

# Temperature and life history: experimental heating leads female tree swallows to modulate egg temperature and incubation behaviour

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## Summary

1. Life-history decisions are strongly affected by environmental conditions. In birds, incubation is energetically expensive and affected significantly by ambient temperature. We reduced energetic constraints for female tree swallows (*Tachycineta bicolor*) by experimentally heating nests during incubation by an average of 6.9 °C to test for changes in incubation behaviour.

2. Females in heated boxes (hereafter 'heated females') increased time spent incubating and maintained higher on-bout and off-bout egg temperatures. This indicates that female energetic constraints, not maximizing developmental conditions of offspring, determine incubation investment. Furthermore, this result suggests that embryonic developmental conditions in unmanipulated nests are suboptimal.

3. We found individual variation in how females responded to experimental heating. Early-laying (i.e. higher phenotypic quality) females with heated nests increased egg temperatures and maintained incubation constancy, while later-laying (lower quality) heated females increased incubation constancy. Changes in egg temperature were due to changes in female behaviour and not due directly to increases in internal nest-box temperatures.

4. Behaviour during the incubation period affected hatching asynchrony. Decreased variation in egg temperature led to lower levels of hatching asynchrony, which was also generally lower in heated nests.

5. Our study finds strong support for the prediction that intermittent incubators set their incubation investment at levels dictated by energetic constraints. Furthermore, females incubating in heated boxes allocated conserved energy primarily to increased egg temperature and increased incubation attentiveness. These results indicate that studies investigating the role of energetics in driving reproductive investment in intermittent incubators should consider egg temperature and individual variation more explicitly.

**Key-words:** embryonic development, environmental variation, life-history tradeoffs

## Introduction

Levels of parental investment can be determined and constrained by environmental conditions (Schultz 1991; Reid *et al.* 2003). In birds, temperature can affect many life-history traits, such as egg volume (Barkowska, Pinowski & Pinowska

2003), clutch size (Cooper *et al.* 2005a), nestling development (Chaplin, Cervenka & Mickelson 2002), nestling growth (McCarty 2001b; Dawson, Lawrie & O'Brien 2005), metabolic rate (Broggi *et al.* 2004), nestling parasite load (Dawson, Hillen & Whitworth 2005), winter range (Canterbury 2002) and body condition (Blem 1981). One aspect of avian life history that is particularly affected by temperature is incubation (Conway & Martin 2000a,b; Weathers *et al.* 2002; Cresswell *et al.* 2003a; Nooner, Dunn & Whittingham 2005). Previous observational and experimental studies have found that the

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maintenance of proper temperatures during embryonic development is costly and incubation can have later influences on current and future reproductive bouts (Williams 1996; Turner 1997; Thomson, Monaghan & Furness 1998; Reid Monaghan & Ruxton 2000a; Cresswell *et al.* 2004).

For many years, the prevailing wisdom among ornithologists was that incubation was not energetically expensive, based on the assumption that incubating parents use basal metabolic heat and excess heat generated during non-incubation activities to warm eggs with minimal direct costs (King 1973). However, maintaining egg temperatures against heat loss has been found to be more costly than originally believed (Turner 1997; Williams 1996) and a body of evidence demonstrates that incubation activity entails significant energetic costs that can vary depending on nest microclimate, parental behaviour and species (Reid *et al.* 2002b; Tinbergen & Williams 2002). This is particularly true when incubation occurs intermittently, characterized by a variable temperature regime, which can have important implications for both embryonic development and parental investment in rewarming and maintaining egg temperature (Reid, Monaghan & Nager 2002a). Furthermore, a thorough understanding of how environmental conditions, particularly temperature, affect life-history trade-offs will be essential to predicting the effect of global climate change on avian reproduction (McCarty 2001a). Previous experimental approaches to examine the energetic costs of incubation have focused on increasing incubation costs during incubation by increasing clutch size (Moreno & Sanz 1994; Visser & Lessells 2001; Engstrand & Bryant 2002; Reid *et al.* 2002b; Hanssen *et al.* 2005; Niizuma *et al.* 2005; de Heij, van den Hout & Tinbergen 2006; Dobbs, Styrsky & Thompson 2006). The sensitivity of incubation to temperature facilitates experimental manipulations of environmental conditions, particularly through modifying thermal conditions within nests. A smaller group of studies have experimentally reduced costs by warming nests (Bryan & Bryant 1999; Reid *et al.* 2000a; Cresswell *et al.* 2004; Magrath, van Overveld & Komdeur 2005), thus allowing for a test of how possible savings, rather than increased costs, are allocated into current and future life-history needs.

Here we expand upon previous manipulations by increasing the magnitude of the thermal change *and* by assessing how females reallocate energy gained from experimental heating. We do this by explicitly considering the role of egg temperature, an important but generally understudied component of incubation investment. Maintenance of proper egg temperature is critical as egg temperature levels affect rewarming costs for females (White & Kinney 1974; Haftorn 1978; Webb 1987; Reid *et al.* 2002b; Olson, Vleck & Vleck 2006; Voss, Hainsworth & Ellis-Felege 2006) and developmental conditions for embryos (Olson *et al.* 2006). A proper consideration of egg temperature may reflect incubation investment more effectively than time spent incubating on its own, as the temperature of eggs reflects the actual energetic transfer of heat. Egg temperature has been shown to respond to experimental manipulations intended to investigate the costs of incubation. For example, females incubating enlarged clutches of European starlings (*Sturnus vulgaris*) raised average egg temperature

slightly, but this was likely due to reduced cooling of enlarged clutches (Reid, Monaghan & Ruxton 2000b), although a similar clutch-enlargement study in black-tailed gulls (*Larus crassirostris*) found that egg temperatures decreased (Niizuma *et al.* 2005).

We conducted an experimental increase in nest temperature to investigate how female tree swallows (*Tachycineta bicolor*), a uniparental (female) incubator where the other sex does not feed their mate, allocate resources. We predicted that females whose nests were heated during incubation (hereafter 'heated females') could allocate conserved energy into three pathways: (i) increased on-bout and off-bout egg temperature, (ii) increased incubation constancy or attentiveness, and (iii) increased self-maintenance. We predicted that heated females would increase egg temperatures relative to control females, as previous work found that an energetic constraint during incubation led to decreased egg temperatures (Ardia & Clotfelter 2007). We also predicted that heated females would increase attentiveness (total time spent incubating), based on the assumption that attentiveness is constrained by parental energy reserves and not by maximal embryonic development conditions. For example, Bryan and Bryant (1999) heated great tit (*Parus major*) nests and found that heated females increased time spent incubating by 55 min per day (3.8%), while heated female pectoral sandpipers (*Calidris melanotos*) increased attentiveness by 52 min (3.2%) (Cresswell *et al.* 2004).

Finally, we predicted that heated females would shift patterns of incubation behaviour to favour embryonic development (Voss *et al.* 2006) relative to a greater investment in self-maintenance by control females. Voss *et al.* (2006) proposed a new model of assessing allocation of time during the incubation period by comparing the time eggs are at equilibrium temperature vs. time off and reheating eggs. Time spent incubating ( $t_{\text{equil}}$ ) is considered as investment in embryonic development, while time off eggs ( $t_{\text{cool}} + t_{\text{heat}}$ ) is considered time invested by females in self-maintenance. Analysis of tree swallow incubation behaviour using this approach found that females are constrained by incubation costs and tend to invest in self-maintenance over embryonic investment, reflected in more time spent off and reheating eggs relative to time eggs spent maintaining eggs at equilibrium temperatures (Voss *et al.* 2006). Here we use this model to examine shifts in allocation as a consequence of our manipulation. We predicted that experimental heating would lead females to shift the allocation of time to show greater time spent in embryonic investment (increased  $t_{\text{equil}}$ ) and reduced time off eggs ( $t_{\text{cool}}$ ) relative to control females.

In addition to absolute change in conditions, we also predicted that our experimental manipulation would lead to reduced variability in embryonic development conditions, as heated females would make fewer foraging trips and thus have fewer off-bouts. Variability in temperature during incubation can affect incubation period (Hepp, Folk & Manlove 2005) and growth rates (Deeming & Ferguson 1991; Olson *et al.* 2006). For example, periodic cooling of zebra finch (*Taeniopygia guttata*) eggs led to lower growth rates (Olson *et al.* 2006), while

increased temperature fluctuation was related to smaller egg mass in great tits (Pendlebury & Bryant 2005). Many climate change models predict greater variability in temperature in the coming decades, which may have energetic consequences on avian reproduction (Pendlebury MacLeod & Bryant 2004). Thus, we tested (i) whether heating reduced variation in incubation behaviour and egg temperature, and (ii) whether reduced variability affected hatching asynchrony, an important factor affecting nestling condition and survivorship (Magrath 1989; Slagsvold & Lifjeld 1989a; Beissinger & Stoleson 1996; Eikenaar, Berg & Komdeur 2003).

Finally, we analysed our results within the context of individual variation, as individuals may differ in how they respond to reduced incubation costs. In previous work, we found that reducing foraging efficiency affected later-laying, presumably lower-quality, individuals more than higher-quality, presumably higher-quality, individuals (Ardia & Clotfelter 2007). In tree swallows, clutch initiation date is a measure of individual quality, as it is the best predictor of annual reproductive success (Stutchbury & Robertson 1988; Winkler & Allen 1996). Individuals arrive at breeding sites at similar times (Stutchbury & Robertson 1987) but differ in their timing of breeding based in large part on their ability to gather resources necessary for breeding, not due to differences in environmental conditions as conditions improve as the season progresses in tree swallows (Winkler & Allen 1995; Winkler & Allen 1996; Ardia 2005; Nooker *et al.* 2005). Here we tested whether lower-quality (i.e. later-breeding) individuals would benefit more from reducing an energetic constraint and predicted that heated lower-quality females would show a greater change in incubation behaviour.

## Methods

### GENERAL FIELD METHODS AND NEST HEATING EXPERIMENT

The experiment was conducted from May to July 2006 in a series of open fields (81 ha) in Amherst, Massachusetts (42°22'N, 72°31'W), on which 153 nest boxes were placed in April 2004. Nests were checked daily during egg laying to determine clutch initiation date and clutch size. Nests were visited daily near the end of the incubation period to determine actual hatching dates, hatching success, and the number of eggs that hatched on each day. On nestling day 1 (hatching = 0), body mass (to the nearest 0.1 g) of each nestling was measured using a digital scale.

Four days after clutch completion, each breeding female was captured, weighed, and measured. Body mass was measured with a digital scale to the nearest 0.1 g. Head-bill length and ninth primary wing feather length were also recorded for another study. Plumage was used to distinguish between second-year (SY) and after second-year (ASY) females. Females were allocated to two treatment groups, balanced for clutch initiation date and clutch size: heated nests and control nests. Balance between treatments was maintained during the nestling period. On incubation day 6, for each heated nest, a Peltier thermoelectric heating device (All Electronics Corporation, Van Nuys, CA, USA) connected to a 115 amp-hr deep cycle marine battery (Kirkland Signature, Issaquah, WA, USA) stored in a plastic tub beneath each nest box was installed at each box. The

heating device operated for 15 min of every hour. The Peltier device was placed in a three-sided protective wooden shed, which was installed on all boxes before birds arrived to breed, after removing the side of the nest box to facilitate heat flow. Control nests also received Peltier devices, which were wired the same as heated boxes and connected to empty plastic tubs to mimic any potential disturbance associated with placement of heaters. Heaters were maintained on boxes for 4 days and removed on incubation day 10. All females were recaptured 12–13 days after clutch completion (2–3 days after removal of heaters) to measure change in body mass.

Insect availability was recorded using a 2-m aerial insect sampler powered by a Robbins and Myers 1650 r.p.m. (12.95 m s<sup>-2</sup>) 1/12 HP motor (Dayton, OH, USA) (McCarty & Winkler 1999) to collect daily samples of aerial insect abundance during the breeding season; sampling began 13 days before the first swallow clutch being initiated and continued 21 days after the last swallow fledged. The sampler was located in the same open field in which nests were located; habitat was homogenous throughout and females were observed travelling across the entire study area to forage. After removing seeds and other debris, insect samples were dried in a drying oven for 48 h and weighed to the nearest 0.1 mg on an analytical balance. For each female, insect availability and average daily air temperature were averaged over the length of the incubation period. Insect biomass showed no trend over the breeding season ( $P = 0.6$ ,  $R^2 = 0.002$ ,  $N = 94$ ).

### INCUBATION BEHAVIOUR AND EGG TEMPERATURE

Once a breeding attempt was discovered, a data logger (Thermochron iButton DS1921, Dallas Semiconductor, Dallas, TX, USA; accuracy  $\pm 1.0$  °C; 17 mm  $\times$  6 mm, 3.0 g) was placed in each nest cup adjacent to the eggs, so as to not interfere with heat transfer among eggs. This data logger was set to record temperature at 4-min intervals for the entire incubation period (24 h day<sup>-1</sup>) in order to monitor incubation behaviour of females. The ability of the loggers to record on-bout and off-bout lengths was validated via visual observations (Daniel R. Ardia, unpublished data). The data loggers themselves do not affect female behaviour, as there were no differences in behaviour between control nests with data loggers and without (Hartman & Oring 2006; Weidinger 2008; Daniel R. Ardia, unpublished data). Onset of incubation from temperature profiles was determined using previously established criteria (Ardia, Cooper & Dhondt 2006). To record internal nest-box temperature, an additional data logger was placed in a plastic fob 6–8 cm above the top of the nest on the back of the nest box; the fob was at least 4 cm from the sides and top of the box. Ambient temperature was recorded via a data logger in the shade under a centrally located nest box. Before placement in the field, each iButton data logger was compared against two independent instruments: a HOBO U12 thermocouple thermometer (Onset Corporation, Bourne, MA, USA) and a Roetemp TM99-A Thermometer (Roetemp, San Diego, CA, USA). Data loggers that deviated by more than 1 °C from these additional thermometers were not used in the study.

Incubation behaviour was characterized as incubation constancy (percentage of time spent incubating eggs) over the entire periods mentioned below. Incubation rhythms of females were analysed for three periods: (i) a 48-h period covering incubation days 4–5, before allocating them to treatment groups, (ii) a 96-h period covering incubation days 6–10 during the heating (or control) treatment, and (iii) a 48-h period covering incubation days 10–12, post-heating treatment. The program RHYTHM (1.0) was used to determine off-bouts after a visual rechecking of the output, with a minimum off-bout

duration of 5 min and a minimum off-bout change in temperature of 4 °C (Cooper & Mills 2005b). Incubation constancy was calculated by subtracting the time consumed by off-bouts from the total period under observation.

Egg temperature was measured by placing an artificial egg in nests for a 24-h period between incubation days 9–10, the last day of the heating treatment. A 13-mm long plastic egg (Berenice's Crafts, www.berenicecrafts.com) was filled with wire-pulling lubricant (Clear-Glide, Ideal Industries, Sycamore, IL, USA). This fluid closely mimics the thermal properties of an egg (Margaret A. Voss, unpublished data). In the centre of each plastic egg, the probe of a HOBO U12 type-T thermocouple thermometer (Onset Corporation, Bourne, MA, USA) was placed recording at 30-s intervals. Two measures of incubation egg temperature were measured: average maximum temperature during on-bouts and average minimum temperature during off-bouts.

Allocation of time between embryonic investment and self-maintenance was examined using a model outlined by Voss *et al.* (2006). Time allocated to incubation ( $t_{\text{equil}}$ ), egg cooling ( $t_{\text{cool}}$ ), and reheating eggs ( $t_{\text{heat}}$ ) was measured for individual incubation cycles using data from egg temperature probes over a 24-h sample period (Voss *et al.* 2006).

## STATISTICAL ANALYSES

Before beginning analyses, variables were tested for assumptions of normality using Shapiro–Wilk's  $W$  (all variables  $W \geq 0.97$ ,  $P \geq 0.20$ ). Hatching asynchrony was calculated as the ratio of the largest nestling body mass to the smallest nestling body mass on nestling day 1 (all nestlings hatched by day 1). Effect of heating treatment on internal nest-box temperature was assessed by comparing the change in temperature between paired boxes before and following heating, due to slight variation in temperature among nest boxes at the site (Ardia, Perez & Clotfelter 2006).

Change in incubation behaviour was tested by examining change in incubation behaviour over time (pre-heating, heating, post-heating) using repeated measures ANOVA (SAS 1988), with stage of incubation period as the repeated measure for each female. General linear models (PROC GLM) (SAS 1988) were used to examine factors affecting incubation constancy during the heating treatment, incubation period, mean off-bout length, on-bout egg temperature, off-bout egg temperature, change in female body mass, the degree of hatching asynchrony, and coefficient of variation (CV) in off-bout length and egg temperature. For model compositions, see Table 1. For all models, the following covariates were included: female residual body mass at the end of the incubation period, clutch initiation date, clutch size, average ambient temperature and insect availability during incubation and female age (SY vs. ASY). All two-way interaction terms were included initially in each model and then removed sequentially by highest  $P$  value for those interactions with  $P > 0.20$ ; removal of interactions did not change the significance of main effects. Except where noted, means are reported as least square means (LSM) with standard errors, which are calculated to include the effects of covariates. Differences were considered statistically significant at  $P < 0.05$ .

## Results

### AMBIENT TEMPERATURE AND EFFECTIVENESS OF HEATING TREATMENT

In the study area, average ambient daily temperatures were below the minimum critical threshold for embryonic development

( $\sim 26^\circ$ ). Over the incubation period for all females, average daily temperatures were  $15.9^\circ \pm 6.9$  SD (max =  $36.5^\circ$ , min =  $0.5^\circ$ ). Those birds breeding before the median clutch initiation date experienced colder average temperatures than did birds breeding after the median clutch initiation date ( $t_{36} = 2.9$ ,  $P = 0.006$ ; first half of breeding season:  $13.1^\circ \pm 6.1$ , max =  $34^\circ$ , minimum =  $0.5^\circ$ ; second half of breeding season:  $18.9^\circ \pm 6.1$ , max =  $36.5^\circ$ , minimum =  $8.1^\circ$ ). Boxes heated experimentally had internal temperatures raised relative to control boxes (average change in temperature  $6.65^\circ \text{C} \pm 0.30$ ,  $N = 15$ ). Heated boxes had higher internal temperatures than control boxes ( $t_{32} = 15.4$ ,  $P < 0.001$ ; mean internal temperature  $\pm$  standard deviation; heated boxes  $25.0^\circ \pm 1.5$ , maximum  $27.4^\circ$ , minimum  $21.1^\circ$ ,  $N = 15$ ; control boxes  $18.1^\circ \pm 1.1$ , maximum  $19.3^\circ$ , minimum  $15.2^\circ$ ,  $N = 19$ ). Heated boxes had higher internal temperatures when ambient temperatures were warmer ( $\beta = 1.1$ ,  $P = 0.003$ ,  $R^2 = 0.51$ ).

### INCUBATION CONSTANCY

Heated females increased the amount of time spent incubating following heating, while control females showed no change (repeated-measures effect of heating  $F_{1,98} = 8.6$ ,  $P = 0.004$ ; Fig. 1). In control females, there was no change in incubation constancy over the incubation period (effect of time  $F_{2,52} = 1.02$ ,  $P = 0.36$ ), but there was a significant interaction between heating treatment and changes in incubation over time (interaction between time and heating treatment  $F_{2,52} = 6.3$ ,  $P = 0.003$ ; Fig. 1). When covariates are included in a general linear model, heating treatment continues to predict incubation behaviour. Females in the heated treatment spent more time incubating than control females (LSM percentage of time incubating; control  $0.70 \pm 0.19$ ,  $N = 19$ ; heated  $0.78 \pm 0.26$ ,  $N = 15$ ; Table 1) and had shorter average off-bouts [LSM off-bout length (min); control:  $15.9 \pm 1.3$ ,  $N = 19$ ; heated:  $9.7 \pm 1.8$ ,  $N = 15$ ]. There was no difference between control and heated females in variation in off-bout length (LSM coefficient of variation; control:  $97.8 \pm 12.5$ ; heated:  $107.7 \pm 15.1$ ). However, second year females were more variable in their off-bout length than were after second year females (least square mean coefficient of variation; SY:  $142.1 \pm 17.9$ ,  $N = 11$ ; ASY:  $82.7 \pm 11.9$ ,  $N = 24$ ). In addition, increasing ambient temperatures led to less within-female variation in off-bout length ( $\beta = -28.5$ , Table 1).

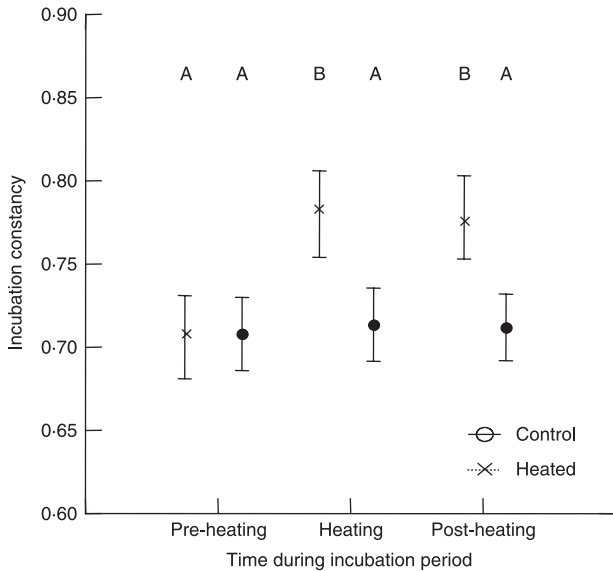
Individual variation, assessed by timing of breeding, affected the response of breeding females to the heating treatment. Incubation constancy increased as the season progressed in both treatment groups, but later-laying heated females showed a greater increase in incubation constancy than earlier-laying heated females, while later-laying control females showed a lesser increase relative to earlier-laying control females (Fig. 2, Table 1).

### EGG TEMPERATURE

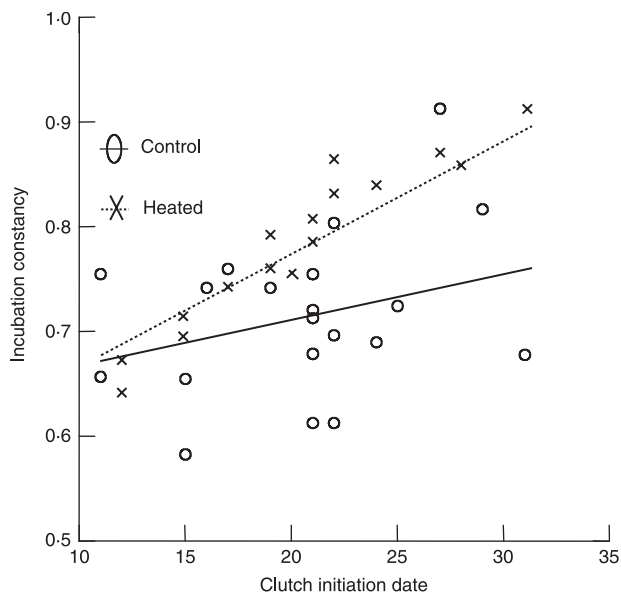
Heating treatment led to increased egg temperatures: females in the heated treatment maintained eggs at higher temperatures

**Table 1.** General linear model analysis of factors predicting incubation behaviour, egg temperature and hatching asynchrony in tree swallows subjected to an experimental heating manipulation during incubation

Predictors	Dependent variable							
	Incubation constancy	Incubation bout length	Coefficient of variation: off-bout length	On-bout egg temperature	Coefficient of variation: on-bout egg temp	Off-bout egg temperature	Coefficient of variation: offbout egg temp.	Hatching
Asynchrony								
Heating treatment	<b><i>F</i> = 7.99</b> <b><i>P</i> = 0.0001</b>	<b><i>F</i> = 8.11</b> <b><i>P</i> = 0.001</b>	<i>F</i> = 2.18 <i>P</i> = 0.15	<b><i>F</i> = 13.33</b> <b><i>P</i> = 0.001</b>	<b><i>F</i> = 4.82</b> <b><i>P</i> = 0.04</b>	<b><i>F</i> = 7.1</b> <b><i>P</i> = 0.01</b>	<b><i>F</i> = 4.55</b> <b><i>P</i> = 0.04</b>	<b><i>F</i> = 6.01</b> <b><i>P</i> = 0.02</b>
Clutch initiation date (CID)	<b>-0.028</b> <b><i>F</i> = 5.03</b> <b><i>P</i> = 0.02</b>	<b>-1.08</b> <b><i>F</i> = 5.31</b> <b><i>P</i> = 0.02</b>	-3.27 <i>F</i> = 0.59 <i>P</i> = 0.47	0.21 <i>F</i> = 1.00 <i>P</i> = 0.33	1.74 <i>F</i> = 1.21 <i>P</i> = 0.29	-0.43 <i>F</i> = 0.33 <i>P</i> = 0.57	2.63 <i>F</i> = 0.05 <i>P</i> = 0.85	0.00 <i>F</i> = 0.03 <i>P</i> = 0.88
Female age	<i>F</i> = 0.60 <i>P</i> = 0.41	<i>F</i> = 0.48 <i>P</i> = 0.53	<b><i>F</i> = 6.66</b> <b><i>P</i> = 0.02</b>	<i>F</i> = 1.03 <i>P</i> = 0.33	<i>F</i> = 1.03 <i>P</i> = 0.33	<i>F</i> = 0.39 <i>P</i> = 0.56	<i>F</i> = 0.66 <i>P</i> = 0.45	<b><i>F</i> = 5.61</b> <b><i>P</i> = 0.03</b>
Average ambient temperature during incubation	0.004 <i>F</i> = 0.09 <i>P</i> = 0.77	-0.520 <i>F</i> = 0.11 <i>P</i> = 0.68	<b>-28.5</b> <b><i>F</i> = 4.67</b> <b><i>P</i> = 0.04</b>	-0.46 <i>F</i> = 0.57 <i>P</i> = 0.46	1.11 <i>F</i> = 0.22 <i>P</i> = 0.66	0.09 <i>F</i> = 0.03 <i>P</i> = 0.91	0.90 <i>F</i> = 0.20 <i>P</i> = 0.66	-0.02 <i>F</i> = 0.30 <i>P</i> = 0.58
Female residual body mass	<i>F</i> = 0.78 <i>P</i> = 0.39	<i>F</i> = 1.28 <i>P</i> = 0.26	<i>F</i> = 0.82 <i>P</i> = 0.37	<b><i>F</i> = 5.47</b> <b><i>P</i> = 0.02</b>	<i>F</i> = 1.07 <i>P</i> = 0.31	<i>F</i> = 0.93 <i>P</i> = 0.34	<i>F</i> = 0.31 <i>P</i> = 0.58	<i>F</i> = 0.42 <i>P</i> = 0.52
Coefficient of variation: min. egg temperature								<b>1.41</b> <b><i>F</i> = 8.59</b> <b><i>P</i> = 0.01</b>
Significant interactions								
CID X Heating treatment	<b><i>F</i> = 5.34</b> <b><i>P</i> = 0.03</b>			<b><i>F</i> = 5.96</b> <b><i>P</i> = 0.02</b>		<b><i>F</i> = 4.71</b> <b><i>P</i> = 0.03</b>		
Degrees of freedom:	1,26	1,26	1,24	1,20	1,21	1,19	1,20	1,11
<i>N</i>	34	34	34	29	29	29	29	21

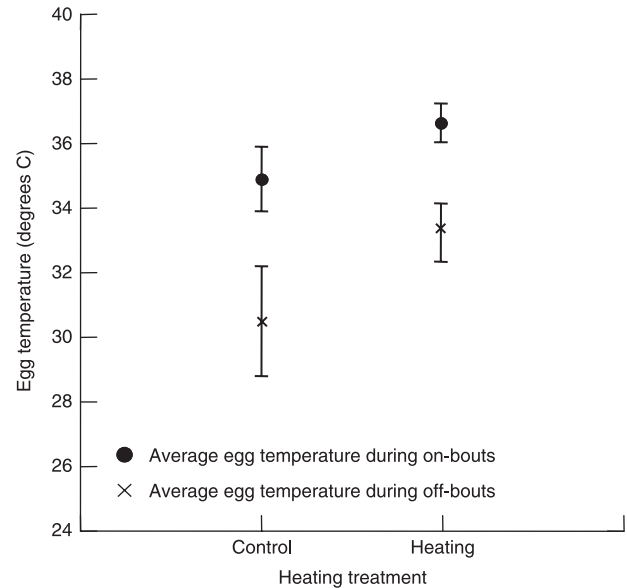


**Fig. 1.** The effect of experimental heating of nest boxes on incubation constancy of female tree swallows. 'Pre-heating', days 2–4 of incubation period; 'Heating', days 6–11 of incubation period; 'Post-heating', days 11–13 of incubation period. Values represent least square means correcting for covariates; error bars represent standard error and letters refer to significant differences among least square means. Control treatment  $N = 19$ ; heated treatment  $N = 15$ .



**Fig. 2.** Incubation constancy of female tree swallows during experimental heating period over days 6–11 of incubation period. See Table 1 for model construction. Clutch initiation date refers to the number of days from 1 May.

during on-bouts than did control females (Fig. 3; Table 1) and showed lower within-female variation in egg temperature during on-bouts (LSM coefficient of variation; control:  $18.4 \pm 2.3$ ; heated:  $9.9 \pm 3.0$ ; Table 1). Females with greater residual body mass maintained higher on-bout egg temperatures (Table 1). No other covariates predicted on-bout egg temperature or within-female variation. During off-bouts, egg temperatures



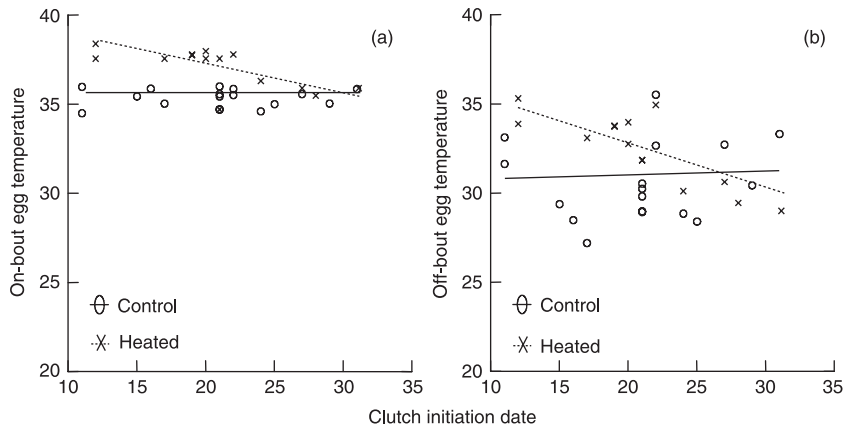
**Fig. 3.** The effect of experimental heating treatment on egg temperature of female tree swallows over days 6–11 of incubation period. Values represent least square means correcting for covariates; error bars represent standard error. Control treatment  $N = 17$ ; heated treatment  $N = 13$ .

in heated nests were higher than in control nests (Fig. 3). Finally, heated females showed less variability in off-bout egg temperature (LSM coefficient of variation; control:  $20.3 \pm 2.1$ ; heated:  $12.7 \pm 2.8$ ) (Table 1).

The effect of timing of breeding on off-bout egg temperature differed among treatments. Control females showed no change in average off-bout egg temperature over the season, but early-breeding heated females maintained higher on-bout and off-bout egg temperatures than did later-breeding heated females (Fig. 4; Table 1). This pattern was opposite to changes in ambient temperatures, which increased as the season progressed ( $\beta = 0.34$ ,  $P < 0.001$ ,  $R^2 = 0.56$ ). In addition, within heated boxes only we examined whether the degree of heating affected egg temperature changes. Changes in on-bout egg temperature were not due to increases in ambient temperature within boxes, as there was no direct relationship between the increase in internal box temperature and on-bout egg temperatures ( $F_{1,11} = 0.01$ ,  $P = 0.94$ ,  $N = 13$ ) or between mean internal temperature of heated boxes and on-bout egg temperature ( $F_{1,11} = 0.77$ ,  $P = 0.40$ ,  $N = 13$ ). There was a weak relationship between change in internal box temperature and off-bout egg temperature ( $F_{1,11} = 3.39$ ,  $P = 0.09$ ,  $R^2 = 0.16$ ).

#### ALLOCATION BETWEEN SELF-MAINTENANCE AND EMBRYONIC INVESTMENT

Heating treatment changed the allocation of time spent maintaining eggs at equilibrium temperature vs. time eggs spent cooling or being heated ( $t_{28} = -3.8$ ,  $P < 0.001$ ). Control females showed a pattern of incubation [ $t_{\text{equil}} - (t_{\text{cool}} + t_{\text{heat}})$ ] that favoured self-maintenance (mean  $-2.74 \pm 0.91$ ;  $N = 17$ ), while heated females favoured allocation of time to embryonic investment



**Fig. 4.** On-bout and off-bout egg temperatures of female tree swallows during experimental heating over days 6–11 of incubation period. Clutch initiation date refers to the number of days from 1 May.

(mean  $1.54 \pm 0.41$ ;  $N = 13$ ). There was no difference between treatments in the skew of distributions of  $t_{\text{equil}} - (t_{\text{cool}} + t_{\text{heat}})$ : control =  $1.32 \pm 0.76$ , heated =  $1.29 \pm 0.61$ ,  $t_{28} = 0.67$ ,  $P = 0.51$ .

#### HATCHING ASYNCHRONY

Heating nests led to reduced mass variation of 1-day old nestlings, a proxy of hatching asynchrony (Table 1; LSM hatching asynchrony; control  $1.61 \pm 0.1$ ,  $N = 15$ ; heated  $1.41 \pm 0.1$ ,  $N = 9$ ). In addition, younger (SY) females had higher hatching asynchrony than did older females (ASY) (Table 1; SY  $1.56 \pm 0.1$ ,  $N = 9$ ; ASY  $1.30 \pm 0.06$ ,  $N = 16$ ). Incubation variability affected hatching asynchrony; females with more variable off-bout egg temperatures had nestlings with higher hatching asynchrony (Table 1).

## Discussion

#### EFFECT OF HEATING ON EGG TEMPERATURE AND RE-ALLOCATION OF RESOURCES

Experimental heating of tree swallow nests revealed that reducing an energetic constraint during incubation leads to re-allocation of resources into greater investment in embryonic investment; heated females increased time incubating as well as both on-bout and off-bout egg temperature. In addition, differences in phenotypic quality affected their responses to the temperature manipulation; higher-quality individuals allocated resources to both increasing egg temperature and incubation attendance, while lower-quality females increased attentiveness only, but kept on-bout egg temperature similar to control females. Our manipulation revealed that investment in incubation is energetically constrained, and suggests that incubation investment in intermittent incubators is limited by resource availability and is below the optimal level for embryonic development. Our results are complementary to a previous study in which we applied an energetic constraint (via feather clipping) and found reduced incubation investment by females. In this study, instead of imposing a manipulated constraint, we tested whether incubation investment itself is naturally constrained by experimentally reducing energetic requirements, thus giving insight into how conserved energy is allocated.

Following a reduction in the energetic cost of incubation, females can allocate conserved energy to either self-maintenance or embryonic investment. If the amount of time that unmanipulated females spend incubating reflects an optimization of embryonic development conditions, then an experimental manipulation would not be expected to increase incubation constancy. However, previous heating experiments found that heating nests during incubation lead to increased incubation constancy (Bryan & Bryant, 1999; Cresswell *et al.* 2004) and other studies support the contention that energetic demands set incubation constancy (Tulp & Schekkerman 2006). Our results corroborate those findings by applying a larger magnitude heating ( $6.9^\circ\text{C}$  increase) with a corresponding greater increase in incubation constancy (8%). Our results indicate that incubation investment is limited in large part by energy resources available to incubators, suggesting that most embryos develop in suboptimal conditions. The pattern we observed between heated and control females *after the heaters were removed* also supports our contention that energy reserves influence incubation investment. Heated females continued to maintain greater incubation constancy after the heating treatment ended relative to control females suggesting that heating affected energy balance and allowed females to continue high levels of incubation investment.

Our egg temperature results are supported by behavioural responses of females, specifically a shift in time allocation during incubation by heated females. Previous work investigating the allocation of time to these activities suggested that tree swallows allocated more time to self-maintenance than to heating eggs (Voss *et al.* 2006). Here we find that heated females shifted their allocation to favour time spent maintaining eggs at equilibrium temperature relative to time eggs cooled and time spent rewarming eggs. In addition, heated females increased residual body mass and absolute body mass in response to the heating treatment suggesting that saved energy was allocated in part to self-maintenance (Perez *et al.*, in review) and females in greater body condition, reflected in higher residual body mass, maintained higher egg temperatures.

This increased incubation time was due in part to increased duration of on-bouts rather than an increase in the number of incubation bouts. Many studies have shown that parents end their incubation bout when their body reserves fall below a

certain level (Haftorn & Ytreberg 1988; Weathers & Sullivan 1989; Chaurand & Weimerskirch 1994; Reid, Monaghan & Ruxton 1999). Changing the energetics of incubation such as through reducing cooling loss through insulation led to longer incubation bouts (Cresswell *et al.* 2003), suggesting that our heating experiment led to similar responses in our birds. Another pathway of response to environmental conditions is through shorter off-bout intervals; heated birds in this study had reduced off-bout lengths. Birds frequently decrease the length of off-bouts in response to low ambient temperatures (Vleck 1981; Biebach 1986). Vleck (1981) suggested that such behaviour may serve the adaptive purpose of decreasing the period of egg cooling, thus reducing the cost of rewarming and preventing an overall increase in the length of the incubation period.

The extent to which embryos may experience suboptimal development conditions was revealed in a recent experiment conducted across a wide taxonomic range of birds that shifted eggs among species that differed in egg temperature (Martin *et al.* 2007). Martin *et al.* (2007) found that eggs incubated at higher egg temperatures had reduced incubation periods, while eggs shifted to lower egg temperatures had extended incubation periods. Our results corroborate this finding within a species by demonstrating egg temperatures are lower than expected and vary predictably among individuals. Further evidence of embryonic development conditions occurring at suboptimal levels is the increase in on-bout egg temperatures following heating treatment. This change in egg temperature is not due to direct changes in the interior nest-box temperature as the average (25°) and maximum air temperature (27.4°) within nest boxes were below the lowest egg temperature measured, even in control nests (30.5°). In addition, there was no direct relationship between the experimental change in internal nest-box temperature and the increase in egg temperature. Moreover, late-laying females, which had warmer nest boxes than early-laying females, did not increase egg temperature to the same extent as did early-laying females. These results provide strong evidence that changes in egg temperature were not due to direct changes in nest-box temperature; rather, the increase in egg temperature following heating reflects an explicit response by heated females to modulate egg temperature.

The strategy of modifying egg temperature in incubating females suggests egg temperature is a parallel and independent pathway for females to modify incubation investment. Heated females shifting excess energy reserves to embryonic investment can allocate those resources to a combination of increased attentiveness and to raising egg temperature. The optimal developmental egg temperature for most bird species, with the exception of penguins, is above 35 °C; however, most species maintain their eggs at temperatures below 35 °C (Drent 1975; Haftorn 1978, 1983; Webb 1987). Our results indicate that this is due to trade-offs between parental self-maintenance and incubation leading to the adoption of developmentally suboptimal incubation temperatures in most species (Webb 1987). Further studies should investigate the role of egg temperature in mediating and integrating the response of incubating birds to natural and experimental changes in energetic demands.

#### EFFECT OF TIMING OF BREEDING

Our temperature manipulation affected females differently based on their phenotypic quality, which further supports our conclusion that incubation investment reflects individual sensitivity and strategies in response to changing environmental conditions. They are also consistent with findings from a previous study, in which we imposed an energetic constraint on tree swallows via feather clipping and found that later-laying, lower-quality females were less able to cope with the handicap and showed a greater decrease in incubation attendance than early-laying, higher-quality females (Ardia & Clotfelter 2007). In this study, our manipulation reduced an energetic constraint and individual tree swallows differed in how they allocated 'excess' resources. Early-breeding heated females, even though ambient temperature conditions were colder, responded by maintaining higher egg temperatures than did later-laying heated females. By contrast, later-laying females increased attentiveness but not egg temperature. Thus, higher-quality heated females appear to parcel excess energy reserves into both modifying egg temperature and maintaining time on eggs, while lower-quality heated females allocated resources only to increasing attentiveness. This may be due to seasonal changes, differences in clutch size, as well as differences in the cost of increasing egg temperature relative to increasing attentiveness. More research is needed to further elucidate the mechanisms underlying this pattern.

When considering the role of within-individual variation, one aspect of incubation behaviour that affects both development conditions of eggs and costs to females through rewarming eggs is variation in off-bout length. Overall, we found that in control females, egg temperature was less variable (18.4% CV) than time spent off eggs (97.8% CV). Our heating manipulation had no effect on variation in off-bout duration, but heating led to decreased variation in egg temperature (9.9% CV). This reduced variation may reflect both increased quality of development conditions and reduced rewarming costs for females.

The effect of variation in incubation conditions on nestlings was reflected in hatching asynchrony, the degree of size difference between offspring at or near hatching. The causes, consequences, and possible adaptive value of hatching asynchrony in birds are an area of uncertainty and debate (Slagsvold & Lifjeld 1989b; Stoleson & Beissinger 1999; Cook *et al.* 2003, 2005; Cooper *et al.* 2005a; Slagsvold & Wiebe 2007). Our results suggest that hatching asynchrony is dependent in part on female investment in incubation. Heated females had nests with lower levels of hatching asynchrony. These results are not due to differences in onset of incubation or clutch size as our manipulation did not begin until the incubation period had started. Informative covariates, such as absolute egg temperature and total level of attentiveness, did not predict hatching asynchrony. Rather, the only predictor of hatching asynchrony we found was the level of within-individual variation in egg temperature; as variation in egg temperature increased, levels of hatching asynchrony also increased. This suggests that female modulation of incubation investment

during the incubation period can affect timing of development. This may arise because the majority of bird species incubate at temperatures that are suboptimal for embryonic development and an increase in incubation temperature can increase the rate of development, shortening the incubation period possibly leading to a greater mass at hatching (Hainsworth *et al.* 1998). Furthermore, mass at hatching in tree swallows is known to be correlated with mass early in the nestling period (Clotfelter, Whittingham & Dunn 2000), thus providing a direct effect of incubation investment on offspring condition.

Overall, our study finds strong support for the prediction that intermittent incubators set their incubation investment at levels dictated by energetic constraints. Furthermore, females incubating in heated boxes allocated conserved energy primarily to increased egg temperature and increased incubation attentiveness; but there were interesting differences among individuals in how they responded. Our results indicate that studies investigating the role of energetics in driving life-history investment should consider egg temperature and individual variation more explicitly.

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