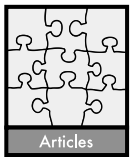

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Carotenoid diet and nestling provisioning in urban and rural great tits *Parus major*

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Considering the importance of dietary constraints for the widely held view of carotenoid pigmentation as an honest quality indicator, there is surprisingly little data on carotenoid availability in different natural diets or along environmental gradients. Here we investigate the carotenoid availability in the main diet of breeding great tits *Parus major*, living in urban and rural environments with known differences in carotenoid pigmentation. Carotenoid availability for nestling great tits was investigated in two respects: (1) quantity and quality of diet (i.e., caterpillar abundance and their carotenoid concentration), and (2) parental feeding frequency. First, caterpillars were generally more abundant in the urban environment and the four common *Lepidoptera* (i.e., caterpillars) genera studied were also heavier here. Second, as determined by HPLC analysis of the caterpillar genera, carotenoid concentration was significantly lower in the urban caterpillars. Furthermore, all except one of the caterpillar genera had higher lutein/zeaxanthin ratio in urban areas, which is in accordance with earlier studies of carotenoid composition in great tit yolk. Third, parental feeding frequency was about twice as high to urban broods compared to rural broods. This result may simply reflect the higher caterpillar abundance (shorter search time) in the urban environment. Poor food quality (low carotenoid concentration) seems therefore to be compensated by quantity in the urban environment. As a consequence the carotenoid availability seems to be similar for nestlings in the two environments.

Carotenoids are important as provitamins, antioxidants and pigments, synthesized *de novo* by plants, some fungi and microorganisms, while animals must obtain them through the diet (Goodwin 1984, Latscha 1990). In birds, carotenoids play many important roles by acting as colour pigments in feathers, free radical scavengers and immuno-stimulants (e.g., Lozano 1994, von Schantz et al. 1999). As free radical scavengers, carotenoids are assumed to protect lipids, proteins and DNA from oxidative damage (Bianchini et al. 2000), which can be induced internally (e.g., free radical production through elevated ATP synthesis during growth or infection) or directly by external factors (e.g., anthropogenic pollution). Due to trade-offs with these immunological functions, variation in plumage pigmentation may provide'honest' information (to conspecifics) about individual health and condition (e.g., Lozano 1994, Johnsen et al. 2003, Saks et al. 2003).

Considering that limited dietary access is the foundation (Endler 1983, Hill 1990) of the widely assumed quality advertisement of carotenoid colour signals (see e.g., Hill 1991, Olson and Owens 1998), the lack of quantitative data on carotenoid availability in the wild is surprising. Even among birds, where carotenoid signalling is particularly well documented (Hill 2006), there is only a handful species for which the natural dietary sources of carotenoids have been analysed. Moreover, these are mainly marine organisms (rich in 'red' keto-carotenoids such as canthaxanthin and astaxanthin) and some terrestrial plant parts (seeds, fruit, rich in'yellow' lutein and zeaxanthin), whereas the enormous variety of prey of insectivorous songbirds is virtually unstudied in this respect (McGraw 2006, but see Olson 2006). One pioneering study is the analysis by Partali et al. (1987) of the caterpillar prey that great tits *Parus major* and other *Parus* species (both with and without carotenoid-pigmented plumage) rely on during

the breeding season. The great tit nestling plumage pigments (lutein and zeaxanthin) were here traced back to the caterpillars and ultimately the leaves on which these feed, with no metabolic modification or strong selectivity in either step. Contrary to the account of this study by McGraw (2006), however, the caterpillar carotenoid concentration were not compared between habitats; instead, the paler nestling plumage pigmentation in a more coniferous habitat was attributed to lower abundance of caterpillars in this environment (see also Slagsvold and Lifjeld 1985). A more recent study by Eeva et al. (1998) showed a correlation between great tit nestling coloration and an index of caterpillar abundance, in this case along a pollution gradient which was not confounded by any obvious habitat variation. Apart from these and a few other studies (see McGraw 2006), the dietary sources of avian carotenoid physiology and pigmentation have not been described, let alone quantified and related to habitat variation.

Caterpillars are rich in carotenoids, basically because they are feeding on the primary carotenoid producers (Partali et al. 1987). Carotenoid access is therefore unlikely to be limiting for caterpillars and may be expected to show no or very slight variation in concentration within or among taxa or habitats. However, in plants it has been shown that carotenoids decrease as a response to environmentally induced oxidative stress (Ekmekci and Terzioglu 2005). An intriguing possibility in our study is therefore that urban foliage and thereby also the caterpillars, contain less carotenoids than the corresponding tree and caterpillars in the rural environment.

In great tits, the importance of caterpillars for successful breeding is well-known (van Balen 1973, Perrins 1991, Naef-Daenzer and Keller 1999, Rytkönen and Krams 2003, but see also Barba and Gil-Delgado 1990). Hatching dates for great tits are highly synchronized with the peak for caterpillars, and disturbance of this synchronization as a result of, for example, global warming leads to reduced breeding success (Visser et al. 1998, 2006). Consequently, not only breeding will be affected by variation in caterpillar accessibility, but also several carotenoid dependent traits, such as pigmentation (Partali et al. 1987).

In great tits, we have previously found that birds in an urban environment (Göteborg city parks) have less chromatic yellow breast plumage compared to rural birds (Isaksson et al. 2005), similar to the results of Hõrak et al. (2000) and Eeva et al. (1998). These studies have all implied a conflict between (irreversible) carotenoid deposition in plumage and elevated defenses against external stressors (e.g., oxidative air pollution from urban traffic or a metal-polluting industry, respectively). In Isaksson et al. (2005) the urban great tits indeed also showed signs of increased antioxidant activity, measured as the ratio of oxidized to reduced glutathione (a cellular antioxidant system). To increase protection from oxidative stress, antioxidant systems can be up-graded either through increased production of internal antioxidants or through increased intake of dietary antioxidants, like carotenoids.

The aims of the present study was to investigate carotenoid availability in spring, and its effect on great tit nestlings in urban versus rural environments, with documented differences in carotenoid plumage coloration and antioxidant use. In spring the parent great tits are dependent on the carotenoid-rich caterpillar supply for successfully raising their brood. We explore carotenoid availability in two ways, (1) diet quantity and quality (i.e., caterpillar abundance, carotenoid concentration, content and composition), and (2) feeding behaviour of parent great tits. We use high performance liquid chromatography (HPLC) to analyse the carotenoid content in four genera of *Lepidoptera* caterpillars (*Oporinia, Operophthera, Amphiphyra* and *Erannis*), at least two of which are known to be included in great tit diet (Kluvijer 1950, Grieco 2002). Given any differences in caterpillar quantity or quality (e.g. carotenoid concentration and/or composition) between the urban and rural environment, we might expect either (1) compensatory feeding effort in the poorer (low-availability) habitat, (2) fewer or lower quality nestlings, or (3) a combination of (1) and (2).

Methods

Study species and study area

The great tit *Parus major*, is one of the most common passerines in the Swedish countryside as well as in urban parks and gardens, mainly due to its willingness to breed in nest boxes, and to utilize food supplemented by humans during winter. From an omnivorous diet during winter, great tits switch to an insect-based diet, such as different *Lepidoptera* caterpillars when these become available in spring and summer. Green caterpillars mainly from the genera *Panolis*, *Operophthera*, and dark caterpillars from *Erannis* have been recorded in the great tit diet (Kluvijer 1950, Grieco 2002).

During chick feeding, caterpillars are of great importance for successful breeding (van Balen 1973), with parents usually bringing a single 45–50 mg caterpillar per visit (Kluvijer 1950). Both great tits and blue tits *Parus caeruleus* are usually single-prey loaders and show a linear relationship between feeding frequency and food delivered (Naef-Daenzer et al. 2000, Biard et al. 2005). Great tit feeding activity is more frequent in the morning than in the afternoon (Kluvijer 1950).

The urban areas in the current study (Slottsskogen and Änggårdsbergen) are within the city limit of

Göteborg in Sweden (population size ca 600,000). The rural areas (Råön, Gräppås and Högås) are located approximately 40–50 km south of Göteborg. The air pollution levels (2003, urban vs. rural e.g., NO_2; 42.2 μg/m^3 vs. 5.6 μg/m^3, SO_2; 4.1 μg/m^3 vs. 1.0 μg/m^3) in Göteborg have been shown to be high enough to have negative health impacts on humans (e.g. Kindbom et al. 2001, Forsberg et al. 2003) and also on birds (Isaksson et al. 2005). The forests in the two study environments are composed of similar tree species, mainly oak (*Quercus* spp.), pine (*Pinus* spp.) and birch (*Betula* spp.).

Caterpillar collection and carotenoid extraction

To get an approximate estimate of caterpillar abundance, we collected and counted all caterpillars during one hour long active searches in each environment (Murakami et al. 2005). The collections were made in all study areas (see above) the same day on two occasions (the 18th and 31st of May (2005), rural: n = 6 (3 areas × 2), urban: n = 4 (2 areas × 2)). All caterpillars were immediately placed in separate air-tight tubes in the field, then weighed and stored separately in 1 ml ethanol at −20° C until further analysis. The most collected *Lepidoptera* caterpillars in both areas were identified to one of four genera: *Oporinia, Operophthera, Amphipyra* and *Erannis* (Nordström et al. 1941, Imby 1989).

The carotenoid concentration was analysed in 20/18 (rural/urban) *Oporinia* spp., 13/14 *Operophthera* spp., 5/8 *Amphipyra* spp., and 6/6 *Erannis* spp., with the differences in sample sizes reflecting differences in relative abundance. Each caterpillar was defrosted and homogenized with an electric homogenizer in the ethanol in which it was stored. The sample was then centrifuged and the liquid transferred to a new tube. To protect the carotenoids during saponification we added 100 μl ascorbic acid (10%). Saponification was made by adding 150 μl 5.4 M KOH to the sample, followed by incubation 30 min at 70° C on a Eppendorf thermomixer (300 rpm). After addition of 100 μl of dH_20, 200 μl n-hexane and 200 μl diethylether, samples were vortexed (90 s), centrifuged (5 min at 1,000 rpm) and the carotenoid-rich hexane phase transferred to a new tube. Another 200 μl hexane was added and the procedure was repeated. The hexane was evaporated (approximately 30 min) under nitrogen in a Speed Vac (ThermoSavant, France). The remaining carotenoids were dissolved in 90 μl of the mobile phase (70:30, acetonitrile/methanol) and 10 μl THF. All chemicals (ethanol, ascorbic acid, KOH, n-hexane, diethylether, acetonitrile, and acetone) were obtained from VWR International AB (Stockholm, Sweden).

HPLC analysis

Immediately following extraction 10–40 μl of each sample was injected into a reverse-phase C-18 HPLC column (ODS-AL, 150 × 4.0 mm i.d., YMC Europe GmbH, Schermbeck, Germany), fitted on a Thermo-Finnigan (San Jose, USA) HPLC system with PS400 ternary pump, AS3000 autosampler and UV6000 UV/VIS diode-array detector. Column temperature was maintained at 30° C and the flow rate at 0.6 ml/min, 2D at 450 nm and 470 nm were obtained and analysed with ChromQuest 4.0 software, and peaks were identified and quantified by comparison to simultaneously analysed standards of lutein (β,ε-carotene-3,3′-diol), zeaxanthin (β, β-carotene-3,3′-diol), and beta-carotene (β, β-carotene) from Roche vitamins (Basel, Switzerland) and by visual inspection of the spectral absorptance curve (Britton et al. 1995).

Breeding and feeding frequency

The study was conducted during late spring (May–June) 2005. Nests were checked every second day to determine incubation date and hatching date. The feeding frequency was recorded when nestlings were 3–4 d (n = 40) old and 8 d old (n = 39). Each nest was observed for 30 min, during the first h of daylight (5.00 h–8.30 h, local time) simultaneously in both urban and rural areas, visited in random order by the same two observers. The observers were hidden at least 30 m from the nest to minimize disturbance. If birds seemed disturbed (as determined by warning calls) the trial was terminated and repeated from a new location later the same morning (urban = 4, rural = 5). Nestling body mass was measured three times (on day 3–4, 8 and 13–14), using a Pesola spring balance (±0.1 g) and they were all individually marked by claw clips. At day 13–14 we also measured tarsus length, using a sliding calliper (±0.01 mm).

Data analyses

All carotenoid concentrations were calculated as microgram per gram caterpillar (μg/g). Identified stereo isomers (9-cis-lutein, 9-cis and 13-cis-zeaxanthin) were pooled with the respective parent carotenoid, and referred to as total lutein and total zeaxanthin, respectively. The measure total carotenoid concentration also included beta-carotene, violaxanthin, neoaxanthin, and two unidentified yellow carotenoids, (Table 2), while peak areas less than 1% were excluded from the analysis.

We used two-factor ANOVA:s with interaction for the caterpillar analyses. In these models environment (urban vs. rural) and species were fixed factors, with

Table 1. Two-factor ANOVAs with (a) caterpillar mass, (b) total carotenoid concentration, and (c) lut/zx ratio as dependent variables.

	(a) Caterpillar mass			(b) Tot. carotenoid conc.			(c) Lut/zx ratio		
	df	F	P	df	F	P	df	F	P
Environment (urban/rural)	1	4.18	0.044	1	7.76	0.007	1	12.25	<0.001
Genus	3	4.69	0.005	3	5.91	0.001	3	13.18	<0.001
Genus × Environment	3	2.73	0.049	3	2.51	0.064	3	2.78	0.047

caterpillar mass, carotenoid concentration, total lutein/total zeaxanthin (lut/zx) ratio, and carotenoid content per caterpillar as dependent variables (see Table 1). Six individual caterpillars from different genera had no detectable zeaxanthin (urban n = 4; rural n = 2), and were therefore excluded from the model with lut/zx as the dependent variable.

For analysis with nestlings we used mixed models with environment as factor and rearbox nested within environment as a random factor. We also used means per brood to be able to investigate covariates. Regarding analysis with feeding frequency, we used the mean between the feeding rate at day 4 and day 8. Interactions with P-values exceeding 0.25 were excluded, using backward elimination and the final models are presented in the Results (see Quinn and Keough 2002). Two clutches were removed from two analyses, one because it was a confirmed outlier (more than twice the standard deviation from the mean) for mean offspring body mass on day 13, and one because the third mass measurement (day 13–14) was not obtained. One chick in five rural and in three urban broods, two in one rural brood, and one whole brood in each environment died before fledging. Body condition was calculated as log mass/(3 × log tars) (Andersson 1994). All factors were considered fixed and all data were tested for model assumptions (deviation from normality (Kolmogorov-Smirnov) and homogeneity of variances (F-test)). Total carotenoid concentration and lut/zx ratio were both log-transformed to achieve normality. The significance level was set to $P < 0.05$ and all values are presented as means ± SE. Statistical analyses were performed in JMP 5.1 (SAS Institute 2003, North Carolina).

Results

Caterpillar abundance and body mass

We found significantly more caterpillars in urban compared to rural environment (rural: 20.33 ± 16.44 caterpillars/h (n = 6); urban: 94 ± 20.14 caterpillars/h (n = 4), t-tests; environment, t = 2.83, P = 0.022). There was a significant difference in caterpillar mass between environments (rural: 44.02 ± 6.32 mg, LSM 3.42 ± 0.12; urban: 48.17 ± 6.62 mg, LSM 3.75 ± 0.11), and among caterpillar genera (Table 1a).

Caterpillar carotenoid concentration, content and composition

The most common carotenoids in all four *Lepidoptera* genera was lutein, followed by zeaxanthin (Table 2). Three other carotenoids (β-carotene, violaxanthin and neoaxanthin) were also identified, together with two unidentified yellow pigments. Except for unusually high levels of β-carotene in rural *Erannis* spp. (ca 6 μg/g, almost as much as zeaxanthin), these three carotenoids were present in similar, small amounts in all caterpillar genera (Table 2 and Fig. 1c). Interestingly, however, rural caterpillars had significantly higher total carotenoid concentration (rural: 32.27 ± 1.87 μg/g (n = 44); urban: 26.89 ± 1.83 μg/g (n = 46), Table 1 and Fig. 1a), and in addition relatively more zeaxanthin as shown by the lower ratio of lutein to zeaxanthin (rural: 7.05 ± 0.72 (n = 42); urban: 15.21 ± 2.04 (n = 42), Table 1 and Fig. 1b). Caterpillar genera also had a significant effect on both carotenoid concentration

Table 2. Carotenoid composition in four genera of *Lepidoptera* caterpillars. Mean concentration (μg/g caterpillar) ± SE.

	Erannis (brown)		*Operophthera* (green)		*Oporina* (green)		*Amphipyra* (green)	
	Urban (n = 6)	Rural (n = 6)	Urban (n = 14)	Rural (n = 13)	Urban (n = 18)	Rural (n = 20)	Urban (n = 8)	Rural (n = 5)
Lutein	17.42 ± 1.11	22.44 ± 4.13	23.99 ± 3.64	26.02 ± 1.02	23.32 ± 2.41	19.27 ± 0.76	16.06 ± 2.3	21.81 ± 3.01
Zeaxanthin	3.96 ± 1.26	7.18 ± 1.64	2.01 ± 0.56	7.99 ± 1.01	1.74 ± 0.33	2.57 ± 0.26	1.22 ± 0.21	1.11 ± 0.54
Violaxanthin	1.26 ± 0.25	1.53 ± 0.49	1.88 ± 2.28	2.11 ± 0.26	0.67 ± 0.13	0.50 ± 0.13	0.33 ± 0.11	0.73 ± 0.37
Neoaxanthin	0.73 ± 0.17	0.74 ± 0.24	1.29 ± 0.38	1.33 ± 0.17	0.43 ± 0.11	0.44 ± 0.12	0.28 ± 0.17	0.37 ± 0.23
Betacarotene	1.72 ± 0.68	5.81 ± 2.49	1.26 ± 0.35	1.90 ± 0.15	0.56 ± 0.18	0.57 ± 0.10	0.12 ± 0.09	0.33 ± 0.22
Unknown	0.72 ± 0.12	0.92 ± 0.18	0.80 ± 0.20	1.62 ± 0.18	0.75 ± 0.16	0.92 ± 0.11	0.34 ± 0.07	0.36 ± 0.19

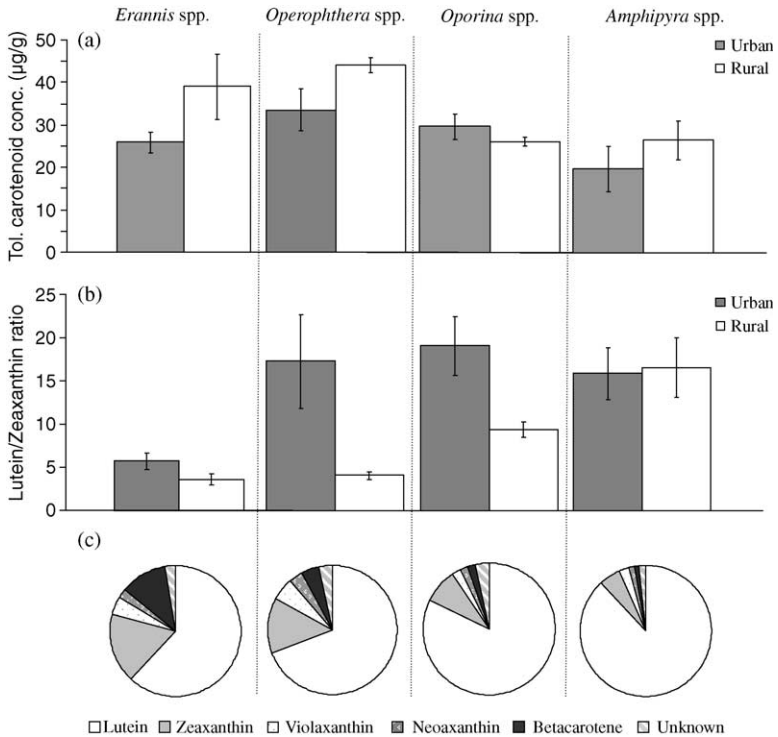

Fig. 1. (a) Total carotenoid concentration (µg/g caterpillar), (b) Lutein/Zeaxanthin ratio, and (c) carotenoid composition in the four caterpillar genera (*Erannis* spp., *Operophthera* spp., *Oporina* spp., and *Amphipyra* spp.).

and lutein/zeaxanthin ratio (Table 1). Because of the higher mass of urban caterpillars (see above), total carotenoid content per caterpillar (independent of genus) were similar between the two environments (rural: 1.18 ± 0.14 mg/caterpillar ($n = 44$) urban: 1.16 ± 0.12 mg/caterpillar ($n = 46$), Two-way ANOVA: environment; $F_{1,85} = 0.06$, $p = 0.802$, genus; $F_{3,85} = 0.56$, $P = 0.637$, genus × environment; $F_{3,82} = 1.26$, $P = 0.295$).

Feeding frequency and nestling condition

There was no significant difference in clutch size (one-way ANOVA: environment: $F_{1,37} = 2.88$, $P = 0.098$, see also Table 3) or hatching date (one-way ANOVA: environment: $F_{1,35} = 2.82$, $P = 0.102$) between urban and rural populations. Urban great tits had twice as high feeding frequency to hatchlings as did rural birds (mean feeding frequency: rural 7.18 ± 1.17 visits; urban 15.8 ± 1.89 visits; ANCOVA: environment, $F_{1,22} = 22.51$, $P < 0.001$; brood size, $F_{1,22} = 0.002$, $P = 0.96$; environment × brood size, $F_{1,22} = 2.81$, $P = 0.11$; hatching date, $F_{13,22} = 2.55$, $P = 0.026$). The feeding frequency per chick in the two environments was on average; 1.48 ± 0.25 vs. 2.60 ± 0.25 (rural vs. urban) visits per chick every half an hour (ANOVA: environment, $F_{1,36} = 5.74$, $P = 0.022$).

This was consistent over time since feeding frequency soon after hatching (day 3–4) was significantly correlated with feeding frequency on day 8 ($r = 0.544$, $n = 36$, $P < 0.001$). Mean offspring mass (per brood) on day 4 was likewise correlated with mean mass on day 8 ($r = 0.073$, $n = 36$, $P < 0.001$) and day 13 ($r = 0.386$, $n = 35$, $P < 0.001$). Despite the striking difference in feeding effort, there was no (urban-rural) difference in body mass when nestlings were 13–14 days old (environment: $F_{1,35} = 0.05$, $P = 0.830$, rearbox (nested within environment): $F_{35,172} = 6.49$, $P < 0.001$) or condition (environment:

Table 3. Mean values of condition, body mass, tarsus, clutch size and fledging success for all nestlings growing up in urban vs. rural environment. Sample sizes are shown in parenthesis.

	Urban	±SD (n)	Rural	±SD (n)
Condition[1]	0.30	0.01(107)	0.30	0.01 (125)
Body mass[1]	17.23	1.05 (107)	17.03	1.48 (125)
Tarsus[1]	22.79	0.72 (107)	22.48	0.73 (126)
Clutch size	7.75	1.74 (20)	6.95	1.13 (19)
Fledging success[2]	91%	0.23 (19)	92%	0.13 (19)

[1]Only 13 day old nestlings are included.
[2]Broodsize at last visit/ broodsize at first visit.

$F_{1,35} = 0.11$, $P = 0.739$; rearbox (nested within environment): $F_{35,172} = 8.11$, $P < 0.001$). To investigate how brood size and nestling age influenced body mass and condition we also used mean brood values, respectively (ANCOVA body mass: environment, $F_{1,31} = 1.46$, $P = 0.236$; nestling age, $F_{1,31} = 1.19$, $P = 0.283$; brood size, $F_{1,31} = 0.52$, $P = 0.475$; condition: environment, $F_{1,31} = 0.03$, $P = 0.868$; nestling age, $F_{1,31} = 0.02$, $P = 0.876$; brood size, $F_{1,31} = 2.73$, $P = 0.11$ see Table 3). However, urban great tits produced on average one more fledgling than did rural pairs (mean brood size; rural 5.32 ± 0.36 fledglings, urban 6.37 ± 0.36 fledglings, one-way ANOVA; $F_{1,36} = 4.379$, $P = 0.044$).

Based on the difference in total carotenoid concentration, caterpillar mass and parents feeding frequency we calculated amount carotenoids each chick received during half an hour ((carotenoid concentration × caterpillar mass × feeding rate)/brood size). Interestingly, a combination of the higher feeding frequency and the heavier caterpillars compensated for the lower carotenoid concentration in the urban habitats (carotenoid availability per chick: 0.54 ± 0.13 vs. 0.63 ± 0.10 (rural vs. urban) μg carotenoids, ANOVA: environment, $F_{1,36} = 0.32$, $P = 0.576$).

Discussion

To our knowledge, the present study is the first to investigate taxonomic and environmental variation in carotenoid content of natural animal prey of a songbird. The main findings were: (1) urban caterpillars was more abundant, heavier, and had lower concentrations of carotenoids with relatively less zeaxanthin compared to rural caterpillars, and (2) urban parents fed their nestlings up to twice as frequent as did rural parents. Consequently, carotenoid availability for nestlings seems to be similar between the two environments, provided that uptake efficiency is similar.

A synchronisation between caterpillar peak and chick-feeding is important to successfully raise a brood (e.g., van Balen 1973). In previous studies, caterpillar abundance has been shown to peak earlier due to increased temperatures in late spring temperatures (Visser et al. 1998, 2006). This results in a mistiming with reproduction, which is initiated by temperatures in early spring. In cities such as Göteborg, human activities and urbanization increase the overall temperatures, a phenomenon called urban heat island effects (UHI) (see e.g., Eliasson and Holmer 1990, Haeger-Eugensson and Holmer 1999). Thus, we expected urban broods to breed earlier, but hatching dates were similar between urban and rural environments. Dates for caterpillar peak were not recorded. Thus, since the caterpillars in the urban areas were both heavier and of higher abundance at the time of collection, there may be a slight difference in caterpillar peak in favour for the urban breeding birds (see below). The abundance was approximately four times higher in urban environments compared to rural environments. This result may, however, be somewhat uncertain due to limitations in the data collection (i.e., only on two occasions), and would benefit from a more comprehensive data collection. Having said that, we are confident that there is an abundance difference, which most likely is due to a so far unexplored finer-scale habitat differences.

The substantial difference in carotenoid concentration between urban and rural caterpillars is a unique and interesting result. The median total carotenoid concentration in the present study was 29 μg/g, which is tenfold the caterpillar concentration (3.3 μg/g) in Partali et al. (1987). Given the variation between caterpillar taxa in this study, it is possible that other, less carotenoid-rich taxa and/or leaf diets were included in the pooled 15 g sample in Partali et al. (1987). More likely, however, is that the caterpillars were kept alive for longer and thereby had less half-digested leaves in their gut at the time of extraction.

Interestingly, rural caterpillars also had relatively more zeaxanthin than urban caterpillars in all except one genus (*Amphiphyra*). This is concordant with our previous studies showing that the lut/zx ratios in great tit egg yolk were lower in the rural environment, although there were no differences in the total carotenoid concentration (Isaksson et al. in press). The difference in carotenoid composition between environments may therefore be a direct (passive) reflection of the dietary content (caterpillar → great tit). The variation may nevertheless have fitness consequences since zeaxanthin appear to be a more effective free radical scavenger (Mortensen and Skibsted 1997, Sujak et al. 1999). A potential explanation for this pattern, and also for the lower carotenoid concentration, is a direct reflection of the carotenoid content of caterpillar host plants. Higher plants are rich in lutein, zeaxanthin, violaxanthin, neoaxanthin, betacarotene and antheraxanthin (Young 1993), of which we found all except antheraxanthin in the caterpillars. As a response to high oxidative stress, for example by pesticides, plant chlorophylls and total carotenoid content have been shown to decrease (Ekmekci and Terzioglu 2005). Moreover, zeaxanthin are included in the xanthophyll cycle, whereas lutein is mainly acting as a light harvesting molecule in the green foliage (for review see; Demming-Adams et al. 1996). We therefore speculate that leaves may have reduced carotenoid content and maybe also a different relationship between lutein and zeaxanthin due to oxidative air pollution and that this is reflected by the

caterpillars feeding on them. We are currently investigating this possibility.

Feeding frequency was higher in the urban habitat, which is probably an effect of the greater caterpillar abundance (i.e., less search time, see for example; Cuthill and Houston 1997, Naef-Daenzer et al. 2000), which resulted in one more fledgling per brood compared to rural families. Contrary to our expectation at the onset of this study, it thus appears that the urban environment has minimal or even positive effects on reproductive effort and reproductive success in great tits. On the other hand, there is a possibility that there are negative effects on other fitness parameters not measured here. For example, for future studies it would be useful to investigate population dynamics (e.g., age structure, mortality, emigration) and life-history strategies in relation to environmental stress (e.g., Visser and Lessells 2001, McNamara et al. 2004).

Moreover, not only quantity is important for nestling survival also quality of the diet (Goss-Custard 1977, Krebs and Avery 1985, Ramsay and Houston 2003, Brommer 2004). Since great tits are single prey loaders and the urban caterpillars had a lower carotenoid concentration, urban nestlings will presumably get a lower carotenoid intake unless parents increase the feeding frequency or that the caterpillars are heavier (i.e., similar content per caterpillar). This was indeed precisely what we found and, consequently the carotenoid availability (i.e., carotenoid concentration and prey abundance) for great tit nestlings seems to be similar in the urban and rural environments.

Previously, we have shown that urban nestlings have a paler (i.e., less carotenoids incorporated in plumage) yellow breast (Isaksson et al. 2005). There are at least two possible explanations: (1) differences in carotenoid availability, and (2) differences in carotenoid usage. In both Fitze et al. (2003) and Isaksson et al. (2006), variation in nestling plumage coloration was strongly environmentally dependent but unrelated to body mass and condition, which suggests that food quality (i.e. carotenoid concentration) rather than quantity is the more important limitation on carotenoid pigmentation. Therefore, even though we found similar carotenoid availability as such between the environments, the present data support that quality (i.e., carotenoid concentration and types) may be a reason for the reduced pigmentation (see Isaksson et al. 2005, 2006), possibly through a nonlinear efficiency in carotenoid uptake in relation to intake. This result may have implications for foraging efficiency theory regarding colour communication (e.g., Endler 1980, Hill 1990, 1991), which suggests a linear relationship between food (i.e., carotenoid) intake and plumage pigmentation. Differences in carotenoid uptake and usage, however, need to be further investigated to evaluate the role of environmental constraints on carotenoid availability.

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References

Andersson, S. 1994. Costs of sexual advertising in the leeking Jackson's widowbird *Euplectes jacksoni*. – Auk 96: 1–10.

Barba, E. and Gil-Delgado, J. A. 1990. Seasonal variation in nestling diet of the great tit *Parus major* in orange groves in eastern Spain. – Ornis Scand. 21: 296–298.

Bianchini, F., Elmstahl, S., Martinez-Garcia, C., van Kappel, A. L., Douki, T., Cadet, J., Ohshima, H., Riboli, E. and Kaaks, R. 2000. Oxidative DNA damage in human lymphocytes: correlations with plasma levels of alpha-tocopherol and carotenoids. – Carcinogenesis 21: 321–324.

Biard, C., Surai, P. F. and Møller, A. P. 2005. Effects of carotenoid availability during laying on reproduction in the blue tit. – Oecologia 144: 32–44.

Britton, G., Liaaen-Jensen, S. and Pfander, H. 1995. Carotenoids: handbook. – Birkhäuser Verlag, Berlin.

Brommer, J. E. 2004. Immunocompetence and its costs during development: an experimental study in blue tit nestlings. – Proc. R. Soc. B 271: 110–113.

Cuthill, I. C. and Houston, A. I. 1997. Managing time and energy. – In: Krebs, J. R. and Davies, N. B. (eds). Behavioural ecology: an evolutionary approach. Blackwell, pp. 97–120.

Demming-Adams, B., Gilmore, A. M. and Adams III, W. W. 1996. In vivo functions of carotenoids in higher plants. – FASEB 10: 403–412.

Eeva, T., Lehikoinen, E. and Ronka, M. 1998. Air pollution fades the plumage of the great tit. – Funct. Ecol. 12: 607–612.

Ekmekci, Y. and Terzioglu, S. 2005. Effects of oxidative stress induced by paraquat on wild and cultivated wheats. – Pest. Biochem. Physi. 83: 69–81.

Eliasson, I. and Holmer, B. 1990. Urban heat island circulation in Göteborg, Sweden. – Theor. Appl. Climatol. 42: 187–196.

Endler, J. A. 1980. Natural and sexual selection on colour patterns in *Poecilia reticulata*. – Evolution 34: 76–91.

Endler, J. A. 1983. Natural and sexual selection on color patterns in poeciliid fishes. – Envir. Biol. Fishes. 9: 173–190.

Fitze, P. S., Kölliker, M. and Richner, H. 2003. Effects of common origin and common environment on nestling plumage coloration in the great tit (*Parus major*). – Evolution 57: 144–150.

Forsberg, B., Modig, L., Svanberg, P.-A. and Segerstedt, B. 2003. Hälsokonsekvenser av ozon- en kvantifiering av det marknära ozonets korttidseffekter på antalet sjukhusinläggningar och dödsfall i Sverige. – Rapport på uppdrag av Statens folkhälsoinstitut, Sweden. [in Swedish]

Goodwin, T. W. 1984. The biochemistry of the carotenoids. Vol II. Animals. – Chapman and Hall, London.

Goss-Custard, J. D. 1977. The energetics of prey selection by redshank, *Tringa totanus* (L.) in relation to prey density. – J. Anim. Ecol. 46: 1–19.

Grieco, F. 2002. How different provisioning strategies result in equal rates of food delivery: an experimental study of blue tits *Parus caeruleus*. – J. Avian Biol. 33: 331–341.

Haeger-Eugensson, M. and Holmer, B. 1999. Advection caused by the urban heat island circulation as a regulating factor on the nocturnal urban heat island. – Inter. J. Climatol. 19: 975–988.

Hill, G. E. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. – Anim. Behav. 40: 563–572.

Hill, G. E. 1991. Plumage coloration is a sexually selected indicator of male quality. – Nature 350: 337–339.

Hill, G. E. 2006. Environmental regulation of ornamental coloration. – In: Hill, G. E. and McGraw, K. (eds). Bird coloration: function and evolution. Vol II. Harvard University Press, Cambridge, pp. 507–560.

Hõrak, P., Vellau, H., Ots, I. and Møller, A. P. 2000. Growth conditions affect carotenoid-based plumage coloration of great tit nestlings. – Naturwiss. 87: 460–464.

Imby, L. 1989. Fjärilar i Sverige. – Rabén & Sjögren, Stockholm. [in Swedish]

Isaksson, C., Örnborg, J., Stephensen, E. and Andersson, S. 2005. Plasma glutathione and carotenoid coloration as potential biomarkers of environmental stress in great tits. – EcoHealth 2: 138–146.

Isaksson, C., Uller, T. and Andersson, S. 2006. Parental effects on carotenoid-based plumage coloration in nestling great tits, *Parus major*. – Behav. Ecol. Sociobiol. 60: 556–562.

Isaksson, C., Johansson, A. and Andersson, S. 2008. Egg yolk carotenoids in relation to habitat and reproductive investment in the great tit, *Parus major*. – Physiol. Biochem. Zool. in press.

Johnsen, A., Delhey, K., Andersson, S. and Kempenaers, B. 2003. Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects. – Proc. R. Soc. B 270: 1263–1270.

Kindbom, K., Svensson, A., Sjöberg, K. and Phil Karlsson, G. 2001. Trends in air concentration and deposition at background monitoring sites in Sweden-major inorganic compounds, heavy metals and ozone. – IVL report B 1429.

Kluvijer, H. N. 1950. Daily routines of the great tit, *Parus m. major* L. – Ardea 38: 99–135.

Krebs, J. R. and Avery, M. I. 1985. Central place foraging in the European bee-eater, *Merops apiaster*. – J. Anim. Ecol. 54: 459–472.

Latscha, T. 1990. Carotenoids- their nature and significance in animal feeds. – F-Hoffmann-LaRoche Ltd., Basel, Switzerland.

Lozano, G. A. 1994. Carotenoids, parasites, and sexual selection. – Oikos 70: 309–311.

McGraw, K. 2006. Mechanics of carotenoid-based coloration. – In: Hill, G. E. and McGraw, K. (eds). Bird coloration: function and evolution. Vol II. Harvard University Press, Cambridge, pp. 177–242.

McNamara, J. M., Welham, R. K., Houston, A. I., Daan, S. and Tinbergen, J. M. 2004. The effects of background mortality on optimal reproduction in a seasonal environment. – Theor. Pop. Biol. 65: 361–372.

Mortensen, A. and Skibsted, L. H. 1997. Importance of carotenoid structure in radical-scavenging reactions. – J. Agric. Food Chem. 45: 2970–2977.

Murakami, M., Yoshida, K., Hara, H. and Toda, M. J. 2005. Spatio-temporal variation in Lepidopteran larval assemblages associated with oak, *Quercus crispula*: the importance of leaf quality. – Ecol. Entom. 30: 521–531.

Naef-Daenzer, B. and Keller, L. 1999. Foraging performance of great and blue tits (*Parus major*, *P. caeruleus*) in relation to caterpillar development and its consequences for nestling growth and fledging weight. – J. Anim. Ecol. 146: 708–718.

Naef-Daenzer, L., Naef-Daenzer, B. and Nager, R. G. 2000. Prey selection and foraging performance of breeding great tits *Parus major* in relation to food availability. – J. Avian Biol. 31: 206–214.

Nordström F., Wahlgren E. and Tullgren A. 1941. Svenska Fjärilar-Systematisk bearbetning av Sveriges storfjärilar Macrolepidoptera. – Nordisk Familjeboks förlag Stockholm. [in Swedish]

Olson, V. A. 2006. Estimating nutrient intake in comparative studies of animals: an example using dietary carotenoid content in birds. – Oikos. 112: 620–628.

Olson, V. A. and Owens, I. P. F. 1998. Costly sexual signals: are carotenoids rare, risky or required? – Trends Ecol. Evol. 13: 510–514.

Partali, V., Liaaenjensen, S., Slagsvold, T. and Lifjeld, J. T. 1987. Carotenoids in food-chain studies. 2. The food-chain of *Parus* spp. monitored by carotenoid analysis. – Comp. Biochem. Physiol. B 87: 885–888.

Perrins, C. M. 1991. Tits and their caterpillar food supply. – Ibis 133: 49–54.

Quinn, G. and Keough, M. 2002. Experimental design and data analysis for biologists. – Cambridge University Press.

Ramsay, S. L. and Houston, D. C. 2003. Amino acid composition of some woodlands anthropods and its implications for breeding tits and other passerines. – Ibis 145: 227–232.

Rytkönen, S. and Krams, I. 2003. Does foraging behaviour explain the poor breeding success of great tits *Parus major* in northern Europe? – J. Avi. Biol. 34: 288–297.

Saks, L., Ots, I. and Hõrak. 2003. Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. – Oecologia. 134: 301–307.

Slagsvold, T. and Lifjeld, J. T. 1985. Variation in plumage coloration of the great tit *Parus major* in relation to habitat, season, and food. – J. Zool. 206A: 321–328.

Sujak, A., Gabrielska, J., Grudzinski, W., Borc, R., Mazurek, P. and Gruszecki, W. I. 1999. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage:

the structural aspects. – Arch. Biochem. Biophys. 371: 301–307.
Young, A. J. 1993. Occurrence and distribution of carotenoids in photosynthetic systems. – In: Young, A. and Britton, G. (eds). Carotenoids in photosynthesis. Chapman and Hall, London, pp. 16–71.
van Balen, J. H. 1973. A comparative study of the breeding ecology of the great tit *Parus major* in different habitats. – Ardea 61: 1–93.
Visser, M. E. and Lessells, C. M. 2001. The costs of egg production and incubation in great tits (*Parus major*). – Proc. R. Soc. B 268: 1271–1277.
Visser, M. E., van Noordwijk, A. J., Tinbergen, J. M. and Lessells, C. M. 1998. Warmer springs lead to mistimed reproduction in great tits (*Parus major*). – Proc. R. Soc. B 265: 1867–1870.
Visser, M. E., Holleman, L. J. M. and Gienapp, P. 2006. Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. – Oecologia 147: 164–172.
von Schantz, T. S., Bensch, S., Grahn, M., Hasselquist, D. and Wittzell, H. 1999. Good genes, oxidative stress and condition-dependent sexual signals. – Proc. R. Soc. B 266: 1–12.