Effects of Sibling Competition on Growth, Oxidative Stress, and Humoral Immunity: A Two-Year Brood-Size Manipulation

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ABSTRACT

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signi cantly weaker in chicks whose BSs were increased. However, they assessed only one component of oxidative stress (the antioxidant capacity), and this does not fully characterize the resulting redox balance (Costantini 2008; Costantini and Verhulst 2009; Monaghan et al. 2009). Therefore, more experimental studies presenting integrated measures of oxidative stress are needed to provide a more comprehensive view of oxidative stress as a life-history trait potentially mediating trade-offs (Hõrak and Cohen 2010).

Although von Schantz et al. (1999) proposed that an activation of the immune system might generate an increase in oxidative stress, strong evidence for this is largely still lacking (Costantini 2008). While some studies have reported that immune activation directly elicits oxidative damage in chicks (Costantini and Dell'Omo 2006) and adult birds (Bertrand et al. 2006; Torres and Velando 2007; van de Crommenacker et al. 2010), other studies have failed to demonstrate a causal link between these two parameters (Alonso-Alvarez et al. 2004; Perez-Rodiguez et al. 2008). Therefore, more studies are needed to fully understand the reciprocal relationships between oxidative stress and immune function (Costantini 2008; Costantini and Møller 2009; Monaghan et al. 2009) and the effect that early developmental conditions have on these traits and their interaction. One reason for the lack of consistency between different studies might be that most studies are performed during only one breeding season, and often without a known "ecological context," for example, if the study was conducted in a

Table 1: Effects in European starling (*Sturnus vulgaris*

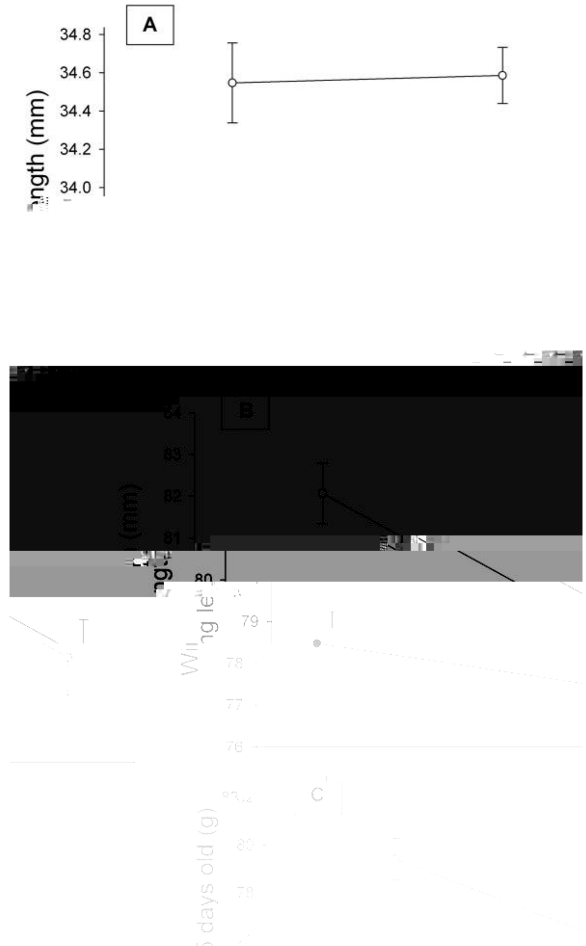

Figure 1. Effects of brood-size manipulation (decreased vs. enlarged

Table 2: Effects in European starling (*Sturnus vulgaris*) chicks, using generalized linear mixed models, of brood-size manipulation (group: decreased or enlarged brood) and year (2007 or 2008) on plasma total antioxidant capacity (TAC), total oxidant status (TOS), plasma oxidative status (ratio between TOS and TAC), and immunoglobulin Y (IgY) levels

	Group			Year			Group × Year			Parameter Estimates ± SE			
Dependent Variable	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	Intercept	Group	Year	Group × Year
TAC (mmol Trolox equivalent L^{-1})	51.66	5.47	.02	61.54	2.43	.12	67.96	5.86	.02	−1.66 ± .08	.01 ± .12	.33 ± .10	.40 ± .16
TOS (μmol H_2O_2 equivalent L^{-1})[a]	58.50	.26	.61	77.23	46.85	.0001	79.34	2.76	.10	2.31 ± .04	.05 ± .06	.22 ± .05	.14 ± .08
Plasma oxidative status[a]	56.27	.66	.42	73.38	62.82	.0001	76.57	.00	.98	1.09 ± .04	.03 ± .06	.34 ± .05	.00 ± .08
IgY level[b]	60.11	6.08	.02	67.12	50.49	.0001	74.38	.11	.74	3.45 ± .26	.76 ± .38	2.05 ± .35	.18 ± .55

Note. Parameter estimate values are given for the following fixed effects: for group, decreased; for year, 2007; and for group × year, decreased × 2007. IgY level data are presented in arbitrary units.

[a]Data were $\log_{10}$ transformed before statistical analysis.

[b]Data were inverse transformed before statistical analysis.

each experimental group of each year (data not shown; LME, $P > 0.05$ in all cases).

Discussion

With this study, we aimed to assess the combined effects of annual variation in year quality and developmental conditions on biomarkers of oxidative stress and humoral immune function in nestling European starlings. First, we showed that chicks raised in experimentally enlarged broods had lower TAC, but only during one of the study years (2007). BS manipulation did not influence TOS, but TOS was higher, independent of BS, in 2007. Consequently, plasma oxidative status was higher in 2007 than in 2008. Second, IgY level, which is a marker of humoral immune function, was higher in 2008 and, unexpectedly, was higher in chicks in experimentally enlarged broods compared with those in smaller broods. Finally, TAC and TOS were positively correlated only in 2007, while there were no relationships between biomarkers of oxidative stress and immune function (IgY) in either year. Our results therefore demonstrate that year effects can confound BS effects when assessing the impacts of sibling competition on chicks' growth and diverse physiological traits such as oxidative stress. This emphasizes the influence of year effects—that is, the "ecological context"—over BS on oxidative stress, and it raises the question of the nature of the physiological mechanisms that could be responsible for variations in oxidative stress.

Broods of European starling chicks were consistently manipulated during two consecutive years (2007 and 2008). Significant differences in morphological traits (tarsus and wing lengths), body mass, and daily growth rates were observed between the two study years; all of these traits had significantly lower values in 2007 than in 2008. Various parameters such as food quality and quantity, parasite pressure, or weather conditions could, acting alone or in synergy, influence chicks' morphological traits. Although the proximal factors responsible for these interyear differences were not controlled for in our study and remain to be identified, we defined 2007 as being a year of lower quality than 2008.

In this study, TAC was lower only in experimentally enlarged broods that were raised during a low-quality year (2007). On the other hand, while TOS did not vary with increasing BS, it was significantly affected by year. Indeed, chicks raised during a low-quality year showed a higher TOS than those raised during a higher-quality year (2008). Consequently, plasma oxidative status, defined as the ratio between pro- and antioxidants, was significantly higher in 2007 than it was in 2008, independent of BS. These results do not support our a priori predictions, and they contrast with the results of some previous studies. For example, oxidative stress was higher in larger broods of wild kestrels (Costantini et al. 2006)esultreonl-247.4hultr show trandapac.3(ha-)]Tm63.t8.9(in)-.5(ollween)-3olobem(s)]TJ T* [(ongths),n

Figure 2. Effects of brood-size manipulation (decreased vs. enlarged broods) on plasma concentrations of (*A*) total antioxidant capacity (TAC), (*B*) total oxidant status (TOS), (*C*) plasma oxidative status (ratio between TOS and TAC), and (*D*) immunoglobulin Y in 15-d-old European starling (*Sturnus vulgaris*) chicks raised during two consecutive years, 2007 (*solid circles*) and 2008 (*empty circles*). Values represented are means ± SE.

peroxidation (marker of oxidative stress–derived damage) in captive green finches (*Carduelis chloris*) secondary to the activation of the immune system. This further illustrates the activation of the endogenous antioxidant machinery consecutive to the induction of a free-radical attack. We tried to address this question by examining the relationship between TOS and TAC between years. In 2007, there was a significant positive relationship between the two parameters, implying that chicks with the highest TOS also had the highest TAC, while in 2008 the latter relationship was not significant. Therefore, the antioxidant machinery might have been activated only during the low-quality year, when the production of prooxidant compounds was higher. Although the latter mechanism might help to buffer the increasing production of oxidants and limit oxidative stress during a low-quality year, one can only suppose it might entail an energetic cost (Cohen et al. 2008; reviewed in Costantini 2008). In this study, the TOS was significantly higher during the low-quality year than during the higher-quality year. Although chicks showed similar antioxidant levels between years, the positive relationship between TOS and TAC that was observed during the low-quality year might suggest that the antioxidant machinery was activated during that year to counteract the high TOS levels and to prevent the individual from sustaining high oxidative stress. While such an upregulation could contribute to limiting oxidative stress, it might have induced an energetic cost.

If there is resource-allocation trade-off between immunity and growth, we predicted that chicks raised under more stressful developmental conditions, such as resource limitation or increasing competition in larger broods, should exhibit lower immune capacity (Naguib et al. 2004). In this study, humoral immunity was accordingly lower during a low-quality year, which could be, among other factors, attributable to lower nutritional conditions during that year. Using a feeding regime manipulation, Brzek and Konarzewski (2007) showed that food-restricted nestling sand martins (*Riparia riparia*) exhibited

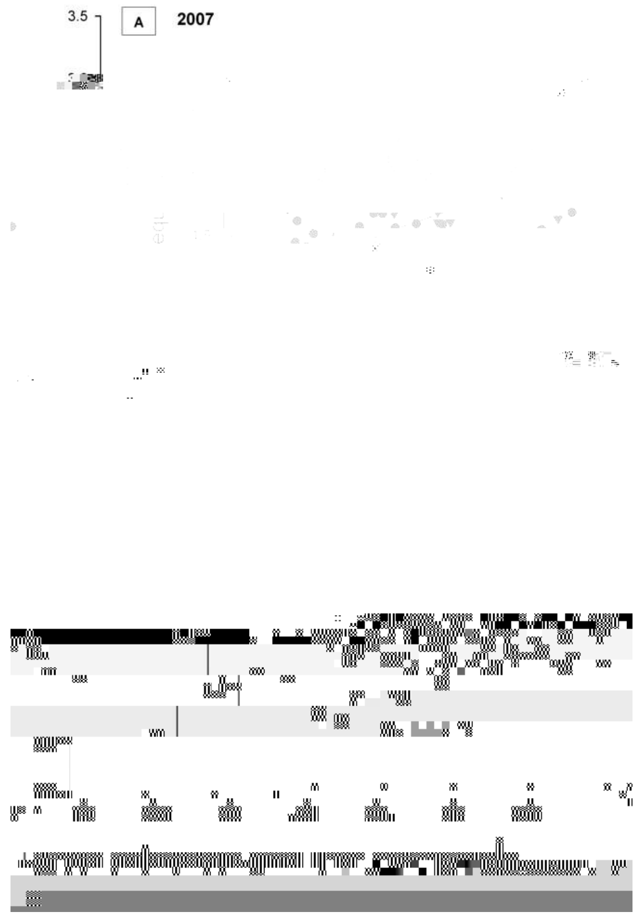

Figure 3. Relationship between total oxidant status and total antioxidant capacity in 15-d-old European starling (*Sturnus vulgaris*) chicks raised in (*A*) 2007 and (*B*) 2008 in two different experimental environments, decreased broods (*triangles*) and enlarged broods (*circles*).

a significantly weaker T-cell-mediated immune swelling response than nestlings fed ad libitum. However, in this study, chicks raised in experimentally enlarged broods showed higher IgY levels than did chicks raised in smaller broods during both years, contradicting our prediction. Other studies have also found a positive relationship between BS and immune function. For example, the T-cell-mediated immune response of domesticated zebra finch chicks raised in experimentally large broods was significantly stronger than that measured in chicks raised in small broods (Tschirren et al. 2009). Likewise, late-hatched barn swallow nestlings had lower body mass but a stronger humoral immunity (plasma immunoglobulin concentration) and a stronger T-cell-mediated immune response than their early-hatched siblings (Saino et al. 2001). However, these latter results are contradictory to those of cross-fostering experiments describing lower T-cell responses following a BS increase in great tits (Hõrak et al. 1999) and zebra finches (Naguib et al. 2004; Alonso-Alvarez et al. 2006). One alternative explanation for our results might relate to parasite load, on the basis of the fact that immunoglobulin levels could reflect not only immune capacity but also an immune response to an ongoing infection (reviewed in Morales et al. 2004), while parasite exposure was shown to increase with increasing BS in great tits (Norris et al. 1994) and tree swallows (*Tachycineta bicolour*; Shutler et al. 2004). Accordingly, small ground finches (*Geospiza fuliginosa* have been upregulated during a low-quality year to cope with the subsequent increased production of oxidants, and this mechanism might be energetically costly. Future studies should take ecological context into account when inferring about proximal factors explaining variations in oxidative stress. Whether the structural and physiological costs imposed by sibling competition will lead to long-term effects on chicks' survival and/or tness remains to be documented.

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