Maternal effects reduce oxidative stress in female nestlings under high parasite load

Greet De Coster, Liesbeth De Neve, Simon Verhulst and Luc Lens

Mothers can adjust the phenotype of their offspring to the local environment through a modification of their egg investment and/or nestling provisioning. However, offspring health may be severely impaired if the conditions experienced by nestlings do not match with those anticipated by the mother. If maternal effects differentially affect the sexes or if one sex is more strongly affected by an environmental stressor, fitness benefits may also differ between male and female offspring. Here, we study maternal effects in male and female great tit *Parus major* nestlings by means of an ectoparasite treatment before egg-laying combined with a partial cross-foster experiment between broods of infested and uninfested nests. Nestlings that were raised in their own nest experienced the same conditions before and after cross-fostering (either in parasite infested or uninfested nests), while cross-fostered ones experienced different conditions (either changing from infested to uninfested or the other way around). We measured effects on nestling plasma levels of oxidative stress [reactive oxygen metabolites (ROMs) and total antioxidant capacity (OXY)], body condition (body size and mass) and post-fledging survival. Daughters, but not sons, from matching conditions showed the lowest ROM and high OXY levels when exposed to parasites, while there was no effect of parasite exposure in any of both sexes in case of a mismatch. In contrast, body condition and post-fledging survival were not (or only slightly) affected by any of the experimental treatments. Results of this study show that maternal effects can affect oxidative stress levels of nestlings in a sex-specific way and that the outcome depends on the exposure to environmental stressors, such as parasites.

Environmental conditions, such as abiotic conditions, food availability and the exposure to parasites, typically vary in time and space. As a consequence, an organism’s phenotype might not be optimally adapted to the environmental conditions it experiences, because as a rule a phenotype is formed before selection takes place. Yet, mothers can improve offspring fitness by adjusting their phenotype to the local environmental conditions through maternal effects (Marshall and Uller 2007). Maternal effects may arise in various ways and at different times in the life cycle: prenatal effects are often mediated by an adjustment of egg investment (e.g. hormones; reviewed by Groothuis et al. 2005, Gil 2008), while postnatal effects usually occur through an adjustment of parental care, such as offspring food provisioning (Clutton-Brock 1991). A key prediction is that offspring will perform better in the environment anticipated by their mother, in comparison to other environments (Marshall and Uller 2007). However, mothers may not always prepare their offspring for the correct environment e.g. because of the time-lag between maternal adjustment and selection on the offspring, potentially resulting in offspring exhibiting poor phenotype-environment matching (DeWitt et al. 1998, Marshall and Uller 2007). Apart from an adjustment to the local (non-maternal) environment, mothers can also adjust offspring phenotype to their own (prevailing) phenotype. For instance, mothers may adjust offspring begging behaviour to their own expected provisioning rate via differential androgen investments in eggs (Kölliker et al. 2000, Hinde et al. 2009). Furthermore, the fitness benefits of maternal effects might differ between male and female offspring through a sex-specific investment of resources or because the same amount of investment has sex-specific consequences (Groothuis et al. 2005, Badyaev et al. 2006a, b, De Neve et al. 2008, Jones et al. 2009).

Maternal effects can act on a broad range of offspring morphological and physiological traits (Naguib and Gil 2005, Marshall 2008, Todd et al. 2011). However, to the best of our knowledge, evidence for maternal effects on offspring oxidative stress is still lacking, despite the fact that persistent oxidative stress can contribute to ageing and various disorders (Harman 1956, Finkel and Holbrook 2000). Oxidative stress is defined as the rate at which oxidative damage to biomolecules is generated after the exposure to reactive species that are e.g. produced in the body as a result of oxidative metabolism (Finkel and Holbrook 2000, Costantini and Verhulst 2009). Organisms have evolved antioxidants, which are obtained from diet or can be produced endogenously, to defend against oxidative
stress (Halliwell and Gutteridge 2007). The large transfer of maternal antioxidants to egg yolk to protect developing offspring against oxidative stress (Sorai and Speake 1998, Blount et al. 2000, McGraw et al. 2005) and the great variability in oxidative stress levels (reviewed by Monaghan et al. 2009, Metcalfe and Alonso-Alvarez 2010) and in maternal antioxidant transfer in relation to environmental conditions (Blount et al. 2002, Royle et al. 2003) suggest that oxidative stress may be modified by maternal effects.

Here, we investigate maternal effects on nestling oxidative stress (reactive oxygen metabolites and antioxidant capacity), body condition (body size and mass) and post-fledgling survival in the great tit Parus major. In particular, we study whether these characteristics are negatively affected when nestlings are reared by foster parents in a foreign nest through a cross-foster experiment. Being raised in a foster nest – hence being exposed to a new nest, new parents and/or new pathogens – may not only disrupt the match between offspring phenotype, maternal phenotype and environment, but may also induce a stress response that deteriorates individual performance (Berthouly et al. 2007). In addition, we manipulated parasite exposure, starting before egg-laying, by means of hen fleas Ceratophyllum gallinae. When blood-sucking ectoparasites such as fleas bite their hosts, they produce small wounds along which oral secretion is introduced. These secretions have antigenic properties that induce immunological responses in the host (Benjamins et al. 1960, Baron and Weintraub 1987), including the great tit (De Coster et al. 2010). Hen fleas have multiple negative effects on behavioural, physiological and reproductive traits of great tits (Richner et al. 1993, Christe et al. 1996b). Yet, mothers that are exposed to hen fleas before egg-laying are able to reduce the deleterious effects on nestling mortality and condition (Heeb et al. 1998, Buechler et al. 2002), indicating the occurrence of parasite-induced maternal effects. Furthermore, the sensitivity to fleas is sex-specific with male nestlings being more negatively affected (Tschirren et al. 2003). By combining a cross-foster experiment with a parasite treatment, we did not only maximize differences between pre- and post-hatching environments of exchanged nestlings, but were also able to study whether, and to what extent, effects of cross-fostering were larger in stressful environments.

It has previously been shown that environmental stressors, such as parasite exposure and infection (Saino et al. 2002, Costantini 2008, Sorci and Faivre 2009) can result in oxidative stress because of the resulting upregulation of the immune system, which is the main physiological defence mechanism against parasites (Zuk and Stoehr 2002). The induction of an immune response may affect oxidative stress levels for at least three reasons (reviewed by Costantini and Møller 2009). First, reactive metabolites are generated during inflammatory immune responses to kill pathogens. However, these molecules might also damage host tissues, resulting in oxidative damage (Sorci and Faivre 2009). Second, the induction of an immune response increases metabolic activity (Demas et al. 1997) and can hence generate oxidative species (Finkel and Holbrook 2000). Third, mounting an immune response (Lochmiller and Deerenberg 2000), but possibly also the adjustment of other physiological and behavioural traits under parasite exposure (Richner et al. 1993, Christe et al. 1996b), may be energetically costly. This may result in a depletion of antioxidant defences to prevent or limit tissue damage if resources are limiting (von Schantz et al. 1999). Hence, effects of parasites on oxidative stress may mainly become apparent in organisms in (energetically) stressful conditions (van de Crommenacker et al. 2011b). Furthermore, there is some evidence for sex-specific variation in oxidative stress (Alonso-Alvarez et al. 2004, Wiersma et al. 2004, van de Crommenacker et al. 2011a), which may be related to sex-specific differences in the susceptibility to parasites (Poulin 1996, Schalk and Forbes 1997, Tschirren et al. 2003, Klein 2004).

Our experimental design allowed to test whether 1) the health status of cross-fostered nestlings is more strongly negatively affected than that of nestlings that develop in their own nest, 2) negative effects of parasite exposure are larger in cross-fostered nestlings, and 3) effects differ between sons and daughters.

Material and methods

Study area and pre-laying treatment

The study was conducted in spring 2009 in a population of great tits breeding in nest boxes in a forest near Gent, Belgium (for details see De Coster et al. 2010). Before the start of the breeding season, all nest boxes were thoroughly brushed to remove nest material and parasites from the previous breeding season. At an advanced stage of nest building (4.2 ± 0.5 d (±SE) before the first egg was laid), an ectoparasite treatment was performed with hen fleas collected from previous year’s nest material. All nests (n = 48) were first put in a closed plastic bag to prevent loss of humidity, and heat-treated for 3 min in a 700 Watt microwave oven to kill all nest organisms (Richner et al. 1993). Afterwards, half of the nests (n = 24) were inoculated with 40 hen fleas placed inside the nest cup (Heeb et al. 1996); the remaining 24 nests were left parasite-free. Only first clutches were included.

Post-laying treatment

Uninfested nests (P−) received two additional heat-treatments, i.e. after the start of egg-laying (3 eggs present at most) and during cross-fostering (see below). At the same time, infested nests (P+) were also transported to a microwave but infested with 20 extra fleas per nest (instead of being heat-treated) at each occasion. During these treatments, nests were temporarily replaced by previously heat-treated nest material so that eggs and nestlings could remain in their own nest box to minimize potential stress. A partial cross-foster experiment (Fig. 1) was carried out two days after hatching. Half broods were reciprocally swapped between pairs of infested (9.0 ± 0.3 nestlings) and uninfested (9.0 ± 0.4 nestlings) nests with the same hatching date. When cross-fostering, all nestlings were marked with a non-toxic permanent colour marker pen (Pentel Maxiflo NLF50) to allow identification, weighed and ranked according to their body mass. In each pair of nests, the heaviest young of each nest and then every second
Post-hatching sampling and measurements

A total of 382 nestlings (94 P+P+; 107 P−P−; 87 P−P+; 94 P+P−) were ringed at the age of 6 d, and when nestlings were 15-d old, a blood sample (150 μl) was collected in heparinized capillary tubes via brachial vein puncture. Blood was stored under cool conditions in the field and centrifuged (10,000 g for 5 min) later that day. Plasma was separated from the cells and frozen at −20°C. Blood cells were used to sex the nestlings following the protocol of Griffiths et al. (1998). This PCR-based technique involves amplification of homologous fragments of chromohelicase (CHD) gene located on both Z and W sex chromosomes. Immediately after blood sampling, nestlings were weighed and tarsus and wing lengths were measured, and the latter two were combined in one measure of body size by means of a principal component analysis. As the first principal component (PC1) for each sex separately was highly correlated with PC1 for both sexes pooled (p = 92.9%; p < 0.0001), the latter was used as a measure of body size (Costantini et al. 2010). After the breeding season (July 2009–February 2010), 31 first-year birds (8 P+P+; 6 P−P−; 10 P−P+; 7 P+P−; 8.2% of fledglings) were recaptured with mist nets with efforts spread across the study area. All recaptured birds were captured at least once before October 2009, suggesting that our recapture effort was adequate to recapture most first-year birds residing in the forest.

Oxidative stress analysis

Oxidative stress results from an imbalance between reactive species and antioxidants. Valid inference should therefore be based on a measure of both components (Costantini and Verhulst 2009). After the breeding season, oxidative stress levels were quantified in blood plasma using two complementary assays which are known to accurately reflect oxidative stress levels in birds and mammals (Brambilla et al. 2001, Costantini and Dell’Omo 2006): the OXY-Adsorbent test and the d-ROMs test (Diacon, Grosseto, Italy) measuring total plasma antioxidant capacity (hereafter OXY) and reactive oxygen metabolites (ROMs; primarily hydroperoxides), respectively. The OXY-Adsorbent test quantifies the ability of the antioxidant barrier, including both exogenous and endogenous antioxidants, to resist the oxidant action of hypochlorous acid (HClO). Analyses were carried out following Costantini and Dell’Omo (2006) (volume: oxidant HClO-based solution 200 μl, chromogen 5 μl, calibrator 5 μl, sample 5 μl; dilution: calibrator 1:100, sample 1:100; incubation 10 min at 37°C). Reactive oxygen species are very reactive with organic molecules, generating ROMs after an oxidizing attack. ROMs also have oxidizing power, but are fairly stable and can therefore be quantified. Analyses of the d-ROMs test were carried out following the manufacturer’s protocol (buffer 400 μl, chromogen 4 μl, calibrator 10 μl, sample 20 μl, incubation 90 min at 37°C). At the end of both procedures, the absorbance of the obtained complex was measured with a spectrophotometer at wavelengths 505 and 546 nm, after which the mean of both values was calculated as a measure of OXY (in mM HClO neutralized per plasma volume) and ROMs (in Carratelli Units with 1 CARR U equivalent to 0.08 mg dl⁻¹ H₂O₂), respectively (but see also below). Plasma samples were randomly assigned to assays. The inter-assay variation at 505 and 546 nm were 8.3 and 6.5% for the OXY-test, and 6.2 and 5.8% for the d-ROMs-test, respectively. Lipemic plasma had a higher absorbance than non-lipemic plasma in the d-ROMs test and plasma colour (yellow, orange or red) affected absorbance in both tests (all p < 0.01), with differences in plasma colour probably...
a result of haemolysis during blood sampling. Therefore, and also to correct for differences between assays, residual ROMs and OXY were calculated from a linear mixed model (see below for random effects) with lipemic state (only for ROMs) and plasma colour and assay ID (for both ROMs and OXY) as explanatory variables. These residual measures were used as response variables in the statistical analyses instead of the original ROM and OXY measures.

**Statistical analysis**

We first tested whether nestling plasma ROMs and OXY were related to body size and body mass by means of general linear mixed models (LMMs), thereby also including sex and the two-way interaction with sex whenever significant (models 1–4; Supplementary material Appendix 1, Table A1).

We then tested whether nestling plasma ROMs, OXY, body size and body mass differed between matching and mismatching pre- and post-hatching environments by means of LMMs. Models also included sex and post-hatching treatment wherever these factors were significant (models 5–8; Supplementary material Appendix 1, Table A1).

We also tested whether post-hatching treatment and sex effects (and two-factor interactions) on ROMs, OXY, body size and body mass differed between individuals exposed to matching or mismatching environments by means of contrast statements (models 9–12; Supplementary material Appendix 1, Table A1). To correct for multiple testing, a sequential Bonferroni-type correction was applied to the p-values (Holm 1979). Three-factor interactions were not modelled due to lack of power as a consequence of our complex experimental design in relation to the sample size. In particular, the power for detecting the observed differences (Results section) at the 5% level of significance is 35 and 50% for ROM and OXY levels, respectively (Verbeke and Molenberghs 2000). All models with ROMs as response variable (models 5 and 9; Supplementary material Appendix 1, Table A1) were controlled for body size as both variables were related (see models 1 and Results).

Finally, we tested whether post-hatching treatment and sex effects (and two-factor interaction) on post-fledging survival differed between individuals exposed to matching or mismatching environments (model 13, Supplementary material Appendix 1, Table A1), whether OXY and ROM levels were related to post-fledging survival and whether this relation was affected by nestling sex (model 14, Supplementary material Appendix 1, Table A1). We therefore applied two generalized linear mixed models with logit link and adaptive Gaussian quadrature. As body mass and laying date are known to affect post-fledging survival (Naef-Daenzer et al. 2001, Verhulst and Nilsson 2008), both variables and their interaction term were added as covariates.

To ascertain that any possible sex effect was not simply caused by parasitized-induced changes in nest sex ratio or by partial cross-fostering inducing a sex-ratio shift, we fitted two generalized linear models with logit link. Sex ratio in the nest of origin or rearing was thereby considered as the response of interest and pre-hatching or post-hatching treatment as explanatory variable, respectively (models 15–16, Supplementary material Appendix 1, Table A1).

All mixed models contained nest of origin and nest of rearing as random factors to account for similarities between nestlings hatched and/or reared in the same nest. Effects of nest of origin were nested within nest of rearing (Kunz and Ekman 2000). We used restricted maximum likelihood (REML) parameter estimation for LMMs to obtain unbiased estimates of variance components, and likelihood ratio test statistics to test if variances differed significantly from zero (Verbeke and Molenberghs 2000). Fixed effects were estimated from the most parsimonious model obtained after the sequential removal of non-significant effects. Degrees of freedom for LMMs were estimated following the method described by Kenward and Roger (1997). All statistical analyses were performed in SAS 9.2 (SAS Inst. 2002–2003, Cary, NC, USA).

**Results**

**Variation in oxidative stress**

ROM levels were lower if pre- and post-hatching environments matched (F$_{1,39} = 4.52$, p = 0.040) and were also lower in daughters (F$_{1,364} = 4.61$, p = 0.032). Subsequent analyses showed that these results were mainly caused by the fact that the effect of the post-hatching treatment differed between both sexes in matching environments (F$_{1,355} = 7.68$, p = 0.012; Fig. 2): daughters showed significantly lower ROM levels than sons in infested nests (F$_{1,352} = 15.37$, p = 0.0002; Fig. 2), but not in uninfested ones.

![Figure 2. Effect of matching and mismatching pre- and post-hatching parasite environments on reactive oxygen metabolite (ROM) levels (+SE) for female and male nestlings. Darker bars refer to environments that involved more parasite infestations. Asterisks denote significant differences (*p<0.05; **p<0.01) within (represented by arrows) and between (asterisks in between bars) the sexes for a particular combination of pre- and post-hatching environments. For ease of visual interpretation original instead of residual dROM levels are depicted.](image-url)

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Figure 2. Effect of matching and mismatching pre- and post-hatching parasite environments on reactive oxygen metabolite (ROM) levels (+SE) for female and male nestlings. Darker bars refer to environments that involved more parasite infestations. Asterisks denote significant differences (*p<0.05; **p<0.01) within (represented by arrows) and between (asterisks in between bars) the sexes for a particular combination of pre- and post-hatching environments. For ease of visual interpretation original instead of residual dROM levels are depicted.
(F_{1,361} = 0.03, p = 0.86; Fig. 2). However, when pre- and post-hatching environments were different, no sex-specific differences were found in relation to post-hatching treatments (F_{1,361} = 0.17, p = 0.68; Fig. 2). When comparing ROM levels of daughters among environments, we found that the lowest ROM levels occurred in parasitized daughters developing in matching environments (Fig. 2). These levels tended to be lower than those of unparasitized daughters in matching environments (F_{1,160} = 3.80, p = 0.053; Fig. 2), and were significantly lower than those of parasitized (F_{1,137} = 6.05, p = 0.031; Fig. 2) and unparasitized (F_{1,189} = 10.30, p = 0.005; Fig. 2) daughters in mismatching environments. In sons, ROM levels tended to differ between parasitized and non-parasitized individuals developing in matching environments (F_{1,196} = 3.53, p = 0.062; Fig. 2), but not among other groups (all p > 0.23). Neither nest of origin nor nest of rearing explained a significant part of the total variability in ROMs (both p > 0.33). Finally, ROM levels negatively covaried with body size (estimate ± SE: −1.67 ± 0.62, F_{1,250} = 7.29, p = 0.0073) while correcting for offspring sex (p = 0.021). This effect was mainly caused by a negative relation between body size and ROM in daughters (estimate ± SE: −1.90 ± 0.84, F_{1,320} = 5.06, p = 0.025), as a similar relation in sons was not significant (estimate ± SE: −1.41 ± 0.89, F_{1,325} = 2.50, p = 0.12). Body mass was not related with ROM levels (p = 0.55).

With respect to OXY levels, the effect of the post-hatching treatment differed between both sexes when pre- and post-hatching environments matched (F_{1,370} = 8.21, p = 0.009): OXY levels of sons were higher than those of daughters in uninfested nests (F_{1,370} = 5.85, p = 0.032; Fig. 3), whereas OXY levels of daughters tended to be higher in infested nests (F_{1,370} = 2.75, p = 0.098; Fig. 3). Comparing OXY levels among environments within each sex, OXY levels of daughters were higher in infested than in uninfested nests (F_{1,370} = 5.02, p = 0.026; Fig. 3), whereas OXY levels of sons tended to be lower in infested nests (F_{1,370} = 3.31, p = 0.069; Fig. 3). In contrast, when pre- and post-hatching environments did not match, the effect of the post-hatching treatment did not depend on the sex (F_{1,370} = 0.00, p = 0.99), neither did OXY levels differ between matching or mismatching environments when averaged over both sexes and post-hatching treatments (F_{1,370} = 0.38, p = 0.54). Neither nest of origin nor nest of rearing explained a significant part of the total variability in OXY (both p = 1). Body size was not related to OXY levels (p = 0.79). Yet, the interaction between body mass and sex on OXY levels was marginally significant (p = 0.055) with OXY levels of female daughters tending to increase with body mass (estimate ± SE: 4.72 ± 2.42, F_{1,373} = 3.80, p = 0.052), while such an effect was not observed in sons (p = 0.44).

**Variation in nestling condition and post-fledging survival**

Overall, nestlings from matching and mismatching environments did not differ in body size (F_{1,445} = 0.28, p = 0.60). However, daughters were smaller than sons (F_{1,328} = 94.17, p < 0.0001), and this sexual dimorphism tended to be larger in case of mismatching pre- and post-hatching environments mainly due to smaller daughters in mismatching environments (F_{1,326} = 3.49, p = 0.063; Fig. 4). Daughters also weighed less than sons (F_{1,329} = 112.78, p < 0.0001), however, this dimorphism was not affected by the matching of pre- and post-hatching environments (F_{1,433} = 1.17, p = 0.29). Neither body size nor mass were affected by the post-hatching treatment (all p > 0.56). Nest of origin (22 and 20%) and nest of rearing (17 and 23%) explained a significant part of the total variance in body size and mass, respectively (all p < 0.017).

Figure 3. Effect of matching and mismatching pre- and post-hatching parasite environments on total plasma antioxidant capacity (OXY) levels (+SE) for female and male nestlings. Darker bars refer to environments that involved more parasite infestations. Reported p-values are those for the interaction between post-hatching treatment and sex for matching and mismatching environments. The asterisks denote significant differences (p < 0.05) within (represented by arrows) and between (asterisks in between bars) the sexes for a particular combination of pre- and post-hatching environments. For ease of visual interpretation original instead of residual OXY levels are depicted.

Figure 4. Interactive effect of sex and the pre- and post-hatching parasite environments on nestling’s body size (±SE).
Finally, post-fledging survival tended to be higher in daughters (F1,289 = 3.19, p = 0.075), but this trend was independent of the level of matching of pre- and post-hatching environments, the post-hatching treatment or OXY and ROM levels (all p > 0.11).

Sex ratios within nests of origin were not affected by the parasite treatment, nor was there any relation between the parasite treatment and the sex ratio after partial cross-fostering (both p > 0.41).

Discussion

Being raised in a foster nest may reduce an organism’s condition and health status. Here, we found that ROM levels of great tit nestlings were higher after cross-fostering. This was mainly caused by the fact that daughters that were raised in their own nest showed lower ROM levels, but only if they were exposed to parasites, than daughters from all other treatment combinations. These daughters also showed lower ROM levels and tended to show higher OXY levels than sons under matching high parasite pressure. Oxidative stress levels were hence lowest (i.e. lowest ROM levels and high OXY levels) in nestlings that developed in their own parasite-infested nests. On the contrary, under matching low parasite pressure, there was no difference between the sexes in ROMs, but daughters showed lower OXY levels than sons. Sons that stayed in their own nest hence experienced slightly less oxidative stress than when reared under low parasite exposure. Oxidative stress levels of nestlings reared in a foster nest were relatively high and did not differ between the sexes or parasite treatments. Also, other measures of nestling health status, such as body mass, were not, or only slightly, affected by cross-fostering and parasite exposure.

The observation that oxidative stress levels are somewhat lower when offspring are reared by their own mother suggests the occurrence of maternal effects, though our results showed that the outcome of such maternal effects depends on offspring sex and environmental conditions (here, whether or not exposed to parasites). As parasitized mothers had already been exposed to parasites before egg-laying, the maternal effect may be caused by a parasite-induced modification of egg investment to help offspring coping with high parasite loads (Buechler et al. 2002, Gasparini et al. 2002, Tschirren et al. 2004). Only daughters seemed to benefit from such a parasite-induced maternal effect, suggesting that maternal investment in egg yolk, nestling susceptibility to such investment, or costs induced by maternal adjustments, differed between sexes (DeWitt et al. 1998, Groothuis et al. 2005, Badyaev et al. 2006a, b, De Neve et al. 2008). Mechanisms underlying such a sex-specific parasite-induced maternal effect remain hypothetical, but may be related to antioxidant or testosterone deposition, since both substances have been related to maternal parasite and antigen exposure (Saino et al. 2002, Tschirren et al. 2004), sex-specific investment (Silverin and Sharp 1996, Verboven et al. 2005, Badyaev et al. 2006b) and oxidative stress levels (Chainy et al. 1997, Zhu et al. 1997, Alonso-Alvarez et al. 2007). Apart from testosterone, other steroid hormones such as oestrogen and glucocorticoid have also been shown to affect oxidative stress (Zhu et al. 1997, Borrás et al. 2003, Viña et al. 2006, Costantini et al. 2011), but it is yet unclear whether, and to what extent, their concentrations vary with maternal parasite exposure and differ between sexes.

A parasite-induced maternal effect may also be caused by an increase in parental food provisioning in response to nest parasites (Christe et al. 1996a, Bouslama et al. 2002), possibly mediated by increased nestling begging intensity (Christe et al. 1996a). This behavioural adjustment may not only directly affect offspring body condition and health status but also the amount of antioxidants that the latter receive with food. Furthermore, the observation that food distribution is more unequal among nestlings of infested nests (Christe et al. 1996a) and the fact that hen fleas reduce body mass and size of great tit nestlings (Richner et al. 1993, Christe et al. 1996a) most strongly in males (Tschirren et al. 2003) indicate that higher oxidative stress levels in sons might also be a result of sex-specific differences in food intake, despite the absence of evidence that parents can effectively discriminate between daughters and sons while feeding (Michler et al. 2010).

A parasite-induced maternal effect that protects great tit offspring from the adverse effects of parasites has previously been suggested as nestlings from flea-exposed mothers were heavier and grew faster than those of unexposed ones in the presence of fleas (Heeb et al. 1998, Buechler et al. 2002). However, in our study, effects of poor phenotype-environment matching and the parasite treatment were not observed on nestling body mass or size. Furthermore, post-fledging survival was not affected by any of both treatments, nor was it related to oxidative stress levels. These results suggest that negative consequences of parasite exposure on the offspring were rather low and the lack of carry-over effects of parasites during development. Similarly, a recent study in Sechelles warblers Acrocephalus sechellensis found no relation between malaria infection and body condition, despite increased oxidative stress in infected birds (van de Crommenacker et al. 2011b). Earlier, it has been suggested that the expression of parasite-induced maternal effects on nestling condition may be context-dependent (Gallizzi et al. 2008), e.g. stronger under harsh environmental conditions when low food availability might prevent parents to compensate for adverse energetic effects of parasite exposure by increasing their food provisioning rate to nestlings (Duва и and Allander 1996). However, the high number of fledglings per nest and high mean fledgling mass compared to previous breeding seasons in the same study area (De Coster unpubl.) suggest that environmental conditions were relaxed during our study. Under such conditions, adverse effects of increased parasite loads on nestling body condition can be expected to be masked, in spite of the observed effect on oxidative stress levels. Alternatively, as oxidative damage accumulates with age and effects are linked with ageing and the development of age-related diseases (Harman 1956, Finkel and Holbrook 2000), negative effects of oxidative stress might only become visible in older birds.

In addition to maternal effects, flea infestation may also have triggered a physiological defence mechanism that is stronger, or only present, in daughters. For example, an elevated free radical production in daughters exposed to parasites might have led to increased antioxidant levels...
result in increased metabolic rate (Romero 2004, Berthouly 2007a) and hence more oxidative stress (Finkel and Holbrook 2000). Furthermore, the mismatch between parental and offspring phenotypes induced by nestling exchange may also have caused negative effects in offspring, such as higher oxidative stress levels. In favour of this hypothesis is the fact that inflammatory immune responses of great tit nestlings are lower after cross-fostering (Berthouly et al. 2007). Also, in domesticated canaries Serinus canaria, cross-fostered nestlings grow slower than those raised by their own parents, because of the disruption of the prenatal signals which enable parents to adjust the begging behaviour of their offspring to their own provisioning behaviour (Hinde et al. 2010). Because of the significance of offspring begging and parental feeding in reducing negative effects of parasites (Christe et al. 1996a, Bouslama et al. 2002; see also above), such a parental-offspring phenotype mismatch may hence also explain the observed results. Future studies should therefore investigate whether such mechanism can also (or rather) explain the observed oxidative stress levels by cross-fostering whole clutches between tetrads of nests, that is by exchanging half of the nestlings between pairs of infested and uninfested nests and the other half between nests subjected to the same treatment.

**Acknowledgements** – We are grateful to A. d’Ursel and A. Beck for allowing us access to the forest, H. Matheve and D. Hendriks for help with fieldwork, E. Mulder for laboratory assistance, G. Verbeke (Univ. of Hasselt, Belgium) for statistical advice. This study was conducted with permission from the Animal Ethics Committee of Ghent Univ. (ECP 08/05). GDC was supported by a doctoral grant and by FWO research community WO.037.10N from the Research Foundation Flanders (FWO). SV was supported by a Vici-grant from the Netherlands Organisation for Scientific Research (NWO).

**References**


Supplementary material (Appendix J5551 at <www.oikosoffice.lu.se/appendix>). Appendix I.
Supplementary material

J5551
### Appendix 1

**Table A1** Overview of all fitted full models. Non-significant effects were sequentially removed to obtain parameter estimates. Terms included in final models are underlined.

<table>
<thead>
<tr>
<th>Model identity</th>
<th>Statistical model</th>
<th>Response variable</th>
<th>Explanatory variables</th>
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<td>size</td>
<td>matching sex treatment</td>
</tr>
<tr>
<td>8</td>
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<td>mass</td>
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<tr>
<td>9</td>
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<td>ROM</td>
<td>within matching/mismatching: sex treatment sex*treatment size</td>
</tr>
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<td>10</td>
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<td>mass</td>
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<tr>
<td>13</td>
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<td>Survival</td>
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<td>ROM OXY sex ROM<em>sex OXY</em>sex mass date mass*date</td>
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<td>Sex ratio</td>
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</tr>
<tr>
<td>16</td>
<td>GLM</td>
<td>Sex ratio</td>
<td>within nest of rearing: treatment</td>
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LMM: general linear mixed model  
GLMM: generalized linear mixed model  
GLM: generalized linear model