.1°C accuracy; 0.02°C resolution; Onset Corporation, model #27-10K4A74I), capable of fitting inside an artificial tree swallow egg similar to the protocol described by Ardia et al. 2010. We centered the thermistor in a hollow, 12–13 mm wide and 18–20 mm long, handmade clay egg filled with wire pulling lubricant (Ideal Industries© ClearGlide™; adapted from Ardia et al. 2010). After modification, we activated the sensor, compared its recordings to the unmodified probe on the other cable to ensure it was accurately recording temperatures, and conducted heating and cooling cycles in the laboratory that would mimic female incubation behavior. To install the probe, the artificial egg was fed through the side of the nest and placed in the middle of the nest cup where it was secured to the nest material using a twist tie. We programed the data loggers to simultaneously record ambient and egg temperature every 30 s throughout the incubation period. We wirelessly downloaded temperature data every 4–6 d using a universal optic USB base station, allowing us to collect data without disturbing the nest. In order to quantify incubation period (e.g. days from the onset of incubation until the first egg hatched), we monitored the nest daily beginning ten days after we installed the logger and visually confirmed approximate incubation onset and hatch time using the logger temperature profile.

Female behavior

We quantified female incubation behavior (on- and off-bouts) using a file converter software program (Rhythm) in conjunction with Raven, a bioacoustical analysis program that can be used to analyze temperature time series data (Cooper and Mills 2005). We only quantified female behavior and incubation temperatures once females began full incubation. We defined the onset of full incubation as the first day that the female incubated overnight and consistently throughout the next day which was obvious from visual examination of the logger temperature profile. We categorized off-bouts as a minimum temperature drop of at least 1.5°C for at least 4 min and on-bouts as anytime in between designated off-bouts. These settings produced on- and off-bout behaviors similar to tree swallow nest attendance previously observed with temperature loggers (Ardia et al. 2010), and were confirmed using visual inspection of temperature profiles. In order to address each one of our hypotheses, we calculated a suite of endpoints, at three temporal scales depending on the resolution and temporal variability of the endpoint. At the finest temporal scale, we calculated the change in egg temperature during every off-bout taken by each of the 55 nesting females and the associated ambient temperature (n = 22 816 paired observations) as previous research showed the rate of change in egg temperature is strongly determined by ambient temperature (Conway and Martin 2000). At the intermediate temporal scale, we calculated daily averages for each of the 55 nests (a total of 734 daily averages) for ambient and incubation temperature, rainfall, number of off-bouts, on- and off-bout duration (min), time spent on and off the nest (h), daily incubation constancy (total time spent incubating divided by the total time of the active period; observed from 06:30 ± 00:03 to 20:23 ± 00:04 for all 55 nests). At the broadest scale we calculated the total number of off-bouts and overall averages for ambient and incubation temperature, incubation period, and time spent < 26°C (physiological zero) for each of the 55 nests based on all data recorded throughout the entire incubation period (n = 55). We report all descriptive parameters in Table 1.

Statistical analysis

We calculated non-linear correlation coefficients to describe the relationships between average incubation temperature,

Table 1. Mean values, standard error (SE) and ranges of measured characteristics associated with egg incubation of 55 tree swallow *Tachycineta bicolor* nests in Roane and Loudon Counties in eastern TN, from April to June 2012.

Characteristic	Mean ± SE	Range
Daily average observations		
Ambient temperature (°C)[a]	21.91 ± 0.13	8.12–29.49
Incubation temperature (°C)[a]	33.67 ± 0.11	17.97–39.16
Rainfall (cm)[a]	0.10 ± 0.04	0–5.61
No. off-bouts per day[b]	30.92 ± 0.50	3–67
Off-bout duration (min)[b]	10.07 ± 0.46	4.03–225.72
On-bout duration (min)[b]	25.27 ± 0.79	7.44–180.38
Incubation constancy[b]	0.69 ± 0.01	0.10–0.95
Total time off per active period (h)[b]	4.34 ± 0.08	0.08–14.14
Total time on per active period (h)[b]	9.37 ± 0.09	0.32–16.47
Off-bout temperature drop (°C)[b]	2.83 ± 0.03	1.50–8.70
Overall nest box observations[c]		
Ambient temperature (°C)	22.04 ± 0.33	15.13–27.29
Incubation temperature (°C)	33.65 ± 0.32	24.11–37.97
On-bout temperature (°C)	34.14 ± 0.29	25.63–38.01
Incubation period	12.74 ± 0.40	9–17
Nest initiation date (Julian day)	121.90 ± 1.74	98–154
Clutch size	5.16 ± 0.09	4–7
Total no. off-bouts	414.05 ± 19.62	160–723
Time eggs spent < 26°C (h)	20.13 ± 5.12	0–219.64

[a] Calculated from 24 h daily averages from the 55 monitored nest boxes (n = 734).

[b] Calculated from daily active period (06:30–20:00) averages from the 55 monitored nest boxes (n = 734).

[c] Calculated from individual nest boxes throughout the entire incubation period (n = 55).

time spent under 26°C (physiological zero), and incubation period. In addition, we used generalized linear mixed models and an information theoretic approach (Burnham and Anderson 1998) to investigate how abiotic factors influenced three aspects of female incubation behavior (number of off-bouts, average duration of off-bouts, and incubation constancy) and how female behavior influenced egg temperature. The earliest incubating female was a notable outlier that had the lowest incubation temperature and the longest incubation period. To ensure that this single individual did not drive statistical outcomes, we conducted all of the correlations, modeling and model ranking with and without this data point. We found that the shape and significance of variable correlations, the directionality of model coefficients, and model ranking outcomes remained largely unchanged with and without this individual. Because there was no evidence that this individual overly influenced statistical relationships and since it was exposed to the coldest ambient temperatures and thus provided valuable biological insights, we retained this data point in all models.

Model building

To avoid problems with multicollinearity, we did not include explanatory variables that were correlated with one another ($p \leq 0.05$; Neter et al. 1996), unless the hypothesis was directed towards the interaction between two variables (e.g. ambient temperature and duration of off-bout). Unfortunately, we did not have enough second year (SY; $n = 4$) females nesting early in the season and were therefore unable to include female age in our models as it was confounded by ambient temperature and Julian date.

Abiotic factors and female behavior

In order to understand the relationships between weather factors (i.e. ambient temperature and rainfall) and female behavior, we developed 20 a priori models (Table 2) that represented our hypotheses. We used daily averages for ambient temperature, rainfall, and their interaction as the explanatory variables that might influence the number of off-bouts, average off-bout duration, average on-bout duration, and daily incubation constancy. Because abiotic factors change between days and across the incubation period, we used daily averages or counts ($n = 734$) for abiotic and female behavioral endpoints and included female as a random effect in each of these models.

Female behavior and egg temperature

In order to understand the influence of female behavior on daily incubation temperature, we developed 9 a priori models using the following explanatory variables: average ambient temperature, incubation constancy, and clutch size. For this particular set of models, we chose incubation constancy as our female behavioral endpoint because it combines off-bout length and number of off-bouts to reflect the proportion of time a female spent incubating during the active period (06:30–20:00). In all of our models, we used daily averages for ambient and incubation temperature and included female as a random effect.

To determine the relationship between change in egg temperature during a single off-bout and ambient temperature during that off-bout, we developed 5 a priori models using every individual off-bout taken by each of the 55 nesting females ($n = 22\,816$ off-bouts) and included female as a random effect.

Model fitting and ranking

We ranked models using Akaike's information criterion corrected for small sample size (AIC_c) calculated in SAS 9.2 (SAS Inst., Cary, NC, USA) using PROC GLIMMIX (SAS 9.2). Models explaining duration of off-bouts, number of off-bouts, change in incubation temperature during an off-bout, and daily incubation temperature were fitted with a log link function, whereas the models explaining incubation constancy were fitted with an id link function. For each analysis, we compared the relative likelihood of each model using Akaike model weights (w_i; Burnham and Anderson 2002). We considered models that were assigned any part of the upper 90% of the Akaike weight to fall within the confidence set of models in each analysis (Burnham and Anderson 2002). We report parameter estimates and their associated standard errors for top-ranked models when the confidence set was composed of a single model, or model averaged estimates (Burnham and Anderson 2002) when more than one model occurred in the confidence set.

Results

Average incubation temperature of natural tree swallow nests ($n = 55$) was 33.65° ± 0.32°C and incubation period averaged 12.74 ± 0.40 d. We described a large range in average incubation temperatures (24.11–37.97°C) and incubation periods (9–17 d: Table 1). As predicted, incubation period declined as average egg temperature increased ($r = 0.42$, $p < 0.01$; Fig. 1A). In addition, the time that eggs spent under 26°C (physiological zero) was positively related to incubation period ($r = 0.57$, $p < 0.01$; Fig. 1B) and negatively related to average incubation temperature ($r = -0.77$, $p < 0.01$; Fig. 1C).

Abiotic factors and female behavior

We observed significant variation in female incubation behavior (Table 1) and found support for our hypotheses that abiotic factors influenced female behavior and that female behavior influenced nest temperature (Table 2, 3). We did not observe model uncertainty in AIC_c ranking of models ($w_i > 0.90$) explaining incubation constancy. However, in the case of duration of off-bouts, duration of on-bout, and number of off-bouts, the upper 90% of AICc model weight was distributed over ≥ 2 models (Table 2, 3). In both cases, we model averaged the top-ranked models that encompassed ≥ 90% of the model weight to produce the final model.

Explanatory variables present in the top-ranked or final averaged model varied by behavioral endpoint (Table 2). The best fitting model explaining variation in incubation constancy only included ambient temperature, where incubation constancy increased with ambient temperature (Table 3, Fig. 2A). Our final averaged model describing the

Table 2. A priori generalized linear models explaining variation in female behavior and incubation temperature in tree swallows *Tachycineta bicolor*.

Response variable	Models	AIC_c	ΔAIC_c	AIC_c weight
Female behavior [a]:				
Total no. off-bouts	= β_0 + ambient temp + rainfall	6471.67	0.00	0.56
	= β_0 + ambient temp × rainfall	6472.61	0.94	0.35
	= β_0 + ambient temp	6475.38	3.71	0.09
	= β_0 + rainfall	6500.72	29.05	0.00
	= β_0	6508.43	36.76	0.00
Ave off-bout duration	= β_0 + ambient temp × rainfall	4280.45	0.00	0.64
	= β_0 + ambient temp + rainfall	4282.97	2.52	0.18
	= β_0 + ambient temp	4283.02	2.57	0.18
	= β_0	4356.89	76.44	0.00
	= β_0+ rainfall	4358.73	78.28	0.00
Ave on-bout duration	= β_0 + ambient temp	5529.61	0.00	0.59
	= β_0 + ambient temp + rainfall	5531.05	1.44	0.29
	= β_0 + ambient temp × rainfall	5532.80	3.19	0.12
	= β_0 + rainfall	5560.33	30.72	0.00
	= β_0	5560.43	30.82	0.00
Daily incubation constancy	= β_0 + ambient temp	−1230.64	0.00	0.96
	= β_0 + ambient temp + rainfall	−1224.42	6.22	0.04
	= β_0 + ambient temp × rainfall	−1218.37	12.27	0.00
	= β_0	−1157.49	73.15	0.00
	= β_0 + rainfall	−1150.58	80.06	0.00
Change in incubation temperature during an off-bout [b]:	= β_0 + ambient temp × duration of off-bout	74464.23	0.00	1.00
	= β_0 + ambient temp + duration of off-bout	74612.20	148.47	0.00
	= β_0 + ambient temp	75918.77	1454.54	0.00
	= β_0 + duration of off-bout	76062.09	1597.86	0.00
	= β_0	77510.01	3045.78	0.00
Daily incubation temperature [a]:	= β_0 + incubation constancy × ambient temperature	4044.68	0.00	0.63
	= β_0 + incubation constancy × ambient temperature + clutch size	4046.57	1.89	0.24
	= β_0 + incubation constancy × ambient temperature + incubation constancy × clutch size	4048.59	3.91	0.09
	= β_0 + incubation constancy + ambient temperature	4051.14	6.48	0.02
	= β_0 + incubation constancy + ambient temperature + clutch size	4052.96	8.28	0.01
	= β_0 + incubation constancy × clutch size + ambient temperature	4054.75	10.07	0.00
	= β_0 + ambient temperature	4068.48	23.80	0.00
	= β_0 + incubation constancy	4082.14	37.46	0.00
	= β_0	4121.64	76.96	0.00

[a] Explanatory variables calculated from daily active period (06:30–20:00) averages from the 55 monitored nest boxes (n = 734).
[b] Explanatory variables calculated from every off-bout taken by the 55 nesting females throughout incubation (n = 22 816).

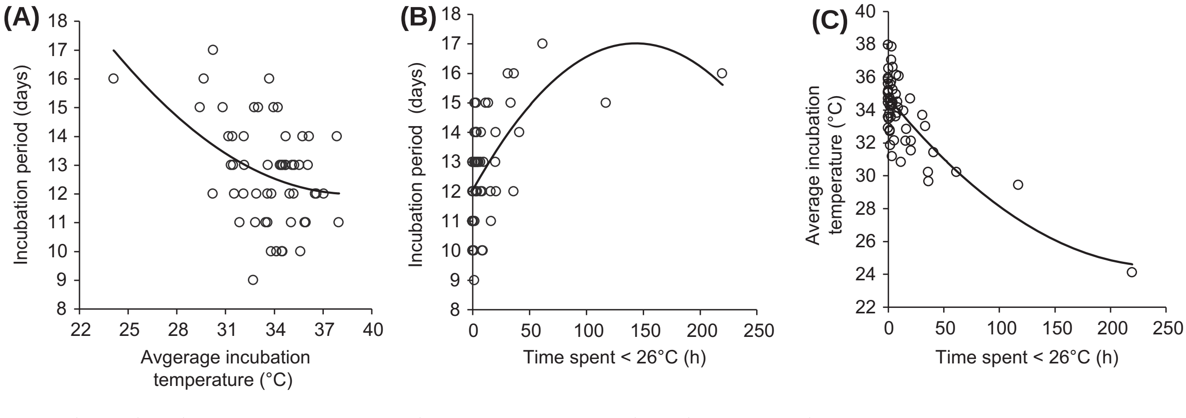

Figure 1. Relationships between (A) average incubation temperature and incubation period ($r = 0.42$, $p < 0.01$, $n = 55$), (B) time spent below physiological zero (26°C) and incubation period ($r = 0.57$, $p < 0.01$, $n = 55$), and (C) time spent below physiological zero (26°C) and incubation temperature ($r = -0.77$, $p < 0.01$, $n = 55$) in tree swallows *Tachycineta bicolor* nesting near Kingston, TN, USA. Note that significant statistical relationships were similar when the single outlier was dropped from the models.

Table 3. Parameter estimate for top-ranked or final averaged models for tree swallows *Tachycineta bicolor* nesting in Roane and Loudon Counties in eastern TN, from April to June 2012.

Response variable	β	Estimate	SE
Number of off-bouts [a]	Ambient temperature	−0.0142	0.0004
	Rainfall	−0.0863	0.0008
	Ambient temperature × rainfall	0.00693	0.00002
Ave off-bout duration [a]	Ambient temperature	−0.064	0.007
	Rainfall	−1.353	0.612
	Ambient temperature × rainfall	0.065	0.030
Ave on-bout duration [a]	Ambient temperature	0.034	0.006
	Rainfall	0.035	0.117
	Ambient temperature × rainfall	−0.003	0.005
Daily incubation constancy [a]	Ambient temperature	0.012	0.001
Change in incubation temperature during an off-bout [b]	Duration of off-bout	0.002	0.0008
	Ambient temperature	−0.031	0.0004
	Duration of off-bout [a] × ambient temperature	0.00009	0.00002
Daily incubation temperature [a]	Daily incubation constancy	0.9577	0.2881
	Ambient temperature	0.0345	0.0079
	Clutch size	−0.0024	0.0098
	Daily incubation constancy × ambient temperature	−0.0343	0.0118
	Daily incubation constancy × clutch size	0.0012	0.0095

[a] Explanatory variables calculated from daily active period (06:30–20:00) averages from the 55 monitored nest boxes (n = 734).
[b] Explanatory variables calculated from every off-bout taken by the 55 nesting females throughout incubation (n = 22 816).

total number of off-bouts included ambient temperature, rainfall, and their interaction (Table 3). The predicted number of off-bouts was greater when conditions were either warm and wet or cold and dry and lowest when ambient conditions were warm and dry (Fig. 2B). In the case of average duration of off-bouts, our final averaged model included ambient temperature, rainfall, and their interaction (Table 3). During precipitation events, the predicted off-bout duration was ~5 times shorter at cold temperatures (9°C) than at warm temperatures (30°C). However, this relationship was reversed when rainfall was absent; under dry conditions the predicted off-bout duration was ~4 times longer when ambient temperatures were ~9°C than when temperatures were ~30°C (Fig. 2C). Finally, our final averaged model describing average on-bout duration included ambient temperature, rainfall, and their interaction (Table 2 and 3). Based on the cumulative weight of the 3 averaged models, the relative importance of ambient temperature (1.0) was over twice that of rain (0.41), and almost ten times that of the interaction between the ambient temperature and rain (0.12). Although the interaction was included in the final model, the predicted on-bout duration was primarily driven by ambient temperature. on-bout durations were the highest during warm and dry conditions followed by warm and wet, then dramatically decreased when conditions were cold and dry or cold and wet.

Female behavior and egg temperature

We documented a 22°C range in daily average egg incubation temperatures (Table 1). The upper 90% of AIC$_c$ model weight was distributed over 3 models describing the

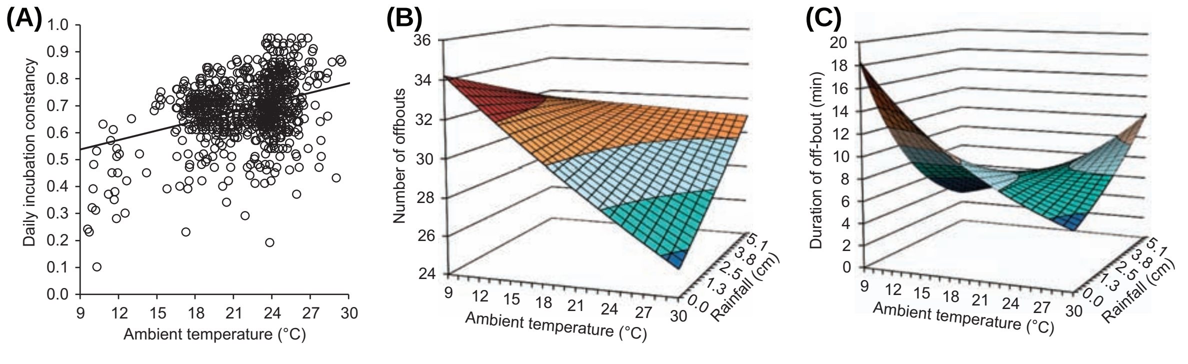

Figure 2. Relationships between explanatory ambient conditions and female behavioral response variables. (A) Predicted regression lines generated from top-ranked models describing the relationship between ambient temperature and incubation constancy is plotted along with observed raw data values. (B) The predicted relationships among number of off-bouts, rainfall, and ambient temperature generated from the top-ranked AIC model. (C) The predicted relationships among duration of off-bouts, rainfall, and ambient temperature generated from the top-ranked AIC model. Note: values plotted and used as covariates in models were daily averages (n = 734) and each color band corresponds to two off-bouts in (B) and two minutes in (C).

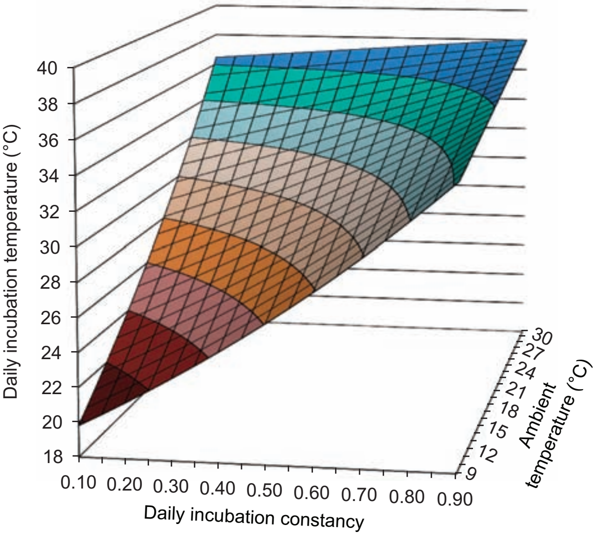

Figure 3. Predicted relationships among average incubation temperature, average ambient temperature, and incubation constancy generated from the top-ranked AIC model. For the figure, the other two variables in the model were held constant at the observed population average. Note: values in models consist of daily temperature averages and incubation constancy (ratio of time spent on the nest during the active period; 06:30–20:00) for each of the 55 nesting females throughout incubation (n = 734) and each color band corresponds to two degrees.

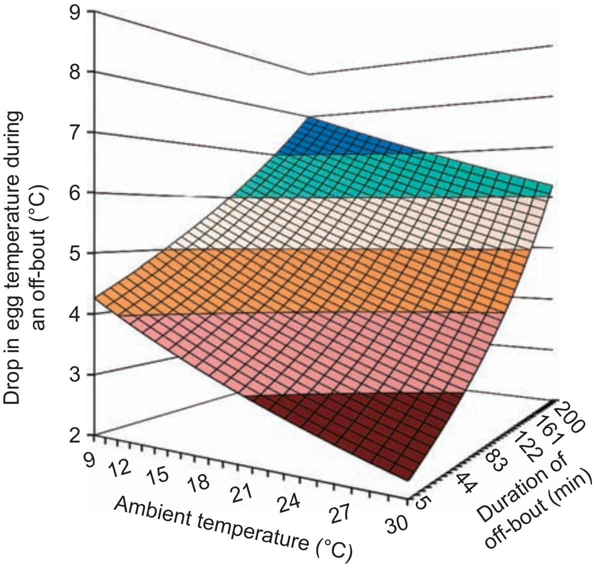

Figure 4. Predicted relationships among absolute change in egg temperature, duration of off-bout, and ambient temperature generated from the top-ranked AIC model. The greatest changes in egg temperatures were observed when females took longer off-bouts during cooler ambient conditions. Note: values in models consist of every off-bout taken by the 55 nesting females and each color band corresponds to one degree.

relationship between female incubation constancy, ambient temperature, and incubation temperature. After model averaging, our final model describing incubation temperature included clutch size, daily incubation constancy, ambient temperature, and two interaction terms: daily incubation constancy by ambient temperature and daily incubation constancy by clutch size. Daily incubation constancy, ambient temperature, and their interaction explained the most variation in incubation temperature, as all 3 models include these terms (Table 2). Based on the cumulative weight of all models including each of these 5 covariates, the relative importance of incubation constancy and ambient temperature and their interaction (1.0) was about three times that of clutch size (0.34), and over ten times that of the interaction between the incubation constancy and clutch size (0.09). The final averaged model predicted a positive relationship between incubation constancy and incubation temperature with higher ambient temperatures exacerbating this effect (Fig. 3).

The top ranked model that described the change in egg temperature during an off-bout included duration of the off-bout and ambient temperature during the off-bout (Fig. 4). Our model (Table 3) predicted that off-bout duration has a positive effect on change in incubation temperature during an off-bout, with smaller egg temperature changes occurring during shorter off-bouts taken at higher ambient temperatures (Fig. 4).

Discussion

Fluctuations in weather can have substantial impacts on parental investment towards incubation, and subsequently influence embryonic development and reproductive success (Conway and Martin 2000, Olson et al. 2006). Our study is the first to report the natural variation in incubation behavior of female tree swallows and its relationship to egg temperatures experienced by developing embryos throughout the entire incubation period. We report substantial individual variation in incubation behaviors and average egg temperatures within our study population (Table 1). Average egg temperatures in our population ranged from 24.11–37.97°C, with daily average egg temperatures ranging from 17.97–39.16°C. Previous studies have shown small (< 1°C) variations in average incubation temperature can affect reproductive success and a multitude of offspring phenotypic characteristics in birds (reviewed by DuRant et al. 2013). Additional concomitant research in this system indicates that suboptimal incubation temperatures are associated with reduced nestling growth and fledging success in Tree Swallows (Coe et al. unpubl.). Together, these results support recent evidence that variation in incubation temperature, which is largely governed by female incubation behavior, may play a role in producing phenotypic variation within a population.

Our study demonstrated that females shifted priority between self-maintenance and incubation demands depending on various weather conditions. We show that the interaction between ambient temperature and rainfall was strongly associated with female incubation behavior, which may explain the inconsistent findings among previous studies that only focused on ambient temperature. Females took the fewest off-bouts under warm and dry conditions compared to when conditions were either cold and dry or warm and wet. Additionally, when conditions were cold and dry or when it was warm and raining, females took longer recesses than when it was warm and dry or cold and wet. Patterns describing female incubation behavior under various weather conditions differ among studies (reviewed by Conway and Martin 2000), and we suspect these discrepancies may exist because these studies focused on a single weather variable rather than the interactive effects of weather conditions. Alternatively, differences among previous studies may be attributed to species- and habitat-dependent differences in food availability, foraging efficiency, or foraging strategies, which may influence the magnitude of the effect of weather conditions on incubation behavior. For example, aerial insect abundance has been shown to decrease rapidly when ambient temperatures fall below 18.5°C (Winkler et al. 2013) and during rainfall (Bryant 1975, Nooker et al. 2005). Since tree swallows are aerial insectivores, lower ambient temperatures during dry conditions may reduce foraging efficiency during each off-bout and females may compensate by taking more, longer off-bouts in order to meet energy demands. However, when low ambient temperatures are combined with precipitation, the cost of foraging may exceed the benefit. Alternatively, when it is warm and dry, foraging efficiency is presumably high, thus allowing female swallows to spend less time away from the nest than during warm rains when aerial insect abundance drops.

The effects of weather conditions on female incubation behavior ultimately influenced incubation temperature. Our findings are consistent with previous studies that showed parental incubation behavior influenced nest microclimate (Deeming and Ferguson 1992, Zicus et al. 1995, Martin et al. 2007). However, our models also indicate that ambient temperature and incubation constancy interactively influence egg temperatures, an observation that expands our understanding of nest microclimate dynamics. Small reductions in incubation constancy resulted in large changes to egg temperatures at low ambient temperatures, compared to much smaller changes in egg temperatures at high ambient temperatures, (Fig. 3) because greater incubation constancy (e.g. short and few recesses) and warm ambient temperatures reduce the amount of egg cooling during recesses. Additionally, the greatest constancy observed by a single female when the ambient temperatures were < 12°C was 0.60, suggesting limitations to a female's capacity to maintain optimal incubation temperatures during challenging conditions.

Even though developing avian embryos depend on a high and stable thermal environment (Webb 1987, Nilsson 2006), demanding environmental conditions might force incubating parents to shift between favoring self-maintenance and maintaining an optimal incubation environment. In our population, females took longer and more frequent off-bouts (i.e. lower incubation constancy) when conditions were either hot and rainy or cold and dry than when ambient conditions were hot and dry or cold and rainy. However, because the rate of egg cooling is greater when ambient temperatures are cooler (Conway and Martin 2000), longer recesses at low ambient temperatures caused greater drops in egg temperature (Fig. 4). Given that we showed females modulated the number of off-bouts, off-bout duration, and incubation constancy under different environmental conditions, our findings suggest an interesting predicament for females reproducing early in the breeding season. Although breeding early in the season has certain benefits such as low interspecific nest site competition, decreased predation risk, (Dunn 1977, Nilsson 1984), and greater reproductive success (Daan et al. 1990, Verhulst and Tinbergen 1991), cool ambient temperatures and rainfall events commonly occur which could present costs to incubating females and affect offspring development. For example, we found the first nest of the season experienced the lowest average ambient temperature (15.13°C) and egg temperature (24.1°C), spent ~55% of the incubation period below physiological zero, and had an incubation period that was also 40% longer than the population average. However, this female displayed on- and off-bout patterns similar to those observed by later season nests during the rare occurrence of warm, early season ambient temperatures suggesting behavioral modulation in response to the weather. Such behavioral responses to variation in weather conditions may have implications for altricial and precocial reproduction as low incubation temperatures could influence egg viability and offspring phenotype (Olson et al. 2006, 2008, Nord and Nilsson 2011, reviewed by DuRant et al. 2013). However, it is possible that different life-history strategies and developmental patterns (e.g. altricial vs precocial) allow for greater deviations in incubation temperature than others, but the relative importance of incubation versus post-hatch parental care requires further research.

Taken together, our data have implications for understanding avian reproduction and life-history strategies. In a number of songbird species, breeding earlier in the season is associated with numerous benefits including producing offspring that are larger at fledging, and are more likely to be recruited into the breeding population compared to smaller offspring which are common among later season nesting (Wheelwright et al. 2003, Müller et al. 2005, Tarof et al. 2011). Additionally, breeding earlier rather than later in the reproductive season has the additional advantages of predator avoidance, reduced competition, increased probability of double-brooding, and increased chances of successfully producing a replacement brood (Dunn 1977, Nilsson 1984, Monroe et al. 2008, Verhulst and Nilsson 2008). Tree swallows double-brood at our study site and typically only the earliest breeders produce a successful second brood. However, our study highlights the fact that early breeding in tree swallows may be constrained by more prevalent low ambient temperatures and rainfall compared to later in the season. Conditions experienced early in the season necessitate long foraging times, consequently increasing the drop in egg temperature during a recess and the cost of rewarming the eggs upon return. As a result, reproductive success or the phenotypic quality of offspring produced by these early nests could be reduced compared to birds who initiate nests later in the season. Indeed, our forthcoming work indicates that cooler egg temperatures reduce fledging success in this population (Coe et al. unpubl.). Thus, females must balance the benefits of early nest initiation with potential costs which may be more complex in areas with low food abundance, during unpredictable weather events, or in the face of other direct disturbances that might influence female incubation behavior.

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