

Responses to increased costs of activity during incubation in a songbird with female-only incubation: does feather colour signal coping ability?

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Abstract Individuals differ in their ability to cope with energetically demanding situations while caring for the current brood, and they can signal this ability by their colouration. We examined the impact of handicapping (clipping of wing and tail feathers) on an energetically demanding care behaviour (incubation) in female Great Tits (*Parus major*). We hypothesised that the intensity of carotenoid-based breast feather colouration signals the ability to cope with impaired flight ability and the consequent increased energetic demands. If this is the case, females with more intensely coloured feathers should cope better with the handicap compared with less intensely coloured females, i.e. the impact of handicapping on mass loss and nest attentiveness should be negatively correlated with colouration. Handicapped females lost more weight than control females but did not decrease nest attentiveness to a greater extent, suggesting that females take the costs of handicapping on themselves. Females in poor condition were more severely influenced by handicapping. Intensity of female breast feather colouration did not correlate with either change in nest attentiveness or body mass loss during incubation. Intensity of breast feather colouration therefore does not appear to signal female ability to cope with this energetically demanding situation during incubation.

Keywords Feather colouration · Female ornaments · Great Tit · Handicapping · Incubation behaviour · Nest attentiveness

Zusammenfassung Reaktionen auf erhöhte Kosten bei der Bebrütung von Singvögeln mit Inkubation ausschließlich durch das Weibchen: Ist die Gefiederfarbe ein Anzeichen für bessere Stressbewältigung? Individuen unterscheiden sich in ihrer Fähigkeit mit energetisch ungünstigen Situationen während der Brutpflege umzugehen und sie zeigen dies anhand ihrer Gefiederfarbe. Wir untersuchten die Auswirkung einer zusätzlichen Belastung (dem Stutzen von Flügel- und Schwanzfedern) auf die Energie aufwändige Inkubation bei Weibchen der Kohlmeise (*Parus major*). Wir nahmen dabei an, dass die Intensität der auf Karotinoiden basierender Färbung der Brustfedern die Fähigkeit anzeigt, mit der energetisch kostspieligen Einschränkung der Flugfähigkeiten umzugehen. Sollte dies der Fall sein, sollten intensiver gefärbte Weibchen besser mit der zusätzlichen Belastung umgehen können, als weniger stark gefärbte Weibchen. Dementsprechend sollten der Masseverlust und die Nestattraktivität negativ mit der Gefiederfärbung korreliert sein. Weibchen mit gestutzten Federn nahmen stärker ab als die Weibchen der Kontrollgruppe, hatten aber nicht deutlich unattraktivere Nester, was darauf hindeutet, dass beeinträchtigte Weibchen die Mehrkosten durch die zusätzliche Belastung auf sich nehmen. Bereits schwache Weibchen wurden durch die zusätzliche Belastung stärker beeinträchtigt. Die Intensität der Färbung des Brustgefieders korrelierte weder mit Nestattraktivität noch mit Gewichtsverlust während der Inkubation. Das deutet darauf hin, dass die Färbung des Brustgefieders nicht auf die Fähigkeit der Stressbewältigung eines Weibchens während der Inkubation schließen lässt.

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Introduction

One of the basic tenets of evolutionary biology is that individuals differ in their ability to survive and cope with

challenging environmental conditions. This ability can be influenced by the quality, age and condition of the individual (Fox et al. 2001). Individual quality and condition can be signalled to potential mates or rivals by various types of ornaments, including those based on carotenoids (Searcy and Nowicki 2005). Carotenoid-based colouration is widespread in animals, including feathers and bare parts in birds (Olson and Owens 2005). Carotenoids cannot be synthesised by animals, must be obtained from food, and thus are potentially in short supply (Olson and Owens 1998). Full expression of carotenoid-based colouration is costly, and carotenoids are involved in a number of trade-offs with important physiological functions, including immune function and the level of oxidative stress (von Schantz et al. 1999; McGraw 2006). Consequently, intensity of carotenoid-based colouration is expected to indicate individual quality, condition, and/or capability of parental effort (Møller et al. 2000; Griffith et al. 2006).

The role of feather ornaments as indicators of quality, condition, and parental effort has been traditionally studied in males (reviewed in Griffith and Pryke 2006). However, there has been a recent surge of interest in the function and evolution of female ornaments (reviewed in Amundsen 2000; Amundsen and Pärn 2006; Kraaijeveld et al. 2007; Clutton-Brock 2009). Recent studies have demonstrated that female ornaments might work as badges of status enabling better access to resources (Murphy et al. 2009; Griggio et al. 2010) or as signals of good parenting abilities (Linville et al. 1998; Siefferman and Hill 2005; but see Smiseth and Amundsen 2000; Griggio et al. 2010). It has been even demonstrated that breeding success might be correlated with female ornament expression (Morales et al. 2007; Bitton et al. 2008), and males might base their mate choice at least partly on the degree of female ornamentation (Griggio et al. 2009; but see Murphy et al. 2009). However, studies examining female ornaments during reproduction in birds have been carried out during the nestling period, while incubation was almost completely neglected (Amundsen 2000; Amundsen and Pärn 2006; but see Hanssen et al. 2006).

Incubation is a very important part of the breeding cycle in birds, and parental effort during incubation can have strong consequences for the reproductive success of the pair (Deeming 2002). Normal embryo development requires eggs to be kept within a narrow range of temperatures (Webb 1987). Nonoptimal temperatures can lead to reduced hatchability and longer incubation periods (Lyon and Montgomerie 1985; Webb 1987; Martin 2008). At the same time, incubation is energetically demanding for the incubating individual (Williams 1996; Thomson et al. 1998; Tinbergen and Williams 2002), who has to split its time between warming the eggs and foraging for itself. Hence, the ability to cope with energetically challenging

situations during incubation can be very important for the reproductive success of the pair. In species with female-only incubation, females can signal this ability by their carotenoid-based feather colouration, and males might accordingly base their mate choice on the intensity of the female's colouration (Amundsen and Pärn 2006; Kraaijeveld et al. 2007).

Handicapping is a useful and widely employed method to study the effects of energetically challenging situations on bird behaviour (Harrison et al. 2009). Birds can be handicapped by adding weights (Wright and Cuthill 1989; Griggio et al. 2005), taping their feathers (Senar et al. 2002a) or feather clipping (Slagsvold and Lifjeld 1988; Sanz et al. 2000). The last method is particularly suitable because it simulates events that can happen in the wild due to attacks by predators, and hence represents a risk to which birds might have become adapted (Slagsvold and Lifjeld 1990). Broken or missing feathers are among the most commonly encountered natural handicaps in free-ranging birds (Dawson et al. 2001). The ability to cope with such a handicap might therefore reveal an important component of individual quality (Harding et al. 2009).

In our study, we examined the effects of handicapping (feather clipping) on incubation behaviour in the Great Tit (*Parus major*), a small, short-lived songbird with female-only incubation. In particular, we determined whether females differed in their responses to this energetic constraint in relation to the intensity of their yellow, carotenoid-based feather colouration. We predicted that impaired flight ability caused by handicapping would (1) extend the time females spent foraging off the nest, and hence decrease the time they spent on the nest, and/or (2) lead to higher body mass loss during incubation compared with controls. Moreover, if carotenoid-based feather colouration of the Great Tit females indicates ability to cope with such an energetic constraint, females with more intense feather colouration can be expected to be less affected by the challenge (Smiseth and Amundsen 2000; Doutrelant et al. 2008).

Methods

General field work

This work was conducted on three adjacent nest-box plots which are ca. 1 km apart in a broad-leaved forest dominated by oak (*Quercus petraea*) on Velký Kosíř in the east of the Czech Republic (49°32'N, 17°04'E). There are 300 nest-boxes in total placed about 1.5 m above the ground. Besides Great Tits, these nest-boxes are inhabited by Collared Flycatchers (*Ficedula albicollis*), Blue Tits (*Cyanistes caeruleus*), Nuthatches (*Sitta europea*) and Coal Tits (*Periparus ater*). Fieldwork was carried out in 2008

from early April until May. We checked nest-boxes daily to record the laying of the first egg and the final clutch size. Day 0 was the day when the last egg was laid. Eggs in our population usually start to hatch on days 11–13, and hatching lasts for 2–3 days.

Cross-fostering

We wanted to isolate the direct effects of female incubation behaviour (i.e. egg warming) on hatching success and incubation period length, excluding any genetic or maternal effects. Therefore, we matched pairs of nests by their age and clutch size and exchanged clutches between pairs of nests. Clutches were exchanged as soon as, or immediately after, egg laying ended. We took the whole clutch from a nest, weighed it on a digital balance to the nearest 0.01 g, and swapped it within the dyad (in 67 out of 82 nests on day 1, range 0–3). Nests were always exactly matched by the date when the last egg was laid. There was no difference in clutch size in 52 nests, a difference of one egg in 28 nests, and of two eggs in two nests. The transfer of eggs took on average 8 min (range = 3–14 min).

Nest attentiveness

During incubation, we monitored the percentage of time incubating females spent on eggs, i.e. nest attentiveness. We deployed temperature data loggers by inserting a probe through the nest wall into the bottom of the nest cup. A second probe was mounted under the nest-box. We measured inner and outer temperatures from 5 a.m. until 10:40 p.m. in 16-s intervals. In the nest temperature recordings, the time when the incubating female is away from the nest is recognizable by downward spikes. The temperature drops quickly when the female leaves the clutch (off-bout) and then starts to increase sharply when she returns (on-bout; Fig. 1). Consequently, it is easy to tell the difference between an attended and an empty nest (e.g. Zimmerling and Ankney 2005). From the pattern of nest temperatures, we calculated nest attentiveness throughout the day. To get ambient temperature for every nest, we took the outer temperature for the start of each on-bout and off-bout and averaged it across the day. The data loggers were deployed on day 3 or 4 of incubation, and the nest attentiveness was measured on the subsequent day (i.e. on days 4–5). Four days after experimental treatment (see below), we measured nest attentiveness again in the same way (i.e. on days 9–10).

Experimental treatment

The day after nest attentiveness was measured for the first time, we captured females in the nest-box (i.e., on days

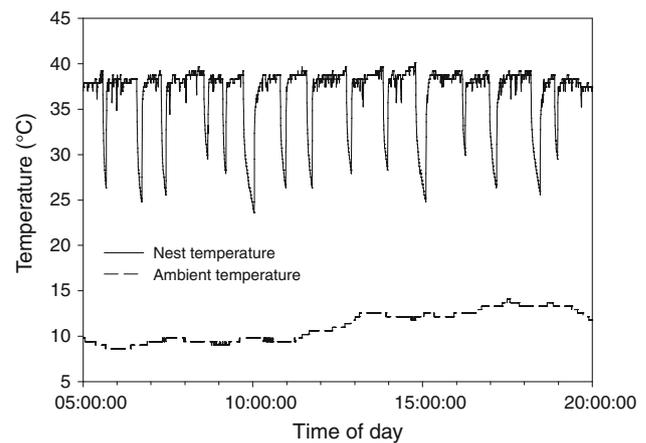


Fig. 1 Graph of a typical incubation profile for the Great Tit

5–6) and weighed them on a spring Pesola balance (to the nearest 0.25 g). We handicapped every first and second female and left every third female as a control. In experimental females, we clipped primaries 5, 6 and 8 (out of the total of 10 primaries, counted from the outside) on both wings, together with the four central tail feathers (out of the total of 12 tail feathers). We clipped the feathers as close to their bases as possible. This methodology was modified from Slagsvold and Lifjeld (1990). We handled control females in the same way as experimental females, except that we did not clip the feathers. We returned all of the females back to the nest-box through the entrance. Then we covered the entrance and waited for about one minute before leaving. The effect of handicapping was temporary and lasted until the post-breeding moult. Experimental and control females did not differ significantly in their initial body weight ($F_{1,75} = 1.2$, $P = 0.283$).

Females and clutches

The day after nest attentiveness was measured for the second time, we captured females in the nest-box again (i.e., on days 10–11). We aged them (one year old or older, Svensson 1992), weighed them on a spring Pesola balance (nearest 0.25 g), and measured their tarsus by a digital calliper (nearest 0.01 mm). We took 10–15 yellow feathers from the upper right part of the breast for later spectrophotometric analysis. Experimental and control females did not differ significantly in their tarsus length ($F_{1,54} = 0.14$, $P = 0.706$). After this day we checked nest-boxes daily to determine hatching success. We removed eggs that did not hatch and dissected them to determine the cause of hatching failure, i.e. eggs with no sign of embryo development or an apparent dead embryo. We defined hatching success as percentage of fertilised eggs that hatched. Since we were interested in the effects of incubation behaviour on hatching success, we excluded eggs with no sign of embryo

development from the analyses. Some unhatched eggs disappeared from the nest before we were able to dissect them. We removed these nests from the analyses of hatching success and thus the sample size was reduced. We calculated incubation period as the time from laying the last egg to hatching the first egg (Lyon and Montgomerie 1985).

Laboratory analyses

We quantified reflectance spectra of yellow feathers sampled from the breast using standard procedures (Andersson and Prager 2006). We used 10–15 feathers from each bird, which is sufficient to obtain reliable values from our species (Quesada and Senar 2006). We used an AvaSpec-2048 fibre optic spectrometer together with an AvaLight-XE xenon pulsed light source and a WS-2 white reference tile. The probe was used both to provide light and to sample the reflected light, and was held perpendicular to the feather surface. We took five readings, each from a different part of each set of feathers. Feathers were arranged on a black, nonreflective surface so that they overlapped extensively.

We obtained the reflectance (%) in the wavelength region of 320–700 nm in 1-nm increments (Fig. 2). We calculated so-called carotenoid chroma because it has been demonstrated that it is positively correlated with the amount of carotenoids deposited in feathers in the Great Tit (Isaksson et al. 2008; Isaksson and Andersson 2008; see also Andersson and Prager 2006). Carotenoid chroma is a preferable index of the concentration of carotenoids in feathers in unsaturated carotenoid-based colours (Andersson and Prager 2006). Carotenoids present in Great Tit breast feathers (lutein, zeaxanthin) absorb maximally at around 450 nm (Andersson and Prager 2006), and the colour of our Great

Tits was unsaturated, because we still had reasonable reflectance around 450 nm (see Fig. 2). We calculated carotenoid chroma as $(R_{700} \text{ minus } R_{450})$ divided by R_{700} , where R_{700} is the reflectance at 700 nm and R_{450} is the 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, took another five readings and again averaged the carotenoid chroma calculated from them. We calculated the repeatability of these two average carotenoid chroma estimates using the intraclass correlation coefficient (Lessels and Boag 1987), which was high ($r_i = 0.85$, $P < 0.001$, $n = 55$). As previous studies also used other characteristics derived from reflectance spectra, we also calculated brightness (R_{avg}), hue (λ_{R50}), and UV chroma (see Montgomerie 2006). We calculated brightness (R_{avg}) and hue (λ_{R50}) according to Andersson and Prager (2006, p. 78). R_{avg} is the reflectance averaged over the interval from 320 to 700 nm. λ_{R50} is the wavelength halfway between R_{max} and R_{min} , where R_{max} is the maximum reflectance and R_{min} is the minimum reflectance between 320 and 700 nm. We also calculated UV chroma as the reflectance between 320 and 400 nm divided by the reflectance between 320 and 700 nm. Experimental and control females did not differ significantly in either of the four colour characteristics: carotenoid chroma ($F_{1,54} = 0.02$, $P = 0.883$), brightness ($F_{1,54} = 0.78$, $P = 0.381$), hue ($F_{1,54} = 1.49$, $P = 0.227$), and UV chroma ($F_{1,54} = 1.43$, $P = 0.237$).

Statistical analyses

We analysed the effects of experimental treatment on desertion rate (likelihood-ratio test), change in nest attentiveness and female mass, incubation period length (general linear models), and hatching success (generalised linear models with a binomial error distribution and a logit link). We analysed all data using JMP software, with the exception of hatching success, where we used SAS. Binomial models were fitted as the number of eggs that hatched/clutch size. We confirmed that the data met the assumptions of general linear models where these were used (Grafen and Hails 2002). We also checked that data in the binomial model were not overdispersed (deviance/ $df = 1.30$).

Initial models included treatment and relevant other factors as predictors, which are apparent from Tables 1 and 2. In the analyses of the change in nest attentiveness (attentiveness before treatment minus after treatment) and body mass (mass after treatment minus before treatment), we also fitted interactions of treatment with female initial condition and breast carotenoid chroma (see Table 1). We did this because we wanted to know whether females differed in their response to handicapping based on their

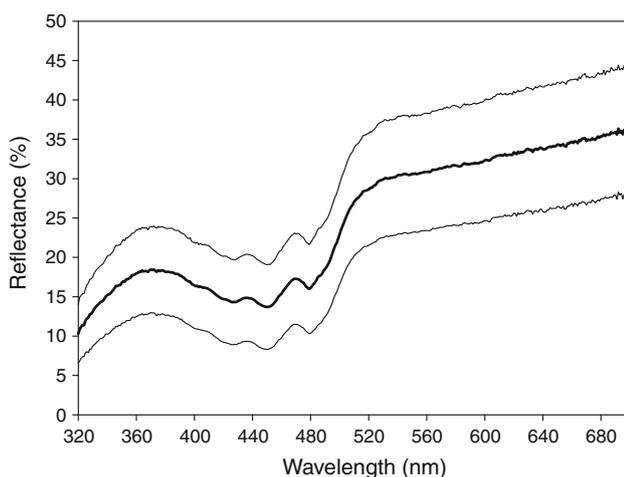


Fig. 2 Average (\pm SD) reflectance spectrum of yellow breast feathers of female Great Tits, as measured in 1-nm increments ($n = 56$)

initial condition and yellow colouration. In the analyses of incubation period length and hatching success, we fitted only the interaction of treatment with female breast

carotenoid chroma (see Table 2). We also re-ran all the models with other colour characteristics (hue, brightness, UV chroma) instead of carotenoid chroma (see Table 3).

Table 1 Models explaining the changes in nest attentiveness and female body mass during incubation

Factor	Change in nest attentiveness (%) ^a				Change in body mass (g) ^b			
	<i>F</i>	<i>df</i>	<i>P</i> ^c	Estimate (SE) ^d	<i>F</i>	<i>df</i>	<i>P</i> ^c	Estimate (SE) ^d
Intercept				−4.71 (2.370)				−0.36 (0.116)
Treatment	0.1	1, 49	<i>0.818</i>	0.35 (1.531) (handic.)	10.0	1, 52	<i>0.003</i>	−0.46 (0.144) (handic.)
Date of experiment	7.4	1, 49	<i>0.009</i>	0.84 (0.308)	0.3	1, 49	0.601	+
Female carotenoid chroma	<0.1	1, 46	0.952	+	1.6	1, 50	0.213	−
Female age	3.6	1, 48	0.064	Older > 1 year old	3.1	1, 51	0.086	Older > 1 year old
Female condition	2.2	1, 47	0.144	+	5.8	1, 52	<i>0.020</i>	−0.41 (0.140)
Female condition × treatment	0.1	1, 44	0.719		5.2	1, 52	<i>0.026</i>	0.40 (0.175) (handic.)
Female carotenoid chroma × treatment	0.6	1, 45	0.461		0.6	1, 48	0.425	

Final models: ^a $F_{2,49} = 3.7, P = 0.031, R^2 = 0.13, n = 52$

^b $F_{3,52} = 5.9, P = 0.002, R^2 = 0.25, n = 56$

^c *P* values of the final models are shown in italics

^d Sign (+ or −) or text in the estimate shows the direction of the nonsignificant effect; exact parameter estimates are listed only for variables retained in final models, including treatment, whatever its significance

Table 2 Models explaining incubation period length and hatching success

Factor	Incubation period (day) ^a				Hatching success (logit scale) ^b			
	<i>F</i>	<i>df</i>	<i>P</i> ^c	Estimate (SE) ^d	χ^2	<i>df</i>	<i>P</i> ^c	Estimate (SE) ^d
Intercept				13.43 (0.358)				3.74 (0.584)
Treatment	0.8	1, 44	<i>0.372</i>	0.21 (0.236) (handic.)	0.3	1, 39	<i>0.570</i>	0.44 (0.771) (handic.)
Date of experiment	24.1	1, 44	<i><0.001</i>	−0.23 (0.047)	0.1	1, 35	0.925	−
Temperature-indep. nest attentiveness	4.6	1, 44	<i>0.037</i>	−7.14 (3.325)	0.4	1, 37	0.515	+
Clutch size	1.5	1, 43	0.232	+	1.8	1, 38	0.186	+
Female carotenoid chroma	0.3	1, 42	0.567	+	0.3	1, 36	0.592	−
Female carotenoid chroma × treatment	0.1	1, 41	0.730		3.5	1, 34	0.063	

Final models: ^a $F_{3,44} = 10.3, P < 0.001, R^2 = 0.41, n = 48$

^b $n = 41$

^c *P* values of the final models are shown in italics

^d Sign (+ or −) in the estimate shows the direction of nonsignificant effects; exact parameter estimates are listed only for variables retained in final models, including treatment, whatever its significance

Table 3 Tests of the effects of brightness (R_{avg}), hue (λ_{R50}), and UV chroma, together with their interaction with handicapping, on changes in nest attentiveness and body mass, incubation period length, and hatching success

	Attentiveness (%)			Body mass (g)			Incubation period (day)			Hatching success (logit scale)		
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	χ^2	<i>df</i>	<i>P</i>
R_{avg}	<0.1	1, 45	0.838	0.3	1, 49	0.59	0.4	1, 42	0.543	0.7	1, 35	0.407
$R_{avg} \times$ treatment	0.3	1, 44	0.564	0.1	1, 48	0.753	0.7	1, 41	0.406	<0.1	1, 34	0.879
λ_{R50}	<0.1	1, 45	0.874	1.2	1, 49	0.286	0.1	1, 42	0.778	0.1	1, 35	0.769
$\lambda_{R50} \times$ treatment	0.1	1, 44	0.795	0.2	1, 48	0.701	0.2	1, 41	0.621	0.5	1, 34	0.472
UV chroma	0.8	1, 45	0.386	0.9	1, 49	0.336	<0.1	1, 42	0.870	<0.1	1, 35	0.930
UV chroma × treatment	0.1	1, 44	0.733	1.1	1, 48	0.299	0.9	1, 41	0.353	2.5	1, 34	0.115

Colour characteristics were tested while added in turn to the full models presented in Tables 1 and 2 (without carotenoid chroma)

Date of experiment was set so that the day of first experiment = 1. Body condition for each female was calculated as the residual from the linear regression of initial body mass on tarsus length. We always retained treatment in the final model as our main factor of interest whatever its statistical significance (see Grafen and Hails 2002). Other predictors were removed from the models starting with interactions. We removed nonsignificant predictors until we ended up only with factors significant at $\alpha = 0.05$. In the tables, we give F , df , and P values of nonsignificant predictors immediately before they were removed from the model. Data are presented as mean \pm SD.

Nest attentiveness is strongly affected by ambient temperature in the Great Tit (Kluijver 1950). Thus, when using attentiveness as a predictor in the analyses of incubation period length and hatching success, we adjusted for variation in ambient temperature among nests during sampling as follows. We fitted a regression of nest attentiveness on ambient temperature separately for both measurements (i.e., before and after treatment). In both cases there was a significant negative relationship (linear regression: before treatment $F_{1,75} = 13.3$, $P = 0.001$, $R^2 = 0.15$; after treatment $F_{1,50} = 18.6$, $P < 0.001$, $R^2 = 0.27$). We calculated the residual nest attentiveness and averaged the residuals from these two regressions. In this way, we obtained temperature-independent attentiveness for each female as a predictor variable.

When analysing observational data on mass decrease, it is necessary to take into account the problem of the regression toward the mean. Regression toward the mean occurs in repeated-measures analyses where subsets of population are compared based on their initial measurements. Thus, for instance, it follows from this effect that initially heavy individuals will lose more mass than initially light individuals. However, since regression to the mean will affect both experimental and control groups, experimental studies are not subject to this problem (Kelly and Price 2005). Accordingly, in our study, we interpret only the difference in mass loss between handicapped and control females, not the pattern in control females itself, which may be subject to the problem of the regression to the mean. However, this does not seem to be the case, because our results are the same even when the data is mathematically adjusted according to Kelly and Price (2005: Equation 6; results not shown).

Results

Altogether we performed cross-fostering on 82 nests. Five females deserted their nests after cross-fostering, leaving 77 females for our experiment (54 experimental and 23 control). There was a strong tendency for experimental females to desert their nests more often after treatment

compared to control females (18 experimental and three control, $\chi^2 = 3.68$, $P = 0.055$, $n = 77$). Clutch size in our population was 10.4 ± 1.20 eggs ($n = 82$). Carotenoid chroma of yellow breast feathers was 0.64 ± 0.06 (range: 0.44–0.75), brightness was 0.24 ± 0.038 (0.17–0.33), hue was 501.3 ± 3.95 (495.0–505.6), and UV chroma was 0.14 ± 0.008 (0.12–0.16, $n = 56$ in all four cases).

Nest attentiveness

Nest attentiveness before the treatment was $76.6 \pm 4.77\%$ ($n = 77$), and did not differ between experimental and control females ($F_{1,75} < 0.1$, $P = 0.875$). On average, nest attentiveness decreased between the first and second measurements by $1.4 \pm 5.52\%$ ($n = 52$). Treatment had no influence on the amount of change in nest attentiveness. However, although our nests were highly synchronised and differed by less than 14 days, there was a significant effect of date. In the first nests of the breeding season, nest attentiveness increased by about 5%, whereas in the last nests, it decreased by about 5% (Table 1). No other factor had any influence on the change in nest attentiveness (Table 1, Fig. 3a).

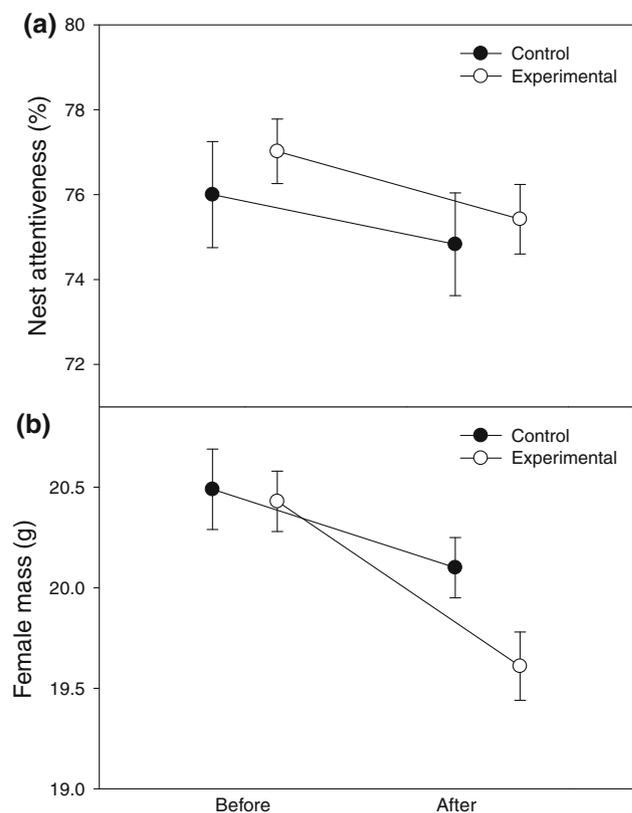


Fig. 3a–b Nest attentiveness (**a**; mean \pm SE) and body mass of incubating females (**b**) in control and experimental nests before and after handicapping (feather clipping)

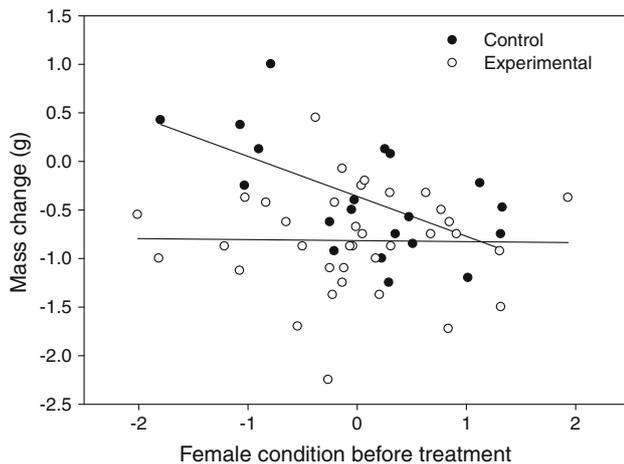


Fig. 4 Body mass change of incubating Great Tit females in relation to female condition before the experiment (mass residuals in relation to tarsus), shown separately for control and experimental (clipped feathers) nests. More negative values of mass change mean higher mass loss over incubation

Body mass loss

Female body mass before the experiment was 20.37 ± 0.88 g ($n = 77$). Body mass loss between the first and the second weighings was 0.66 ± 0.58 g ($n = 56$). Mass loss was significantly higher in experimental females (0.81 ± 0.52 g, $n = 36$) than in control females (0.38 ± 0.59 g, $n = 20$; simple effect of treatment: $F_{1,54} = 8.0$, $P = 0.007$, Fig. 3b). Mass loss was, however, also related to the initial condition of the female, and the relationship differed between experimental and control females, as evidenced by the significant interaction between treatment and initial female condition (Table 1, Fig. 4). No other factors were significant (Table 1).

Incubation period

Length of the incubation period was 11.8 ± 0.97 days ($n = 48$). Treatment had no effect on the length of the incubation period. It was negatively related to season and temperature-independent nest attentiveness; other factors were not significant (Table 2).

Hatching success

Overall hatching success was $91.1 \pm 11.52\%$ ($n = 51$). There was no effect of treatment on hatching success; similarly, no other factor was significant (Table 2).

It follows from the above results that female carotenoid-based feather colouration expressed as carotenoid chroma was not correlated with her ability to cope with energetic handicap during incubation (see also Table 1). Similarly, no other colour characteristic (hue, brightness or UV

chroma) was correlated with female coping ability, incubation period length or hatching success (Table 3).

Discussion

Handicapping had no influence on female incubation behaviour, length of incubation period or hatching success. However, during incubation, handicapped females lost more body weight overall than control females. Females in poor condition were more severely influenced by handicapping. Intensity of female breast feather colouration did not correlate with either female incubation behaviour, body mass loss during incubation, incubation period length or hatching success.

It seems that most of the costs of handicapping were channelled to female mass loss. This agrees with a previous study of the Great Tit, where handicapped females kept feeding rates to the nestlings unchanged at the cost of deteriorating their own body conditions (Sanz et al. 2000). Similar results were obtained in a study of the Tree Swallow *Tachycineta bicolor*, where the costs of handicapping were paid through the loss of female body mass, while nestling condition was unaffected (Winkler and Allen 1995). However, in incubating Tree Swallows, handicapped females both lost more mass than control females and also slightly decreased nest attentiveness (Ardia and Clotfelter 2007). In some other species, handicapping did not influence female body mass or body condition, but it did influence feeding rate and consequently nestling condition and growth, e.g. in Antarctic Petrels *Thalassoica antarctica*, Leach's Storm-petrels *Oceanodroma leucorhoa*, Cory's Shearwaters *Calonectris diomedea*, and tropical House Wrens *Troglodytes aedon* (Sæther et al. 1993; Mauck and Grubb 1995; Navarro and González-Solís 2007; Tieleman et al. 2008). The two species where females invested in the current brood at the expense of their own conditions (Great Tits and Tree Swallows) are both short-lived, with low probabilities of future reproduction, which selects for increased investment into the current breeding attempt. On the contrary, long-lived species with a high probability of future reproduction, including Antarctic Petrels, Leach's Storm-petrels, Cory's Shearwaters, and tropical House Wrens, are expected to reduce any increases of investment into the current brood in order to maximise their own survival (Roff 1992; Ghalambor and Martin 2001).

Our experimental treatment affected females that were in poor condition disproportionately more than those in good condition (see Fig. 4). The importance of a good overall female state for successful incubation in the Great Tit is further supported by our finding that handicapped females deserted their clutches more often than control

females. Similar relationships between female condition and nest desertion have been also found in other species (Wiggins et al. 1994; Yorio and Boersma 1994; Merilä and Wiggins 1997; but see Bleeker et al. 2005). An obvious explanation for this pattern is that incubation is energetically demanding and females in poor condition, caused by low body mass or impaired flight abilities, are not able to withstand the energetic stress (Williams 1996; Thomson et al. 1998; Tinbergen and Williams 2002).

Intensity of yellow breast feather colouration was not related to the ability of females to cope with the handicap. One might ask how female colouration could help prevent a change in body mass. Handicapping is a standard way of testing whether an individual is of higher quality, i.e. is better able to cope with a challenging situation. Our experimental approach was motivated by a widespread finding that individual quality often shows up only under unfavourable conditions (e.g. brood size manipulations, various forms of handicapping, food restrictions; e.g. Ardia and Clotfelter 2007; Doutrelant et al. 2008). We conjecture that handicapped females could overcome the handicap by working harder. On a mechanistic basis, this means putting more energy into flight to get resources (self-maintenance) and simultaneously caring for the clutch (incubating) without these functions being compromised. Of course, this higher effort is expected to bear costs, e.g. a higher metabolic rate and higher oxidative stress generated by heavy work. This can presumably be achieved only by higher-quality individuals. There certainly was a variation among females in the degree of their body mass loss (see Figs. 3, 4), i.e. in their ability to cope. We were interested in whether this variation could be ascribed to female colouration, and found out that this was not the case.

We would like to mention three potential problems when generalising our results. First, costs of the manipulation could also have been observed after hatching. This might have been particularly true during nestling feeding, when females have to fly more. Previously, all studies examining female feather colouration during reproduction in birds have been carried out during the nestling period (Amundsen and Pärn 2006; but see Hanssen et al. 2006). Several of them investigated the function of yellow breast feather colouration in Great and Blue Tits, but generated mixed results. Some found a positive relationship between the intensity of female yellow colouration and breeding success, whereas others found no or even a negative relationship (correlative studies: Senar et al. 2002b; Mänd et al. 2005; Hidalgo-Garcia 2006; experiment: Doutrelant et al. 2008). Hence, the information content of female yellow colouration might differ between parts of the breeding cycle, i.e. incubation versus feeding of young. Second, the colouration of the females that deserted just after the manipulation is missing. It is possible that these deserting females had low

carotenoid chroma values and were of inferior quality. Consequently, if we were left with only higher-quality individuals, our test of the indicator potential of the carotenoid-based colouration in females would have been weakened. Third, males feed females during incubation in the Great Tit. If we found better coping ability in more colourful females, we would not be sure whether they cope better because they are able to work harder, or because they are more helped by their males. However, male incubation feeding is not a source of potential bias in our study, because we found no effect of female colour on the ability to cope with energetic stress. Moreover, we studied this problem for three years in a nearby population, and there was no effect of female colour on male incubation feeding (Matysioková and Remeš 2010).

While bearing the abovementioned reservations in mind, our results are not consistent with a role for feather carotenoids as indicators of female quality or capacity for extra parental effort, as has been demonstrated by several other studies (see above). Differences in the results of multiple studies investigating feather ornaments in the same species are known to occur due to population differences in the information content of the ornamental traits (Dunn et al. 2008; Galván and Moreno 2009) and different expressions of ornaments in different populations and subspecies (Hill 2002). Great Tit subspecies differ strongly in the intensity of yellow breast colouration (Harrap and Quinn 1996). However, there is no work quantifying differences in yellow colouration and in the functional ecology of feather ornaments among populations of the Great Tit. Nevertheless, it is at least possible that different populations are subject to different constraints on the expression of yellow colouration, and that the information content of feather ornaments varies in space. Only rigorous studies conducted in an explicitly comparative framework may provide answers to the heterogeneity of studies conducted so far (Senar et al. 2002b; Mänd et al. 2005; Hidalgo-Garcia 2006; Doutrelant et al. 2008; this study).

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