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Effect of sibling competition and male carotenoid supply on offspring condition and oxidative stress

Sylvain Losdat, Fabrice Helfenstein, Benoît Gaude, and Heinz Richner
Evolutionary Ecology Lab, Institute for Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

Early developmental conditions have major implications for an individual's fitness. In species where offspring are born simultaneously, the level of sibling competition for food access is intense. In birds, high sibling competition may subject nestlings to decreased growth rate as a result of limited food and increased levels of oxidative stress through high metabolic activity induced by begging behaviors. We manipulated the level of sibling competition in a natural population of great tits and assessed the consequences for nestling body condition and resistance to oxidative stress. In a full factorial design, we both augmented brood size to increase sibling competition and supplemented the male parents with physiological doses of carotenoids thereby doubling the natural carotenoid intake, aiming at increasing the males' investment in current reproduction and thereby decreasing sibling competition. Nestling body mass was reduced by the brood enlargement and enhanced by the carotenoid supplementation of fathers. Nestling resistance to oxidative stress, measured as total antioxidant defenses in whole blood, was not influenced by the treatments. Because nestlings experience high metabolic activities, an absence of an effect of sibling competition on free radicals production seems unlikely. Nestling body mass decreased and resistance to oxidative stress tended to increase with initial brood size, and hence these correlational effects suggest a trade-off between morphological growth and development of the antioxidant system. However, the result of the experimental treatment did not support this trade-off hypothesis. Alternatively, it suggests that nestling developed compensatory mechanisms that were not detected by our antioxidant capacity measure. *Key words:* antioxidant capacity, body condition, brood size manipulation, carotenoid supplementation, *Parus major*, sibling competition. *[Behav Ecol 21:1271–1277 (2010)]*

The ecological conditions that individuals experience during their early development have crucial implications for their future development, survival, and reproduction (Roff 1992; Stearns 1992). There is growing evidence that availability of dietary antioxidants at early life stages constrains the development of several fitness-determining traits (Surai 2002; Blount 2004; Catoni et al. 2008). For example, an experimental increase of dietary antioxidant availability to either laying females or directly to young has been shown to enhance offspring growth (Fenoglio et al. 2002; de Ayala et al. 2006; Cucco et al. 2006), boost the immune system (Saino et al. 2003), reduce susceptibility to pathogens (O'Brien and Dawson 2008), and reduce the occurrence of oxidative damage (Lin et al. 2005; Noguera et al. 2010). Taken together, these results suggest that investment in these antioxidant-demanding processes is constrained by the risk of oxidative stress, that is, an imbalance between free radicals and antioxidants in favor of the former (Sies 1991).

In this context, sibling competition, which occurs as a consequence of several offspring being born simultaneously and which generally results in young engaging in vigorous physical struggles to access the limited food provided by the parents (Mock and Parker 1997; Wright and Leonard 2002), can also be expected to generate oxidative stress. Indeed, studies in birds have shown that competitive ability, that is, a nestling's ability to physically exclude its rival siblings from distributed food and to monopolize the food source and/or to attract the parents' attention, is determined by pre- and postnatal antioxidant availability (Berthouly et al. 2007; Helfenstein, Berthouly, et al. 2008; Noguera et al. 2010). This is to be expected because in birds begging behaviors typically consist of nestlings stretching out and flapping wings to rise above their rival siblings and reach the adult parent (Kölliker et al. 1998; Neuenschwander et al. 2003). Normal metabolism produces free radicals against which all organisms have evolved several lines of defense (Surai 2002; Halliwell and Gutteringe 2007). However, antioxidant systems are not infallible (Halliwell and Gutteringe 2007; Niess and Simon 2007), and oxidative stress is a likely consequence of sibling competition and energetically costly begging behaviors (Leech and Leonard 1996; Bachman and Chappell 1998; Kilner 2001). An important evolutionary consequence is that, in large families where competition is by definition intense, offspring potentially face a trade-off in the allocation of antioxidants either to compensate for the consequences of competitive behaviors or to other antioxidant demanding fitness-related functions (e.g., immunity; Costantini and Møller 2009).

Surprisingly, although oxidative stress and its evolutionary consequences are of growing interest to evolutionary ecologists (Costantini 2008; Monaghan et al. 2009), very few studies investigated whether sibling competition generates oxidative stress and/or reduces offspring resistance to free radical attacks. We are aware of 2 studies only, one showing that growing in enlarged families enhances oxidative stress in captive zebra finches *Taenopygia guttata* (Alonso-Alvarez et al. 2007) and another one demonstrating that begging vocalization is antioxidant demanding in wild yellow-legged gulls *Larus michahellis* (Noguera et al. 2010).

Address Correspondence to S. Losdat. E-mail: sylvain_losdat@yahoo.fr.

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In this study, we aimed at manipulating the level of sibling competition in great tit *Parus major* broods by experimentally increasing brood size. Manipulating brood size reduces offspring condition (e.g., Roulin et al. 1999; Velando and Alonso-Alvarez 2003; Jacot and Kempenaers 2007) as a potential consequence of both less food being distributed per capita and offspring having to compete more vigorously to obtain their share of a scarcer resource. However, even when parents adjust their feeding effort to offspring number (and therefore the feeding rate per capita remains unchanged (Christe et al. 1996; Saino et al. 1997; Sanz and Tinbergen 1999; Magrath et al. 2007), brood size enlargement has been shown to promote intense sibling competition, also in great tits (Neuenschwander et al. 2003). In this species, increasing brood size enhances sibling competition, regardless of the possible effects on parental effort (Neuenschwander et al. 2003). Increasing brood size may also change the thermal environment, the perceived competition and other factors related to food scrambles. Experimentally manipulating the size of great tit broods thus appears as an appropriate procedure for investigating the effect of sibling competition on oxidative stress.

The intensity of sibling competition thus depends on the degree of parental investment, which in turn depends on available resources (Martin 1995). Carotenoid pigments are a large family of antioxidant molecules involved in numerous physiological functions in animals (Møller et al. 2000; Blount 2004). Although their role as in vivo antioxidants is currently debated (Costantini and Møller 2008), they do participate in the protection of DNA, proteins, and biological membranes from oxidative stress (Møller et al. 2000; Krinsky 2001). Additionally, carotenoids, particularly β-carotene and their derived products (retinoids, vitamin A) may act in a hormone-like fashion to up- and downregulate the immune system (Bendich 1989; Koutsos et al. 2003; Chew and Park 2004; Hartley and Kennedy 2004). In particular, they have been shown to alleviate the costs of immune activity in favor of lower oxidative damage or higher body condition (Hõrak et al. 2006; 2007). For these reasons, carotenoids are also expected to help males to face a pathogen infection, maintain good body condition, and sustain full reproductive activities. However, carotenoids cannot be synthesized de novo by animals and thus have to be ingested with the food (Partali et al. 1987; Olson and Owens 1998). For many species of birds, including great tits, they are a limiting resource whose availability varies in space and time (Olson and Owens 1998; Møller et al. 2000; Isaksson and Andersson 2007). Therefore, carotenoid availability is expected to set the level of parental effort during current reproduction (Stearns 1992) and supplementing males with carotenoids should increase their feeding effort and thus reduce sibling competition.

In this study, we conducted a 2×2 full factorial experiment on great tits, in which we aimed to modify sibling competition by increasing the natural brood size on the one hand and decreasing sibling competition by supplementing males with carotenoids on the other hand. We predicted that nestlings from augmented broods should be more susceptible to oxidative stress (as measured by the in vitro Kit Radicaux Libres (KRL) test in which the total antioxidant defenses in whole blood are assessed as the time needed to hemolyse 50% of the red blood cells exposed to a controlled free radical attack) and show reduced body mass and size.

MATERIALS AND METHODS

This experiment was conducted during spring 2008 in a natural population of great tits breeding in nest-boxes in a forest near Bern, Switzerland (lat 46°7′N, long 7°8′E). Nest-boxes were regularly visited from the beginning of the breeding season to determine laying and hatching dates and record brood size at hatching. The laying date in the study population streched from the 17 April to the 18 May.

Brood size manipulation

Two days posthatch (day 0 = hatching date), all nests were randomly assigned to be either augmented with 2 nestlings of the same age or to remain unchanged. We experimentally manipulated the brood size of half the nests ($n = 26$) and visited all other nests but left them unchanged ($n = 24$). The additional nestlings used for increasing brood size came from nests of the same population that were not included in this study.

Carotenoid supplementation of male parent

Seven days posthatch, all males were caught at the nest using electronic traps triggered from a distance using a remote control and randomly assigned to be carotenoid supplemented or to receive a placebo. This resulted in a fully crossed, fully randomized design with respect to both brood size manipulation and carotenoid supplementation. Males were force-fed with either one fresh alive *Calliphora* spp larva coated with a blend of corn oil, lutein, zexanthin, and β-carotene (carotenoid supplemented) or with one larva coated with corn oil only (placebo). Carotenoids were provided in the relative proportions found in the natural diet of great tits (80% lutein, 3% zeaxanthin, and 17% β-carotene according to Partali et al. (1987)). Males were captured again on Day 11 and the carotenoid supplementation was repeated. On each occasion, we provided 4 times the daily amount of carotenoids that males obtain naturally (Helfenstein, Losdat, et al. 2008), that is, 0.29 mg of total carotenoids per supplementation occasion.

Because carotenoids are lipid-soluble antioxidants that birds can store in their liver (Surai 2002), our mode of supplementation thereby effectively doubled the average daily intake of carotenoids over the entire experimental period. Of the 50 males initially captured, 3 carotenoid supplemented and 3 placebo males could not be recaptured on Day 11 and therefore received a single dose of carotenoids only. Because their inclusion in the data set can render the analysis more parsimonious at worst, they were kept in the data set.

Morphological measurements

We sampled 324 nestlings on Day 15 posthatch from 50 nests. We measured their body mass (±0.1 g) and tarsus length (±0.5 mm) and collected a 7 μl blood sample from the brachial vein.

Nestling resistance to oxidative stress

Nestling ability to resist oxidative stress was assessed using the KRL test (Brevet Spiral V02023, Couternon, France; http://www.nutriteck.com/sunyatakrl.html) adapted to bird physiological parameters (osmolarity, temperature; Alonso-Alvarez, Bertrand, Devevey, Gaillard, et al. 2004; Alonso-Alvarez, Bertrand, Devevey, Prost, et al. 2004). This assay provides a quantitative measure of the whole blood resistance to oxidative stress as it assesses the time required to hemolyse 50% of red blood cells of the sample when exposed to a controlled free radical attack. Briefly, 7 μl of whole blood were immediately diluted in 255.5 μl of KRL buffer (150 mM Na^+, 120 mM Cl^-, 6 mM K^+, 24 mM $HCO3^-$, 2 mM Ca^{2+}, 340 mOsM, pH·7.4) and stored at 4 °C before analysis which took place 6.2 ± 4 h after blood collection. We loaded 80 μl of KRL-diluted whole blood into wells of a 96-well microplate. We

Table 1

Linear mixed models testing for an effect of the brood size manipulation and the carotenoid supplementation of the male parent on nestling body mass and nestling tarsus length

Effect	Estimate ± SE	F_{df}	P
Nestling body mass (square-root transformed)			
Intercept	4.66 ± 0.18	—	—
Laying date	−0.0001 ± 0.04	$<0.01_{1,43}$	0.97
Initial brood size	−0.11 ± 0.02	$25.36_{1,44}$	<0.001
Brood size manipulation[a]	−0.67 ± 0.22	$8.55_{1,44}$	0.005
Male parent carotenoid treatment[b]	0.10 ± 0.05	$4.41_{1,44}$	0.04
Brood size manipulation × carotenoid treatment[c]	−0.01 ± 0.09	$0.01_{1,41}$	0.91
Brood size manipulation × initial brood size	0.06 ± 0.03	$4.28_{1,44}$	0.04
Carotenoid treatment × initial brood size	−0.04 ± 0.03	$1.97_{1,42}$	0.17
Nestling tarsus length			
Intercept	21.01 ± 0.38	—	—
Laying date	−0.001 ± 0.03	$0.06_{1,44}$	0.81
Initial brood size	−0.13 ± 0.05	$7.85_{1,46}$	0.007
Brood size manipulation[a]	−0.65 ± 0.16	$17.15_{1,46}$	0.0001
Male parent carotenoid treatment[b]	−0.1 ± 0.16	$0.41_{1,45}$	0.53
Brood size manipulation × carotenoid treatment[c]	−0.07 ± 0.1	$0.52_{1,43}$	0.47
Brood size manipulation × initial brood size	−0.06 ± 0.32	$0.04_{1,42}$	0.84
Carotenoid treatment × initial brood size	−0.01 ± 0.1	$0.008_{1,41}$	0.93

Models included the nest as a random factor to account for the nonindependence of nestlings raised in the same nest (random parameter not shown). F and P values of terms not retained in the final model are those just before removal; SE, standard error.

[a] Relative to the control-brood group.

[b] Relative to the placebo group.

[c] Relative to the placebo group and the control-brood group.

subsequently added to each well 136 µl of a 150 mM solution of 2,2-azobis-(amidinopropane) hydrochloride (a free radical generator; 646 mg of [2,2′-azobis-(amidinopropane) hydrochloride] diluted in 20 ml of KRL buffer (Wahl et al. 1998)). The microplate was subsequently read with a microplate reader spectrophotometer (PowerWave XS reader; Witec Ag, Switzerland) at 40 °C. The rate of hemolyse was determined by the change in optical density measured at 540 nm (Alonso-Alvarez, Bertrand, Devevey, Gaillard, et al. 2004; Alonso-Alvarez, Bertrand, Devevey, Prost, et al. 2004; Bertrand et al. 2006). This assay reflects the current availability of antioxidant defenses as well as the past oxidative insults experienced by red blood cells (Esterbauer and Ramos 1996; Brzezinska-Slebodzinska 2001).

Statistical analyses

We used linear mixed-effect models to analyze nestling body mass (square-root transformed), tarsus length, and whole blood resistance to oxidative stress (log-transformed). We used the morphological raw values because other models using either principal component analysis scores of all morphological variables, residuals of body mass versus tarsus length, or tarsus length included as a covariate were less parsimonious while giving qualitatively similar results.

Explanatory variables in the initial models were the brood size manipulation, the carotenoid treatment of the male parent, the initial brood size, and all 2-way interactions. Including a quadratic term for brood size did not improve the fit of the models and was thus discarded from all models. We also included the laying date as a covariate to correct for seasonal effects. The nest identity was included as a random factor to correct for the nonindependence of nestlings raised in the same nest. We tested the fit of our models by checking the residuals for normality and homoscedasticity and by plotting the residuals against the predicted values. Nonsignificant interactions were backward eliminated using a stepwise elimination procedure based on the Akaike Information Criterion (AIC) and maximum likelihood. Model parameters and tests of fixed effects are derived from restricted maximum likelihood procedures. Tests are 2-tailed with a significance level set to $\alpha = 0.05$. All analyses were performed with R 2.11.1 (R Development Core Team 2008).

RESULTS

Nestling body condition

Nestlings from control broods were heavier than those from enlarged broods (brood size manipulation: $F_{1,44} = 8.55$, $P = 0.005$, Table 1, Figure 1), and nestlings reared by carotenoid-supplemented males were heavier than those reared by placebo males (carotenoid treatment: $F_{1,44} = 4.41$, $P = 0.04$, Table 1, Figure 1). The effect of brood size enlargement did not depend on carotenoid treatment and vice versa (brood size manipulation × carotenoid treatment: $F_{1,41} = 0.01$, $P = 0.91$, Table 1). As expected from the addition of an equal number of nestlings to broods of initially varying sizes, we found a significant interaction between brood size manipulation and initial brood size where small broods suffered more from brood enlargement than large ones (brood size manipulation × initial brood size: $F_{1,44} = 4.28$, $P = 0.04$, Table 1).

Nestlings grew longer tarsi in control broods than in enlarged ones (brood size manipulation: $F_{1,46} = 17.15$, $P < 0.001$; Table 1). However, nestling tarsus length was unaffected by the carotenoid treatment of the male parent (Table 1). Nestling body mass and tarsus length showed a negative relationship with initial brood size (Table 1).

Nestling resistance to oxidative stress

Nestling resistance to oxidative stress was not influenced by our treatments (Table 2). Nestling resistance to oxidative stress showed a positive relationship with laying date (laying date: $F_{1,40} = 4.50$, $P = 0.04$) and tended to increase with initial brood size ($F_{1,40} = 3.05$, $P = 0.069$; Table 2, Figure 2).

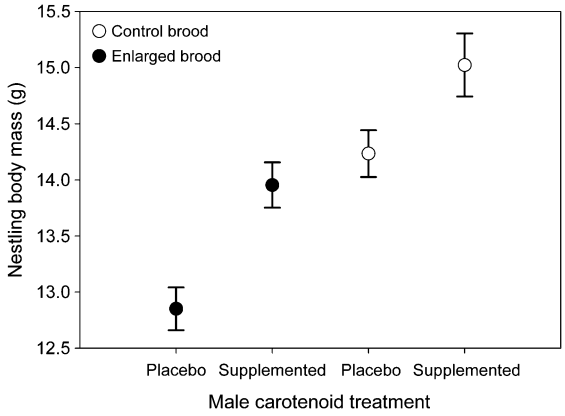

Figure 1
Nestling body mass on Day 15 posthatch in relation to the brood size manipulation and carotenoid supplementation of the male parent. Values are means ± standard error. Both treatments were significant, but their 2-way interaction was not (Table 1). Supplementing the male parent with carotenoids fully compensated for the nutritional stress induced by more intense sibling competition because nestlings reared by carotenoid-supplemented males in enlarged broods grew as heavy as nestlings reared by placebo males in control broods (13.95 ± 0.2 g and 14.23 ± 0.2 g, respectively, Tukey-adjusted post hoc test: $z = -1.60$, $P = 0.38$).

Within-nest variance

More intense sibling competition should lead to increased within-nest variance in nestling body mass. Although nonsignificant (within-nest coefficient of variation; $F_{1,\ 45} = 1.74$, $P = 0.089$), we found a trend in the predicted direction, that is, nestling body mass showed greater variance in enlarged broods than in control ones, thus reflecting more intense scramble competition. There was, however, no effect of the carotenoid treatment nor of the initial brood size and the interactions between these variables (all $F < 1.65$, $P > 0.21$).

DISCUSSION

Experimentally manipulating sibling competition had strong effects on nestling growth but did not affect their resistance to oxidative stress. Nestlings reared in enlarged broods grew smaller and lighter, whereas those reared by carotenoid-supplemented males grew heavier.

Our finding that increasing brood size lowers body mass and growth rate is in line with previous studies (e.g., Kilner 2001; Neuenschwander et al. 2003; Velando and Alonso-Alvarez 2003). Nutritional stress during early development is known to impact growth, physiology, social behavior, and reproduction (Metcalfe and Monaghan 2001) and therefore reduces individual fitness. Such effects result from both nutritional shortage, that is, fewer resources available per nestling and from the consequences of increased begging and social competition (e.g., Kilner 2001; Neuenschwander et al. 2003).

Nestlings reared by carotenoid-supplemented males were heavier. In addition, nestlings reared by carotenoid-supplemented males in enlarged broods grew as heavy as nestlings reared by placebo males in control broods (13.95 ± 0.2 g and 14.23 ± 0.2 g, respectively, Tukey-adjusted post hoc test: $z = -1.60$, $P = 0.38$), suggesting that supplementing the male parent with carotenoids fully compensated for the nutritional stress induced by more intense sibling competition. Owing to the physiological roles played by carotenoids as immunomodulators (Bendich 1989; Chew and Park 2004; Hartley and Kennedy 2004; Costantini and Møller 2009) and antioxidants (Bendich 1989; Surai 2002; Alonso-Alvarez, Bertrand, Devevey, Gaillard, et al. 2004; Hõrak et al. 2007), this result likely reflects short-term effects of carotenoids on the males' ability to adjust food provisioning to nestling needs. Considering the small physiological doses of carotenoids given to males, this result may reflect a threshold effect where males operating at the edge of their physiological possibilities would substantially benefit from a modest carotenoid supplementation.

Contrary to our predictions, modifying the level of sibling competition resulted in no detectable change in our measure of resistance to oxidative stress. A first explanation would be that sibling competition does not generate oxidative stress and thus entails no cost in terms of reduced antioxidant capacity and oxidative damage. However, even though this explanation cannot be excluded with our data, it seems the most unlikely for the following reason. Studies have shown that body mass at fledging significantly determines recruitment probability (Magrath 1991; Naef-Daenzer et al. 2001; Schwagmeyer and Mock 2008), and nestlings are thus expected to optimize body mass at fledging. Metabolic activity is a primary source of free radicals as unavoidable by-products of ATP synthesis, and the extremely fast rate of development characterizing small altricial birds likely subject nestlings to acute oxidative stress (Halliwell and Gutteringe 2007; Costantini and Verhulst 2009). This has been recently exemplified in a study by Hall et al. (2010), which showed that growth is an antioxidant demanding process in

Table 2
Linear mixed model testing for an effect of the brood size manipulation and the carotenoid supplementation of the male parent on nestling resistance to oxidative stress (log-transformed), that is, whole blood resistance to a controlled free radical attack

Effect	Estimate ± SE	F_{df}	P
Intercept	0.11 ± 0.81	—	—
Laying date	0.03 ± 0.01	$4.50_{1,40}$	0.04
Initial brood size	0.07 ± 0.04	$3.05_{1,40}$	0.068
Brood size manipulation[a]	0.05 ± 0.13	$0.15_{1,39}$	0.70
Male parent carotenoid treatment[b]	0.02 ± 0.13	$<0.01_{1,38}$	0.99
Brood size manipulation × carotenoid treatment[c]	−0.12 ± 0.27	$0.20_{1,36}$	0.66
Brood size manipulation × initial brood size	−0.01 ± 0.08	$0.008_{1,35}$	0.93
Carotenoid treatment × initial brood size	0.09 ± 0.07	$1.30_{1,37}$	0.26

The model included the nest as a random factor to account for the nonindependence of nestlings growing in the same nest (random parameter not shown). F and P values of terms not retained in the final model are those just before removal; SE, standard error.

[a] Relative to the control-brood group.

[b] Relative to the placebo group.

[c] Relative to the placebo group and the control-brood group.

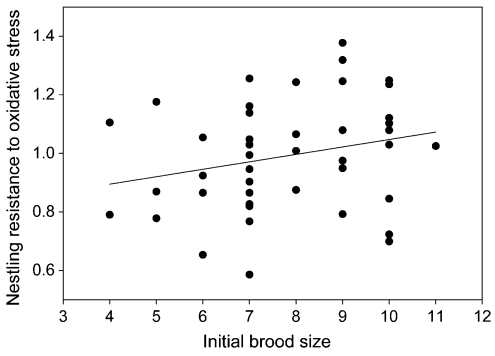

Figure 2
Log-transformed whole blood resistance to a controlled free radical attack in relation to initial brood size. Each point is the mean per nest. The line is the linear regression line.

which oxidative stress likely plays a pivotal role. Moreover, another recent piece of work suggests that oxidative stress is a likely cost of begging behaviors (Noguera et al. 2010). Therefore, we assume that the absence of an effect of our manipulations on nestling resistance to oxidative stress calls for alternative explanations.

First, the method we used to assess past and present occurrences of oxidative stress, that is, whole blood resistance to a controlled free radical attack may need cautious interpretation. Recent studies showed that the antioxidant capacity of a given tissue may not always directly reflect the occurrence and intensity of an oxidative stress (Cohen and McGraw 2009; Costantini and Verhulst 2009). Oxidative stress occurs when the total antioxidants of an individual cannot fully neutralize the free radicals produced so that unquenched free radicals remain in tissues for long enough to cause further reactions. Therefore, the basal homeostatic situation can change either as a consequence of increased free radical production or reduced antioxidant capacity (Monaghan et al. 2009). Antioxidant capacity assesses circulating antioxidant response, but the relationship between enzymatic and circulating antioxidants is unknown (Somogyi et al. 2007; Monaghan et al. 2009). The age of red blood cells may also influence the measure (Senok et al. 1997; Brzezinska-Slebodzinska 2001), and for this reason, we measured all nestlings in all treatments at exactly the same age of 15 days posthatch, and treatment groups were completely randomized with regard to initial brood size. Costantini and Verhulst (2009) also emphasized that levels of antioxidant capacity only reflect the balance between prooxidants and antioxidants in a specific tissue but do not allow direct estimations of either the levels of antioxidants and prooxidants separately or their levels in other tissue and body fluids.

Second, nestlings may have mobilized antioxidants stored in other tissues (e.g., the liver) and/or may have enhanced antioxidant enzyme synthesis, which could have masked the effect of our treatment and alleviated the costs of sibling competition with regard to oxidative stress (Monaghan et al. 2009). These compensatory mechanisms may have restored the nestlings' ability to overcome oxidative stress while being undetected by our measure. Furthermore, competition between siblings for parental care is the first occurrence of social competition an organism encounters after birth (Mock and Parker 1997), and physiological adaptations allowing nestlings to compete in conditions of nutritional stress, such as compensatory antioxidant mechanisms, should be selected for. However, investing more in the antioxidant system through compensatory mechanisms or upregulation of circulating antioxidants (Alonso-Alvarez et al. 2008) likely has metabolic costs (e.g., antioxidant enzyme synthesis) or may deplete antioxidant stores and increase the risk of future oxidative stress (Monaghan et al. 2009).

Interestingly, although this should be cautiously interpreted, we found a positive trend between initial brood size and nestling resistance to oxidative stress, which may be explained by at least 2 mechanisms. First, clutch size is expected to be related to parental capabilities (Daan and Drent 1980; Slagsvold and Lifjeld 1990) and thus to reflect individual genetic and/or phenotypic quality. Parents caring for initially larger broods and supposedly of higher quality may have nestlings with higher resistance to oxidative stress either because antioxidant capacity is heritable (Martin et al. 1996; Costantini and Dell'Omo 2006) or because such parents can provide a better environment for offspring growth and development, which may in turn enhance antioxidant capacities (Costantini and Dell'Omo 2006; Norte et al. 2009). Second, recent work by Alonso-Alvarez et al. (2007) revealed a negative correlation between somatic growth rate and individual resistance to oxidative stress. This finding suggests that the risk of exposure to oxidative stress may trigger a trade-off between investing in antioxidant activity and the need to maintain morphological development during early development of nestlings. In line with Alonso-Alvarez's hypothesis that oxidative stress constrains somatic growth rate, we found initial brood size to be negatively related to nestling growth and body mass. This argues for an evolutionary trade-off between investing energy in morphological development or in antioxidant activity as suggested by Alonso-Alvarez et al. (2007). It needs to be kept in mind, however, that initial brood size is a correlational variable and hence predictions regarding nestling physiological measures or traits could go in several directions. Experimental manipulation of brood size, as done here, shows no support for this trade-off.

To conclude, we found that increased carotenoid availability to male parents during the rearing period has a positive effect on offspring quality. However, neither carotenoid supplementation of the male parent nor increased brood size significantly influenced nestling resistance to oxidative stress. Oxidative stress as a potential cost of sibling competition requires further investigation using direct measures of the consequences of oxidative stress and oxidative damages to biological molecules. In addition, the relative importance of parental quality, growth strategies, and genetic components in determining nestling resistance to oxidative stress should be considered in further studies.

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