2-010.qxd 03.07.02 16:50 Seite 1

Fat-soluble antioxidants in the eggs of great tits *Parus major* in relation to breeding habitat and laying sequence

Peeter Hõrak[1], Peter F. Surai[2] and Anders P. Møller[3]

Incubation in many species of altricial birds starts before completion of the clutch, resulting in asynchronous hatching that may increase the probability of at least some nestlings dying during periods of food scarcity. Females may interfere with this process by enhancing or suppressing the survival probabilities of last-hatched nestlings by differential investment in egg quality with respect to laying order. We investigated the concentrations of fat-soluble antioxidants in the yolks of eggs in an urban and a rural population of great tits *Parus major*. Urban females deposited consistently smaller amounts of carotenoids and vitamin E in their eggs despite laying fewer eggs than rural females. Eggs in both populations showed a 40 % decrease in vitamin E concentration with laying sequence. Last-laid eggs of the rural great tits had 37 % lower concentration of carotenoids than first-laid eggs while no such difference emerged in the city. These results are consistent with the idea of reduced maternal investment in last-laid eggs, as predicted by models of adaptive brood reduction.
Key words: carotenoids; hatching asynchrony; maternal effects; vitamin A; vitamin E.

[1] Institute of Zoology and Hydrobiology, Tartu University, Vanemuise 46, 51014 Tartu, Estonia; [2] Avian Science Research Centre, Scottish Agricultural College, Ayr KA6 5HW, UK; [3] Laboratorie d'Ecologie Evolutive Parasitaire, CRNS UMR 7103, Université Pierre et Marie Curie, Bât. A, 7ème étage, 7 quai St. Bernard, Case 237, F–75252 Paris Cedex 05, France.
Corresponding author: Peeter Hõrak, Institute of Zoology and Hydrobiology, Tartu University, Vanemuise 46, 51014 Tartu, Estonia; e-mail: horak@ut.ee

Among many altricial bird species, nestlings within a brood do not hatch simultaneously but over a period of several days (e.g. Clark & Wilson 1981, Ricklefs 1993, Stoleson & Beissinger 1995). Hatching asynchrony is mainly determined by incubation beginning before clutch completion, resulting in first-laid eggs hatching earlier than last-laid eggs (Lack 1968, O'Connor 1984) and consequent size hierarchies among nestlings that may cause death or slow development of late-hatched, small nestlings (Hahn 1981). The existence of hatching asynchrony has been considered a paradox since it can negatively affect reproductive success, due to death or poor condition at fledging of disadvantaged offspring (Stoleson & Beissinger 1995). At least seventeen different hypotheses have been proposed to explain this phenomenon (reviews in Ricklefs 1993, Stoleson & Beissinger 1995, Stenning 1996).

Asynchronous hatching is usually considered adaptive because of the potential benefits accruing to parents by starting incubation before clutch completion, establishing a hierarchy in reproductive value among their offspring. Alternatively, asynchronous hatching (and accompanying partial brood mortality) may result from parental tactics to obtain benefits from an early start to incubation. In both cases, one might expect that it is in the interest of parents to modify the quality of their eggs with respect to laying sequence. In the case of adaptive brood reduction, parents would benefit from enhancement of the quality of first-laid eggs by supplying these with greater quantities of micro- and macro-nutrients. On the other hand, if asynchronous hatching is an undesirable by-product of parental tactics accruing from benefits of early onset of incubation, then one might predict that it is in the interest of parents to enhance the quality of late-hatching young by supplying last-laid eggs with resources that would aid the youngest hatchling to catch up in competition with older siblings. Mothers can differentiate their investment into egg content by various mechanisms, such as modifying egg size (e.g. Parsons 1975, Meathrel & Ryder 1987, Lamey 1990, Jover et al. 1993), macronutrient content (e.g. Bryant 1978, Bryant & Tatner 1990, Royle et al. 1999), concentrations of fat-soluble antioxidants (Royle et al. 1999, 2001, Blount et al. 2002), immunoglobulins (Blount et al. 2002, Saino et al. 2002), or androgens (Schwabl 1993, 1997, Gil et al. 1999, Royle et al. 2001) in relation to laying sequence.

The aim of this study is to describe variation in egg antioxidant concentration within the laying sequence in great tits *Parus major.* Nestlings hatch usually within one or two days of each other (e.g. Orell 1983, Lebedeva 1994) but may take up to five or more days (Slagsvold & Amundsen 1992). The question of whether hatching asynchrony in the great tit serves an adaptive purpose to facilitate flexible brood reduction is unclear. Hõrak (1995) showed that partial brood mortality was accompanied by a decrease in fledgling weight and recruitment rate (but with increased female survival). On the other hand, great tit parents have been shown to participate actively in brood reduction by expelling the youngest, under-nourished young from the nest (Lebedeva 1994). Furthermore, Amundsen & Slagsvold (1998) suggested that high-quality parent great tits may benefit from hatching synchrony, while low-quality parents benefit from asynchrony.

We describe patterns over the laying sequence in the concentrations in eggs of three antioxidants that are expected to play an essential role in growth and development of nestlings. Because animals cannot synthesise carotenoids and vitamin E, these have been hypothesized to be a limiting resource, such that their availability can constrain the expression of antioxidant activity and immune function (Lozano 1994, von Schantz et al. 1999). Such antioxidant limitation is especially likely to occur in the avian embryo – a closed system which during its development completely relies on the maternally deposited resources. Indeed, it has been shown repeatedly that maternal diet and subsequently the yolk composition play a crucial role in the development and efficiency of the antioxidant systems of avian embryos (reviewed in Surai & Sparks 2001). Furthermore, it has been demonstrated that dietary fat-soluble antioxidants such as carotenoids and vitamins A and E may have important consequences for the maintenance of a functional phenotype in rapidly growing nestlings (reviews in Surai 1999, Møller et al. 2000). Rapid growth is associated with the production of large amounts of free radicals that, if not neutralised, can cause permanent damage to DNA, proteins and lipids, including cell membranes, tissues and organs. Since last-hatched nestlings grow faster, beg more frequently, and show higher levels of activity than early hatched nestlings (Lebedeva 1994, Saino et al. 2001), we would expect last-laid eggs to contain more antioxidants if nestling growth requirements were the factor determining allocation patterns of antioxidants to eggs. Alternatively, if great tit mothers practice adaptive brood reduction, we would predict that first-laid eggs should contain more macro-nutrients and antioxidants than last-laid eggs. In this scenario, first-laid eggs are more likely to produce viable offspring due to hatching asynchrony, and it would be adaptive to enhance their quality by supplying them with extra resources. We test these predictions in two great tit populations, breeding under contrasting environmental conditions in high-quality rural and low-quality urban habitat. Based on our previous findings that carotenoid-based plumage coloration is paler among urban great tits (Hõrak et al. 2001), we hypothesize that dietary carotenoids appear more limiting in our urban study site. Hence, we expect the eggs of urban great tits to contain less carotenoids than these of their rural conspecifics.

To elucidate the possible relationship between egg content and hatching asynchrony, we compare the patterns of hatching asynchrony and brood reduction between these populations on the basis of the prevalence and extent of early nestling mortality and the magnitude of nestling size hierarchies. We predict that if egg antioxidant concentrations decrease in laying sequence then such a decline should be more prominent in the population with higher degree of hatching asynchrony.

Methods

Data were collected in April–May 2000 in two neighbouring (urban and rural) great tit populations breeding in nest boxes in and around Tartu (58° 22' N, 26° 43' E), south-eastern Estonia. The study areas, which are 8 km apart, were described by Hõrak & Lebreton (1998). Only data from first clutches were used. Reproductive parameters of breeding great tits were recorded by regular inspection of nestboxes. Clutches (randomly) chosen for measurement of egg variables in relation to laying sequence were visited on the day of laying of the first egg and on days of expected clutch completion. Eggs were marked individually to enable collection of first and last egg on the day when laying ceased. After collection, eggs were photographed for measurement of volumes as described by Hõrak et al. (1997) and stored at –20 °C until analyses. Data were collected under the licence from the Estonian Ministry of the Environment. Seven clutches from the rural population (clutch size = 11–12 eggs) and 8 clutches from the urban study area (clutch size = 8–10 eggs) were used for comparison of parameters of the first and last egg. Their respective differences were analysed with repeated measures ANOVA with habitat as a factor and laying sequence as a repeated measure. Values are reported as means ± s.d.. All tests are two-tailed with a 5 % significance level.

Because we were unable to measure directly the extent of hatching asynchrony and brood reduction between populations, we used indirect estimates such as the magnitude of nestling size hierarchies and the prevalence and extent of early nestling mortality. The benefits of brood reduction in terms of reducing food competition among nestlings are clearly greater if nestling mortality occurs early. We therefore assumed that adaptive brood reduction is likely to be more prevalent in populations with higher frequency and extent of early nestling mortality (occurring before the 8th day of the nestling period). Likewise, we assumed adaptive brood reduction to be associated with greater magnitude of nestling size hierarchies. To estimate the latter, we used intra-brood coefficient of variation of nestling body mass on the 8th day of the nestling period (see Orell 1993). Since nestling mortality and growth are sensitive to experimental brood size manipulations, only non-manipulated broods were used for the above-mentioned analyses.

Vitamins E (α-tocopherol) and A (retinol) were determined by High Precision Liquid Chromatography (HPLC) as previously described by Surai et al. (2001). In brief, the egg yolk (100–200 mg) was mixed with a 5 % solution of NaCl (0.7 ml) and ethanol (1 ml) was added for protein precipitation. Hexane (5 ml) was then added and the mixture was homogenized for 1 min. The hexane phase, containing vitamins A and E and carotenoids, was separated by centrifugation and collected. The extraction was repeated twice more with 5 ml hexane. Hexane extracts were combined, evaporated and redissolved in a mixture of methanol/dichloromethane (1:1, vol/vol). Samples were injected into HPLC system (Shimadzu Liquid Chromatograph, LC-10AD, Japan Spectroscopic Co. Ltd. with JASCO Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb, type S30DS2, 3μ C_{18} reverse phase HPLC

Table 1. Comparison of reproductive parameters of urban and rural great tits in 2000. Nestling mortality is the number of nestlings that died before day 8 of the nestling period. All data (except for clutch size) are for non-manipulated broods.

Variable	Urban			Rural			Statistic	P
	Mean	s.d.	n	Mean	s.d.	n		
Clutch size	8.89	1.58	74	11.43	1.00	40	10.4[1]	<0.0001
% of broods with early mortality	18		22	30		10	0.56[2]	0.45
Nestling mortality	0.18	0.39	22	0.70	1.60	10	1.04[3]	0.30
CV of nestling mass	0.08	0.06	23	0.17	1.16	9	2.81[3]	0.005
% of hatchlings fledged	81	28	21	82	18	9	0.66[3]	0.50
Fledgling mass (g)	16.25	1.69	20	16.74	1.27	10	0.80[4]	0.43

[1] t-test for separate variances; [2] χ^2-test; [3] Wilcoxon test; [4] t-test

2-010.qxd 03.07.02 16:50 Seite 4

column, 15 cm × 4.6 mm (Phase Separations Ltd., UK). Chromatography was performed using a mobile phase of methanol/water (97:3, v/v) at a flow rate of 1.05 ml/min. Fluorescence detection of retinol involved excitation and emission wavelengths of 325 and 480 nm and detection of vitamin E used excitation at 295 nm and emission at 330 nm. Standard solutions of α-tocopherol and retinol in methanol were used for instrument calibration and tocol was used as an internal standard.

Carotenoids were determined from the same extract using the same HPLC system, but fitted with a Spherisorb, type S5NH2 5µ C_{18} reverse phase HPLC column, 25 cm × 4.6 mm (Phase Separations Ltd., UK). Chromatography was performed using a mobile phase of methanol/water (97:3, v/v) at a flow rate of 1.5 ml/min. Total carotenoids were detected at 445 nm as a single peak using lutein as a standard.

Results

Rural great tits laid clutches more than two eggs larger than their urban conspecifics but the proportion of hatchlings that fledged and fledgling weights did not differ between the study areas (Table 1). Neither did we find any significant differences in the extent or prevalence of early nestling mortality (Table 1). An indirect estimate of hatching asynchrony – coefficient of variation of nestling mass on day 8 – was two times higher in the rural population. In general, 2000 appeared to be a favourable year for breeding great tits. Both rural and urban birds fledged more hatchlings in 2000 (81 % and 82 %; Table 1) compared to the average of the four previous years (rural 1996–99: 69 ± 27 %, n = 70 broods; urban 1996–99: 72 ± 28 %, n = 166 broods, respectively). This difference was significant in the city ($Z_{21,166}$ = 2.02, P = 0.043), but not in the rural population ($Z_{9,70}$ = 1.44, P = 0.147).

Rural great tits had about twice the concentrations of carotenoids in their eggs than their urban conspecifics (47.9 ± 20.7 µg/g v. 21.3 ± 6.0 µg/g; Table 2, Fig. 1). Rural birds also had significantly higher vitamin E concentrations in their eggs than urban birds (154.9 ± 51.1 µg/g v. 119.2 ± 46.7 µg/g; Table 2, Fig. 1). We found no significant between-habitat differences in egg vitamin A content, yolk mass or egg volume (Table 2).

Table 2. Effect of breeding habitat (urban v. rural) and laying sequence (first v. last egg) on egg parameters of great tits in repeated measures ANOVA. A significant habitat × sequence term means that variation of trait values in laying sequence differed between habitats. For direction of the effects, see Figure 1.

Variable	Effect	F_{df}	P
Carotenoids	Habitat	$23.8_{1,13}$	0.0003
	Sequence	$7.4_{1,13}$	0.017
	Habitat × Sequence	$9.3_{1,13}$	0.009
Vitamin E	Habitat	$15.0_{1,12}$	0.002
	Sequence	$19.1_{1,12}$	0.0009
	Habitat × Sequence	$0.3_{1,12}$	0.6
Vitamin A	Habitat	$0.1_{1,12}$	0.8
	Sequence	$2.3_{1,12}$	0.5
	Habitat × Sequence	$0.4_{1,12}$	0.5
Yolk mass	Habitat	$2.5_{2,12}$	0.143
	Sequence	$4.6_{1,12}$	0.056
	Habitat × Sequence	$0.6_{1,12}$	0.5
Egg volume	Habitat	$1.8_{2,13}$	0.2
	Sequence	$0.0_{1,13}$	1
	Habitat × Sequence	$0.5_{1,13}$	0.5

2-010.qxd 03.07.02 16:50 Seite 5

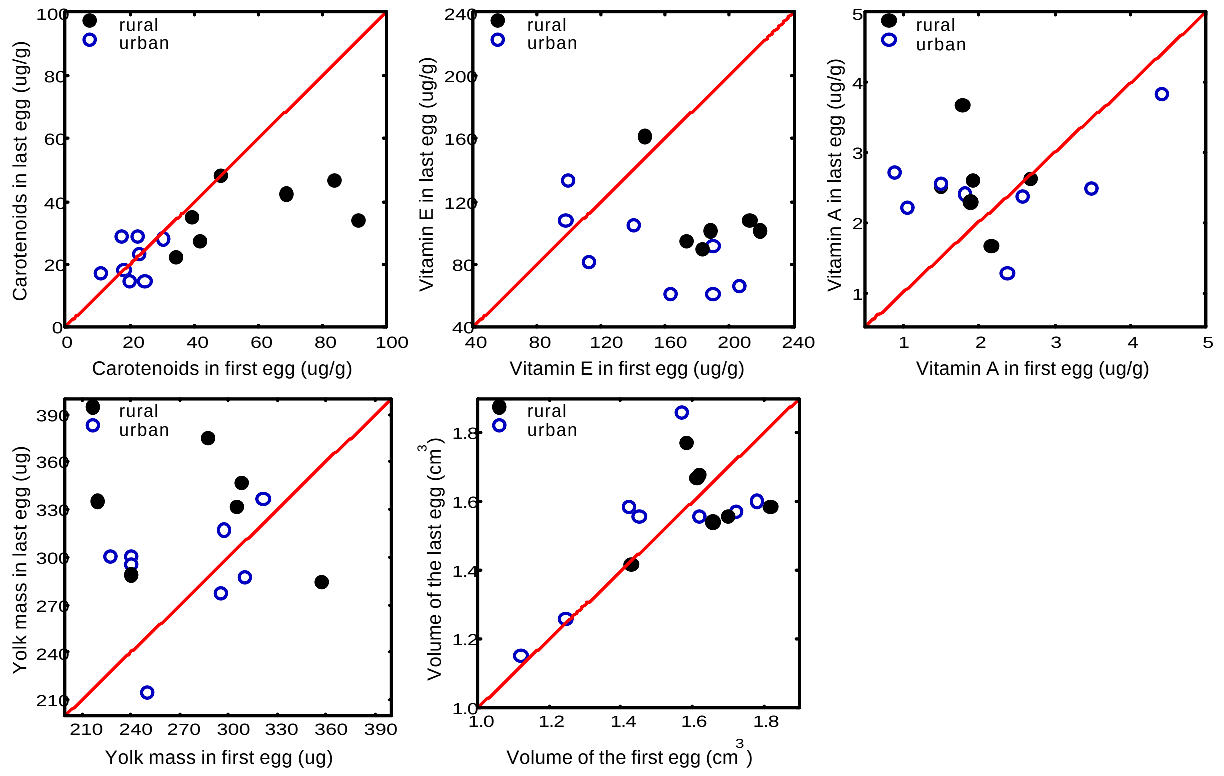

Figure 1. Comparison of egg variables in first-laid v. last-laid eggs in urban and rural great tits. Diagonal lines denote identical values.

Last-laid eggs contained about 40 % less vitamin E than first eggs (Fig. 1). These differences were in similar magnitude in both urban and rural populations as indicated by a non-significant interaction term in Table 2 (Urban: difference = 60.5 ± 64.8 µg/g; Rural: difference = 77.0 ± 47.4 µg/g). In rural great tits, egg carotenoid concentration decreased by 37 % from first to last egg (difference = 21.7 ± 20.0 µg/g; Fig. 1), while no decline in egg carotenoid concentration occurred in the town (difference = 0.2 ± 1.2 µg/g). The habitat-specific pattern in intraclutch variation of egg carotenoid content was confirmed by the significant Habitat × Sequence interaction term in Table 2. Egg yolk mass was generally about 9 % larger in last-laid than in first-laid eggs (difference = 28.5 ± 51.8 µg). This difference, however, was only marginally significant ($P = 0.056$; Table 2). We found no significant differences in vitamin A concentration or egg volume between first and last eggs in either population (Table 2).

Discussion

The coefficient of variation in nestling body mass was twice as large in rural great tits compared to urban birds. If this higher variation in nestling body masses was caused by greater hatching spread then our rural great tits appear more likely to adopt an adaptive brood reduction strategy than their urban conspecifics. However, at present we cannot exclude also the possibility that larger intrabrood variation in nestling body mass of rural birds could have arisen because rural parents had a harder task of rearing a larger brood (independently of any influence of hatching asynchrony) or because of greater intrabrood variation in important egg constituents. Nevertheless, hatching asynchrony would probably be the easiest way to generate sibling size hierarchies and offers most parsimonious explanation for the high intrabrood variation in nestling mass. Notably, although the prevalence and extent of early nestling mortality did not differ significantly between populations, the direction of these differences was consistent with an idea of adaptive brood reduction being more prevalent in the rural population (Table 1).

Compared to urban birds in Tartu, eggs of rural great tits had twice the carotenoid concentration and a 23 % higher vitamin E concentration. This difference was found despite the fact that rural birds on average laid 2.5 more eggs than urban birds (Table 1). These differences are also reflected by parallel differences in the hue of carotenoid-based plumage of female great tits from the two populations (Hõrak et al. 2001). One possible explanation for the differences in egg content would be that female great tits had better access to carotenoid- and vitamin E rich food sources in our rural study area, as compared to the city. Although the conversion of carotenoids from folivorous lepidopteran larvae to carotenoid-based plumage of breeding great tits is well documented (Partali et al. 1987), we do not currently know to what extent caterpillars comprise a significant part of the diet during this period. Thus, we can at best only hypothesise that the food of female great tits in our rural study area contained more carotenoids and vitamins. On the other hand, great tits breeding in Tartu are probably exposed to considerably higher heavy metal (notably cadmium and lead) pollution from traffic exhaust fumes, than their rural conspecifics. Both of these metals are known as strong inducers of oxidative stress (e.g. Ercal et al. 2000, Congiu et al. 2000). Vitamin E is known to be protective against cadmium-induced oxidative damage (Sarkar et al. 1997) and a prophylactic action of β-carotene on Cd toxicity has been shown recently (El-Missiry & Shalaby 2000). Administration of β-carotene to rats, concurrent with cadmium, ameliorated Cd-induced lipid peroxidation in brain and testes and prevented decrease in antioxidant (superoxide dismutase, glutathione S-transferase and reduced glutathione) concentrations. Concentration of α-carotene in mouse kidney and testis was significantly decreased as a result of cadmium administration (Massanyi et al. 1999). Therefore, we cannot exclude the possibility that urban great tits laid eggs of lower antioxidant concentration due to maternal depletion of carotenoids and vitamin E for the scavenging of free radicals.

Our observation that concentrations in eggs of vitamin E and carotenoids declined significantly with laying order is consistent with the idea of great tits being adaptive brood reductionists. Under this scenario, parents would benefit by enhancement of the quality of first-laid eggs by supplying these differentially with greater quantities of micro- and macro-nutrients, because first-laid eggs are more likely to produce viable offspring than last-laid eggs. Notably, carotenoid concentration declined with laying order only in the rural population which, according to the results of the present study, seems to show a greater extent of brood reduction than our urban great tit population. Our observations therefore clearly reject the hypothesis that mothers supply last-laid eggs with higher concentration of antioxidants as to compensate for the higher oxidative stress burden accompanying faster growth and greater activity levels of last-hatched offspring.

The physiological and molecular mechanisms by which such differential transfer of antioxidants to egg yolk occurs are not known and need further investigation. For example, additional supplementation of lesser black-backed gulls *Larus fuscus* with carotenoids did not change the pattern of decline in yolk carotenoid and vitamin E concentration in relation to laying sequence (Blount et al. 2002). When lesser black-backed gulls were induced to lay additional eggs, carotenoid concentrations in the egg yolk started to recover from the third egg up to the sixth or eighth eggs (A. Fidgett and P. F. Surai, unpubl. data).

Despite prominent differences in carotenoid and vitamin E concentrations, last-laid eggs of great tits were not inferior compared to first-laid eggs in all aspects of quality. Egg size (an indicator of an egg's total energy content in the great tit; Ojanen 1981) did not vary with laying sequence (Fig. 1) while yolk size (an indicator of egg lipid content) even tended to increase in last-laid eggs (Fig. 1, Table 2). The latter result also suggests that decline of carotenoid and vitamin E content in relation to laying sequence could hardly be ascribed to deterioration of food supplies in the course of laying. If that were the case, we would expect the last-laid eggs to be smaller and contain smaller yolks than the first-laid eggs. Moreover, such a situation seems unlikely because the availability of nutritious and carotenoid-rich lepidopteran larvae is usually higher at the end of laying period (T. Tammaru pers. comm.). It is thus possible that a tendency of yolk mass to increase in last-laid eggs reflects the generally improved food situation during the formation of these eggs.

In conclusion, we have shown that egg carotenoid concentrations in rural great tits was double that of their

2-010.qxd 03.07.02 16:50 Seite 7

conspecifics in the neighbouring urban population, and that concentration of carotenoids and vitamin E decreased with laying sequence. The latter finding is consistent with the idea of reduced maternal investment in last-laid eggs, predicted from an hypothesis of adaptive brood reduction.

Acknowledgements. We thank Indrek Ots, Ene Sarapuu, Lea Tegelmann and Helen Vellau for field assistance and two anonymous referees for their constructive criticisms of the manuscript. This study was supported by Estonian Science Foundation grant no. 4537 to PH. P. F. S. was supported by the Scottish Executive Rural Affairs Department.

References

Amundsen, T. & Slagsvold, T. 1998. Hatching asynchrony in great tits: a bet-hedging strategy? Ecology 79: 295–304.

Blount, J. D., Surai, P. F., Nager, R., Houston, D. C., Møller, A. P., Trewby, M. L. & Kennedy, M. W. 2002. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. Proc. R. Soc. Lond. B 269: 29–36.

Bryant, D. M. 1978. Establishment of weight hierarchies in the broods of house martin *Delichon urbica*. Ibis 120: 16–26.

Bryant, D. M. & Tatner, M. 1990. Hatching asynchrony, sibling competition and siblicide in nestling birds: studies of swiftlets and bee-eaters. Anim. Behav. 39: 657–671.

Congiu, L., Chicca, M., Pilastro, A., Turchetto, M. & Tallandini, L. 2000. Effects of chronic dietary cadmium on hepatic glutathione levels and glutathione peroxidase activity in starlings (*Sturnus vulgaris*). Arch. Environ. Contam. Toxicol. 38: 357–361.

Clark, A. B. & Wilson, D. S. 1981. Avian breeding adaptations: hatching asynchrony, brood reduction, and nest failure. Q. Rev. Biol. 56: 253–277.

El-Missiry, M. A. & Shalaby, F. 2000. Role of beta-carotene in ameliorating the cadmium-induced oxidative stress in rat brain and testis. J. Biochem. Mol. Toxicol. 14: 238–243.

Ercal, N., Neal, R., Treeratphan, P., Lutz, P. M., Hammond, P. A., Dennery, D. R. & Spitz, D. R. 2000. A role for oxidative stress in suppressing serum immunoglobulin levels in lead-exposed Fischer 344 rats. Arch. Environ. Contam. Toxicol. 39: 251–256.

Gil, D., Graves, J., Hazon, N. & Wells, A. 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. Science 286: 126–128.

Gosler, A. 1993. The great tit. Hamlyn, London.

Hahn, D. C. 1981. Asynchronous hatching in the laughing gull: cutting losses and reducing rivalry. Anim. Behav. 29: 421–427.

Hõrak, P. 1995. Brood reduction facilitates female but not offspring survival in the great tit. Oecologia 102: 514–519.

Hõrak, P., Mänd, R. & Ots, I. 1997. Identifying targets of selection: a multivariate analysis of reproductive traits in the great tit. Oikos 78: 592–600.

Hõrak, P. & Lebreton, J.-D. 1998. Survival of adult great tits in relation to sex and habitat: a comparison of urban and rural populations. Ibis 140: 205–209.

Hõrak, P., Ots, I., Vellau, H., Spottiswoode, C. & Møller, A. P. 2001. Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. Oecologia 123: 166–173.

Jover, L., Ruiz, X. & González-Martín, M. 1993. Significance of intraclutch egg size variation in the purple heron. Ornis Scand. 24: 127–134.

Lack, D. 1968. Ecological adaptations for breeding in birds. Methuen, London.

Lamey, T. C. 1990. Hatch asynchrony and brood reduction in penguins. Pp 399–416 in Davies, L. S. & Darby, L. T. (eds). Penguin Biology. Academic Press, San Diego.

Lebedeva, N. V. 1994. Nidicolous and post-nidicolous mortality of young from asynchronous broods of some passerine birds (Passeriformes). Zool. Zh. 73: 122–131 (in Russian with English summary).

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

Massanyi, P., Bardos, L., Oppel, K., Hluchy, S., Kovacik, J., Csicsai, G. & Toman, R. 1999. Distribution of cadmium in selected organs of mice: effects of cadmium on organ contents of retinoids and beta-carotene. Acta Physiol. Hung. 86: 99–104.

Meatherel, C. E. & Ryder, J. P. 1987. Intraclutch variation in the size, mass and composition of ring-billed gull eggs. Condor 89: 364–368.

2-010.qxd 03.07.02 16:50 Seite 8

Møller, A. P., Biard, C., Blount, J. D., Houston, D. C., Ninni, P., Saino, N. & Surai, P. F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? Poultry Avian Biol. Rev. 11: 137–159.

O'Connor, R. J. 1984. The growth and development of birds. John Wiley & Sons, New York.

Ojanen, M., Orell, M. & Väisänen, R. A. 1981. Egg size variation within passerine clutches: effects of ambient temperature and laying sequence. Ornis Fennica 58: 93–108.

Orell, M. 1983. Nestling growth in the great tit *Parus major* and the willow tit *P. montanus*. Ornis Fennica 60: 65–82.

Parsons, J. 1975. Asynchronous hatching and chick mortality in the herring gull *Larus argentatus.* Ibis 117: 517–520.

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

Ricklefs, R. E. 1993. Sibling comeptition, hatching asynchrony, incubation period, and lifespan in altricial birds. Pp 199–276 in Power, D. M. (ed.). Current Ornithology, Vol. 11. Plenum Press, New York.

Royle, N. J., Surai, P. F., McCartney, R. J. & Speake, B. K. 1999. Parental investment and egg yolk lipid composition in gulls. Funct. Ecol. 13: 298–306.

Royle, N. J., Surai, P. F. & Hartley, I. R. 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? Behav. Ecol. 12: 381–385.

Saino, N., Incagli, M., Martinelli, R., Ambrosini, R. & Møller, A. P. 2001. Immunity, growth and begging behaviour of nestling barn swallows *Hirundo rustica* in relation to hatch order. J. Avian Biol. 32: 263–270.

Saino, N., Ferrari, R., Martinelli, R., Romano, M., Rubolini, D. & Møller, A. P. 2002. Early maternal effects mediated by immunity depend on sexual ornamentation of the father in the barn swallow (*Hirundo rustica*). Proc. R. Soc. Lond. B (in press).

Sarkar, S., Yadav, P. & Bhatnagar, D. 1997. Cadmium-induced lipid peroxidation and antioxidant system in rat erythrocytes: the role of antioxidants. J. Trace Elem. Med. Biol. 11: 8–13.

Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. Proc. Natl. Acad. Sci. USA. 90: 11446–11450.

Schwabl, H., Mock, D. W. & Gieg, J. A. 1997. A hormonal mechanism for parental favouritism. Nature 386: 231–231.

Slagsvold, T. & Amundsen, T. 1992. Do great tits adjust hatching spread, egg size and offspring sex ratio to changes in clutch size? J. Anim. Ecol. 61: 249–258.

Stoleson, S. H. & Beissinger, S. R. 1995. Hatching asynchrony and the onset of incubation in birds, revisited. Pp 191–270 in Power, D. M. (ed.). Current Ornithology, Vol. 12. Plenum Press, New York.

Stenning, M. J. 1996. Hatching asynchrony, brood reduction and other rapidly reproducing hypotheses. Trends Ecol. Evol. 11: 243–246.

Surai, P. 1999. Vitamin E in avian reproduction. Poultry Avian Biol. Rev. 10: 1–60.

Surai, P. F. & Sparks, N. H. C. 2001. Comparative evaluation of the effect of two maternal diets on fatty acids, vitamin E and carotenoids in the chick embryo. Brit. Poultry. Sci. 42: 252–259.

Surai, P. F., Speake, B. K., Decrock, F. & Groscolas, R. 2001. Transfer of vitamins E and A from yolk to embryo during development of the king penguin. Physiol. Biochem. Zool. 74: 928–936.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999. Good genes, oxidative stress and condition-dependent sexual signals. Proc. R. Soc. Lond. B 266: 1–12

Received 26 March 2002
Revision accepted 18 June 2002