

Incubation behaviour of Blue *Cyanistes caeruleus* and Great Tits *Parus major* in a Mediterranean habitat

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Abstract. The incubation stage in avian reproduction could be as costly as the nestling rearing stage. This is particularly true in the case of uniparental incubation, during which both current and future breeding attempts may be compromised. Therefore, the knowledge of the proximate effects that condition the incubation behaviour in free-living bird populations is of great importance in understanding the evolution of avian life history. In this two-year study, we assessed the incubation behaviours of Blue *Cyanistes caeruleus* and Great Tits *Parus major* inhabiting the same Mediterranean area in central Spain through the usage of iButton data loggers. It showed that the incubating behaviour of our tit populations resembles that reported in previous studies, but with peculiarities related to living at lower latitudes, i.e. with a relatively low attentiveness and a shorter active day. Both tit species showed very different incubation strategies, with Blue Tits leaving more frequently the nest (Mean \pm SE number of off-bouts, Blue Tit = 27.14 ± 0.63 , Great Tit = 16.95 ± 0.58) but for shorter periods than Great Tits (off-bout duration, Blue Tit = 8.76 ± 0.22 min, Great Tit = 14.04 ± 0.56 min; on-bout duration, Blue Tit = 22.63 ± 0.60 min, Great Tit = 36.86 ± 0.86 min). Nonetheless, both species provided a similar nest attentiveness, percentage of time of the active day during which the females were actively incubating (Blue Tit = $70.87 \pm 0.57\%$, Great Tit = $70.75 \pm 0.83\%$). Presumably, differences in the cooling rate of clutches, estimated with the iButtons, could be behind the differences in incubation behaviour between species and the greater capacity of Great Tits to adjust their incubation behaviour.

Key words: incubation rhythm, attentiveness, cooling rate, *Cyanistes caeruleus*, *Parus major*, iButton

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INTRODUCTION

The reproduction of birds has classically been divided into three well-differentiated stages: egg production, incubation and care of nestlings (Nilsson et al. 2008); although it has subsequently been proposed that the stage of nest construction should also be included (Mainwaring & Hartley 2013). Until recently, researchers' attention has been focused almost exclusively on the last stage (care of nestlings), because it has been considered to be the most expensive in terms of energy expenditure (Williams 1996). However, the incubation period could be as expensive as nestling rearing, where the metabolic rates of parents

incubating eggs can exceed the metabolic costs of thermoregulation at cool temperatures by 40–50% (reviewed in Tinbergen & Williams 2002). This is particularly true in the case of species with uniparental incubation, usually provided by the female (Conway & Martin 2000a). The high costs of uniparental incubation compromise not only current breeding attempts but also those in the future (Williams 1996, Reid et al. 2000a, Visser & Lessells 2001, Tinbergen & Williams 2002). Optimal embryo-development conditions are also provided by the female through incubation, so this stage is crucial to the hatchability of eggs and the phenotype of nestlings (Cooper et al. 2005, Chalfoun & Martin 2007, Nord & Nilsson 2011).

These embryo-development conditions are behaviourally modified by the females and this behaviour is reflected in the incubation rhythm, which must be the result of the balance between the needs of the female and the needs of the embryos (Cooper & Voss 2013). However, many ecological factors affect the costs of incubation and thus influence the incubation behaviour itself, such as ambient temperature, as it negatively influences the cooling rate of eggs (Webb 1987, Turner 2002) and negatively affects the costs of rewarming and maintaining the temperature of the eggs (de Heij et al. 2008, Nord et al. 2010); daylight duration, as it marks the time available to forage, which would otherwise be used to care for the eggs (Ricklefs & Brawn 2013); age of the embryo, because the cooling rate of eggs increases as the embryos develop and females consequently adjust their incubation behaviour (Cooper & Voss 2013); nest volume, as it influences the microclimate around the eggs in terms of temperature and humidity, and thus affects the attentiveness (Reid et al. 2002b, Deeming 2011, Deeming & Gray 2016) and clutch size, because the costs of warming larger clutches are relatively higher (de Heij et al. 2007, Nord et al. 2010, Nord & Nilsson 2012). On the other hand, increasing clutch size reduces the cooling rate of eggs (Reid et al. 2000b, Boulton & Cassey 2012). Knowing the effect of these factors is essential to understanding the evolution of certain life history traits in birds, such as clutch size (Monaghan & Nager 1997, Reid et al. 2002a) or nest construction (Reid et al. 2002b), but also to understanding the proximate effects underlying the high variation within and among species' incubation behaviours (Conway & Martin 2000b).

The aim of this study was to describe and compare the incubation behaviour of Blue *Cyanistes caeruleus* and Great Tits *Parus major* in a Mediterranean area in central Spain. We expect to clarify to some extent the proximate effects underlying the variation in incubation behaviour between and within these species (Chalfoun & Martin 2007). Although there are several studies describing the incubation behaviour of these species (i.e. Kluijver 1950, Haftorn 1981, Cowie & Novak 1990 or Amininasab et al. 2016), the climatic conditions in this region differ greatly from those in the majority of studies, which have been carried out at higher latitudes. At higher latitudes, temperatures are colder and daylight is longer, features that are likely to influence species' incubation behaviour (Ricklefs & Brawn 2013, Álvarez & Barba 2014a). Both species inhabit the same area

and share a similar ecology, being female-only, intermittent incubators, feeding primarily on lepidopteran caterpillars and occupying the same wooden nest boxes (Perrins 1991). This allowed us to compare their incubation behaviours controlling for some confounding factors such as latitude, food supply and predation risk. We hypothesized that both tit species will show a similar nest attentiveness due to evolutionary processes (Chalfoun & Martin 2007). However, we predicted that each species, due to intrinsic differences between them, will cope with ambient conditions with different incubation behaviours.

METHODS

Study area

The present study was conducted in the locality of San Pablo de los Montes (39°32'44"N, 4°19'41"W, Toledo, central Spain). This region is located in the Toledo Mountains, at a mean altitude of 1400 m asl. The area has a continental Mediterranean climate, with a mean annual temperature of 12.9 °C and a mean annual precipitation of 841.5 mm. Its landscape comprises ten oak forest patches (*Quercus pyrenaica*) in addition to Mediterranean scrubland consisting principally of Butcher's Broom *Ruscus aculeatus*, Flax-leaved *Daphne gnidium*, roses *Rosa* spp., Common Broom *Cytisus scoparius* and heather *Erica* sp. The 10 oak patches were separated from each other by a mean distance of 4.2 km. In autumn 2010, we hung 330 wooden nest boxes on the branches of oak trees at a height of 2.5–3 meters, and at a distance of 30 m from each other (for more details see Bueno-Enciso et al. 2016). Those nestboxes were quadrangular and made of pine wood, with an entrance hole of 32 mm and an internal chamber of 17.5–19.5 × 11.5 × 13 cm (floor surface = 149.5 cm²).

Field work

Field work was carried out in the springs of 2012 and 2013. In this regard, the spring of 2012 was significantly warmer than the spring of 2013 (mean ± SE temperature from 1st April to 1st June in 2012 = 19.90 ± 0.83 °C, and in 2013 = 16.17 ± 0.62 °C; t-value = 2.86, d.f. = 136, p = 0.005). From 1st April (day = 1) onwards we regularly visited all nest boxes to obtain the laying date of the first egg and the clutch size, parameter that is likely to influence the incubation behaviour of these species (Haftorn & Reinertsen 1985; De Heij et al. 2007; Nord et al. 2010, but see also De Heij et al.

2008; Nord and Williams 2015). Once the first egg laid was found, we measured the height of the nest with a ruler (to the nearest 0.01 cm), and then multiplied this height by the area of the nest box as a surrogate of nest volume. In our study area, the composition of nests in both species is similarly constituted mainly by moss and fur, although male Blue Tits frequently incorporate ornamental feathers outside the nest-cup without thermal function (Sanz & García-Navas 2011). After the nestlings had fledged, we removed the old nests from the nest boxes to ensure that every nest measured was of recent construction.

We defined the incubation period as the number of days between the last egg laid and the first signs of hatching, as described in Martin (2002). The mean (\pm SD) duration of the incubation period in each species was: for Blue Tits, 13.92 ± 2.36 days and for Great Tits, 13.85 ± 2.97 days. The tits' incubation behaviour was measured by placing two Thermochron iButton DS1922L data loggers inside the nest box. One was placed touching the eggs beneath them (nest data logger) while the other was stuck to an inner side of the nest box with the aid of brown medical tape (control data logger). Nest data loggers were also covered with brown medical tape to prevent the female from recognising them, as this makes them less showy. All data loggers recorded measurements between days 7 and 9 of the incubation period, and were placed in the nest the day before the measurements were carried out and retrieved the day after. Both nest and control data loggers were programmed to start recording temperatures each 11 s at 00:01 a.m. and finish two hours after sunset on the same day. We downloaded the temperature recordings using the Eclo Express-Thermo software 2007 (www.eclo.pt/expressthermo). The programme Rhythm 1.0 (Cooper & Mills 2005) was used to convert the text files of the temperature into audio tracks, which were imported to the RavenPro 1.5 programme (Charif et al. 2010). We adjusted the programme rhythm 1.0 with a minimum off-bout duration of 2 min and a minimum off-bout change in temperature of 2 °C, after a visual rechecking of some trial outputs in the RavenPro 1.5 programme. This programme was also used to measure the incubation rhythm parameters, which were: 1. Onset — minutes after sunrise that the nest was first left (negative values indicate minutes before sunrise); 2. Offset — minutes after sunset that the nest was last entered (negative values indicate minutes before sunset); 3. Active day — difference in minutes between the

offset and the onset; 4. On-bout — average duration in minutes of the periods during which the females actively warmed the eggs; 5. Off-bout — average duration in minutes of the periods during which the females were off the nest; 6. No off-bout — number of times that the females left the nest per day; and 7. Attentiveness — percentage of time in the active day during which the females were actively incubating. We also divided the drop in the nest data logger temperature by the off-bout duration as a proxy of the cooling rate of the clutch. Although the thermal properties of the iButtons do not mimic those of the eggs, the fact that in both species the nest architecture and the embryo development stage when we measured the incubation behaviour were very similar; and because we placed the iButtons beneath and touching the eggs, the heat provided by females must correlate with the temperature assessed by the nest data loggers (Conway & Martin 2000a).

We validated the ability of the nest data loggers to record changes in the clutch temperature with a subset of 47 nests (25 Blue Tit nests and 22 Great Tit nests). A handy-cam (Sony DCR-SR290E) was placed on a tripod 5 m away from the nest box entrance. We filmed the departures and entrances of the females for 2 hours at midday (from 13 pm to 15 pm) on the same day that we measured the incubation rhythm, when ambient temperatures are highest and could be close to the incubation temperature, making it difficult to recognize the temperature changes in nest data loggers due to the departures and entrances of the incubating females. We then compared the female activity (assessed with the video recordings) with the changes in eggs- temperature (assessed with the nest data loggers) to evaluate the ability of the iButtons to accurately record female incubation behaviour.

Statistical analyses

Mean \pm SE and range of laying day and clutch size are shown in Table 1. In total we measured the incubation rhythms of 33 Blue Tits and 30 Great Tits in 2012 and 53 Blue Tits and 22 Great Tits in 2013. The date on which the incubation behaviours were measured not differ between species (GLMM: Estimate \pm SE = -1.61 ± 1.83 , $F_{1,133} = 0.78$, $p = 0.38$) and in both years (GLMM: Estimate \pm SE = -1.08 ± 1.81 , $F_{1,133} = 0.36$, $p = 0.55$).

To achieve a comprehensive view of the incubation rhythm in each species, we first conducted

a series of Pearson's correlation tests between the incubation variables, similar to Haftorn (1981). We then compared the clutch size and each incubation rhythm parameter between species using Student's *t*-tests. To determine the daily variation in the incubation behaviour of the two species, we divided the daily hours into three blocks: morning, from 6:00 am to 11:59 am; afternoon, from 12:00 pm to 16:59 pm and evening, from 17:00 pm to 21:00 pm. We performed General Linear Mixed Models (GLMM) in which the variables of the incubation behaviour were used as response variables, the year and day block were used as categorical predictors and the calendar date of measurement was used as a covariate. We included the forest patch as the random term. Nest attentiveness model was fitted with a binomial distribution, in which the response variable was a data frame containing two columns, the total time spent actively incubating the eggs and the total time spent outside the nest by females.

The effects of the ecological factors on the incubation behaviour within tit species were examined by performing General Linear Mixed Models (GLMM) in which the incubation rhythm parameters were the response variables, the forest patch was the random term, and year and species were categorical predictors. In all models, species was nested in each predictor variable. The ecological factors were included as covariates and were ambient temperature assessed by control data loggers, daylight, incubation day (i.e. age of the embryo), clutch size and nest volume.

No female incubation behaviour was recorded twice in the same year. However, two Blue Tit females and four Great Tit females were recorded in both study years. We performed all analyses with and without these duplicated females and found no difference in results. Consequently, each incubation behaviour recording was treated as independent and the results presented below include duplicated females. Homoscedasticity, proper distribution employed and dependence were graphically verified with the residuals (Zuur et al. 2009). Models were initially constructed with all the explanatory terms fitted, including interactions. The final models were then selected by means of a backward procedure during which non-significant interactions were progressively eliminated. All analyses were performed in R (R Core Team 2014) with the "lme4" package (Bates et al. 2014). The mean \pm SE (n) is shown in the results.

RESULTS

Validation of the iButton data loggers

Upon viewing the videos, we confirmed that the sudden decreases in the nest data loggers were indeed due to the departures of the incubating females (see Appendix 1, Blue Tits: $r = 0.96$, $p < 0.001$; Great Tits: $r = 0.96$, $p < 0.001$).

Incubation behaviour

In general, both species started the active day after sunrise, with the exception of 4 female Blue (5%) and 5 Great Tits (10%), respectively. All females finished their active day before sunset (Table 1). There was no significant correlation between the onset and the offset for both species (Blue Tits: $r = -0.08$, $n = 86$, $p = 0.45$ and Great Tits: $r = 0.16$, $n = 52$, $p = 0.25$). In both species, attentiveness was negatively correlated with the mean duration of the off-bouts (Blue Tits: $r = -0.51$, $n = 86$, $p < 0.001$ and Great Tits: $r = -0.56$, $n = 52$, $p < 0.001$) and positively correlated with the duration of the on-bouts (Blue Tits: $r = 0.52$, $n = 86$, $p < 0.001$ and Great Tits: $r = 0.50$, $n = 52$, $p < 0.001$). However, the number of off-bouts was negatively related to the attentiveness of Blue Tits ($r = -0.24$, $n = 86$, $p = 0.02$) but not to that of Great Tits ($r = -0.25$, $n = 52$, $p = 0.07$). The cooling rate of the nest data logger positively affected attentiveness in both species; that is, the higher the cooling rate the higher the attentiveness (Blue Tits: $r = 0.26$, $n = 86$, $p = 0.01$ and Great Tits: $r = 0.30$, $n = 52$, $p = 0.03$). The duration of on-bouts in both species was positively correlated with the duration of their previous off-bouts (Blue Tits: $r = 0.24$, $n = 1087$, $p < 0.001$ and Great Tits: $r = 0.21$, $n = 594$, $p < 0.001$).

Comparison between species

In our study area, Great Tits had larger clutch sizes than Blue Tits (Blue Tits: 7.81 ± 0.21 , $n = 86$; Great Tits: 8.69 ± 0.23 , $n = 52$; Student's *t*-test: $t = -2.70$, d.f. = 136, $p = 0.008$) and also had very different incubation behaviours (Appendix 2). Blue Tits had a significantly longer active day than Great Tits (Student's *t*-test: $t = 2.81$, d.f. = 136, $p < 0.01$, Fig. 1A), although the onset and offset of each species did not differ significantly (Onset, Blue Tits: 22.84 ± 1.95 min (86) and Great Tits: 28.29 ± 2.75 min (52); Student's *t*-test: $t = -1.66$; $p = 0.10$. Offset, Blue Tits: -50.96 ± 3.15 min (86) and Great Tits: -59.18 ± 4.25 min (52); Student's *t*-test: $t = 1.57$; $p = 0.12$). Besides the duration of the active day, the Blue Tits' on- and off-bout periods were significantly shorter than those of

Table 1. Description of the incubation behaviour of Blue and Great Tits in the Toledo Mountains (Toledo, Central Spain) during the two years of study. Measurement date is the calendar day on which the iButton data loggers were placed (day 1 = 1April). Negative values for onset or offset indicate time in minutes before sunrise and sunset respectively.

	Blue Tit			
	2012 (N = 33)		2013 (N = 53)	
	Mean \pm SE	Range	Mean \pm SE	Range
Laying day	31.88 \pm 2.03	5–53	35.25 \pm 1.75	17–65
Clutch size	7.82 \pm 0.34	4–12	7.81 \pm 0.27	4–11
Measurement date	49.76 \pm 1.60	28–67	51.28 \pm 1.52	35–81
Time of first leaving	07:22 \pm 0.04	06:51–08:00	07:22 \pm 0.04	06:58–08:30
Time of last entering	20:41 \pm 0.07	19:10–21:35	20:31 \pm 0.06	19:28–21:24
Active day (min)	797.79 \pm 5.52	697.02–844.78	792.57 \pm 4.69	691.92–872.32
Onset (min)	24.49 \pm 2.49	-5.58–52.70	21.79 \pm 2.77	-9.00–83.10
Offset (min)	-45.29 \pm 5.75	-133.03–17.52	-54.49 \pm 3.60	-122.68– -0.73
On-bout (min)	22.09 \pm 0.84	12.38–31.42	22.97 \pm 0.82	12.73–38.18
Off-bout (min)	9.04 \pm 0.41	5.53–15.75	8.58 \pm 0.25	4.03–13.20
No Off-bouts	27.45 \pm 1.03	19–43	26.94 \pm 0.81	16–41
Attentiveness (%)	69.89 \pm 0.85	60.50–79.25	71.47 \pm 0.76	60.27–81.43

	Great Tit			
	2012 (N = 30)		2013 (N = 22)	
	Mean \pm SE	Range	Mean \pm SE	Range
Laying day	31.83 \pm 2.59	9–53	30.45 \pm 2.49	17–59
Clutch size	8.43 \pm 0.27	5–11	9.05 \pm 0.40	4–12
Measurement date	50.57 \pm 2.24	28–69	47.82 \pm 2.17	34–72
Time of first leaving	07:32 \pm 0.06	06:55–08:29	07:21 \pm 0.05	06:54–07:57
Time of last entering	20:27 \pm 0.09	19:16–21:19	20:32 \pm 0.11	19:25–21:33
Active day (min)	770.15 \pm 5.59	711.18–822.79	789.33 \pm 7.32	714.87–853.45
Onset (min)	35.09 \pm 3.69	-7.73–75.52	19.01 \pm 3.25	-4.30–55.07
Offset (min)	-62.09 \pm 5.90	-124.58– -6.23	-55.21 \pm 6.06	-117.62– -9.40
On-bout (min)	39.09 \pm 1.64	23.97–55.95	33.82 \pm 2.20	18.68–57.15
Off-bout (min)	15.97 \pm 0.74	8.30–29.92	11.41 \pm 0.43	8.02–14.65
No Off-bouts	15.27 \pm 0.56	11–21	19.23 \pm 0.96	11–29
Attentiveness (%)	69.22 \pm 1.09	62.39–80.62	72.83 \pm 1.17	63.13–80.69

the Great Tits (Student's t-test_{On-bout}: $t = -10.89$, d.f. = 136, $p < 0.001$ and Student's t-test_{Off-bout}: $t = -10.23$, d.f. = 136, $p < 0.001$, Fig. 1B, 1C), but the relationship between the duration of the on- and off-bouts was the same for both species. However, Blue Tits performed a significantly more bouts than Great Tits (Student's t-test: $t = 10.93$, d.f. = 136, $p < 0.001$, Fig. 1D), which eventually led to the same attentiveness in both species (Blue Tits: $70.87 \pm 0.57\%$ (86) and Great Tits: $70.75 \pm 0.83\%$; Student's t-test: $t = 0.09$, d.f. = 136, $p = 0.93$). Another important trait that differed significantly between species was the cooling rate of the nest data loggers, which was greater in Blue Tits (Student's t-test: $t = 3.10$, d.f. = 136, $p < 0.91$, Fig. 1E).

With regard to daily variation in the incubation behaviour, the interaction between categorical predictors (year and day block) was not significant in any of the models, and nor was the covariate 'calendar date of measurement'. Only Great Tits

were affected by the study year. They performed more but shorter off-bouts, and increased their attentiveness in 2013 with respect to 2012 (Off-bout — GLMM, $F_{1,151} = 25.56$, $p = 0.001$; No off-bout — GLMM, $F_{1,151} = 10.67$, $p = 0.002$, Attentiveness — GLMM, $\chi^2_{1,151} = 6.57$, $p = 0.01$). Apart from the yearly variation in Great Tits, there were some differences between species regarding their daily incubation behaviours (Table 2). Blue Tits performed significantly shorter on-bouts in the morning than during the rest of the day, while the duration of the Great Tits' on-bouts did not vary throughout the day (Table 2). With regard to the off-bouts, both species showed a similar pattern. The duration of the off-bouts continued to lengthen as the day progressed; and the frequency with which these off-bouts took place was the opposite, with the highest number of off-bouts occurring in the morning, and the lowest number occurring in the evening (Table 2). The daily attentiveness pattern in each species

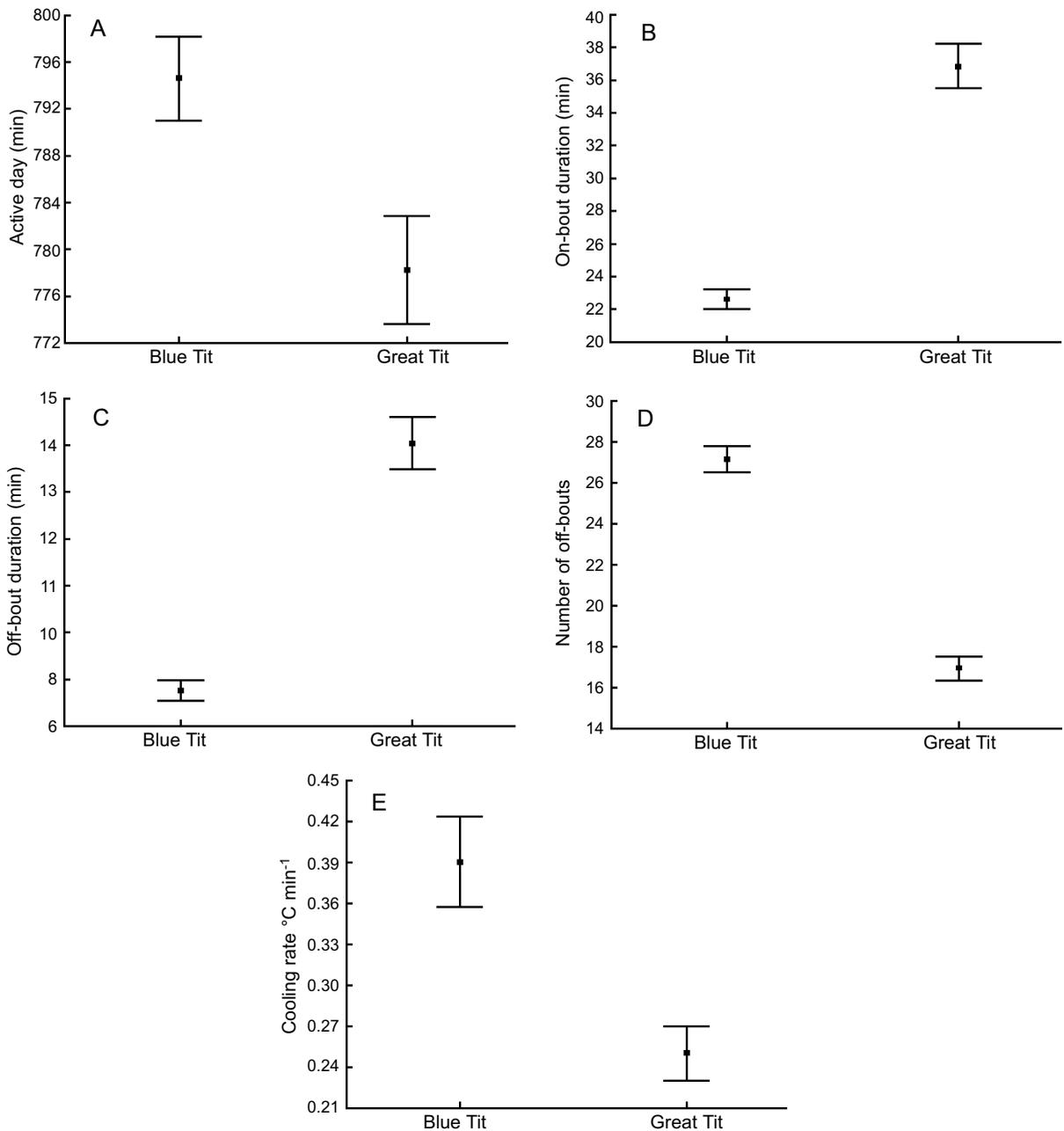


Fig. 1. Differences in the active day (A), on-bout duration (B), off-bout duration (C), number of off-bouts (D) and cooling rate (E) between Blue Tits (n= 86) and Great Tits (n= 52) breeding in Toledo Mountains (Toledo, central Spain). Vertical bars indicate SE.

differed. Blue Tits provided a significantly higher attentiveness during the afternoon, while Great Tits significantly decreased their attentiveness during the evening (Table 2).

Incubation behaviour in an ecological frame

As the former analyses showed, study year only affected incubation parameters in Great Tits. Beside the effects on the duration and number of the on- and off-bouts, and on attentiveness, as

mentioned above, the onset in Great Tits occurred later in 2012 than in 2013 (Table 3).

Incubation day only affected significantly the active day of Blue Tits and the attentiveness of Great Tits (Table 3). Female Blue Tits significantly delayed their offset and increased their active day as their embryos got older, and Great Tits increased their attentiveness as the embryos got older (Table 3).

Table 2. Daily variation in the incubation behaviour of Blue and Great Tits analysed with GLMMs. Results are Mean \pm SE. Average ranks of levels with different superscripts are significantly different (Tukey HSD test < 0.05); factors: Mo= Morning, Af= Afternoon and Ev= Evening.

	Morning	Afternoon	Evening	test	d.f.	p	Tukey HSD test
Blue Tits							
On-bout	23.87 \pm 0.87	27.42 \pm 1.32	28.36 \pm 1.69	F = 4.81	247	0.009	Mo ¹ < Ev ² , Af ^{1, 2}
Off-bout	8.23 \pm 0.26	9.47 \pm 0.33	10.15 \pm 0.36	F = 18.06	247	< 0.001	Mo ¹ < Af ² = Ev ²
No Off-bouts	9.42 \pm 0.29	8.98 \pm 0.30	7.05 \pm 0.29	F = 39.86	247	< 0.001	Mo ¹ = Af ¹ > Ev ²
Attentiveness	71.41 \pm 0.70	72.95 \pm 0.83	71.70 \pm 0.84	$\chi^2 = 12.65$	247	0.002	Mo ¹ = Ev ¹ < Af ²
Great Tits							
On-bout	39.82 \pm 2.35	39.61 \pm 2.14	37.82 \pm 2.30	F = 0.34	151	0.71	Mo ¹ = Af ¹ = Ev ²
Off-bout	12.51 \pm 0.76	14.54 \pm 0.65	16.24 \pm 0.85	F = 9.25	151	< 0.001	Mo ¹ < Ev ² , Af ^{1, 2}
No Off-bouts	6.32 \pm 0.30	6.08 \pm 0.25	4.55 \pm 0.24	F = 18.94	151	< 0.001	Mo ¹ = Af ¹ > Ev ²
Attentiveness	71.51 \pm 1.15	72.14 \pm 0.99	68.32 \pm 1.39	$\chi^2 = 40.61$	151	< 0.001	Mo ¹ = Af ¹ > Ev ²

Both Blue and Great Tits significantly advanced their onset as the ambient temperatures increased, but their active day was not significantly affected by ambient temperature (Table 3). Ambient temperature negatively affected the on-bout duration of Great Tits and positively affected the off-bout duration of Blue Tits (Table 3). Due to these effects, the attentiveness in both species was negatively affected by ambient temperature (Table 3 and Fig. 2A). Finally, the cooling rate of Blue Tit nest data loggers was negatively affected by ambient

temperature (Cooling rate — GLMM, Estimate \pm SE = -0.1 \pm 0.01, $t = -2.85$, $p = 0.01$).

The duration of the daylight only significantly affected the onset and offset of Blue Tits. This species started incubation activity later and finished earlier as the daylight lengthened (Table 3). Daylight also negatively affected the off-bout duration in Great Tits (Table 3). Finally, both species provided significantly more attentiveness as the days got longer (Table 3 and Fig. 2b).

Table 3. Results of the GLMMs analysing the effects of the species (Blue and Great Tits), year and the ecological factors on the incubation behaviour of tits in the Toledo Mountains (Toledo, central Spain). Species were nested in each explanatory variable. The ID of the forest patch was included in all models as the random term. The degrees of freedom in all models were 138.

	Estimate \pm SE	Onset		Estimate \pm SE	Offset	
		Test	p		Test	p
Intercept	-201.60 \pm 95.43		0.04	472.54 \pm 149.76		0.002
Species		F = 4.27	0.04		F = 0.90	0.34
Year		F = 14.76	< 0.001		F = 0.63	0.43
Blue Tit	-3.08 \pm 1.96	$t = 1.57$	0.12	-4.08 \pm 3.12	$t = 1.31$	0.19
Great Tit	-9.80 \pm 2.70	$t = 3.62$	< 0.001	-0.19 \pm 4.39	$t = 0.04$	0.97
Incubation day		F = 0.83	0.44		F = 0.83	0.44
Blue Tit	-1.92 \pm 1.69	$t = -1.13$	0.26	9.21 \pm 2.71	$t = 3.40$	< 0.001
Great Tit	1.01 \pm 1.70	$t = 0.60$	0.55	-0.85 \pm 2.72	$t = -0.31$	0.76
Temperature		F = 7.32	< 0.001		F = 1.33	0.27
Blue Tit	-0.99 \pm 0.35	$t = -2.81$	0.01	-0.83 \pm 0.55	$t = -1.51$	0.13
Great Tit	-1.01 \pm 0.37	$t = -2.77$	0.002	-0.36 \pm 0.58	$t = -0.62$	0.54
Daylight		F = 5.24	0.01		F = 5.24	0.01
Blue Tit	0.49 \pm 0.15	$t = 3.22$	0.002	-0.78 \pm 0.24	$t = -3.16$	0.002
Great Tit	0.05 \pm 0.45	$t = 0.38$	0.71	-0.45 \pm 0.23	$t = -1.97$	0.05
Clutch size		F = 0.06	0.94		F = 0.06	0.94
Blue Tit	0.41 \pm 1.17	$t = 0.35$	0.73	-2.75 \pm 1.89	$t = -1.46$	0.15
Great Tit	0.02 \pm 1.45	$t = 0.02$	0.99	4.97 \pm 2.34	$t = 2.12$	0.04
Nest volume		F = 1.75	0.18		F = 1.75	0.18
Blue Tit	0.02 \pm 0.01	$t = 1.84$	0.07	-0.04 \pm 0.02	$t = -2.04$	0.04
Great Tit	-0.001 \pm 0.01	$t = -0.31$	0.75	-0.04 \pm 0.02	$t = -1.71$	0.09

Continued on the next page...

	Active day			On-bout		
	Estimate ± SE	Test	p	Estimate ± SE	Test	p
Intercept	683.67 ± 168.12		< 0.001	0.87 ± 39.56		0.98
Species		F = 3.29	0.07		F = 1.57	0.21
Year		F = 2.74	0.10		F = 4.76	0.03
Blue Tit	-0.55 ± 3.55	t = 0.16	0.87	0.13 ± 0.82	t = -0.16	0.87
Great Tit	9.49 ± 4.92	t = -1.93	0.06	-2.95 ± 1.15	t = 2.57	0.01
Incubation day		F = 7.06	0.001		F = 1.31	0.27
Blue Tit	11.37 ± 3.04	t = 3.74	< 0.001	-0.48 ± 0.71	t = -0.68	0.50
Great Tit	-1.23 ± 3.05	t = -0.41	0.69	1.05 ± 0.72	t = 1.47	0.14
Temperature		F = 0.40	0.67		F = 3.26	0.04
Blue Tit	-0.03 ± 0.62	t = -0.05	0.96	-0.22 ± 0.15	t = -1.54	0.13
Great Tit	0.58 ± 0.65	t = 0.90	0.37	-0.31 ± 0.15	t = -2.07	0.04
Daylight		F = 1.87	0.16		F = 0.91	0.40
Blue Tit	-0.24 ± 0.27	t = -0.87	0.39	0.09 ± 0.06	t = 1.33	0.19
Great Tit	0.44 ± 0.26	t = 1.73	0.09	-0.02 ± 0.06	t = -0.26	0.80
Clutch size		F = 2.64	0.08		F = 1.21	0.30
Blue Tit	-2.83 ± 2.12	t = -1.34	0.18	0.52 ± 0.50	t = 1.05	0.30
Great Tit	4.90 ± 2.63	t = 1.87	0.06	0.71 ± 0.61	t = 1.15	0.25
Nest volume		F = 5.39	0.01		F = 2.12	0.12
Blue Tit	-0.06 ± 0.02	t = -2.96	< 0.01	0.001 ± 0.01	t = 0.27	0.79
Great Tit	-0.04 ± 0.02	t = -1.42	0.16	-0.01 ± 0.01	t = -2.04	0.04

	Off-bout			No Off-bout			Attentiveness		
	Estimate ± SE	Test	P	Estimate ± SE	Test	P	Estimate ± SE	Test	P
Intercept	49.76 ± 13.25		< 0.001	44.79 ± 28.92		0.12	-2.38 ± 0.37		< 0.001
Species		F = 7.17	0.01		F = 2.80	0.10		$\chi^2 = 0.03$	0.86
Year		F = 21.14	< 0.001		F = 4.30	0.04		$\chi^2 = 11.61$	< 0.001
Blue Tit	-0.10 ± 0.28	t = 0.37	0.71	-0.01 ± 0.61	t = 0.01	0.99	0.01 ± 0.01	z = -1.48	0.14
Great Tit	-2.29 ± 0.39	t = 5.91	< 0.001	2.15 ± 0.85	t = -2.54	0.01	0.07 ± 0.01	z = -5.23	< 0.001
Incubation day		F = 0.58	0.56		F = 1.33	0.27		$\chi^2 = 9.84$	0.007
Blue Tit	-0.26 ± 0.24	t = -1.07	0.29	0.83 ± 0.52	t = 1.58	0.12	0.009 ± 0.008	z = 1.24	0.22
Great Tit	0.03 ± 0.24	t = 0.13	0.90	-0.22 ± 0.52	t = -0.42	0.68	0.02 ± 0.008	z = 2.92	0.003
Temperature		F = 3.60	0.03		F = 1.00	0.37		$\chi^2 = 191.38$	< 0.001
Blue Tit	0.11 ± 0.05	t = 2.29	0.02	0.12 ± 0.11	t = 1.11	0.27	-0.02 ± 0.002	z = -11.31	< 0.001
Great Tit	0.07 ± 0.05	t = 1.40	0.16	0.10 ± 0.11	t = 0.88	0.38	-0.02 ± 0.001	z = -9.05	< 0.001
Daylight		F = 7.74	< 0.001		F = 1.51	0.22		$\chi^2 = 102.10$	< 0.001
Blue Tit	-0.01 ± 0.02	t = -0.24	0.81	-0.08 ± 0.05	t = -1.68	0.10	0.002 ± 5.75e-4	z = 4.01	< 0.001
Great Tit	-0.08 ± 0.02	t = -3.93	< 0.001	0.02 ± 0.04	t = 0.47	0.64	5.21e-3 ± 5.46e-4	z = 9.56	< 0.001
Clutch size		F = 0.42	0.65		F = 0.90	0.41		$\chi^2 = 33.40$	< 0.001
Blue Tit	-0.12 ± 0.17	t = -0.70	0.48	-0.47 ± 0.37	t = -1.29	0.20	0.03 ± 0.005	z = 5.63	< 0.001
Great Tit	0.12 ± 0.21	t = 0.60	0.55	-0.16 ± 0.45	t = -0.35	0.73	8.06e-3 ± 7.11e-3	z = 1.13	0.26
Nest volume		F = 6.46	0.002		F = 1.26	0.29		$\chi^2 = 4.19$	0.12
Blue Tit	-0.001 ± 0.002	t = -0.07	0.95	-0.003 ± 0.003	t = -0.86	0.39	4.09e-5 ± 6.64e-5	z = 0.76	0.45
Great Tit	-0.01 ± 0.002	t = -3.59	< 0.001	0.01 ± 0.004	t = 1.33	0.19	1.22e-4 ± 6.64e-5	z = 1.91	0.06

The clutch size positively affected the offset of Great Tits. That is, this species delayed its offset as their clutches got larger (Table 3). Clutch size also positively affected the nest attentiveness of Blue Tits (Table 3).

Nest volume significantly affected the offset of Blue Tits. Those females with relative large nests advanced their offset with respect to the sunrise and had shorter active days (Table 3). Nest volume negatively affected the length of on- and off-bouts in Great Tits, although this effect was not reflected in their attentiveness (Table 3).

DISCUSSION

The view of the video recordings confirmed that the use of the iButton methodology to assess the incubation behaviour of these tit species was reliable, as demonstrated in other species (Smith et al. 2016).

Almost all females measured began their daily activity after sunrise and all of them finished before sunset, behaviour that has been previously described (Haftorn 1981, Cowie & Novak 1990). This is probably due to the fact that tits are

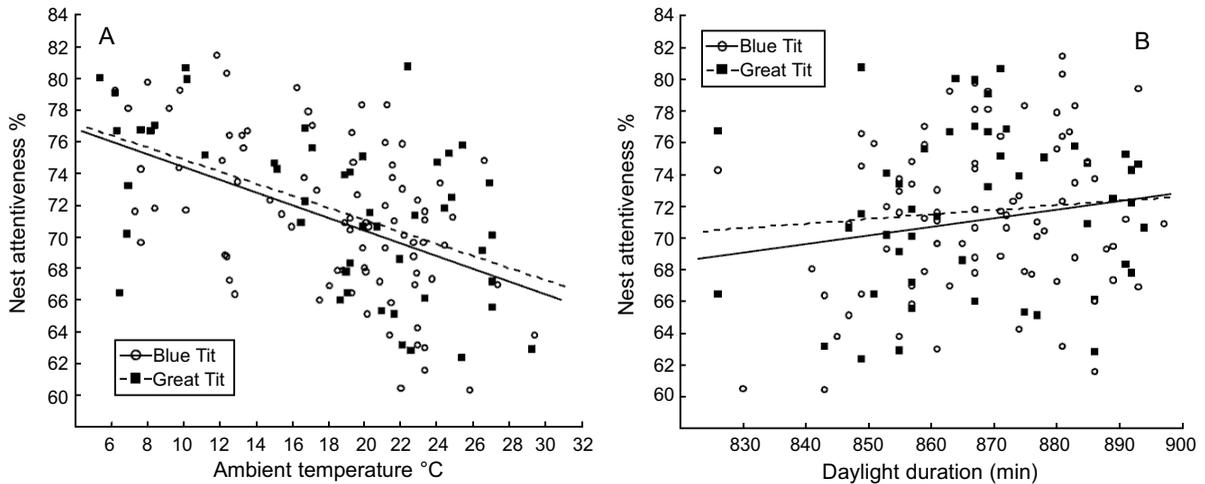


Fig. 2. Effect of ambient temperature (A) and daylight duration (B) on nest attentiveness in two populations of Blue and Great Tits in the Toledo Mountains (Toledo, central Spain).

eyesight predators and consequently need daylight to forage. The result that the onset was not correlated with the offset was also obtained by Haftorn (1981), and suggests that the time of first leaving and the time of last entering are set by females cueing on features other than the length of the active day. However, the on-bout duration was highly correlated with the duration of the previous off-bout in both species, as occurred with the Norwegian population studied by Haftorn (1981). This is probably due to the fact that — under similar conditions of food availability — the female is able to intake a higher quantity of food during long off-bouts and then uses this energy to spend more time caring for the eggs (Conway & Martin 2000b, Kovarik et al. 2009). The cooling rate of nest data loggers was positively related to the attentiveness in both species, probably because of the necessity of females to avoid declines in clutch temperature below the Physiological Zero Temperature (PZT, Conway & Martin 2000a).

The incubation behaviour of our populations of Blue and Great Tits resembles that described for a southern European Great Tit population breeding at a similar latitude (Álvarez & Barba 2014a), characterised by having a relatively low attentiveness and shorter active day in relation to more northern populations (e.g. Haftorn 1981, Cowie & Novak 1990). This is mainly because there are fewer hours of daylight at lower latitudes but the time needed to forage presumably remains constant (Álvarez & Barba 2014a, Shaw & Cresswell 2014).

Besides this latitudinal pattern in the incubation behaviour, both Blue and Great Tits showed

very different incubation strategies in our study area (Fig. 1). However, the attentiveness of the two species was very similar, reinforcing the idea that the global pattern in nest attentiveness is due to evolutionary processes (Chalfoun & Martin 2007). The duration of the on- and off-bouts, as well as their quantity, differ between species. These traits express the adjustments that incubating females make in order to balance their own needs and those of the developing embryos (Cooper & Voss 2013). In this respect, fewer but longer off-bouts may be beneficial for females in terms of energy savings (Conway & Martin 2000b). Blue Tits probably could not lengthen their off-bouts as much as Great Tits because their clutches cool faster than Great Tit clutches, as the cooling rate of nest data loggers suggests. One reason for this could be due to the smaller clutch size of Blue Tits compared to Great Tits. In this respect, clutch size negatively affected the cooling rate of eggs because the neighbouring eggs buffer the heat loss by convection in large clutches (Reid et al. 2000b, Boulton & Cassey 2012). However, clutch size did not significantly affect the duration and number of the bouts in either of the two species (Table 3), a feature that challenges this possibility. Nonetheless, other intrinsic specific characteristics like the smaller body and egg size of Blue Tits compared to Great Tits could be behind the different incubation strategies exhibited by the two species, although we could not test this in this study due to the lack of data. In our study area, Blue Tit weight is about half the weight of Great Tits and their eggs are about 40% smaller than those of Great Tits (authors' unpubl. data). Large

body sizes can store more fat reserves than smaller body sizes, and allow individuals to spend more time without eating. Similarly, small eggs have a higher cooling rate than larger ones (Turner 2002, Chalfoun & Martin 2007) and have less resources to protect embryos from inclement conditions (Martin 2008).

Although both species showed similar daily patterns, there were some differences (Table 3). It could be that the smaller body size of Blue Tits constitutes a greater handicap regarding the energy costs of the continuous nightly incubating period. This may result in a significantly lower duration of on-bouts in the morning. As the day progressed, both species increased the duration of their off-bouts and diminished their frequency, probably due to rising temperatures throughout the day. Blue Tits increased their attentiveness in the afternoon, possibly because in the morning and evening this species shifted their investment to self-maintenance to recover and prepare, respectively, for the continuous nightly incubation period. On the other hand, Great Tits diminished their attentiveness in the evening, which could be a consequence of the warmer ambient temperatures during this period of the day and to accumulate energy reserves to face the continuous nightly incubation period.

The analysis of the ecological factors that may influence tit incubation behaviour suggested that Great Tits had a more flexible incubation rhythm than Blue Tits. Great Tits performed less but longer off-bouts and showed a significantly lower attentiveness in 2012 than in 2013. A possible explanation could rely on the fact that 2012 was warmer than 2013, which may have reduced the costs of incubating in the warmer year (Bryan & Bryant 1999, Nord et al. 2010). Again, the faster temperature loss in Blue Tit clutches may have constrained the response of this species, as it impedes the lengthening in excess of their off-bouts. In 2012, Great Tit onset occurred later than in 2013, despite the warmer conditions in the previous year and the negative effect of the ambient temperature on the onset. Therefore, this yearly variation in the onset of Great Tits should be related to causes other than ambient temperatures.

The incubation day significantly affected the incubation behaviour in both species, although in different ways. This is mainly because the cooling rate of eggs increases as the embryos age, and females shape these new incubating conditions behaviourally (Cooper & Voss 2013). Blue Tits seemed to adjust their incubating behaviour by

lengthening their active day, through a delay in their offset. Great Tits significantly increased their nest attentiveness.

As the ambient temperatures increased, both tit species left the nest for first time earlier in the morning. This effect of ambient temperature on the onset could be due to the fact that on warm mornings, the risk that the egg temperature falls below the PZT is lower (Conway & Martin 2000a) and females can leave their clutches unattended earlier. However, the main effect of ambient temperature on tit's incubation behaviour was its negative effect on their attentiveness. This effect has been previously seen in other observational studies (Haftorn 1981, 1988, Matysioková & Remeš 2010) and thermal manipulation experiments (Haftorn & Reinertsen 1990, Londoño et al. 2008, Álvarez & Barba 2014b). This is likely because in warm conditions the energy costs of maintaining the temperature of eggs and rewarming them decreases (Haftorn & Reinertsen 1985, Nord et al. 2010), and females can shift these energy savings in self-maintenance (Chalfoun & Martin 2007). The cooling rate of Blue Tit nest data loggers was negatively affected by ambient temperature. This could be because the larger clutch and egg size in Great Tits buffers the temperature loss of clutches in this species (Boulton & Cassey 2012).

The effect of daylight on the incubation behaviour of our studied populations of tits resembles that found in a latitudinal gradient (Ricklefs & Brawn 2013). In this respect, birds compensate for the seasonal shortening of the nightly hours by increasing their attentiveness during the day and spend more time in their nests in the early morning and in the late evening. This explains why the active day changes so little seasonally or latitudinally (Álvarez & Barba 2014a). However, in our study area, Great Tits did not change their on- and offset in relation to the duration of daylight. This is why their active day was nearly significantly affected by the daylight.

Female Great Tits delayed their offset as their clutch size increased, but Blue Tits did not. It could be that Great Tit females needed to store more energy reserves before the continuous nightly incubating period when they had relatively large clutches. The clutch size also positively affected the attentiveness in Blue Tits. This could indicate that, in this species, only the best females are capable of laying relatively more eggs and providing them a higher degree of attentiveness.

Finally, nest volume negatively affected the offset and active day of Blue Tits. Large nests may

better preserve the heat of clutches, allowing females to regulate the nest microclimate more efficiently (Reid et al. 2000a), and reducing their time needed to forage by advancing their offset. Meanwhile, Great Tits may reduce the length of their bouts with larger nests. The enhanced thermoregulatory properties of large nests likely allowed female Great Tits to reduce the length of their on-bouts, as the temperature loss in these clutches may be less.

CONCLUSIONS

The incubation behaviour of our Mediterranean populations of tits resembles that previously described but with the peculiarities of southern populations. Both species, despite sharing a similar ecology in the same habitat, showed very different incubation strategies. In this regard, Great Tits seem to have less costly and more flexible incubation behaviour than Blue Tits.

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STRESZCZENIE

[Wzorzec wysiadywania u bogatki i modraszki w warunkach klimatu śródziemnomorskiego]

Nakłady ponoszone przez ptaki na wysiadywanie mogą być równie wysokie, jak te związane z opieką nad pisklętami. Ma to znaczenie zwłaszcza u gatunków, u których wysiadywaniem zajmuje się tylko jeden z rodziców, gdyż nakłady poniesione na wysiadywanie mogą wpływać zarówno na dalsze etapy danej próby rozrodu, jak i kolejne lęgi. Dlatego poznanie czynników wpływających na wysiadywanie ma ogromne znaczenie dla zrozumienia ewolucji historii życiowych.

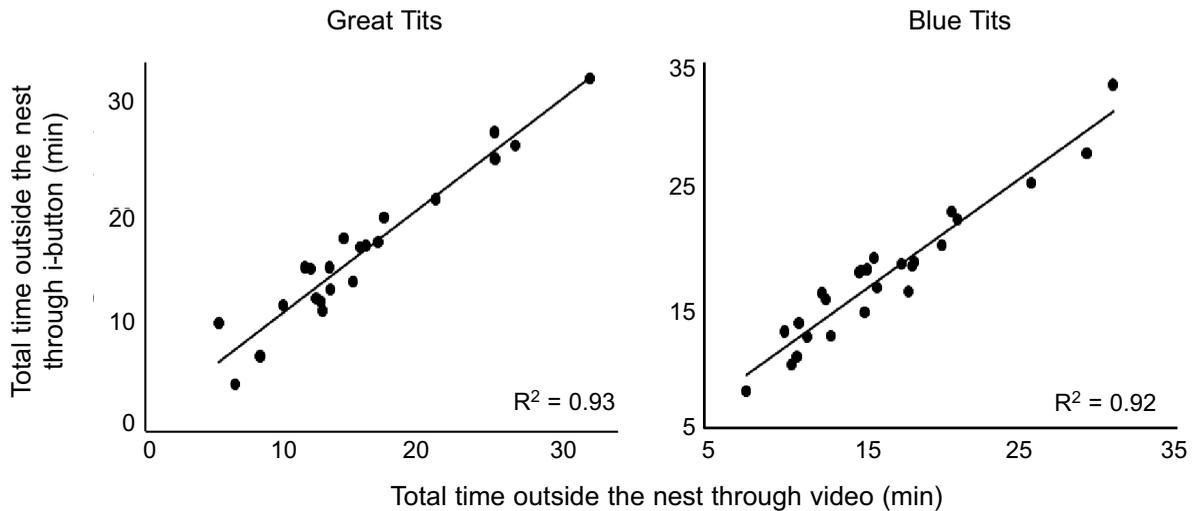
W pracy badano wzorzec wysiadywania dwóch gatunków sikor — bogatki i modraszki zamieszkujących ten sam region śródziemnomorski w centralnej Hiszpanii. Wzorzec wysiadywania określano na podstawie zapisu temperatury dokonywanego za pomocą dwóch rejestratorów temperatury (iButton, model DS1922L): jednego umieszczonego w czarze pod jajami i drugiego przytwierdzonego do wewnętrznej ściany skrzynki lęgowej. Pomiar temperatury, co 11 s, pomiędzy 7 i 9 dniem wysiadywania, rozpoczął się o godz. 00:01 a kończył dwie godziny po zachodzie słońca. Zapisy temperatury z rejestratorów weryfikowano z danymi z 2-godzinnych nagrań wideo (Apendyks 1, 2). Na podstawie zapisu temperatury określano: początek i koniec wysiadywania danego dnia, długość aktywności dobowej związanej z wysiadywaniem, średnią długość sesji wysiadywania, średnią długość czasu spędzanego poza gniazdem, oraz procentowy udział aktywności dobowej spędzany na wysiadywaniu (Tab. 1)

Stwierdzono, że wzorzec wysiadywania w badanych populacjach przypomina wzorce opisane dla tych gatunków w innych pracach, jednak ze zmianami wynikającymi z miejsca prowadzenia badań na niższych szerokościach geograficznych, tj. z krótszą aktywnością dobową oraz krótszym procentowym udziałem aktywności dobowej spędzanej na wysiadywaniu (Tab. 1). Badane gatunki wykazywały różne strategie wysiadywania — modraszki m.in. częściej opuszczały gniazdo, ale czas pobytu poza gniazdem był u nich krótszy niż u bogatki (Fig. 1, Tab. 3). Istniały również pewne różnice we wzorcu wysiadywania w obrębie dnia: u modraszki długość sesji na

gnieździe była krótsza rano niż przez resztę dnia, podczas gdy u bogatki była zbliżona w poszczególnych okresach dnia (Tab. 2). Ogólnie, oba gatunki poświęcały na wysiadywanie w ciągu dnia zbliżoną ilość czasu (procentowy udział aktywności dobowej spędzany na wysiadywaniu), przy czym parametr ten był ujemnie związany z temperaturą otoczenia oraz dodatnio z długością dnia (Tab. 1, 3, Fig. 2).

Autorzy sugerują, że za obserwowane różnice w zachowaniach związanych z wysiadywaniem między dwoma badanymi gatunkami sikor mogą być odpowiedzialne różnice w szybkości ochładzania się ich zniesienia.

Appendix 1. Lineal correlation between the total time outside the nest assessed by means of visual departures of the females using video recordings and sudden decreases in the clutch temperature using iButtons data loggers placed in the nest cup among the eggs Blue and Great Tits.



Appendix 2. Diagrams of the incubation rhythm during daylight of Great Tits (A) and Blue Tits (B) in a population in the Toledo Mountains (Toledo, central Spain). Incubation behaviours were obtained by placing iButton data loggers among the eggs. Off-bouts are highlighted in blue, and their order number is shown in the upper part of the diagrams.

