



Incubation Feeding and Nest Attentiveness in a Socially Monogamous Songbird: Role of Feather Colouration, Territory Quality and Ambient Environment

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Abstract

Parental investment and environmental conditions determine reproductive success in wild-ranging animals. Parental effort during incubation, and consequently factors driving it, has profound consequences for reproductive success in birds. The female nutrition hypothesis states that high male feeding enables the incubating female to spend more time on eggs, which can lead to higher hatching success. Moreover, both male and female parental investment during incubation might be signalled by plumage colouration. To test these hypotheses, we investigated relationships between male and female incubation behaviour and carotenoid and melanin-based plumage colouration, territory quality and ambient temperature in the Great Tit *Parus major*. We also studied the effect of female incubation behaviour on hatching success. Intensity of male incubation feeding increased with lower temperatures and was higher in territories with more food supply, but only in poor years with low overall food supply. Female nest attentiveness increased with lower temperatures. Plumage colouration did not predict incubation behaviour of either parent. Thus, incubation behaviour of both parents was related mainly to environmental conditions. Moreover, there was no relationship between male incubation feeding, female nest attentiveness and hatching success. Consequently, our data were not consistent with the female nutrition hypothesis.

Introduction

Parental investment and environmental conditions during reproduction are key determinants of reproductive output in free-ranging animals. Incubation is one of the key processes in avian reproduction (White & Kinney 1974; Deeming 2002a). Some form of incubation behaviour is present in 99% of all bird species. In species with uniparental incubation, which is usually done by females (Skutch 1957), the incubating individual has reduced time for foraging and self-maintenance (Drent 1975). As incubation is energetically demanding (Williams 1996; Thomson et al. 1998; Tinbergen & Williams 2002), this can

have negative effects on body condition of the incubating parent, subsequent care during the same or next breeding attempt, or survival to the next breeding season (Heinsohn & Cockburn 1994; Heaney & Monaghan 1996; Reid et al. 2000; Visser & Lessells 2001; de Heij et al. 2006).

In many species in which the male does not participate directly in warming the eggs, he feeds the incubating female. This behaviour is called incubation feeding (Lack 1940; Kendeigh 1952). In hornbills, some raptors and some songbirds, the incubating female is completely dependent on incubation feeding (Kendeigh 1952; Poulsen 1970; Verbeek 1972). However, in the majority of species

males provide only a certain part of the daily food intake of incubating females (Davies 1977). The intensity of incubation feeding differs both within and among species (Kendeigh 1952; Conway & Martin 2000), and can be influenced by various factors. Its intensity was found to increase with decreasing ambient temperature (Nilsson & Smith 1988; Smith et al. 1989; Pearse et al. 2004), higher male quality (Lifjeld et al. 1987; Siefferman & Hill 2005), or higher food supply on territory (Zanette et al. 2000). Currently, the most popular hypothesis to explain the occurrence and patterns of incubation feeding is the female nutrition hypothesis (von Haartman 1958; Royama 1966). It claims that incubation feeding is an important source of energy for the incubating female. Consequently, male provides female with an important part of her daily energy intake, that allows her to spend more time on eggs, i.e. to increase her nest attentiveness (Martin & Ghalambor 1999; Tewksbury et al. 2002).

As higher nest attentiveness can lead to higher hatching success (Lyon & Montgomerie 1985; Webb 1987), incubation feeding can significantly affect reproductive performance of birds. However, nest attentiveness can be influenced by other factors in addition to incubation feeding. Females increase nest attentiveness in cold temperatures to keep eggs within temperature limits necessary for successful development of the embryo (Yom-Tov et al. 1978; Webb 1987; Sanz 1997). High-quality territories with superior food supply enable females to spend more time on eggs (Rauter & Reyer 1997; Zanette et al. 2000; Zimmerling & Ankney 2005). Apart from environmental conditions, higher quality females also spend more time on eggs (Ardia & Clotfelter 2007), which is also evidenced by a positive relationship between clutch size and nest attentiveness found in some species of birds (Blagosklonov 1977; Jones 1987; Deeming 2002b).

Individual quality and ability to provide parental care and invest in a given breeding attempt can be signalled by plumage colouration (Hill & McGraw 2006). Carotenoid-based feather colouration is widespread in birds (Olson & Owens 2005). Carotenoids cannot be synthesized by birds, and thus their concentration in feathers is dependent on both food availability and foraging efficiency (McGraw 2006a). Moreover, when deposited into feathers they cannot be used for important physiological functions, including immunological defence or mitigating oxidative stress. Consequently, carotenoids allocated to feathers should indicate individual quality and/or condition (Møller et al. 2000). Although melanin

ornaments are often claimed not to reflect condition (McGraw 2006b), recent evidence suggests that in certain conditions they might be as condition-dependent as carotenoid-based ornaments (Griffith et al. 2006). Potential proximate mechanisms of condition-dependence might include corticosterone-mediated stress (Roulin et al. 2008), oxidative stress (Galván & Alonso-Alvarez 2008, 2009), allocation of calcium among competing physiological functions (Roulin et al. 2006) or hormonal control of melanin deposition (McGraw 2008). Thus, both types of plumage colouration can be important in signalling capability of parental investment.

In our study, we examined male and female incubation behaviour in the Great Tit *Parus major*, a typical socially monogamous passerine with female-only incubation. Our aims were to find out: (1) which factors affect the intensity of male incubation behaviour, i.e. incubation feeding, (2) what is the effect of incubation feeding and other factors on female incubation behaviour, i.e. nest attentiveness, (3) what is the effect of nest attentiveness on hatching success, and (4) whether carotenoid- and melanin-based plumage colouration predicts parental effort during incubation in both males and females. Based on the predictions of the female nutrition hypothesis, we expected to find a positive relationship between incubation feeding and nest attentiveness; we also expected a positive relationship between nest attentiveness and hatching success. We expected that parental effort will be positively related to the intensity of both carotenoid- and melanin-based plumage colourations.

Methods

General Fieldwork

This work was conducted on three adjacent nest-box plots (188 nest-boxes in total), in a deciduous forest near Grygov (49°31'N, 17°19'E) in eastern Czech Republic. The forest is dominated by lime *Tilia* spp. and oak *Quercus* spp. with interspersed ash *Fraxinus excelsior*, hornbeam *Carpinus betulus*, and alder *Alnus glutinosa*. Nest boxes are placed about 1.5 m above ground and besides Great Tit are inhabited by Blue Tit *Cyanistes caeruleus*, Collared Flycatcher *Ficedula albicollis*, and Nuthatch *Sitta europea*. Fieldwork was carried out between 2005 and 2007 from early Apr. until mid-Jun. We checked nest-boxes daily to record the laying of the first egg and final clutch size. Later, we checked which eggs hatched to determine the hatching success. We defined hatching

success as the percentage of eggs that hatched. We used only nests where we knew the exact fate of all the eggs.

Incubation Behaviour

During incubation, we monitored nest attentiveness of females and incubation feeding by males. We obtained one sample from each nest. To determine nest attentiveness, we deployed temperature data loggers (Hobo H8 Temp/External; Onset Computer Corp., Pocasset, MA, USA), by putting their first probe (1.8 m cable) through the nest wall into the bottom of the nest cup. The data logger itself with the second, inner probe was mounted under the nest-box. These probes measured inner and outer temperature at every nest from 06.00 until 12.00 hours (i.e. for 6 h) in 9-s intervals. This interval is the shortest possible to enable the coverage of 6 h of recording with respect to the memory capacity of our data loggers. To determine incubation feeding, we placed video cameras about 5 m in front of the nest-box on the ground and recorded bird activity for 90 min. This is a standard recording period in studies of incubation feeding in songbirds (e.g. Zanette et al. 2000; Badyaev & Hill 2002; Doerr & Doerr 2007). We deployed cameras in the morning, between 07.30 and 12.00 hours. We took ambient temperature during incubation feeding from a local meteorological station. Both data loggers and cameras were deployed early in the incubation period. Median was day 4 (range 1–8) for data loggers and day 3 (1–9) for cameras, where day 0 means the day when the last egg was laid. Female Great Tits were in full incubation already on day 1, as evidenced by no significant effect of the day of incubation on nest attentiveness (see Results). In 80 nests, cameras were deployed on the same morning when data loggers were recording temperature. Setting cameras took very short time (<1 min). However, to check for potential disturbance, we compared nest attentiveness for nests, where cameras and data loggers were recording on the same morning vs. on different days; there was no difference ($F_{1,159} = 0.02$; $p = 0.900$) excluding any potential systematic bias in the data.

On the nest temperature recordings, time when the incubating female is away from the nest, is recognisable by downward spikes (Fig. 1). Temperature drops quickly when the female leaves the clutch (off-bout) and then starts to increase sharply when she returns (on-bout). Consequently, it is easy to make the difference between an attended and an empty nest (Zimmerling & Ankney 2005). From the

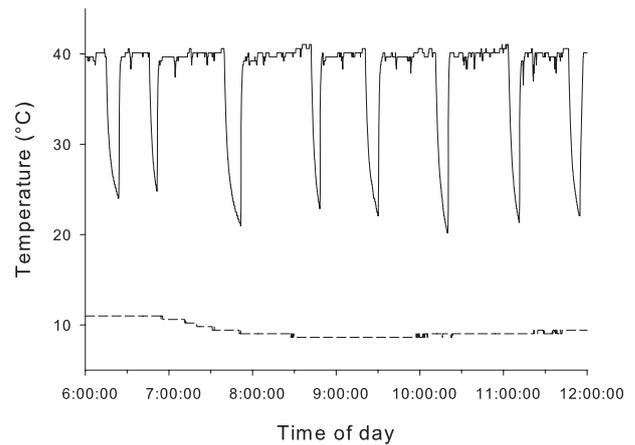


Fig. 1: Graph of a typical incubation profile of the Great Tit (temperature in the nest cup: full line, ambient temperature: dashed line).

pattern of nest temperatures, we calculated nest attentiveness during the 6 morning hours. It was calculated as time (in seconds) the female was inferred to be on the eggs divided by total monitoring time and expressed in percents. To evaluate reliability of temperature data loggers in monitoring nest attentiveness, we calculated the number of minutes on the temperature recording that was wrongly identified as either on-bout or off-bout by direct comparison with video tapes taken at the same time. Only 3.3% of 7200 minutes were misidentified ($n = 80$ nests). To get ambient temperature for every nest, we took outer temperature for the start of each on- and off-bout and averaged it across the 6 h of monitoring.

Adults

During feeding of nestlings (median age of young for females = 7 days, for males = 9 days), we captured parents in the nest-box. We captured females on almost all the nests ($n = 165$). However, because of time constraints, we captured males only on a subset of nests ($n = 109$). We took from 10 to 15 yellow feathers from the upper right part of breast for later spectrophotometric analysis. We photographed the breast by a digital camera (Panasonic DMC-FZ5). When taking the picture of the breast, we held the bird outstretched by its tarsi and beak; we photographed it from a standard distance fixed by a tripod, together with a standard following procedures given by Figuerola & Senar (2000). All these measurements and photographs were taken by VR. We also determined the age of birds based on their plumage as 1 yr old or older (Svensson 1992).

Territory Quality

We characterized territory quality by assessing food supply during incubation of every pair in the vicinity of its nest-box. As the main food consumed by Great Tit during incubation, which takes place in our population mostly in early May, is caterpillars (Betts 1955), we characterized food supply as the amount of caterpillars on trees within the territory. We determined (1) relative food supply on each of the five most numerous tree species by the frass fall method (Zandt 1994) and (2) counted all trees with a diameter above 10 cm at breast height in a circle with 20-m radius around every occupied nest-box. We put three plates (0.15 m²) around an occupied box during incubation under randomly chosen trees, always ca. 5 m from the box in three equidistant directions. We collected fallen frass after 48 h into small plastic bags that were sealed and stored in a cold place. After the field season, we let the contents dry overnight under room temperature and humidity, removed large debris and weighed the rest to the nearest 0.0001 g.

We analysed the amount of frass fallen on the plate in relation to tree species and controlled for canopy height (three categories: low, medium, high), year and date. There was a significant effect of tree species ($F_{4,170} = 11.7$, $p < 0.001$, $n = 179$). Least squares means for the five tree species were 0.075 for oak, 0.060 for hornbeam, 0.031 for lime, 0.026 for alder, and 0.016 for ash (in g/48 h/0.15 m²). We recalculated these means so that ash, species with the least frass, had coefficient of 1. Other species had accordingly higher coefficients: 4.6 for oak, 3.7 for hornbeam, 1.9 for lime and 1.6 for alder. Thus, for example one oak was equivalent to 4.6 individuals of ash, because our data indicated that it had 4.6 times more caterpillars as compared to ash, when controlled for possible confounding effects of sampling. Our results concerning relative food supply on different species of trees agree with previous analyses (Keller & van Noordwijk 1994; Naef-Daenzer 2000). To determine territory quality, we summed the number of trees within 20-m radius around the nest multiplied by their respective coefficients. Tit parents do not limit their foraging exclusively to 20 m around their nest. The distance where the great majority of their foraging takes place is given in literature, variously as within 25 m (Naef-Daenzer 2000), 30 m (Smith & Sweatman 1974) or 45 m from the nest (Naef-Daenzer & Keller 1999) in similar habitats to ours. These figures come from studies done during feeding of the young; comparable

figures for the incubation period are currently lacking. The radius of 20 m was chosen as a compromise between biological plausibility and the workload of counting trees.

Analyses of Samples

We measured the area of the black breast stripe from photos in Adobe Photoshop CS3 Extended. We used the quick selection tool to roughly delimit the breast stripe. Then, we manually finished the selection so that it was as precise as possible and measured its area. We used a ruler photographed together with every bird to adjust the scale of each photo and to obtain absolute area (in cm²). We defined stripe area as the area of the black band between the point of inflexion, where the ventral stripe widens to a throat patch, and the posterior end of the stripe (Figuerola & Senar 2000). All measurements were taken by BM. To assess repeatability, a different observer measured a subsample of photos. Repeatability, calculated as an intraclass correlation coefficient (Lessels & Boag 1987), was high ($r_i = 0.87$, $p < 0.001$, $n = 75$).

According to standard procedures (Andersson & Prager 2006), we quantified reflectance spectra of yellow feathers sampled from the breast. We used 10–15 feathers from each bird, which is enough to obtain reliable values in Great Tit (Quesada & Senar 2006). We used Avantes AvaSpec-2048 fiber optic spectrometer (Avantes BV, Eerbeek, The Netherlands) together with AvaLight-XE xenon pulsed light source and WS-2 white reference tile. The probe was used both to provide light and to sample reflected light stream, and was held perpendicular to feather surface. We took five readings, each from different part of each set of feathers. Feathers were arranged on black, non-reflective surface so that they overlapped extensively.

We needed a spectrophotometric measure of the amount of carotenoids in the breast feathers. Senar et al. (2008) showed that hue, measured by a Minolta colorimeter (Minolta CR200 colorimeter; Konica Minolta, Tokyo, Japan), correlated with lutein content of breast feathers in the Great Tit. Similarly, Isaksson & Andersson (2008) and Isaksson et al. (2008) showed that so called carotenoid chroma correlated positively with feather carotenoids in the Great Tit. Carotenoids present in Great Tit breast feathers (lutein, zeaxanthin) absorb the most at around 450 nm, and theoretical modelling also showed that carotenoid chroma directly reflects the amount of carotenoids in feathers (Andersson & Prager 2006). As we used a spectrophotometric

approach equal to that of Isaksson & Andersson (2008) and Isaksson et al. (2008), and as the hue calculated from spectrophotometric measurement of feathers does not correlate with the amount of carotenoids in feathers (Isaksson et al. 2008), we use here carotenoid chroma. We obtained reflectance (in %) from the wavelength of 320–700 nm in 1-nm increments. We calculated carotenoid chroma as $(R_{700}-R_{450})/R_{700}$, where R_{700} is reflectance at 700 nm and R_{450} reflectance at 450 nm. In statistical analyses, we used the average carotenoid chroma calculated from the five readings from each set of feathers. To assess the repeatability of our measurements, in a subsample of feathers, we arranged feathers anew and took another five readings and again averaged the carotenoid chroma calculated from them. We calculated repeatability of these two average carotenoid chroma estimates, calculated as intraclass correlation coefficient (Lessels & Boag 1987), which was high ($r_i = 0.85$, $p < 0.001$, $n = 55$).

Statistical Analyses

Due to the fidelity of birds to their breeding grounds, some females were sampled in more than 1 yr (22 females two times, 4 females three times, $n = 143$). Thus, we adjusted for this by fitting female identity as a random effect in mixed models for repeated measurements. In contrast, only three males were

sampled two times ($n = 93$). As their treatment as repeated measurements would lead to strongly unbalanced data, we instead excluded at random one observation for each of these three males.

We used general linear mixed models to explain variation in female nest attentiveness (Proc Mixed of SAS 9.1, SAS Institute Inc., Cary, NC, USA). We used generalized linear mixed models with binomial distribution and logit link function for hatching success (Proc Glimmix of SAS 9.1), which was coded as number of eggs hatched/clutch size. Finally, we used generalized linear models with Poisson distribution and log link function for male incubation feeding (the response variable was no. of feedings per 90 min, Proc Genmod of SAS 9.1). These models fit the data well and the overdispersion of data in generalized linear models was reasonable (deviance/df = 1.3 for hatching success and 1.9 for incubation feeding). Chi-square values from the models of incubation feeding are likelihood ratio test statistics for type 3 tests.

To keep the number of predictors low, we included only predictors that have been previously shown to affect our dependent variables. We always started with a set of variables that were either factors of interest (feather colouration and age of adults, nest attentiveness, incubation feeding), characteristics of the environment (laying date, ambient temperature, territory quality) or covariates (year, clutch size, age of clutch, time of day). In the analyses of

Factor	Incubation feeding			Estimate (SE)
	χ^2	df	p	
Intercept				4.44 (0.941)
Year	11.06	2, 81	0.004	0.38 (0.688): 2005 2.42 (0.744): 2006
Clutch size	2.03	1, 80	0.154	+
Laying date	1.63	1, 79	0.202	+
Age of clutch	4.1	1, 81	0.043	-0.22 (0.115)
Time of day	20.78	1, 81	<0.001	-0.38 (0.088)
Ambient temperature	6.35	1, 81	0.012	-0.07 (0.029)
Territory quality	0.29	1, 81	0.592	0.01 (0.002)
Male age	<0.01	1, 76	0.989	1 yr old > older
Male stripe area	0.66	1, 78	0.418	-
Male carotenoid chroma	0.38	1, 77	0.539	+
Territory quality × year	7.69	1, 81	0.021	0.002 (0.0053): 2005 -0.014 (0.0057): 2006
Territory quality × male stripe area	0.70	1, 74	0.404	
Territory quality × male carotenoid chroma	2.21	1, 75	0.137	

Table 1: Results of a generalised linear model explaining male incubation feeding ($n = 90$)

p-values of the final model are in bold. Sign (+ or -) or text in Estimate show the direction of the non-significant effects; exact parameter estimates are listed only for variables retained in the final model.

Table 2: Results of a general linear mixed model explaining female nest attentiveness (n = 143)

Factor	Nest attentiveness			Estimate (SE)
	F	df	p	
Intercept				0.76 (0.034)
Year	2.20	2, 137	0.114	2007 > 2005 > 2006
Clutch size	6.76	1, 140	0.010	0.01 (0.003)
Laying date	1.37	1, 139	0.244	+
Age of clutch	0.04	1, 131	0.852	+
Ambient temperature	38.04	1, 140	<0.001	-0.01 (0.001)
Territory quality	1.83	1, 135	0.179	-
Female age	0.26	1, 134	0.608	1 yr old > older
Female stripe area	2.07	1, 136	0.153	-
Female carotenoid chroma	0.13	1, 132	0.720	-
Male incubation feeding	0.14	1, 133	0.710	-
Territory quality × year	0.18	2, 125	0.832	
Territory quality × female stripe area	1.00	1, 130	0.320	
Territory quality × female carotenoid chroma	0.02	1, 124	0.900	
Territory quality × male incubation feeding	0.12	1, 129	0.730	
Male incubation feeding × year	0.48	2, 127	0.618	

p-values of the final model are in bold. Sign (+ or -) or text in Estimate show the direction of the non-significant effects; exact parameter estimates are listed only for variables retained in the final model.

male incubation feeding, we fitted interactions of territory quality with year, male breast stripe area and male breast carotenoid chroma (Table 1). In the analyses of female nest attentiveness, we also fitted interactions of territory quality with year, female breast stripe area, female breast carotenoid chroma and male incubation feeding, and the interaction of male incubation feeding with year (Table 2). We included these interactions because we wanted to know whether effects of territory quality and male incubation feeding differ with year, and whether the effects of male and female colouration and male incubation feeding depend on territory quality. Variables included into models differed according to the dependent variable and are apparent from Tables 1–3. We did not include male incubation feeding as a predictor in the analysis of hatching success, because the only way incubation feeding could affect hatching success is through nest attentiveness. We also did not include male plumage colouration as a predictor in the analysis of female nest attentiveness, because we did not want to test hypotheses on differential allocation or compensation. Male plumage colouration was not likely to bias the results obtained on the effects of female colouration on nest attentiveness, because there was no assortative pairing in relation to colouration in our population (correlation between mates: carotenoid chroma

Table 3: Results of a generalised linear mixed model explaining hatching success (n = 119)

Factor	Hatching success			Estimate (SE)
	F	df	p	
Intercept				0.21 (1.167)
Year	0.15	2, 110	0.860	2005 > 2006 > 2007
Clutch size	4.98	1, 117	0.028	0.27 (0.121)
Laying date	0.01	1, 112	0.927	+
Female age	0.58	1, 116	0.446	1 yr old > older
Female stripe area	0.10	1, 114	0.751	+
Female carotenoid chroma	0.12	1, 113	0.729	-
Nest attentiveness	0.25	1, 115	0.619	+

p-values of the final model are in bold. Sign (+ or -) or text in Estimate show the direction of the non-significant effects; exact parameter estimates are listed only for variables retained in the final model.

$r = -0.15$, $p = 0.121$, $n = 104$; breast stripe size $r = 0.07$, $p = 0.487$, $n = 104$). We subsequently removed one by one the least significant factors until we ended with only statistically significant variables of the final model (Grafen & Hails 2002). In tables, we give F , χ^2 , df and p -values of non-significant predictors, immediately before they were removed from the model. Residuals were always checked to conform to the requirements of a particular model. Denominator df were estimated by Satterthwaite method.

Ethical Note

We used standard methods in capturing and handling birds used in the research of cavity-nesting passerines. We captured adults in the nest-box. We handled them for as short time as possible to minimize any distress. We plucked the smallest number of feathers possible to obtain reliable results based on a previous methodological study (Quesada & Senar 2006). Our temperature probes had no adverse effects on birds.

This study complies with the current law of the Czech Republic. We had all necessary permits for this study and it was overseen by the Ethical Committee of Palacky University.

Results

Altogether we had 176 active nests over the 3 yr. However, we did not obtain all measurements for all the nests and thus, sample sizes for individual analyses differ. Clutch size was 10.6 ± 1.34 eggs (mean \pm SD, range 7–16, $n = 174$), nest attentiveness $75 \pm 6\%$ (range 61–89, $n = 161$), incubation feeding 0.86 ± 1.19 per hour (range 0–5.4, $n = 166$), and hatching success was $94 \pm 9.5\%$ (range = 44–100, $n = 136$).

Incubation Feeding

Male incubation feeding was negatively associated with ambient temperature (Fig. 2a), time of day and age of the clutch. Thus, the intensity of incubation feeding decreased with higher temperatures, later time of the day and advancing age of the clutch. The frequency of incubation feeding increased with territory quality, but only in years with low overall food supply (2005 and 2007; Fig 3). Frass fall amounted to 0.14 (g/48 h/1 m²) in 2005, 0.11 in 2007 and 0.51 in 2006. Thus, year 2006 had about five times more frass compared with years 2005 and 2007. Other effects were not significant (Table 1).

Nest Attentiveness

Nest attentiveness was negatively related to ambient temperature (Fig. 2b) and positively to clutch size. Thus, percentage of time females spent on eggs increased with lower temperatures and a higher number of eggs in the nest. Other factors were not significant (Table 2; Fig. 4a).

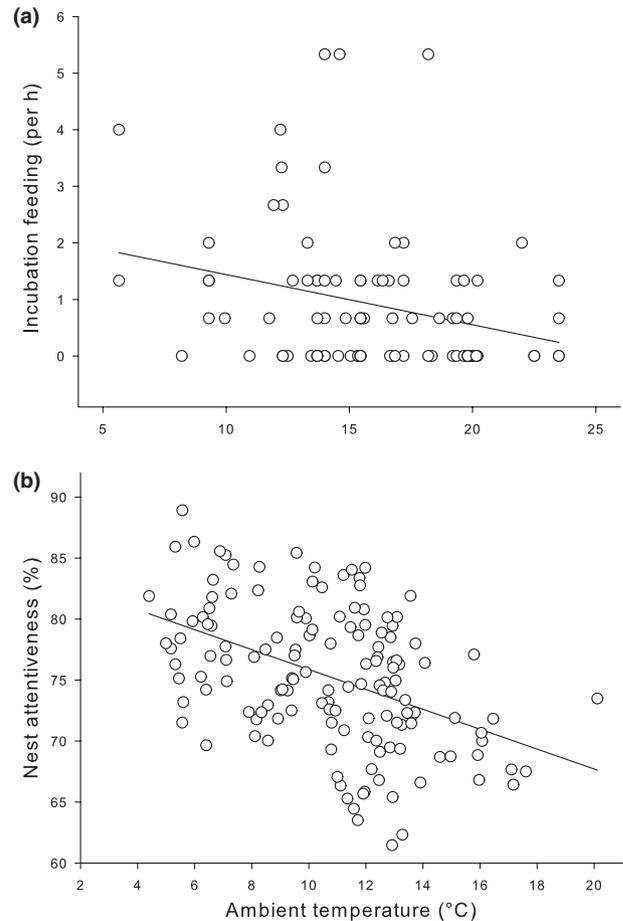


Fig. 2: Relationships of (a) incubation feeding ($n = 90$) and (b) nest attentiveness ($n = 143$) to ambient temperature. Lines are least squares regression fits to the data.

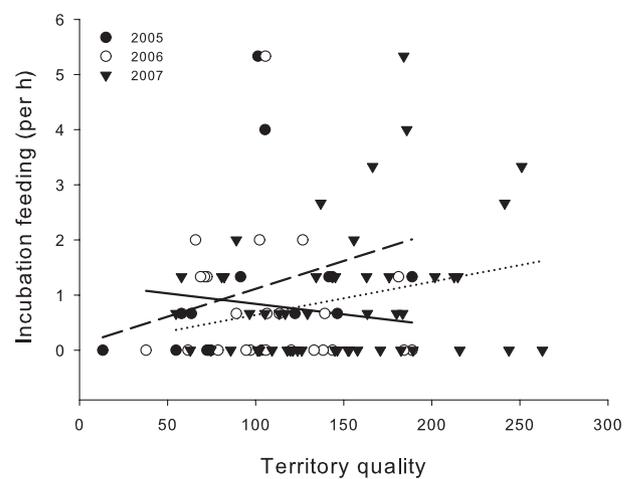


Fig. 3: Relationship between incubation feeding and territory quality (for units see Methods) separately for years 2005 (dashed line), 2006 (full line) and 2007 (dotted line; $n = 90$).

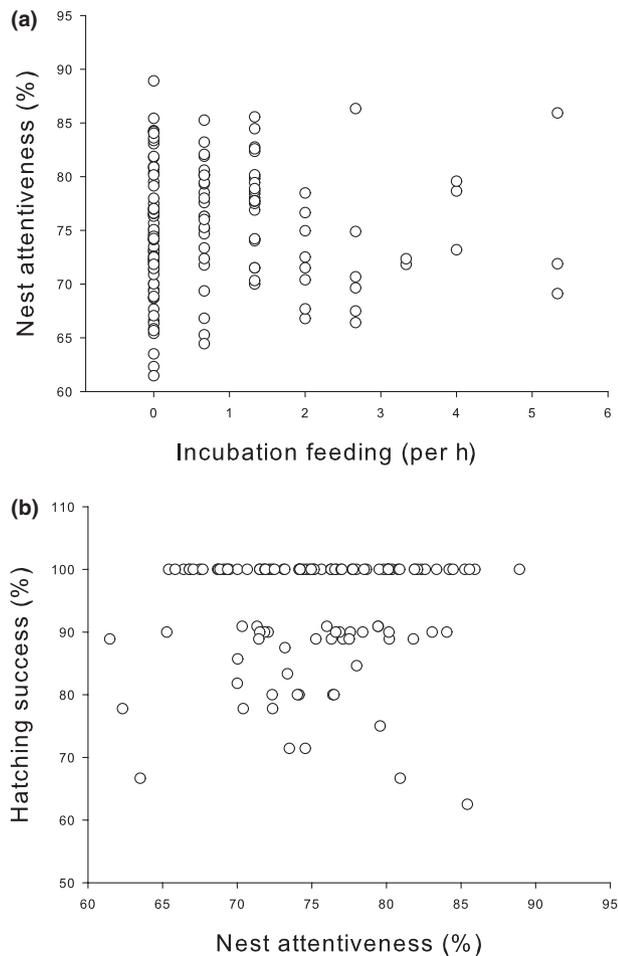


Fig. 4: Relationships between (a) female nest attentiveness and male incubation feeding ($n = 143$) and (b) hatching success and nest attentiveness ($n = 114$). These relationships are predicted to be positive under the female nutrition hypothesis.

Hatching Success

Hatching success was positively related to clutch size; other factors were not significant (Table 3; Fig. 4b).

Discussion

Incubation feeding rate in our population was similar to that observed in other secondary cavity nesters (e.g. Moreno & Carlson 1989; Siefferman & Hill 2005) and varied substantially among males (0–5.4 per hour). Neither of male characteristics (plumage colouration, age) predicted the rate of incubation feeding, whereas environmental conditions and characteristics of the nesting attempt did (ambient temperature, territory quality, time of day and age of the clutch). Incubation feeding did not predict nest attentiveness (Fig. 4a). Nest attentiveness was

associated neither with any of the female characteristics (plumage colouration, age), nor territory quality, but it was associated with ambient temperature and clutch size. Nest attentiveness did not predict hatching success (Fig. 4b).

Predictors of Incubation Behaviour

Carotenoid and melanin-based ornaments can signal male quality and his capacity to invest in a given breeding attempt (Griffith & Pryke 2006). However, in our population of Great Tit neither of the examined colouration traits predicted male incubation behaviour. This agrees with results obtained for the same type of feather colouration in Northern Cardinal *Cardinalis cardinalis* (Jawor & Breitwisch 2006) and Eastern Bluebird *Sialia sialis* (Siefferman & Hill 2003, 2005), although structural feather colour predicted incubation feeding in Eastern Bluebird. The only species where a positive relationship between carotenoid-based feather colouration and incubation feeding was observed is House Finch *Carpodacus mexicanus* (Hill 1991; Badyaev & Hill 2002). Male age did not correlate with incubation feeding in our population, whereas it did so in some other species (Røskaft et al. 1983; Lifjeld & Slagsvold 1986). Statistically significant predictors of incubation feeding in our population of Great Tit were ambient temperature, territory quality, time of day and age of the clutch. Thus, male incubation feeding in our population was related to environmental conditions rather than to male characteristics.

All studies examining female parental care and/or breeding success in relation to female plumage colouration have focused on nestling period (Amundsen & Pärn 2006). Our study is the first that focused on the relationship between female plumage colouration and female behaviour during incubation. Neither carotenoid-based nor melanin-based female plumage colouration predicted female nest attentiveness in our population of Great Tit. In species where male does not participate directly in incubating the eggs, he can increase female nest attentiveness, and hence probably hatching success, by higher intensity of incubation feeding (Sedgwick 1993; Tewksbury et al. 2002; Fontaine et al. 2007). However, we did not find such a relationship. Female incubation behaviour in our population was related only to ambient temperature and clutch size and not to male behaviour, female characteristics, or quality of the breeding territory.

Environmental conditions were the main correlates of incubation behaviour in both male and

female Great Tit. In particular, females increased nest attentiveness with colder temperatures (see also Hinde 1952; White & Kinney 1974; Sanz 1997), which was mirrored by increased male incubation feeding. Attentiveness increased on average from 68% at 20°C to 81% at 4°C. Variation between females at the same temperature was about 20% (Fig. 2b). Similarly, male incubation feeding increased from 0.2 to 1.8 per hour with temperature drop from 24 to 6°C, whereas variation among males at the same temperature might have been as high as from 0 to 5 per hour (Fig. 2a).

Male incubation feeding increased with territory quality, but only in years with low overall food supply. Zanette et al. (2000) found out that male Eastern Yellow Robins *Eopsaltria australis* fed their incubating females more in a habitat with higher food supply. The effect of territory food supply on male incubation feeding agrees with previous observations that Great Tit parents on territories with higher food supply have lower energy expenditure during nestling feeding (Tinbergen & Dietz 1994), breed early (Wilkin et al. 2007) or have better growing nestlings that fledge in higher body weight (Naef-Daenzer & Keller 1999). Thus, territories with higher food supply enable parents to invest more in current breeding attempt and rear higher quality young or to save energy. It is remarkable that this effect was apparent only in years with low overall food supply. It seems that in a good year, all males had territories with enough food to supply their incubating mate and male provisioning capacity played a role only in poor years. On the other hand, nest attentiveness was not related to territory quality in our study, whereas in some other species it was (Rauter & Reyer 1997; Zanette et al. 2000). Extra resources obtained by females on good territories could be allocated to self-maintenance rather than to incubation effort. Thus, females foraging in good territories during incubation off-bouts and receiving more food from males during on-bouts might have lost less weight during incubation, suffered less costs to other functions (physiological, behavioural) or increased their parental effort in other parts of the breeding cycle or in future breeding bouts.

Female Nutrition Hypothesis

Incubation feeding did not predict nest attentiveness, which in turn was not related to hatching success (Fig. 4). Thus, our data were not consistent with the female nutrition hypothesis. However, three alternatives should be mentioned here. First, as mentioned

above, females might have allocated extra food obtained from males into self-maintenance instead of incubation effort. Second, female nest attentiveness might respond to the amount or quality of food brought by the male on the nest. Any patterns that could arise from the effects of food load or quality would be missed by our approach. Third, Great Tit male feeds his female also while she is off the nest (Hinde 1952; Royama 1966; de Heij 2006). Incubation feeding off the nest is very difficult to record (Hinde 1952; Nilsson & Smith 1988; Pearse et al. 2004). We were not able to follow females during off-bouts in the dense canopy of our flood-plain forest (B. Matysioková & V. Remeš, unpubl. data). Similarly, most of the previous studies focused exclusively on feeding on the nest, even in species with off-nest incubation feeding occurring (e.g. Hinde 1952; Lifjeld et al. 1987; Nilsson & Smith 1988; Smith et al. 1989; Sanz 1997; Pearse et al. 2004; de Heij 2006; Lloyd et al. 2009; but see Klatt et al. 2008). However, rate of incubation feeding on the nest need not fully correspond to overall incubation feeding rate. In the only study that quantified incubation feeding both on and off the nest, which was done on Scarlet Tanager *Piranga olivacea*, no significant relationship between incubation feeding on and off the nest was found (Klatt et al. 2008; B. Stutchbury, pers. comm.). Thus, if incubation feeding off the nest were more important for the female, we would have missed some important patterns in our population. On the other hand, food load delivered to female when on the nest might be larger, i.e. more important, than that delivered to female outside of nest (Nilsson & Smith 1988). However, these questions remain unresolved.

Conclusions

In summary, our work is one of the first studies that deal with relationships between feather colouration and incubation behaviour in birds. Our data suggest that neither carotenoid-based nor melanin-based colouration predict bird behaviour during incubation, which agrees with previous findings in other species (Siefferman & Hill 2003, 2005; Jawor & Breitwisch 2006; but see Hill 1991). However, it is possible that they play a role in other parts of the breeding cycle, e.g. in nestling period (Senar et al. 2002; Doutrelant et al. 2008; Quesada & Senar 2007; review in Griffith & Pryke 2006), or during non-breeding season (review in Senar 2006). On the contrary, we revealed significant relationships with environmental conditions, including ambient temperature and territory

quality. There was no relationship between incubation feeding, nest attentiveness and hatching success. Consequently, although alternative explanations are possible, our data are not consistent with the female nutrition hypothesis conceived to explain the occurrence and rate of male incubation feeding in birds.

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