

Nestling begging intensity and parental effort in relation to prelaying carotenoid availability

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Carotenoids are antioxidants playing major roles in physiological functions at various stages of an animal's life. Female birds deposit large amounts of carotenoids into their eggs. Carotenoids are, however, a limiting resource, and females are expected to balance carotenoid deposition into the eggs with their utilization for themselves. Carotenoid availability is thus likely to determine both the levels of yolk carotenoids and maternal care during rearing. Carotenoids have been shown to benefit the embryo and the growing nestling, and it can be hypothesized that an increase in carotenoid availability during laying leads to higher nestling condition and competitive ability. We manipulated carotenoid availability to great tit pairs prior to and during egg laying and later partially cross-fostered chicks at hatching. During the rearing period, we measured how carotenoid availability affected nestlings begging behavior and male and female feeding effort. We also manipulated the ectoparasite load, predicting that carotenoid supplementation would help adults and nestling to cope with parasites. Nestlings hatched from eggs laid by carotenoid-supplemented females and raised in small broods begged more intensely. Nestlings in small deparasitized broods also begged more actively. The feeding effort of control females increased with brood size, whereas the feeding effort of carotenoid-supplemented females was high whatever the brood size. Male feeding effort was unaffected by our treatment. Our results support the hypothesis that maternally derived carotenoids increase nestling begging behavior and hence competitive ability. They further suggest that carotenoid availability determines the level of parental investment and can mediate trade-offs between life-history traits. *Key words:* begging, food provisioning, maternally derived carotenoids, parasites, parental care, reproductive investment. [*Behav Ecol* 19:108–115 (2008)]

Life-history theory predicts that female investment in current reproduction has to be balanced against its costs on future reproductive potential or survival (Stearns 1992). This trade-off, and the subsequent reproductive investment realized by a female, will depend on various constraints such as her body condition, the availability and quality of the resources in her breeding environment, and/or the quality of her reproduction partner. Carotenoid pigments are a large family of antioxidant molecules that are involved in numerous physiological functions in animals (Møller et al. 2000; Blount 2004). They are part of the antioxidant barriers of vertebrates protecting DNA, proteins, and biological membranes against oxidative stress arising from normal metabolic processes (Møller et al. 2000; Krinsky 2001). They also participate in the activation of the immune system and in detoxification processes consecutive to the production of reactive oxygen species (oxygen ions, free radicals, and peroxides) during respiratory burst (Chew and Park 2004). However, carotenoids cannot be synthesized *de novo* by animals and have to be ingested with food (Partali et al. 1987; Olson and Owens 1998). Moreover, they are supposed to be a limiting resource in the nature, and their availability may vary in space and time. Carotenoid limitation may also arise from individual differences in foraging skills and capacity to acquire dietary carotenoids and/or from individual differences in the ability to assimilate them (Olson and Owens 1998; Møller et al. 2000). Therefore, carotenoid availability is expected to mediate the trade-off between current and future reproduction

and between reproduction and survival and to set the level of reproductive effort realized by females. An increase in carotenoid availability should then allow a female to invest more in her current reproduction and to increase the amount of care she provides to her progeny. Although, males typically pay a lower cost to reproduction (Trivers 1972), they are also expected to balance their current parental investment with its cost on their future reproduction or survival. Therefore, carotenoid availability is also expected to determine a male's parental effort.

Parental investment is not only limited to parental care provided after the birth or hatch of the offspring (Clutton-Brock 1991) but also comprises maternal investment in the embryo or egg (Mousseau and Fox 1998). Maternal effects can greatly affect offspring development and fitness (Mousseau and Fox 1998). In birds, maternal effects can be mediated by the deposition of various nutrients and biochemical compounds in the egg yolk. Carotenoid pigments are among those biochemicals (Blount et al. 2000). Carotenoids have been shown to play important roles in the development of avian embryos and neonates. More precisely, they have been shown to provide antioxidant protection to embryonic tissues especially during hatching (Surai et al. 1996; Surai and Speake 1998; Blount, Surai, Nager, et al. 2002; McGraw et al. 2005) and promote the maturation of the immune system (Koutsos et al. 2003, 2006; Saino et al. 2003). Moreover, Koutsos et al. (2003) have shown that increased levels of carotenoids in the eggs can improve a nestling's ability to metabolize and efficiently utilize dietary carotenoids. Females thus appear to dramatically influence the development of a number of fitness-related traits in their offspring by depositing varying amounts of carotenoids into their eggs. However, carotenoids being limiting, females are expected to balance carotenoid investment in their eggs with their need to allocate sufficient

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amounts of antioxidants to their own essential physiological functions in order to be able to provide sufficient parental care posthatching and/or to preserve their future reproduction and survival (Blount 2004). An increase in carotenoid availability is thus predicted to lead not only to a higher parental effort after hatching, but also to the deposition of higher amounts of carotenoids into the egg yolk.

In species where large broods hatch simultaneously, sibling competition is by definition potentially intense. In situations of sibling competition, an important component of a nestling's fitness is its ability to obtain food, that is, its competitive ability. In birds, begging behavior typically involves energetically costly postures in which nestlings stretch to reach adult level and flap their wings in order to outcompete their siblings and get food (Kölliker et al. 1998; Neuenschwander et al. 2003). Begging behavior may then reflect a nestling's vigor and competitive ability (Rodríguez-Girones et al. 1996). Through their numerous benefits on nestling condition and health, carotenoids are likely to contribute to nestling competitive ability. So far, a couple of studies have investigated the potential influence of yolk antioxidants (Rubolini et al. 2006) or direct carotenoid supplementation of the chicks (Tschirren et al. 2005) on nestling begging behavior, but they both failed at finding any significant relationship. In this study, we aimed at examining whether an increase in carotenoid availability to the female during egg laying leads to an increase in nestling competitive ability via the investment females realize into their eggs, for example, maternally derived carotenoids.

The great tit (*Parus major*) is a sexually dimorphic passerine with biparental care. Prior to and during laying, we supplemented half of the pairs of a wild population of great tits with carotenoid-enriched food, whereas the other half received nonenriched food. Nestlings were then partially cross-fostered at hatching to disentangle the effects of maternally derived carotenoids from the effects of posthatching environment on nestling development. We expected both males and females that received supplementary carotenoids to increase their parental effort and to feed their nestlings more. Such an effect may be expected to be stronger in females than in males because they are likely to be more limited by natural carotenoid availability. In addition, we predicted that nestlings hatched from eggs laid by carotenoid-supplemented females should have a higher competitive ability and show more intense begging behavior. We also manipulated parasite load in the nest. Parasites have been shown to impair nestling fitness by reducing body condition (Richner et al. 1993; Richner 1998) and growth rate (Nilsson 2003). Hence, nestlings may have to face a trade-off in the allocation of resources such as carotenoids between the defense against parasites and other functions (e.g., growth) that may determine their competitive ability. We thus predicted that increased prelaying carotenoid availability to females would benefit more to flea-infested nestlings.

METHODS

Experimental protocol and data collection

We studied a natural population of great tits *P. major* in 2004 in the Bremgarten forest near Bern, Switzerland. All pairs nested in nest-boxes. Our experimental setup comprised 336 nest-boxes divided into 24 plots separated by approximately 150-m wide "buffer" zone. In early March, we removed old nest material from nest-boxes, which were then thoroughly brushed to eliminate overwintering parasites. Each plot was then randomly assigned to 1 of the 2 diet treatments, supplemented with carotenoid-enriched (C+) or nonenriched food (C-), and to 1 of the 2 parasite treatments, in-

festes with Hen fleas *Ceratophyllus gallinae* (F+) or parasite-free (F-). We applied our treatments on a plot basis to 1) minimize the chance that an individual foraging outside its territory would consume food of a different treatment and 2) minimize the likelihood that individuals redistributing themselves after the start of our treatment would end up in a nest under a different treatment. Individuals might have fed in the buffer zone. However, this border effect would only result in creating additional noise in the data.

Supplemental food was provided in the form of a fat ball suspended under a reversed flowerpot hung at a branch in the close vicinity of the nest-box. Standard commercial fat balls were first heated and melted at approximately 60 °C. Once cooled down, half of the preparation was added with a cocktail of carotenoids. We used a mixture of lutein, zeaxanthin, and β , β -carotene (Roche Vitamins Inc., Switzerland) in the relative proportions found in natural food of great tits (80%, 3%, and 17%, respectively, Partali et al. 1987). The total amount of carotenoids added was 100 mg/kg of food (0.01% w/w). This apparently high concentration of carotenoid was chosen because preliminary observations showed that a number of other small passerines, particularly *Paridae*, were also feeding on this additional food. Thus, experimental birds are likely to have consumed much less food than we actually provided. The food was shaped into small balls, wrapped in cellophane film, and stored at 4 °C in a dark climate chamber to be used within 3 days.

Food supplementation started on 20 March, when breeding territories were established. Nests were visited every fourth day to record the start of nest building and determine laying date. The food was renewed or replaced every second visit to the nest. After the construction of the nest cup, we introduced 40 fleas (*C. gallinae*) collected from old nest material into nests of parasitized plots. This number was known to be sufficient for the establishment of a flea population in the nest (Heeb et al. 1996; Tripet and Richner 1999).

After the laying of the fourth egg, nests were visited daily to determine incubation date and predict hatching date. We stopped supplying food on a nest-by-nest basis as soon as incubation started. Broods were partially cross-fostered at hatching to disentangle the effects of maternally deposited yolk-carotenoids (maternal effect) from posthatching parental effects. Nestlings were exchanged between nests of similar brood size and similar mean brood mass, having different food treatments but within the same flea treatment. Hatchlings were weighed to the nearest 0.01 g using a Sartorius balance and ranked according to body mass. In one of the nests, the cross-foster treatment (stay/swap) was randomly assigned to the lightest chick and then alternated through the mass-based rank list. Eventually, nestlings in their rearing nests had similar rank as in their natal nests ($r = 0.79$, $F_{1,479} = 795.58$, $P < 0.0001$) and brood size was unchanged. Hatchlings were individually marked by plucking downy feathers from their head, back, and wings. We captured both parents when the nestlings were 12 days old (hatching day = day 0) and measured their body mass (± 0.1 g) and tarsus length (± 0.1 mm).

Video recordings

A sample of 80 nests comprising 481 nestlings was randomly chosen to be video recorded when nestlings reached the age of 6 days. The 6-day-old nestlings were individually marked on their heads with small spots of dark-red acrylic paint (Kölliker et al. 1998). Color marking was made blind to the treatments. If parents reacted differently to the markings, this would only result in additional noise in the data but would not confound our results with regard to our treatments. We then filmed the

brood within the nest with a video camera equipped with an infrared light source. The nest-box had a built-in camera in the upper part, allowing to film close-up feeding bouts from above the nest cup (Christe et al. 1996; Kölliker et al. 1998). Of the 2-h 15-min record, we discarded the first 45 min during which feeding rate steadily increased to reach a stable level afterward (Helfenstein F, unpublished data). For every feeding bout, we recorded the sex of the feeding parent, the identity of the fed nestling, and prey size. Prey size was classified as small, intermediate, or large (Kölliker et al. 1998). The food quantity delivered to a nestling per hour was calculated as the product of feedings per hour per nestling and mean prey size (Tschirren et al. 2005). For a subsample of 34 nests comprising 215 nestlings, we also recorded nestling begging intensity before food delivery on a 5-level scale: 0 = calm, 1 = weak gaping, 2 = persistent gaping, 3 = gaping neck fully stretched, 4 = gaping, neck fully stretched, and wing flapping (Kölliker et al. 1998). The observer (F.H.) had no knowledge of the treatment of the natal or rearing nests of the nestlings.

Food consumption

We do not have data on food consumption for 2004. However, we repeated a similar experiment using the same protocol in 2006 and were then able to estimate how much food pairs consumed. In 2006, each new fat ball was weighed when supplied and then weighed again when replaced (mass = 0, if entirely eaten) to the nearest 0.1 g. The difference between these 2 values estimates food consumption per 6-day renewal period.

Statistical procedures

To match modeling assumptions, we used the log-transformed estimated quantity of food provided per nestling per hour to analyze the effects of our treatments on parental feeding effort and food allocation among the nestlings. A generalized linear mixed model (GLMM) included the carotenoid treatment of the natal nest (maternal effect), the carotenoid treat-

ment received by the parents of the rearing nest, the flea treatment, and the sex of the rearing parent as fixed factors. The brood size, the date of video recording, and the rank of the nestlings at hatching in the rearing nest were included as covariates. Only interactions deriving from predictions we could make a priori were included in the model. To account for the hierarchical structure of our experimental design, we included the following random factors: the natal plot nested in the carotenoid treatment of the natal nest and the flea treatment, the rearing plot nested in the carotenoid treatment of the rearing nest and the flea treatment, the dyad (pair of cross-fostered nests) nested in the flea treatment, the natal nest nested in the natal plot and the dyad, the rearing nest nested in the rearing plot and the dyad, and the interaction between the rearing nest and the natal nest. As our data comprised 2 observations per nestling (a feeding frequency by each of the rearing parents), we included the identity of the nestling as a random factor. A more complex set of random effects did not better partitioned the variance in our data and/or led to ill estimation of some covariance parameters and did not change the significance of the fixed effects.

To investigate the influence of our treatments on nestling begging intensity, a potential measure of a nestling competitiveness, we only included effects potentially directly affecting the nestlings phenotype, that is, the flea treatment and the treatment of the natal nest (maternal effect), as fixed factors. We did not include the effect of the carotenoid treatment received by the rearing parents in this analysis to avoid circularity in the interpretation of the results and because we had no a priori predictions for this effect. This GLMM further included the brood size, the date of video recording, and the rank of the nestlings at hatching in the rearing nest as covariates. Only interactions deriving from predictions we could make a priori were included in the model. This model included the same combination of random factors as listed above.

Analyses were conducted with the SAS system version 9.1. All GLMMs assumed normal distribution of the error and used the restricted maximum likelihood estimation method

Table 1

GLMM testing for effects of the natal nest carotenoid treatment (maternal effect), the rearing nest carotenoid treatment and the flea treatment, and random effects deriving from the experimental design on nestling begging intensity

Covariance parameter estimates

Parameter	Estimate \pm SE	Wald's Z	P
Natal plot (maternal effect \times flea treatment)	0	—	—
Rearing plot (rearing nest treatment \times flea treatment)	0.001 \pm 0.024	0.04	0.49
Dyad (flea treatment)	0.033 \pm 0.072	0.47	0.32
Natal nest (natal plot \times dyad)	0.015 \pm 0.014	1.09	0.14
Rearing nest (rearing plot \times dyad)	0.078 \pm 0.075	1.03	0.15
Rearing nest \times natal nest (dyad)	0	—	—
Error	0.135 \pm 0.015	8.87	<0.0001

Type-3 tests of fixed effects

Effect	Estimate \pm SE	F_{df}	P
Maternal effect ^a	0.42 \pm 0.17	6.23 _{1,115}	0.014
Flea treatment ^b	-1.51 \pm 0.40	14.17 _{1,25.2}	0.0009
Brood size	-0.98 \pm 0.43	5.20 _{1,29.4}	0.03
(Brood size) ²	0.05 \pm 0.03	4.29 _{1,28.8}	0.0475
Maternal effect \times (brood size) ^{2a}	-0.01 \pm 0.01	4.62 _{1,113}	0.034
Flea treatment \times (brood size) ^{2b}	0.02 \pm 0.01	10.97 _{1,22}	0.003

The best fit was obtained when including a quadratic term for brood size. Nonsignificant terms are not presented (all $F < 1.31$, $P > 0.25$).

^a Estimate of the carotenoid group relative to the control group.

^b Estimate of the flea-infested group relative to the control group.

and the Kenward–Roger correction for the calculation of fixed effects degrees of freedom (Littell et al. 2006). We used Wald's Z -statistic to assess whether the random factors significantly structured variance in the data. We checked the fit of our models by testing the residuals for normality and homoscedasticity and by plotting the residuals against the predicted values. Unless they appeared in higher order interaction terms, nonsignificant terms were backward dropped using a stepwise elimination procedure. We used 2-tailed type-3 tests for fixed effects with a significance level set to $\alpha = 0.05$.

RESULTS

Food consumption

In 2006, we found no evidence that birds supplemented with either carotenoid-enriched or nonenriched fat balls consumed different amounts of food. Control birds ate an average of 36.54 g of food per 6-day period, and carotenoid-supplemented pairs consumed an average of 37.36 g of food (random effect: plot nested in carotenoid treatment, $\sigma^2 = 34.05 \pm 19.02$, Wald's $Z = 1.79$, $P = 0.04$; fixed effect: carotenoid treatment, $F_{1,9,28} = 0.05$, $P = 0.83$). We estimated that females supplemented with carotenoids were given an average of 0.6 mg of carotenoids per day. This quantity, however, assumes that food was entirely eaten by the focal birds and not by any other animal and is thus overestimated. This number is approximately 40 times inferior to the daily quantities provided in previous studies on 2 tit species (Biard et al. 2005, 2007) and is close to the daily quantities of carotenoids great tits acquire from their food (Partali et al. 1987; Crocker et al. 2002).

Laying date, clutch size, and brood size at hatching

Control and carotenoid-supplemented pairs and flea-infested and parasite-free pairs did not differ in their laying date (random effect: plot, $\sigma^2 = 0.23 \pm 2.61$, Wald's $Z = 0.09$, $P = 0.46$; fixed effects: carotenoid treatment, $F_{1,17,1} = 1.13$, $P = 0.30$; flea treatment: $F_{1,17,1} = 2.04$, $P = 0.17$; interaction: $F_{1,17,1} = 3.12$, $P = 0.10$), clutch size (random effect: plot, $\sigma^2 = 0.40 \pm 0.28$, Wald's $Z = 1.44$, $P = 0.08$; fixed effects: carotenoid treatment, $F_{1,16,9} = 0.05$, $P = 0.83$; flea treatment: $F_{1,16,9} = 1.49$, $P = 0.24$; interaction: $F_{1,16,9} = 0.05$, $P = 0.83$), and brood size at hatching (random effect: plot, $\sigma^2 = 0.26 \pm 0.26$, Wald's $Z = 0.96$, $P = 0.17$; fixed effects: carotenoid treatment, $F_{1,14,7} = 0.03$, $P = 0.86$; flea treatment: $F_{1,14,7} = 3.20$, $P = 0.09$; interaction: $F_{1,14,7} < 0.01$, $P = 0.99$). These results confirm that our experimental setup did not lead to a redistribution of high-quality individuals into the carotenoid-supplemented or the parasite-free plots. They also guaranty that despite a seasonal increase in the amount of carotenoids deposited into the egg yolk, this latter effect does not confound our treatments.

Nestling begging intensity

Begging intensity was affected by interactions between the brood size on the one hand and the carotenoid treatment of the natal nest (maternal effect) and the presence of fleas on the other hand (Table 1). Nestlings in small broods begged more intensely when they benefited from additional maternally derived carotenoid in their eggs (Figure 1a). Within parasite-free broods, nestlings in small broods begged more intensely than nestlings in large broods. Within flea-infested broods, nestlings from broods of intermediate sizes begged the less intensely (Figure 1b).

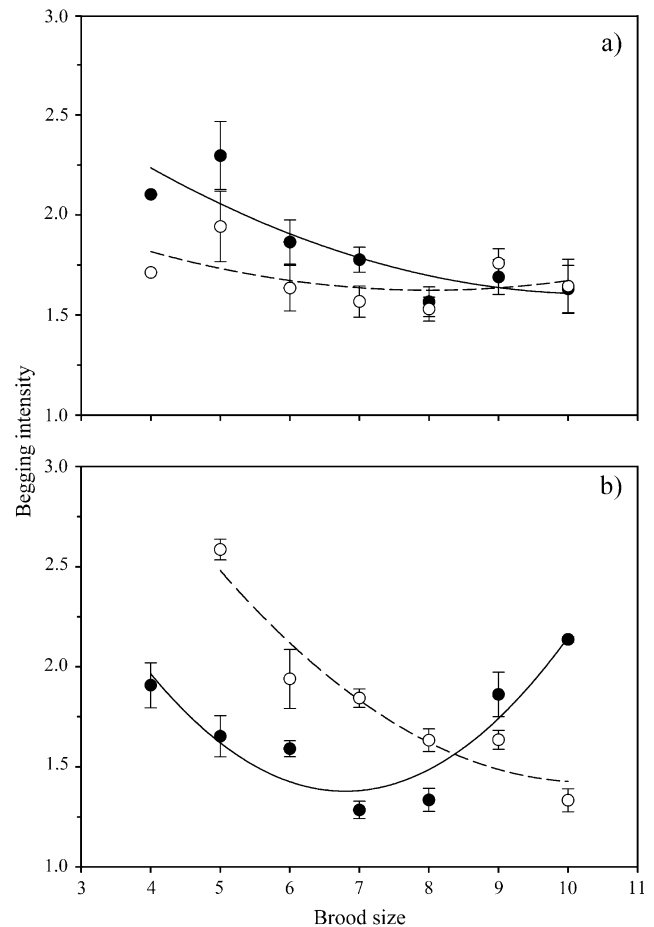


Figure 1

Mean nesting begging intensity (mean predicted values \pm SE) in relation to the brood size of the rearing nest and according to (a) whether the natal female received supplementary carotenoids (maternal effect, filled circles and continuous line) or not (open circles and dashed line) and (b) the flea treatment (flea-infested nests: filled circles and continuous line; noninfested nests: open circles and dashed line). Results remain qualitatively unchanged when removing the broods with only 4 nestlings.

Feeding frequency

We found a second-order interaction between sex of the rearing parent, carotenoid treatment received by the rearing parent, and brood size to be significant (Table 2). Males did neither increase their feeding effort in large broods (Figure 2b; slope estimates \pm standard error [SE] from partial models controlling for other effects of the full model; control group: 0.08 ± 0.06 , $F_{1,43,5} = 1.66$, $P = 0.20$; carotenoid group: 0.05 ± 0.06 , $F_{1,31,2} = 0.81$, $P = 0.38$) or when supplemented with carotenoids (least-square means, log-transformed frequency; control group: 1.22 ± 0.06 ; carotenoid group: 1.31 ± 0.07 , Tukey post hoc test, $t_{24,8} = 0.94$, $P = 0.35$). In contrast, non-supplemented females increased their feeding effort in large broods (slope = 0.13 ± 0.05 , $F_{1,44,2} = 8.54$, $P = 0.0055$), and females supplemented with carotenoids having small broods increased their feeding effort to the extent that feeding no longer covaried with brood size (slope = -0.03 ± 0.06 , $F_{1,32,9} = 0.23$, $P = 0.63$; Figure 2a).

The analysis also reveals a marginally nonsignificant interaction between the sex of the feeding parent and the maternal effect (Table 2). Males showed a tendency to feed more nestlings hatched from control eggs (least-square means of

Table 2

GLMM testing for effects of the natal nest carotenoid treatment (maternal effect), the rearing nest carotenoid treatment and the flea treatment, and random effects deriving from the experimental design on the log-transformed quantity of food delivered per nestling per hour by each rearing parent

Covariance parameter estimates

Parameter	Estimate \pm SE	Wald's <i>Z</i>	<i>P</i>
Natal plot (maternal effect \times flea treatment)	0	—	—
Rearing plot (rearing nest treatment \times flea treatment)	0.013 \pm 0.013	0.99	0.16
Dyad (flea treatment)	0.002 \pm 0.018	0.10	0.46
Natal nest (natal plot \times dyad)	0	—	—
Rearing nest (rearing plot \times dyad)	0.06 \pm 0.025	2.39	0.009
Rearing nest \times natal nest (dyad)	0	—	—
Nestling identity	0	—	—
Error	0.258 \pm 0.012	20.91	<0.0001

Type-3 tests of fixed effects

Effect	Estimate \pm SE	<i>F</i> _{df}	<i>P</i>
Maternal effect ^a	-0.06 \pm 0.05	0.02 _{1,889}	0.88
Rearing nest treatment ^a	0.22 \pm 0.40	5.89 _{1,79.4}	0.0575
Sex of the rearing parent ^b	-0.84 \pm 0.25	3.09 _{1,875}	0.08
Brood size	0.07 \pm 0.04	0.16 _{1,77.4}	0.03
Nestling's mass-based rank	-0.02 \pm 0.01	7.84 _{1,890}	0.005
Maternal effect \times sex of the rearing parent ^c	0.13 \pm 0.07	3.65 _{1,875}	0.056
Rearing nest treatment \times sex of the rearing parent ^c	0.96 \pm 0.34	7.93 _{1,875}	0.005
Rearing nest treatment \times brood size	-0.02 \pm 0.06	3.08 _{1,76.5}	0.09
Sex of the rearing parent \times brood size	0.07 \pm 0.03	0.03 _{1,875}	0.86
Rearing nest treatment \times sex of the rearing parent \times brood size ^c	-0.14 \pm 0.05	9.12 _{1,875}	0.003

Except when appearing in significant higher order interaction, nonsignificant terms are not presented (all $F < 3.34$, $P > 0.07$).

^a Estimate of the carotenoid group relative to the control group.

^b Estimate of the female parent relative to the male parent.

^c Estimate of the female parent from the carotenoid group relative to all other groups.

log-transformed frequency \pm SE; carotenoid group: 1.24 \pm 0.05; control: 1.30 \pm 0.05), whereas females tended to feed more nestlings that benefited from additional maternally derived carotenoids (carotenoid group: 1.03 \pm 0.05; control: 0.96 \pm 0.05).

Nestling body mass at day 6

We found no significant effect of our treatments on nestling body mass at day 6 (Table 3). Interestingly, however, nestling body mass increased with brood size. Nestling body mass was also positively correlated with the date at which chicks reached 6 days of age and negatively correlated with their mass-based rank at hatching.

Adult body condition

Parent body mass differed between sexes (random effects: plot, $\sigma^2 = 0$, nest, $\sigma^2 = 0$; sex effect, $F_{1,138} = 11.91$, $P = 0.0007$; least-square means \pm SE, males: 17.63 \pm 0.09 g, females: 17.20 \pm 0.08 g) was marginally positively correlated with tarsus length ($F_{1,138} = 3.75$, $P = 0.055$) and negatively correlated with the date at which nestlings reached the age of 6 ($F_{1,138} = 6.19$, $P = 0.014$). However, parent body mass was not affected by our carotenoid or flea treatments or any interaction between the main fixed effects (all $F < 2.79$, $P > 0.10$).

DISCUSSION

Our results provide evidence supporting the hypothesis that maternally derived carotenoids may increase nestling begging activity and potentially nestling competitive ability. We found that, in small broods, nestlings hatched from eggs laid by

females supplemented with carotenoids begged more intensely. We also found that nestlings of small broods had a lower body mass. The interaction between our carotenoid supplementation and brood size together with the positive relationship between nestling body mass and brood size suggests that maternally derived carotenoids may help low-quality nestlings to beg more intensely as a possible means to elicit higher parental effort (Kölliker et al. 1998; Neuenschwander et al. 2003). This interpretation is supported by correlational and experimental studies showing that individuals of low phenotypic/genetic quality produce smaller clutches and broods (Coulson and Porter 1985; Pettifor et al. 1988, 2001; Hanssen et al. 2003). Nestlings of small broods are therefore expected to be of lower quality themselves. In agreement with this hypothesis, we also found nestlings of small broods to benefit more from deparasitization.

In the present study, we did not directly quantify the carotenoid concentration in egg yolk. However, previous experiments in several species have shown that carotenoid-supplemented females deposit higher levels of carotenoids into the eggs than control females (Blount, Surai, Houston, et al. 2002; Blount, Surai, Nager, et al. 2002; Bertolotti et al. 2003; Biard et al. 2005). Moreover, 2 similar experiments have been carried out on the same population in 2005 and 2006, which confirm that carotenoid-fed females deposit higher levels of carotenoids into their eggs than control-fed females (Berthouly et al. 2007). Hence, the higher begging intensity of nestlings produced by carotenoid-supplemented female raised in small broods is likely to be at least partially due to a higher carotenoid transfer into the eggs by carotenoid-supplemented females. Maternally derived carotenoids may have improved nestling phenotype and consequently nestling begging activity and competitive ability through several

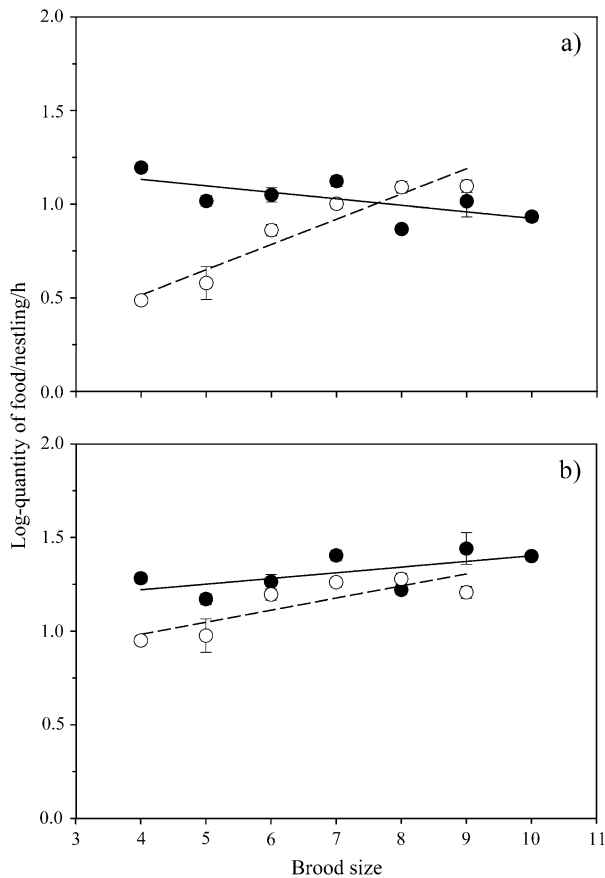


Figure 2 Log-transformed amount of food (mean predicted values \pm SE) provided per hour to an individual nestling in relation to the brood size in the rearing nest and according to whether the female (a) and the male (b) rearing parent received supplementary carotenoid during the laying period (filled circles and continuous line) or not (open circles and dashed line).

processes at various stages of the embryo’s or chick’s development. In the chicken, Surai et al. (1996) have shown that carotenoids are transferred from the yolk and yolk sac membrane to the embryo and that these maternally derived caro-

tenoids confer an antioxidant protection to growing embryo’s tissues. The antioxidant properties of carotenoids are also hypothesized to be crucial during hatching, an event characterized by a high metabolic activity and oxidative stress (Surai and Speake 1998). Posthatch, maternally derived carotenoids enhance chick protection against lipid peroxidation (Surai et al. 2003), decrease parameters of systemic inflammation (Koutsos et al. 2006), increase nestling ability to raise an immune response (Saino et al. 2003; Berthouly et al. 2007; Biard et al. 2007), and increase nestling ability to utilize dietary carotenoids (Koutsos et al. 2003). In our study, higher concentrations in yolk carotenoids may have improved embryonic growth, better protected the chicks against oxidative stress during hatching, and/or later enabled nestlings to better assimilate and metabolize dietary carotenoids. For all these reasons, nestlings hatched from carotenoid-enriched eggs may have been better able to compete for food as shown by their higher begging activity. Yet, increased carotenoid availability may also have influenced the mothers’ condition and thereby the size and/or the composition of their eggs, for example, the content of vitamins, antibodies, or hormones (Schwabl 1996; Royle et al. 1999; Grindstaff et al. 2003), all compounds likely to affect nestling condition and thus competitive ability.

A previous study on the same population of great tits investigating the impact of direct carotenoid supplementation to nestlings failed to find a significant effect on nestling begging activity (Tschirren et al. 2005). Although directly supplementing nestlings with carotenoids modified the coloration of their breast feathers, the authors did also not find parents to discriminate between carotenoid- and control-fed offspring. Such a discrepancy with our results may arise for several reasons. First, their modest sample size and the use of nonparametric method may have prevented them from detecting an effect. Second, whereas we provided laying females with a cocktail of carotenoids comprising lutein, zeaxanthin, and β,β -carotene to mimic the natural carotenoid composition of great tit diet, Tschirren and colleagues fed nestlings with lutein and zeaxanthin only. β,β -Carotene, which does not contribute to nestling plumage coloration, has recently been found to influence nestling immunity, whereas lutein and zeaxanthin do not (Fitze et al. 2007). This raises the question of a potential role for β,β -carotene alone in determining nestling condition, begging activity, and competitive ability. Finally, our results may highlight the prominent influence of maternal effects on offspring development relative to parental

Table 3 GLMM testing for effects of the natal nest carotenoid treatment (maternal effect), the rearing nest carotenoid treatment and the flea treatment, and random effects deriving from the experimental design on the nestling mass at day 6

Covariance parameter estimates

Parameter	Estimate \pm SE	Wald’s Z	P
Natal plot (maternal effect \times flea treatment)	0	—	—
Rearing plot (rearing nest treatment \times flea treatment)	0	—	—
Dyad (flea treatment)	0.544 \pm 0.194	2.80	0.0025
Natal nest (natal plot \times dyad)	0.190 \pm 0.067	2.85	0.0022
Rearing nest (rearing plot \times dyad)	0.225 \pm 0.099	2.25	0.012
Rearing nest \times natal nest (dyad)	0	—	—
Residual	0.534 \pm 0.041	13.01	<0.0001

Type-3 tests of fixed effects

Effect	Estimate \pm SE	F_{df}	P
Date of age 6	0.16 \pm 0.03	39.86 _{1,60.4}	<0.0001
Nestling’s mass-based rank	-0.31 \pm 0.02	253.98 _{1,400}	<0.0001
Brood size	0.15 \pm 0.06	5.05 _{1,38}	0.03

Except when appearing in significant higher order interaction, nonsignificant terms are not presented (all $F < 1.24$, $P > 0.27$).

effects arising at later developmental stages (Mousseau and Fox 1998).

In species with more than one offspring, begging intensity is likely to be a function of the level of food requirement and condition status of an individual nestling on the one hand and the level of sibling competition on the other hand (Godfray and Johnstone 2000). Food requirements are likely to increase and condition status to decrease in small broods if parent and offspring quality covary positively with brood size. Inversely, sibling competition increases with increasing brood size. Interestingly, we found that in parasitized nests, the highest levels of begging were found in very small and very large broods. In the contrary, in parasite-free nests, the lowest levels of begging were found in large broods. These results suggest that the presence of parasites may intensify sibling competition in large broods.

Our results also support the hypothesis that carotenoid availability can modulate female investment in reproduction with supplementary carotenoids enabling female great tits to increase their parental effort. Among pairs, which received control food, females having large brood fed their nestlings more than females having small broods. This result is in agreement with the hypothesis that individuals producing small broods are of lower quality. In contrast, carotenoid-supplemented females having small broods increased their feeding effort to such an extent that feeding did no longer covary with brood size. Increased carotenoid availability prior and during laying may have enabled low-quality, small-brood females to respond to the more intense begging of nestlings hatched from eggs laid by carotenoid-fed females (Neuenschwander et al. 2003). Although the presence of nestlings produced by carotenoid-supplemented females may have increased the overall begging level in control nests as well, low-quality control females may have been unable to increase their feeding effort. It is interesting to note that parent body condition was unaffected by our carotenoid supplementation. This further suggests that additional carotenoids enabled low-quality females to sustain a high feeding effort and possibly a higher metabolic activity without impairing their own condition.

Females typically pay a greater cost to reproduction than males (Trivers 1972). Particularly, female great tits build the nest, pay a high metabolic cost during egg laying (Nilsson and Råberg 2001), and incubate the eggs and the hatchlings. Females are thus likely to be energetically constrained during the nestling-rearing period. They may then be expected to favor offspring that provide them with the highest fitness return, that is, nestlings that benefited from additional maternally derived carotenoids. Indeed, we found a marginally nonsignificant tendency (Table 2, $P = 0.056$) in female parents to feed more nestlings hatched from eggs laid by carotenoid-supplemented females. Although nestlings produced by carotenoid-fed females appear to be better able to compete for food and to receive more food from female parents, we did not find them to be heavier by the time video recordings were made, that is, 6 days posthatch. However, significant differences in body mass are revealed later in nestling development with further results showing that nestlings produced by carotenoid-fed females end up being heavier at 14 days of age (Berthouly et al. forthcoming).

Until nestlings are born, males pay a much smaller cost to reproduction than females and may be much less energetically constrained during the nestling-rearing period. This may explain why male feeding effort was unrelated to brood size and why males did not significantly increase their provisioning when supplemented with carotenoids. Furthermore, if males have more energy to spend in parental effort, they may be expected to either feed all nestlings equally or even privilege lower quality nestling in order to maximize their repro-

ductive success. This hypothesis is supported by the male tendency to feed more nestlings hatched from control eggs.

Overall, our findings provide further evidence that antioxidant pigments such as carotenoids are of particular importance early in the life of an individual for the development of fitness-related trait such as its competitive ability and later, as adults, to cope with the costs of reproduction.

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