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Sex-related effects of maternal egg investment on offspring in relation to carotenoid availability in the great tit

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Summary

1. Maternal carotenoids in the egg yolk have been hypothesized to promote maturation of the immune system and protect against free radical damages. Depending on availability, mothers may thus influence offspring quality by depositing variable amounts of carotenoids into the eggs. Sex allocation theory predicts that in good quality environments, females should invest into offspring of the sex that will provide larger fitness return, generally males.

2. In a field experiment we tested whether female great tits bias their investment towards males when carotenoid availability is increased, and whether male offspring of carotenoid-supplemented mothers show higher body condition. We partially cross-fostered hatchlings to disentangle maternal effects from post-hatching effects, and manipulated hen flea *Ceratophyllus gallinae* infestation to investigate the relationship between carotenoid availability and resistance to ectoparasites.

3. As predicted, we found that carotenoid-supplemented mothers produced males that were heavier than their sisters at hatching, while the reverse was true for control mothers. This suggests that carotenoid availability during egg production affects male and female hatchlings differentially, possibly via a differential allocation to male and female eggs.

4. A main effect of maternal supplementation became visible 14 days after hatching when nestlings hatched from eggs laid by carotenoid-supplemented mothers had gained significantly more mass than control nestlings. Independently of the carotenoid treatment, fleas impaired mass gain of nestlings during the first 9 days in large broods only and reduced tarsus length of male nestlings at an age of 14 days, suggesting a cost to mount a defence against parasites.

5. Overall, our results suggest that pre-laying availability of carotenoids affects nestling condition in a sex-specific way with potentially longer-lasting effects on offspring fitness.

Key-words: fleas, maternal effects, sex-specific resource allocation, yolk antioxidants.

Introduction

It has been suggested that early life conditions can have short- and long-term effects on fitness components such as morphology, physiology, health and behaviour (Desai *et al*. 1995; Anisman *et al*. 1998). In birds, pre- and postnatal conditions have been shown to influence fitness-related traits of offspring in various ways. On the one hand, the quality of the neonatal nutrition can determine an individual's capacity to assimilate dietary antioxidants later in life and influence the expression of its sexual ornaments (Kitaysky *et al*. 2001; Ohlsson *et al*. 2002; Blount *et al*. 2003b). On the other hand, maternal investment into egg quality, i.e. egg size and/or egg composition, has been shown to affect nestling growth and development of immune functions (Grindstaff, Brodie & Ketterson 2003; Saino *et al*. 2003; Andersson *et al*. 2004). Extent and impact of such maternal effects on offspring performance depend not only on the mother's condition at breeding (Saino *et al*. 2002; Love *et al*. 2005) and on the quality of the mate (Saino *et al*. 2002), but also on the quality of the environment, for example the quality of the diet during breeding (Biard, Surai & Møller 2005). Females are thus expected to influence phenotypic traits and ultimately fitness of their offspring via the adjustment of the resources transferred into the eggs (Royle *et al*. 1999; Saino *et al*. 2002; Tschirren, Richner & Schwabl 2004; Verboven *et al*. 2005).

Carotenoids are a family of antioxidant pigments that play important physiological roles in both the protection against

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the damage to biological molecules caused by free radicals and in the activation of the immune system (Surai & Speake 1998; Møller *et al*. 2000; Alonso-Alvarez *et al*. 2004; McGraw & Ardia 2004). Carotenoids are also supposed to be involved in the regulation of embryonic growth and development. They are hypothesized to control cell differentiation (Royle *et al*. 1999), promote maturation the immune system (Møller *et al*. 2000; Koutsos *et al*. 2003; Saino *et al*. 2003), and provide antioxidant protection to the embryonic tissues (Surai & Speake 1998; Blount *et al*. 2002a; McGraw, Adkins-Regan & Parker 2005). Carotenoids are not synthesized de novo, but ingested with food, and their availability is supposed to be limited in nature (Partali *et al*. 1987; Olson & Owens 1998). Trade-offs in the allocation of carotenoids to different physiological functions can thus be expected.

Female birds transfer varying amounts of carotenoids into their eggs and thus influence offspring quality. This decision is likely to be influenced by their mates' quality (Saino *et al*. 2002), their own condition (Saino *et al*. 2002; Love *et al*. 2005), and the habitat quality at the time of breeding, for example carotenoid availability (Blount *et al*. 2002b; Bortolotti *et al*. 2003; Tschirren *et al*. 2004; Biard *et al*. 2005; Verboven *et al*. 2005). Consistent with this view, eggs laid by females previously fed with carotenoid-enriched food showed higher carotenoid levels than eggs laid by control females (Blount *et al*. 2002a,b; Biard *et al*. 2005; Karadas *et al*. 2005). A transfer of larger amounts of carotenoids into the eggs may not only have direct beneficial effects on nestling condition, but also improve the ability of nestlings to metabolize and efficiently utilize carotenoids later in life (Koutsos *et al*. 2003).

Fitness return per unit investment for daughters and sons differs in many organisms. In sexually dimorphic species, sex-related differences in susceptibility to harsh conditions (Tschirren, Fitze & Richner 2003) are expected to lead to different reproductive values of sons and daughters in a given environment (Trivers & Willard 1973). The more susceptible sex will generally have the greater variability in reproductive success and thus provide higher fitness returns in habitats of good quality (Arnold 1994). Thus by investing more into the sex that provides greater fitness return under given environmental conditions (i.e. quality of the diet, the mates, and the parasite load), mothers may increase their own reproductive success (Trivers & Willard 1973; Sheldon *et al*. 1998; Albrecht 2000; Radford & Blakey 2000; Badyaev, Hill & Whittingham 2002; Whittingham, Dunn & Nooker 2005). In the great tit, where males are bigger, more colourful and more impaired by parasitic hen fleas *Ceratophyllus gallinae* than females (Tschirren *et al*. 2003), male offspring may provide a larger fitness gain than females when carotenoid availability is high. Mothers should then bias their investment toward sons by transferring more resources into male eggs (i.e. carotenoids), or by producing more sons than daughters (McGraw *et al*. 2005).

In a wild great tit *Parus major* population, we tested the prediction that sons hatched from eggs laid by carotenoid-supplemented mothers would be in better condition than sons hatched from control eggs, whereas the contrary was predicted for daughters. At hatching, nestlings were partially cross-fostered to separate the effects of maternally transmitted carotenoids from the effects of the post-hatching environment on nestling performance. We also manipulated parasite load in the nests. Parasites have been shown to impair nestling fitness by reducing body condition (Richner, Oppliger & Christe 1993; Tschirren *et al*. 2003), growth rate (Nilsson 2003) and activate a costly immune response (de Lope, Møller & de la Cruz 1998). Hence defence against parasites may compete for limited resources with other functions, resulting for example in an allocation trade-off for carotenoids between plumage coloration and detoxification. Furthermore the effect of parasites on nestlings is sex-specific in great tits: parasite-exposed males show reduced body mass and shorter tarsi while females do not seem affected (Tschirren *et al*. 2003). Thus, by a controlled flea infestation, we aimed at manipulating the trade-off in carotenoid allocation between immune system and other physiological functions in order to increase the detectability of the effects of maternally transmitted carotenoids on the differential performance of male and female offspring.

Methods

FOOD SUPPLEMENTATION AND PARASITE TREATMENT

The study was carried out in 2004 in a population of great tits breeding in nest boxes in the Bremgartenwald, a forest near Bern, Switzerland. Our study area contained 336 nest boxes distributed over 24 study plots. In early March, before the start of nest building, we removed old nesting material from nest boxes and brushed them thoroughly to eliminate parasites. At the same time we hung a feeding dish within 2 m of each nest box. We then randomly assigned a food and a flea treatment to each plot, leading to four plot types: (1) plots of flea-infested nests supplemented with carotenoid-enriched food (F+ C+); (2) plots of flea-infested nests supplemented with non-enriched food (F+ C–); (3) plots of parasite-free nests supplemented with carotenoid-enriched food (F– C+); and (4) plots of parasite-free nests supplemented with non-enriched food (F– C–). We provided supplemental food in the form of standard commercial fatballs mixed with sunflower seeds (provided by Erbo Agro AG , Bützberg, Switzerland). In order to dissolve the fat and to denaturate most carotenoids naturally present in the seeds, the fatballs were crumbled, slowly heated to 60 °C and maintained at that temperature for approximately 10 min (treatment as recommended by Roche AG; personal communication to AB). After cooling, we added 0·08 mg of lutein, 0·003 mg of zeaxanthin, and 0·017 mg of beta-carotene per gram (provided by Roche AG) to one half of the food mixture. Relative concentrations of the three carotenoids (i.e. 80% of lutein, 3% of zeaxanthin, and 17% of β,β-carotene) correspond to the ratio found in the natural diet of great tits (Partali *et al*. 1987). The mixture was then shaped into either carotenoid-enriched or nonenriched balls that were stored in a dark climatic chamber at 4 °C. Based on their daily food requirements, we estimated that the maximal daily quantities of carotenoids ingested by each bird was about eight times higher than the quantities they would have ingested if their diet consisted of lepidopteran larvae only (Partali *et al*. 1987; Crocker *et al*. 2002). However, great tits also eat seeds and buds, especially during winter. *Alnus incana* leaves contain 500 times more carotenoids than lepidoteran larvae (Partali *et al*. 1987), and thus plant seeds are also likely to contain much higher quantities of carotenoids than insects. Moreover the concentration

of carotenoids in egg yolks are nine times higher than the concentration found in lepidopteran larvae indicating that great tits obtain carotenoids from other food types. Thus, we believe that the quantities of carotenoids we provided are well within the range of the natural variations in carotenoid availability to birds in the wild. In order to minimize the influence of our food treatment on breeding habitat choice, we started supplementation after breeding territories had been established, i.e. on 20 March. Indeed, known correlates of female quality such as laying date, clutch and brood size were not significantly different between the two groups (respectively: $P = 0{\cdot}832$; $P = 0{\cdot}434$; $P = 0{\cdot}269$), which supports the assumption that female quality was randomized over the two treatments. While the provided food was eaten by the target pairs, as confirmed by direct observations, it was also partly also consumed by other bird species, and the exact amount eaten by each individual could thus not be determined precisely. C+ and C– fatballs seemed to be consumed at similar rates.

After the construction of the nest cup we infested half of the nests (12 plots) with 40 fleas *Ceratopyllus gallinae* collected from old nest material. This amount is known to be sufficient for the establishment of a flea population in the nest (Heeb *et al*. 1996; Tripet & Richner 1999). We used regular nest checks to determine the start of the incubation and the hatching date. Food supplementation was stopped on the first day of incubation.

At hatching, nestlings were weighed and partially cross-fostered to disentangle the effects of maternally derived carotenoids from post-hatching environmental effects. Cross-fostering was carried out on the first day of hatching in order to minimize the effects of the post-hatching environment on nestling condition. Hatchlings were exchanged between two nests of similar clutch size and similar average brood mass, and having different food treatments (C+ nestlings ↔ C– nestlings) but sharing the same flea treatment (C+ F+ ↔ C– F+ and C+ F– ↔ C– F–). Hatchlings were ranked according to their body mass in the nest of origin. The lightest hatchling was randomly assigned either to stay in the nest of origin or to be exchanged to the partner nest by tossing a coin. Cross-foster status (exchange/stay) was then alternated through the mass-based rank list of hatchlings. At the time of cross-fostering, only two thirds of the eggs of the nest had hatched yet. Thus nestlings hatched 1 or 2 days after the first hatchlings automatically remained in their nest of origin. Our design did not modify the original brood size. We individually marked hatchlings by partially removing tuft feathers from the heads, backs and wings. Nestlings were weighed 9 and 14 days after hatching. At day 15 we also measured their tarsus length. Nestlings were permanently marked 9 days after hatching with a unique metal ring. When nestlings were 14 days old we took a blood sample from a small puncture of the leg vein. The blood was stored in EDTA in an Eppendorf tube at –30 °C until analysis. Blood was used to sex the nestlings (see Griffiths *et al*. 1998 for the sexing technique). A total of 437 nestlings from 98 nests were identified, sexed and measured.

TOTAL YOLK CAROTENOIDS

In order to keep broods at their original size, we did not collect eggs in 2004. In 2005 and 2006 we repeated a similar food supplementation experiment and collected the third and the fifth (minimal clutch size) egg in a random sample of nests (2005: $n = 23$; 2006: $n = 107$), which allowed us to verify the assumption that carotenoid supplementation effectively led to higher carotenoid levels in the eggs. Total carotenoid concentrations were assessed using high performance liquid chromatography following the protocol described in (Surai & Speake 1998; Karadas *et al*. 2005). Carotenoid supplementation significantly increased total carotenoid concentration in the egg yolk thus confirming the above assumption. Carotenoid-fed mothers deposited a higher quantity of carotenoid into the egg yolk than control mothers [2005 ($P = 0{\cdot}0031$) (mean ± 1 SE): C+ females: $26{\cdot}16 \pm 11{\cdot}08$ μg g^{-1} of egg yolk; C– females: $16{\cdot}40 \pm 8{\cdot}7$ μg g^{-1} of egg yolk; 2006 ($P = 0{\cdot}01$): C+ females: $25{\cdot}79 \pm 9{\cdot}91$ μg g^{-1} of egg yolk; C– females: $20{\cdot}27 \pm 9{\cdot}21$ μg g^{-1} of egg yolk] (See Berthouly *et al.* 2007). The concentrations found in the eggs collected in 2005 and 2006 were well within the range measured in other great tit populations (Royle *et al*. 1999; Grindstaff *et al*. 2003; Saino *et al*. 2004; Tschirren *et al*. 2004), and thus the carotenoid dosing did not induce unusually high carotenoid concentrations in the eggs.

STATISTICAL PROCEDURES

The effects of the carotenoid supplementation on body mass at hatching were tested using mixed models. Flea treatment and feeding treatment of the genetic mother were included as main factors. Sex, clutch size, laying date of the first egg, and hatching sequence were included as covariates. Hatching sequence corresponds to day of hatching as hatching is asynchronous. Nestlings could either have hatched on the day where the first hatching occurred in the nest, or 1–2 days thereafter [i.e. two modalities: hatched the first day (A) or hatched the second or the third day (B)]. This factor was assumed to correct for the age of the nestlings, and most importantly to account for a part of the variance due to the cross-fostering. The nest of origin (random effect) was nested within parasite and carotenoid treatments.

We also used linear mixed models to investigate the effect of the carotenoid supplementation on mass gain between hatching and the age of 9 days, and between day 9 and day 14. We did not perform a single repeated measure analysis to investigate the effect of our treatments on the mass gain over both periods: first, the mass gain between age 0 and age 14 is not linear and second, the heterocedasticity in the residuals could not be suitably modelled, i.e. variance at age 0 was much less than at age 9 and 14 days. We thus performed two distinct analyses with the dependent variables being the body mass at day 9 or at day 14 and included the body mass at hatching or the body mass at day 9 as a covariate, respectively. Thus, by including previous body weight as a covariate, the dependent variable represents a measure of mass gain over the given time period. Flea treatment and feeding treatment of the genetic mother were the main factors in the models. We also included the following fixed effects and covariates: the feeding treatment of the rearing female, sex of the nestling, brood size, hatching date and hatching sequence. Nest of origin and nest of rearing were added as random factors and nested within parasite and carotenoid treatments. The analyses of the tarsus length at day 15 also included flea treatment and feeding treatment of the genetic mother as main factors in the models, and feeding treatment of the rearing female, sex of the nestling, brood size of the nest of rearing, hatching date and hatching sequence as covariates.

Effects of carotenoid supplementation and flea infestation on clutch and brood size were analysed with Generalized Linear Models with Poisson errors and log link, corrected for dispersion parameters different from 1 if necessary. We included the laying date of the first egg as a covariate in the two models. The effects of the carotenoid supplementation and flea infestation on hatchability were investigated using logistic regression with binomial distribution and logit link function. To control for the effect of clutch size we included it as a covariate in the model. The effect of carotenoid supplementation on total yolk carotenoid concentration was analysed with a mixed model, including the carotenoid treatment, clutch size, laying date and egg rank as fixed effects or covariates. The nest was included as a random factor.

Table 1. Summary of the linear mixed model for the body mass at hatching. Nest of origin was included as a random factor and nested both within the feeding treatment of the genetic mother and the flea treatment. Nonsignificant interactions were backward eliminated

Parameter	Estimation ± 1 SE	*F*-value	*P*-value
Feeding treatment of the genetic mother (C+)		$0{\cdot}43_{1,96}$	0·51
Flea treatment (F+)		$0{\cdot}09_{1,96}$	0·76
Sex		$0{\cdot}04_{1,378}$	0·84
Laying date	0·009 ± 0·005	$2{\cdot}99_{1,96}$	0·087
Hatching sequence (A)	0·160 ± 0·02	$91{\cdot}88_{1,378}$	< 0·0001
Clutch size		$0{\cdot}01_{1,96}$	0·91
Carotenoid treatment × sex	0·028 ± 0·01	$6{\cdot}43_{1,378}$	0·012

Analyses of mass gain were performed using JMP IN 5·1 (Sall, Creighton & Lehmann 2005). Logistic Regressions, Generalized Linear Models and the analyses of body mass at hatching were performed using R (Maindonald & Braun 2003; R Development Core Team 2006). Residuals were tested for normality, and dependent variables were transformed if necessary. All nonsignificant interactions were backward eliminated using a stepwise elimination procedure. Tests are two-tailed with a significance level set to $\alpha = 0{\cdot}05$.

Results

TOTAL YOLK CAROTENOIDS

Treatment effects on clutch size, brood size and hatchability

Clutch size was independent of feeding ($t_{199} = 0{\cdot}807$, $P = 0{\cdot}42$) and flea treatments ($t_{199} = 1{\cdot}76$, $P = 0{\cdot}080$). The proportion of eggs hatched was not affected by these treatments ($t_{194} = 1{\cdot}275$, $P = 0{\cdot}20$; $t_{194} = 1{\cdot}648$, $P = 0{\cdot}10$, respectively) even after controlling for clutch size. Feeding treatment and the parasite infestation did not influence brood size (respectively: $t_{199} = 0{\cdot}955$, $P = 0{\cdot}34$; $t_{199} = 1{\cdot}208$, $P = 0{\cdot}23$).

NESTLING BODY MASS

Nestling body mass at hatching

Male hatchlings from carotenoid-fed females were heavier than control males, while the opposite was observed for females (Fig. 1), as revealed by a significant interaction between feeding treatment of the genetic mother and the sex of the offspring (Table 1). Flea treatment did not influence the weight at hatching (Table 1). Body mass at hatching was also significantly influenced by the hatching sequence but not by the laying date (Table 1). As equal numbers of C+ males, C+ females, C– males, C– females hatched the first, the second and the third day ($\chi^2_2 = 1{\cdot}62$, $P = 0{\cdot}44$) the effect of the carotenoid treatment on hatching mass is not confounded by differences in hatching sequence between the sexes.

Nestling mass gain between hatching and day 9

Mass gain between hatching and day 9 was independent of the feeding treatment of the genetic mother (Table 2; Fig. 2).

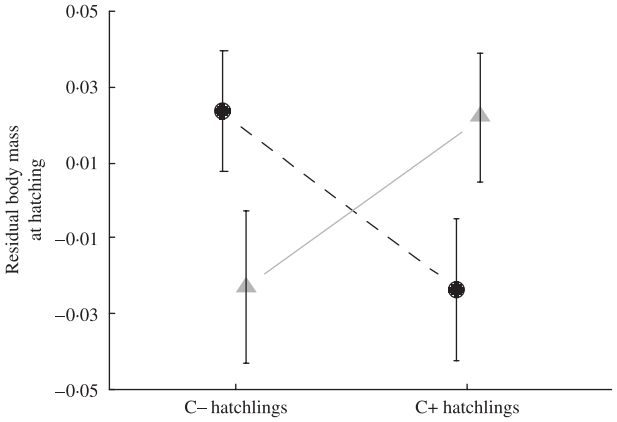

Fig. 1. Mean (± 1 SE) residual body mass of male (grey triangles and solid lines) and female (black points and dotted lines) hatchlings originating from eggs laid by carotenoid-fed (C+ nestlings) or control mothers (C– nestlings). C+ male hatchlings were heavier than C+ female hatchlings, whereas in the C– male hatchlings were lighter than C– female hatchlings.

There was a significant effect of the interaction between the flea treatment and brood size on mass gain between hatching and day 9 (Table 2). Among infested, nests nestling mass gain was more strongly affected by a larger brood size than in parasite-free nests. Males gained significantly more weight than females (Table 2). Birds that hatched early in the season had a higher mass gain than those that hatched later (Table 2). Within a nest, first hatched nestlings had a higher mass gain than later hatched ones, as shown by the significant effect of the hatching sequence on mass gain between hatching and day 9 (Table 2).

Nestling mass gain between day 9 and day 14

Nestlings from carotenoid-fed mothers (C+ nestlings) gained significantly more weight than nestlings from control mothers (C– nestlings) (Table 2) (Fig. 2). Males gained significantly more mass than females (Table 2), and females tended to be more affected by fleas than males, as shown by the marginally significant interaction between flea treatment and sex (Table 2) (Fig. 3). Birds hatched early in the season gained more mass than nestlings hatched later (Table 2). Within nests, firsts hatched

Table 2. Summary of the linear mixed model for mass gain between hatching and day 9, and between days 9 and 14, and for tarsus length at day 15. Nest of origin and nest of rearing were included as random factors and nested within the flea treatment and the feeding treatment of the genetic mother or of the rearing mother, respectively. Nonsignificant interactions were backward eliminated

		Estimation		
	Parameters	± 1 SE	*F*-value	*P*-value
Body mass at day 9	Feeding treatment of the genetic mother (C+)		$1{\cdot}05_{1,46}$	0·31
	Flea treatment (F+)		$0{\cdot}57_{1,46}$	0·45
	Sex (female)	–0·267 ± 0·05	$26{\cdot}60_{1,306}$	< 0·0001
	Feeding treatment of the rearing mother (C+)		$0{\cdot}45_{1,69}$	0·51
	Body mass at hatching	2·373 ± 0·19	$146{\cdot}07_{1,306}$	< 0·0001
	Hatching date	0·153 ± 0·03	$24{\cdot}01_{1,306}$	< 0·0001
	Hatching sequence (A)	1·591 ± 0·18	$76{\cdot}28_{1,306}$	< 0·001
	Brood size	–0·281 ± 0·09	$9{\cdot}22_{1,306}$	0·0026
	Flea treatment × brood size	–0·203 ± 0·09	$4{\cdot}75_{1,306}$	0·030
Body mass at day 14	Feeding treatment of the genetic mother (C+)	0·085 ± 0·03	$5{\cdot}39_{1,96}$	0·022
	Flea treatment (F+)		$2{\cdot}08_{1,21}$	0·16
	Sex (female)	–0·207 ± 0·03	$38{\cdot}97_{1,310}$	< 0·0001
	Feeding treatment of the rearing mother (C+)		$0{\cdot}09_{1,21}$	0·76
	Body mass at day 9	0·604 ± 0·03	$522{\cdot}78_{1,310}$	< 0·0001
	Hatching date	–0·070 ± 0·02	$7{\cdot}84_{1,310}$	0·0054
	Hatching sequence (A)	–0·462 ± 0·12	$14{\cdot}09_{1,310}$	0·0002
	Brood size		$0{\cdot}4240_{1,310}$	0·52
	Flea treatment × sex	–0·060 ± 0·03	$3{\cdot}49_{1,310}$	0·063
Tarsus length at day 15	Feeding treatment of the genetic mother (C+)		$0{\cdot}28_{1,96}$	0·59
	Flea treatment (F+)	–0·125 ± 0·05	$6{\cdot}75_{1,21}$	0·017
	Sex (female)	–0·172 ± 0·02	$54{\cdot}42_{1,307}$	< 0·0001
	Feeding treatment of the rearing mother (C+)		$0{\cdot}46_{1,21}$	0·62
	Body mass at day 14	0·231 ± 0·02	$141{\cdot}65_{1,307}$	< 0·0001
	Hatching date		$2{\cdot}03_{1,307}$	0·15
	Hatching sequence (A)		$1{\cdot}39_{1,307}$	0·53
	Brood size		$0{\cdot}05_{1,307}$	0·82
	Flea treatment × sex	0·054 ± 0·02	$5{\cdot}98_{1,307}$	0·015

nestlings gained significantly less mass between day 9 and 14 than later hatched ones, as shown by the significant effect of the hatching sequence (Table 2).

In summary, food treatment did not influence mass gain between hatching and day 9. However, its effect appeared between day 9 and day 14: C+ nestlings gained significantly more weight than C– nestlings during this period. Flea infestation impaired mass gain between hatching and day 9 in large broods, but this effect was not detectable after day 9. Finally, we found a sex-specific effect of fleas on body mass gain between day 9 and 14: females tended to be more affected by fleas than males at that age.

Tarsus length at day 15

The feeding treatment of the genetic mother did not influence the length of the tarsus (Table 2); however, flea infestation significantly affected tarsus length (Table 2) (Fig. 3). Nestlings raised in infested nests had a smaller tarsus than nestlings raised in parasite-free nests. Fleas had a stronger effect on male than on female tarsus, as shown by the significant interaction between flea treatment and sex (Table 2) (Fig. 3). Finally, hatching sequence, hatching date and brood size did not influence tarsus length (Table 2).

Discussion

Our study shows that carotenoid supplementation affected hatchling body mass in a sex-specific way. Male nestlings hatched from eggs laid by carotenoid-fed mothers were heavier than females, while female nestlings hatched from eggs laid by control mothers were heavier than their brothers. There are at least two possible explanations for this result.

First, carotenoid-fed mothers could have biased their investment towards male eggs by increasing the size of the eggs, and/or the quantity of carotenoids (Verboven *et al*. 2005), hormones or other nutrients transferred into the eggs (Ohlsson *et al*. 2002; Blount *et al*. 2003b). This may result either in a better embryonic development and hence higher body mass at hatching for males than for females in the carotenoid-supplemented group, or in a higher mass gain of males hatched from eggs laid by carotenoid-supplemented mothers during the short period between hatching and first weighing (i.e. in field studies, body mass cannot be consistently measured right after hatching but rather within the time span of a few minutes to a few hours after hatching). Several mechanisms could be responsible for such differences in mass gain: (1) the transfer of higher amounts of carotenoids into male eggs by carotenoid-fed mothers may have enhanced the competitiveness

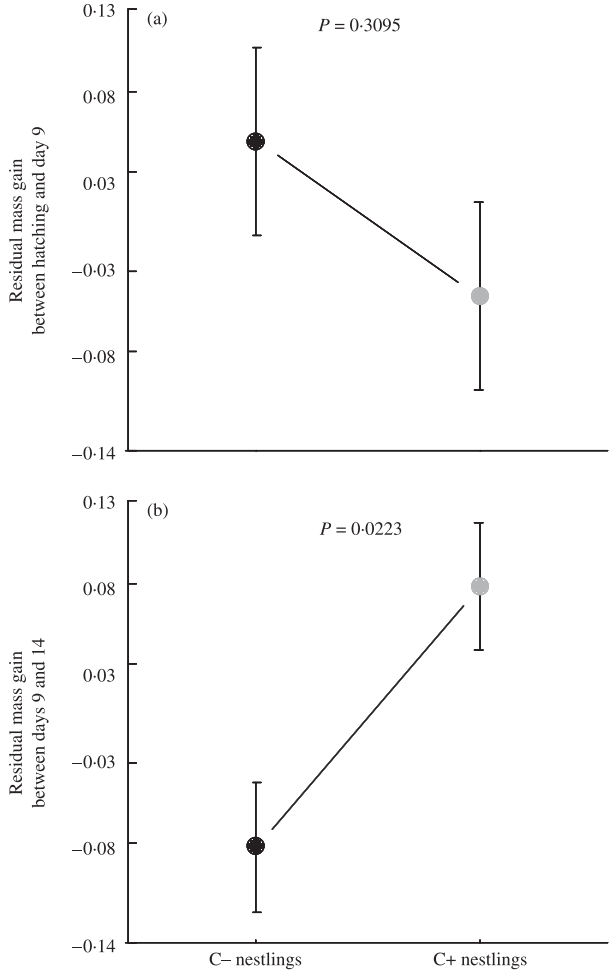

Fig. 2. Mean (± 1 SE) (a) residual mass gain between hatching and day 9 and (b) residual mass gain between days 9 and 14 of C+ nestlings (i.e. hatched from eggs laid by carotenoid-supplemented females) and C– nestlings (i.e. hatched from eggs laid by control females). Between hatching and day 9 there were no differences between C+ nestlings and C– nestlings (a), while between days 9 and 14 C+ nestlings were significantly heavier than C– nestlings (b). *P*-values are derived from the model presented in the Results (Table 3).

of male nestlings, their ability to metabolize the carotenoids, or their resistance to the stress induced by hatching (Ohlsson *et al*. 2002; Blount *et al*. 2003b), and hence males benefiting from larger quantities of carotenoids in the egg yolk sack would have grown faster after hatching than their sisters; (2) an unequal transfer of some other substances into the egg yolk, e.g. androgenic hormones that may have enhanced the competitiveness of male nestlings and thereby increased mass gain; and (3) carotenoid-fed mothers may have laid eggs with larger yolks. Larger yolks may have led to larger yolks sacks of male hatchlings and enabled them to cope with starvation after hatching. Inversely to C+ hatchlings, C– females were heavier than their brothers at hatching. C– mothers might thus have biased their investment towards females as high-quality female nestlings may provide a higher fitness return than male nestlings in environments with lower carotenoid availability (Trivers & Willard 1973).

Second, mothers may not have biased their egg investment toward any sex, but C+ and C– mothers may simply have produced eggs of different quality and thereafter male and female embryos have responded to egg composition differentially, resulting in different embryonic development between C+ and C– males and between C+ and C– females. The two hypotheses are not mutually exclusive and the effect of differential investment of C+ and C– mothers in male and female eggs may be further enhanced by a sex-specific sensitivity to egg composition.

As mentioned above, carotenoid-supplemented females deposited higher amounts of carotenoids into their eggs. Thus, if carotenoids improve embryonic development, hatchling competitiveness, and/or hatchling ability to efficiently utilize carotenoids, it can be expected that increased quantities of carotenoids in the eggs lead to a higher body mass of newly hatched nestlings. However, this was not the case. We found an interaction effect between the feeding treatment and offspring sex on hatchling body mass, but no main effect of the feeding treatment: C+ hatchlings were not heavier than C– hatchlings. This could be due to the fact that control mothers compensated for a lack of carotenoids by laying bigger eggs or depositing other substances such as proteins or growth hormones in higher quantities.

The body mass differences found between C– and C+ males and between C– and C+ females at hatching had no carry-over effect on nestling mass gain. However, body mass at hatching could determine other physiological functions and/or morphological and behavioural traits than mere mass gain. For example, body mass at hatching may influence the efficiency of certain components of the immune system, the quality and the brightness of the plumage, and competitive ability. Moreover cross-fostering may have slightly changed original sex-specific mass hierarchy within a brood, lowering the potential effects of mass rank at hatching. Males gained significantly more mass than females between hatching and day 9, and also between day 9 and day 14, but as mentioned above this was independent of the feeding treatment. However, C+ nestlings gained significantly more mass than C– nestlings between day 9 and day 14, but not between hatching and day 9. Carotenoid-supplemented mothers may have produced eggs of higher quality than control mothers by laying larger eggs and/or by modifying egg contents such as carotenoid and hormone concentrations. In both 2005 and 2006, we found that egg yolks produced by carotenoid-supplemented mothers contained more carotenoids than yolks of control mothers. Thus, the effects of maternal carotenoid supplementation on offspring growth may be partially due to an increase in the carotenoid content of egg yolks. Such an increase in carotenoid quantities in the eggs may have enhanced the ability of nestlings to efficiently utilize carotenoids (Koutsos *et al*. 2003), or could have increased their competitive performance (Berthouly, Helfenstein & Richner 2007; Helfenstein *et al*. unpublished), resulting in a higher mass gain several days after hatching. This result suggests that the effects of maternally transmitted substances on offspring phenotype become only visible later in the nestling period when energy demand is

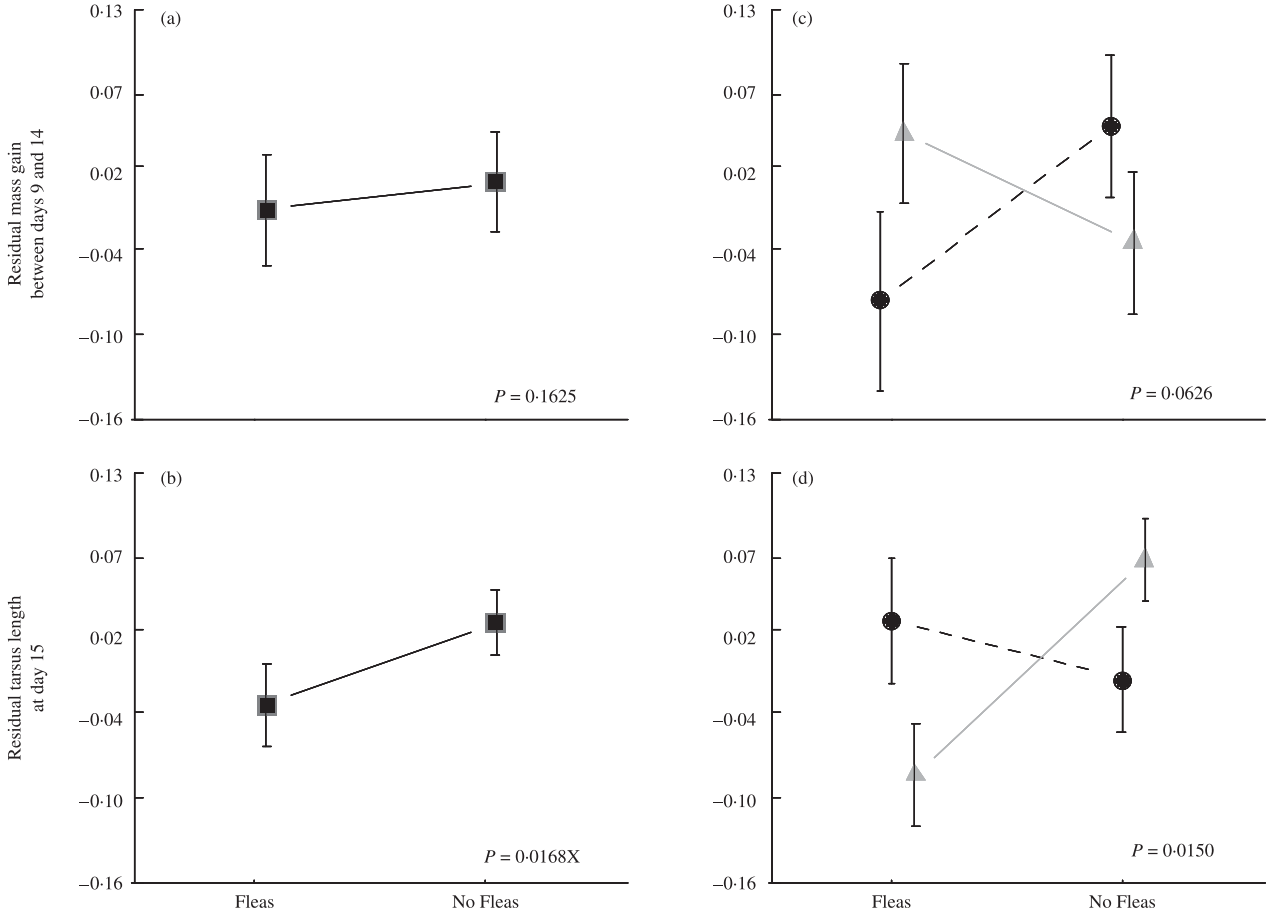

Fig. 3. Mean (± 1 SE) (a) residual mass gain between days 9 and 14, and (b) residual tarsus length at day 15 of nestlings raised in infested nests (Fleas) and nestlings raised in nests free of parasites (No fleas). Mean (± SE) (c) residual mass gain between days 9 and 14 and (d) residual tarsus length at day 15 of males (grey triangle and solid line) and females (black points and dotted lines) raised in infested (Fleas) or parasite-free (No fleas) nests. In infested nests, nestlings tended to have a smaller body mass than nestlings raised in parasite-free nests (a), and body mass of females tended to be more affected by flea infestation than body mass of males (b). Overall, nestlings in infested nests had a significantly smaller tarsus than nestlings in parasite-free nests (c), due mainly to the effect on males (d). *P*-values are derived from the model presented in the Results (Table 3).

higher (e.g. due to plumage development, thermoregulation, development of the immune system). In previous experimental studies on great tits it has also been shown that parasite-induced maternal responses during egg laying become visible 15 days after hatching only (Heeb *et al*. 1998; Gallizzi *et al.* unpublished), suggesting that the quantity and quality of maternally transmitted substances (e.g. hormones, antibodies, antioxidants) may have an increasing influence on nestlings' condition as they grow. In our study, benefits of maternal effects become apparent a few days before fledging, and could thereby influence post-fledging survival (Naef-Daenzer, Widmer & Nuber 2001; Naef-Daenzer & Grüebler, unpublished).

We found that fleas impaired mass gain between hatching and day 9 in large broods, but not between day 9 and 14. In addition fleas tended to reduce the mass gain of females between day 9 and day 14, whereas males tended to be less affected. In contrast, male but not female tarsi were shorter in infested nests. Thus, males and females were both impaired by fleas but the magnitude of the effect and the traits affected were different between the sexes. Overall it suggests that producing a defence against parasites is costly (Gonzalez *et al*. 1999) and that the increased energy requirements under parasite exposure cannot be fully compensated by the parents and may therefore lead to different allocation strategies of limited resources between male and female nestlings. Moreover, in agreement with Tschirren *et al*. (2003), our results suggests that males were more impaired by fleas than females as there was only a tendency for parasitized females to be lighter than nonparasitized females, whereas parasitized males had significantly shorter tarsi than nonparasitized males.

In summary our results show that carotenoid availability during egg-laying influences hatchlings in a sex-specific way, probably through differential allocation to male and female eggs, but also possibly through sex-specific sensitivity to egg composition. When carotenoid availability was increased, male hatchlings were heavier than their sisters, and this may increase the competitiveness and/or stimulate the maturation of the immune system (Møller *et al*. 2000; McGraw & Ardia 2005; McGraw *et al*. 2005). Inversely, when carotenoid availability was not increased, females were apparently

'favoured'. This may decrease the disparity in competitiveness between male and female nestlings and ultimately increase female nestling quality. As a consequence, under poor environmental conditions (e.g. environments with limited carotenoid availability) female nestlings may enjoy a higher reproductive success than male nestlings of mediocre quality. Carotenoid-fed females deposited higher quantities of carotenoids into their eggs, and nestlings hatched from eggs laid by carotenoid-fed females gained more mass between day 9 and 14. It suggests that carotenoid supplementation influenced egg investment and that maternally transmitted substances such as carotenoids influenced body condition several days after hatching, which may have long lasting effects on offspring performance. Further experiments and long-term monitoring of individuals into adulthood are required to investigate the effects of the maternally transmitted carotenoids on adult body condition, competitiveness, immunity, sexual attractiveness and other fitness-correlated traits.

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