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

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Running headline: Maternal effects on oxidative stress

Summary

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.

Keywords: Antioxidants, birds, cross-fostering, ectoparasites, great tit, hen fleas, host-parasite interaction, oxidative status

Introduction

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 in 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 (e.g. Groothuis et al. 2005, Badyaev et al. 2006a, De Neve et al. 2008, Badyaev et al. 2006b, Jones et al. 2009).

52 Maternal effects can act on a broad range of offspring morphological and physiological traits
53 (e.g. Todd et al. 2011, Marshall 2008, Naguib and Gil 2005). However, to the best of our
54 knowledge, evidence for maternal effects on offspring oxidative stress is still lacking, despite
55 the fact that persistent oxidative stress can contribute to ageing and various disorders
56 (Harman 1956, Finkel and Holbrook 2000). Oxidative stress is defined as the rate at which
57 oxidative damage to biomolecules is generated after the exposure to reactive species that are
58 e.g. produced in the body as a result of oxidative metabolism (Costantini and Verhulst 2009,
59 Finkel and Holbrook 2000). Organisms have evolved antioxidants, which are obtained from
60 diet or can be produced endogenously, to defend against oxidative stress (Halliwell and
61 Gutteridge 2007). The large transfer of maternal antioxidants to egg yolk to protect
62 developing offspring against oxidative stress (Blount et al. 2000, McGraw et al. 2005, Surai
63 and Speake 1998) and the great variability in oxidative stress levels (reviewed in Monaghan
64 et al. 2009, Metcalfe and Alonso-Alvarez 2010) and in maternal antioxidant transfer in
65 relation to environmental conditions (Blount et al. 2002, Royle et al. 2003) suggest that
66 oxidative stress may be modified by maternal effects.
67
68 Here, we investigate maternal effects on nestling oxidative stress (reactive oxygen
69 metabolites and antioxidant capacity), body condition (body size and mass) and post-
70 fledgling survival in the great tit (*Parus major* L.). In particular, we study whether these
71 characteristics are negatively affected when nestlings are reared by foster parents in a foreign
72 nest through a cross-foster experiment. Being raised in a foster nest - hence being exposed to
73 a new nest, new parents and/or new pathogens - may not only disrupt the match between
74 offspring phenotype, maternal phenotype and environment, but may also induce a stress
75 response that deteriorates individual performance (Berthouly et al. 2007). In addition, we
76 manipulated parasite exposure, starting before egg-laying, by means of hen fleas

(*Ceratophyllus gallinae* Schrank). When blood-sucking ecoparasites 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 (Baron and Weintraub 1987, Benjamini et al. 1960), including the great tit (De Coster et al. 2010). Hen fleas have multiple negative effects on behavioural, physiological and reproductive traits of great tits (Christe et al. 1996b, Richner et al. 1993). Yet, mothers that are exposed to hen fleas before egg-laying are able to reduce the deleterious effects on nestling mortality and condition (e.g. Buechler et al. 2002, Heeb et al. 1998), 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 (Sorci and Faivre 2009, Costantini 2008, Saino et al. 2002) 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 in 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 (see also van de Crommenacker et al. 2011b). Furthermore, there is some evidence for sex-specific variation in oxidative stress (e.g. 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 (e.g. Tschirren et al. 2003, Klein 2004, Poulin 1996, Schalk and Forbes 1997).

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

Materials 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 Ghent, 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 days (± 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 (see 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

142 the same hatching date. When cross-fostering, all nestlings were marked with a non-toxic
143 permanent colour marker pen (Pentel Maxiflo NLF50) to allow identification, weighed and
144 ranked according to their body mass. In each pair of nests, the heaviest young of each nest
145 and then every second nestling in the mass-based rank was alternately assigned to stay in the
146 nest of origin or to be exchanged between nests, until the clutch size of the smallest nest was
147 reached. Nestlings were kept warm in a warmed padded box during cross-fostering to
148 minimize potential stress. Nestlings remaining in the nest of origin were also handled and
149 removed from their nest to make the treatment of cross-fostered and non-cross-fostered
150 nestlings as similar as possible. Nestlings that remained in their own nest experienced the
151 same conditions before and after cross-fostering (i.e. P+P+ or P-P-), while cross-fostered
152 ones experienced different conditions (i.e. P-P+ or P+P-). Although cross-fostered nestlings
153 were removed from the nest for a longer time (cross-fostered 18.2 ± 0.5 min; non-cross-
154 fostered: 7.8 ± 0.5 min), cross-foster duration had no significant effect on nestling body mass
155 or size (see below) or measures of oxidative stress in any of both groups of nestlings (all $P >$
156 0.20). Four nests were deserted after cross-fostering (3 P+, 1 P-). From the other nests, all but
157 4 nestlings survived until fledging. Nests were collected at the day of fledging and stored at
158 4°C. Previous tests on the same set of nests showed that numbers of flea larvae were
159 significantly increased in flea-infested nests (De Coster et al. 2010), which validates our
160 parasite treatment.

161 FIGURE 1 ABOUT HERE

162

163 Post-hatching sampling and measurements

164

A total of 382 nestlings (94 P+P+; 107 P-P-; 87 P-P+; 94 P+P-) were ringed at the age of 6 days, and when nestlings were 15-days 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 (ρ = 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 (e.g. Brambilla et al. 2001, Costantini and Dell'Omo 2006): the OXY-Adsorbent test and the d-ROMs test (Diacron, Grosseto, Italy) measuring

190 total plasma antioxidant capacity (hereafter OXY) and reactive oxygen metabolites (ROMs;
191 primarily hydroperoxides), respectively. The OXY-Adsorbent test quantifies the ability of the
192 antioxidant barrier, including both exogenous and endogenous antioxidants, to resist the
193 oxidant action of hypochlorous acid (HClO). Analyses were carried out following Costantini
194 and Dell'Omo (2006) (volume: oxidant HClO-based solution 200 µl, chromogen 5 µl,
195 calibrator 5 µl, sample 5 µl; dilution: calibrator 1:100, sample 1:100; incubation 10 min at
196 37ºC). Reactive oxygen species are very reactive with organic molecules, generating ROMs
197 after an oxidizing attack. ROMs also have oxidizing power, but are fairly stable and can
198 therefore be quantified. Analyses of the d-ROMs test were carried out following the
199 manufacturer's protocol (buffer 400 µl, chromogen 4 µl, calibrator 10 µl, sample 20 µl,
200 incubation 90 min at 37ºC). At the end of both procedures, the absorbance of the obtained
201 complex was measured with a spectrophotometer at wavelengths 505 nm and 546 nm, after
202 which the mean of both values was calculated as a measure of OXY (in mM HClO
203 neutralized per plasma volume) and ROMs (in Carratelli Units with 1 CARR U equivalent to
204 0.08 mg/dl H_2O_2), respectively (but see also below). Plasma samples were randomly assigned
205 to assays. The inter-assay variation at 505 nm and 546 nm were 8.3% and 6.5% for the OXY-
206 test, and 6.2% and 5.8% for the d-ROMs-test, respectively. Lipemic plasma had a higher
207 absorbance than non-lipemic plasma in the d-ROMs test and plasma colour (yellow, orange
208 or red) affected absorbance in both tests (all $P < 0.01$), with differences in plasma colour
209 probably a result of haemolysis during blood sampling. Therefore, and also to correct for
210 differences between assays, residual ROMs and OXY were calculated from a linear mixed
211 model (see below for random effects) with lipemic state (only for ROMs) and plasma colour
212 and assay ID (for both ROMs and OXY) as explanatory variables. These residual measures
213 were used as response variables in the statistical analyses instead of the original ROM and
214 OXY measures.

215

216 Statistical analysis

217

218 We first tested whether nestling plasma ROMs and OXY were related to body size and body
219 mass by means of general linear mixed models (LMMs), thereby also including sex and the
220 two-way interaction with sex whenever significant (model 1-4; Table A1).
221 We then tested whether nestling plasma ROMs, OXY, body size and body mass differed
222 between matching and mismatching pre- and post-hatching environments by means of
223 LMMs. Models also included sex and post-hatching treatment wherever these factors were
224 significant (model 5-8; Table A1).
225 We also tested whether post-hatching treatment and sex effects (and two-factor interactions)
226 on ROMs, OXY, body size and body mass differed between individuals exposed to matching
227 or mismatching environments by means of contrast statements (model 9-12; Table A1). To
228 correct for multiple testing, a sequential Bonferroni-type correction was applied to the *P*-
229 values (Holm 1979). Three-factor interactions were not modelled due to lack of power as a
230 consequence of our complex experimental design in relation to the sample size. In particular,
231 the power for detecting the observed differences (see Results section) at the 5% level of
232 significance is 35% and 50% for ROM and OXY levels, respectively (Verbeke and
233 Molenberghs 2000). All models with ROMs as response variable (model 5 and 9; Table A1)
234 were controlled for body size as both variables were related (see model 1 and Results).
235 Finally, we tested whether post-hatching treatment and sex effects (and two-factor
236 interaction) on post-fledging survival differed between individuals exposed to matching or
237 mismatching environments (model 13, Table 1A), whether OXY and ROM levels were
238 related to post-fledging survival and whether this relation was affected by nestling sex (model
239 14, Table 1A). 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 (e.g. 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, 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 (e.g. 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 Institute Inc. 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 ($F_{1,353}$= 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 ($F_{1,361}$ = 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 ±

285 SE: -1.41 ± 0.89; $F_{1,325}$ = 2.50; P = 0.12). Body mass was not related with ROM levels (P =
286 0.55).

287 FIGURE 2 ABOUT HERE

288

289 With respect to OXY levels, the effect of the post-hatching treatment differed between both
290 sexes when pre- and post-hatching environments matched ($F_{1,370}$= 8.21, P = 0.009): OXY
291 levels of sons were higher than those of daughters in uninfested nests ($F_{1,370}$ = 5.85, P =
292 0.032; Fig. 3), whereas OXY levels of daughters tended to be higher in infested nests ($F_{1,370}$
293 = 2.75, P = 0.098; Fig. 3). Comparing OXY levels among environments within each sex,
294 OXY levels of daughters were higher in infested than in uninfested nests ($F_{1,370}$ = 5.02, P =
295 0.026; Fig. 3), whereas OXY levels of sons tended to be lower in infested nests ($F_{1,370}$ = 3.31,
296 P = 0.069; Fig. 3). In contrast, when pre- and post-hatching environments did not match, the
297 effect of the post-hatching treatment did not depend on the sex ($F_{1,370}$= 0.00, P = 0.99),
298 neither did OXY levels differ between matching or mismatching environments when
299 averaged over both sexes and post-hatching treatments ($F_{1,376}$= 0.38; P = 0.54). Neither nest
300 of origin nor nest of rearing explained a significant part of the total variability in OXY (both
301 P = 1). Body size was not related to OXY levels (P = 0.79). Yet, the interaction between
302 body mass and sex on OXY levels was marginally significant (P = 0.055) with OXY levels of
303 female daughters tending to increase with body mass (estimate ± SE: 4.72 ± 2.42; $F_{1,373}$ =
304 3.80; P = 0.052), while such an effect was not observed in sons (P = 0.44).

305 FIGURE 3 ABOUT HERE

306

307 Variation in nestling condition and post-fledging survival

308

309 Overall, nestlings from matching and mismatching environments did not differ in body size
310 ($F_{1,44.3}$ = 0.28; P = 0.60). However, daughters were smaller than sons ($F_{1,328}$ = 94.17; P <
311 0.0001), and this sexual dimorphism tended to be larger in case of mismatching pre- and
312 post-hatching environments mainly due to smaller daughters in mismatching environments
313 ($F_{1,326}$ = 3.49; P = 0.063; Fig. 4). Daughters also weighed less than sons ($F_{1,329}$ = 112.78; P <
314 0.0001), however, this dimorphism was not affected by the matching of pre- and post-
315 hatching environments ($F_{1,43.3}$ = 1.17; P = 0.29). Neither body size nor mass were affected by
316 the post-hatching treatment (all P > 0.56). Nest of origin (22 and 20%) and nest of rearing (17
317 and 23%) explained a significant part of the total variance in body size and mass, respectively
318 (all P < 0.017). Finally, post-fledging survival tended to be higher in daughters ($F_{1,289}$ = 3.19;
319 P = 0.075), but this trend was independent of the level of matching of pre-and post-hatching
320 environments, the post-hatching treatment or OXY and ROM levels (all P > 0.11).
321 FIGURE 4 ABOUT HERE
322
323 Sex ratios within nests of origin were not affected by the parasite treatment, nor was there
324 any relation between the parasite treatment and the sex ratio after partial cross-fostering (both
325 P > 0.41).
326

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 daughters 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 then daughters 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 (e.g. Buechler et al. 2002, Tschirren et al. 2004, Gasparini et al. 2002). 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 (e.g. Groothuis et al. 2005, Badyaev et al. 2006a, De Neve et al. 2008, Badyaev et al. 2006b, DeWitt et al. 1998). 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 (e.g. Saino et al. 2002, Tschirren et al. 2004), sex-specific investment (e.g. Verboven et al. 2005, Badyaev et al. 2006b, Silverin and Sharp 1996) and oxidative stress levels (e.g. Alonso-Alvarez et al. 2007, Zhu et al. 1997, Chainy et al. 1997). Apart from testosterone, other steroid hormones such as oestrogen and glucocorticoid have also been shown to affect oxidative stress (Zhu et al. 1997, Viña et al. 2006, Borrás et al. 2003, 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 (Bouslama et al. 2002, Christe et al. 1996a), 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 (Buechler et al. 2002, Heeb et al. 1998). 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 (Dufva 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, unpublished data) 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 (Costantini 2008, Barja 2002), which may, in turn, result in lower oxidative damage. The fact that daughters from infested nests showed very low ROM levels and increased levels of OXY supports this hypothesis. In contrast, sons tended to show high ROM and low OXY levels in infested nests, suggesting that their antioxidant system was not able to counteract the negative effects of parasites on oxidative stress. Possibly, parasites triggered other defence mechanisms in males. For example, it has been suggested that under harsh nest conditions, male jackdaw (*Corvus monedula*) offspring show increased levels of oxidative stress, while female offspring are more adversely affected in their growth (Salomons et al. 2009). Our results showed similar effects since females, but not males, tended to be smaller under mismatching pre- and post-hatching conditions. However, the negative relation between body size and ROMs and the tendency towards a positive relation between body mass and OXY in daughters (but not in sons) does not support the occurrence of a trade-off between investment in growth and oxidative stress coping. Rather, these relations suggest that all these characteristics reflect nestling condition. Particularly, daughters in good condition seem able to maintain low oxidative stress levels despite simultaneous investments in body size and mass, which is known to lead to increased metabolic activity and free radical production (reviewed in Balaban et al. 2005). Possibly, an elevated production of antioxidants helps daughters to actively buffer against an increased free radical production.

Despite the higher ROM levels in nestlings that developed in a foster nest, parasite exposure did not affect any of the measures of nestling health status when a nestling developed in a foster nest, nor did both sexes respond differently. This suggests that there are no additional

426 costs of post-hatching parasite exposure when reared in a foster nest, presumably because
427 negative effects of parasite exposure were low compared to those of cross-fostering.
428 However, because of our experimental design, all translocated nestlings were exposed to
429 parasites in some life-stage, i.e. before or after hatching. The latter (i.e. parasitized nestlings
430 from mothers that were not exposed to parasites) might be negatively affected because they
431 were not prepared to a parasitic environment by their mother, possibly resulting in a lower
432 parasite tolerance (Heeb et al. 1998). The former (i.e. offspring from parasitized mothers that
433 were reared in an environment without parasites) possibly produced a potentially costly
434 phenotype, which was in vain if the same stressor was not imposed on the offspring (but see
435 Gallizzi et al. 2008). Hence, an alternative explanation is that effects of direct (i.e. only post-
436 hatching) parasite exposure and maternal (i.e. only pre-hatching) parasite exposure are
437 similar.
438
439 In our experimental design, nestlings from matching environments not only experienced the
440 same parasite pressure before and after hatching but were also raised by their own parents
441 and in their own nest, while this was not the case for nestlings from mismatching
442 environments. Hence, high oxidative stress levels in mismatching nestlings may also be a
443 consequence of the stress induced by developing in a foster nest instead of being the
444 consequence of mismatching pre- and post-hatching parasite exposure. Stressful conditions,
445 such as developing in a foster environment, may result in increased metabolic rate (Romero
446 2004, Berthouly et al. 2007) and hence more oxidative stress (Finkel and Holbrook 2000).
447 Furthermore, the mismatch between parental and offspring phenotypes induced by nestling
448 exchange may also have caused negative effects in offspring, such as higher oxidative stress
449 levels. In favour of this hypothesis is the fact that inflammatory immune responses of great tit
450 nestlings are lower after cross-fostering (Berthouly et al. 2007). Also, in domesticated

451 canaries (*Serinus canaria*), cross-fostered nestlings grow slower than those raised by their
452 own parents, because of the disruption of the prenatal signals which enable parents to adjust
453 the begging behaviour of their offspring to their own provisioning behaviour (Hinde et al.
454 2010). Because of the significance of offspring begging and parental feeding in reducing
455 negative effects of parasites (Bouslama et al. 2002, Christe et al. 1996a; see also above), such
456 a parental-offspring phenotype mismatch may hence also explain the observed results. Future
457 studies should therefore investigate whether such mechanism can also (or rather) explain the
458 observed oxidative stress levels by cross-fostering whole clutches between tetrads of nests,
459 that is by exchanging half of the nestlings between pairs of infested and uninfested nests and
460 the other half between nests subjected to the same treatment.
461
462

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464

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473

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675
676

677 **Figure legends**

678

679 **Fig. 1.** Scheme of partial cross-foster protocol. Half broods (N = 24 pairs) were reciprocally
680 swapped (see arrows) between infested nests (P+; black edged) and uninfested nests (P-;
681 white edged). The two nest boxes at the top and at the bottom represent the pre- and post-
682 hatching environment, respectively. Mothers from black nestlings had been infested with
683 parasites before egg-laying, while mother from white nestlings had not. The cross-fostering
684 resulted in two groups: matching (i.e. P+P+ or P-P-) versus mismatching (i.e. P-P+ or P+P-)
685 pre- and post-hatching environments.

686

687 **Fig. 2.** Effect of matching and mismatching pre- and post-hatching parasite environments on
688 reactive oxygen metabolite (ROM) levels (+ SE) for female and male nestlings. Darker bars
689 refer to environments that involved more parasite infestations. Reported P-values are those
690 for the interaction between post-hatching treatment and sex for matching and mismatching
691 environments. Asterisks denote significant differences (*P < 0.05; **P < 0.01) within
692 (represented by arrows) and between (asterisks in between bars) the sexes for a particular
693 combination of pre- and post-hatching environments. For ease of visual interpretation original
694 instead of residual dROM levels are depicted.

695

696 **Fig. 3.** Effect of matching and mismatching pre- and post-hatching parasite environments on
697 total plasma antioxidant capacity (OXY) levels (+ SE) for female and male nestlings. Darker
698 bars refer to environments that involved more parasite infestations. Reported P-values are
699 those for the interaction between post-hatching treatment and sex for matching and
700 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.

Fig. 4. Interactive effect of sex and the pre- and post-hatching parasite environments on nestling's body size (± SE).

Figures

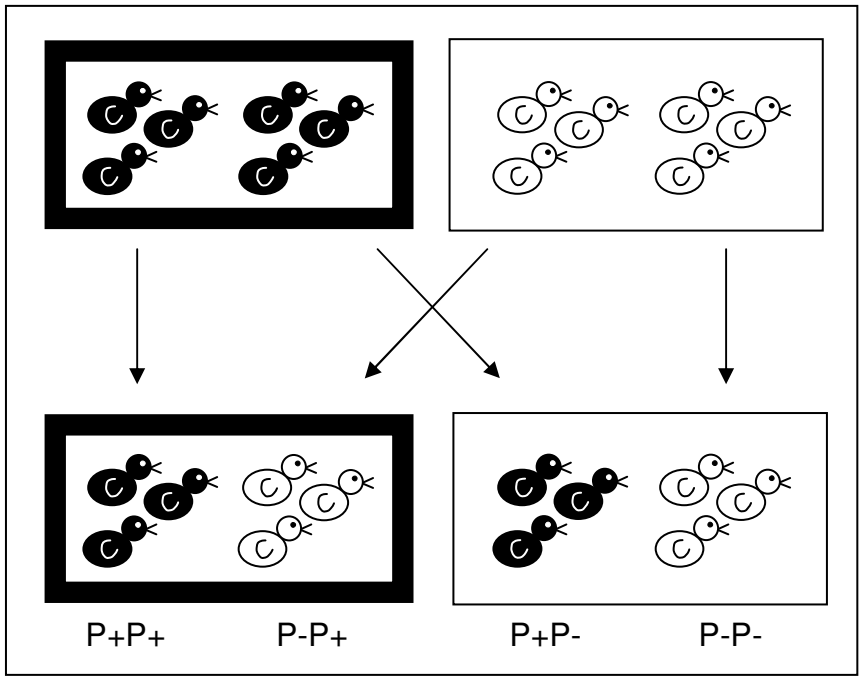

Fig. 1

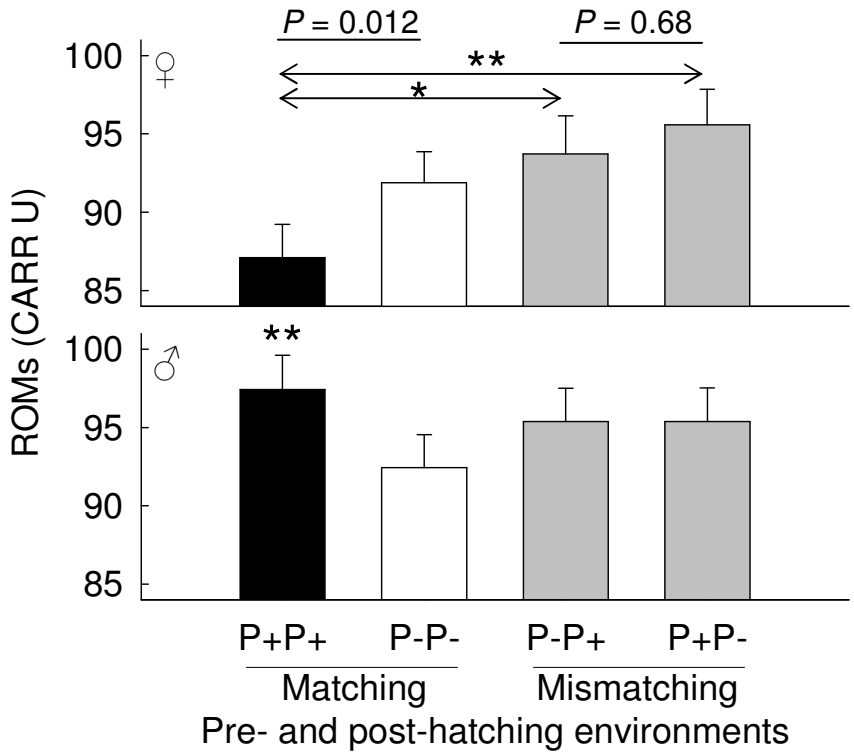
P = 0.012
P = 0.68
**
*
100
95
90
85
♀
♂
**
ROMs (CARR U)
P+P+
P-P-
P-P+
P+P-
Matching
Mismatching
Pre- and post-hatching environments

Fig. 2

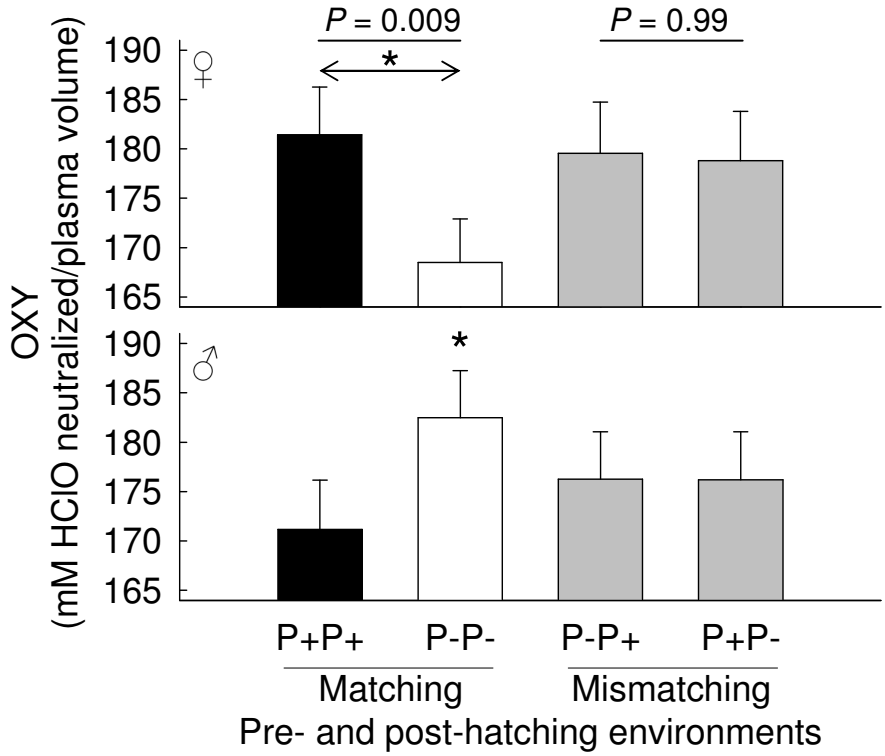
P = 0.009
P = 0.99
*
♀
♂
OXY
(mM HClO neutralized/plasma volume)
190
185
180
175
170
165
P+P+
P-P-
P-P+
P+P-
Matching
Mismatching
Pre- and post-hatching environments

Fig. 3

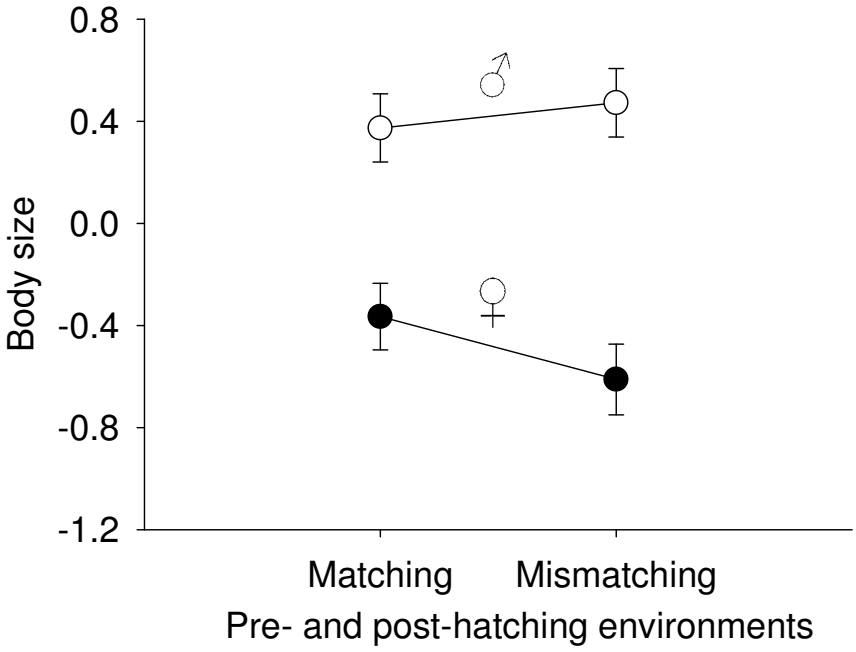
0.8
0.4
0.0
-0.4
-0.8
-1.2
Body size
♂
♀
Matching
Mismatching
Pre- and post-hatching environments

Fig. 4

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.

Model identity	Statistical model	Response variable	Explanatory variables
1	LMM	ROM	size sex size*sex
2	LMM	ROM	mass sex mass*sex
3	LMM	OXY	size sex size*sex
4	LMM	OXY	mass sex mass*sex
5	LMM	ROM	matching sex treatment size
6	LMM	OXY	matching sex treatment
7	LMM	size	matching sex treatment
8	LMM	mass	matching sex treatment
9	LMM	ROM	within matching/mismatching: sex treatment sex*treatment size
10	LMM	OXY	within matching/mismatching: sex treatment sex*treatment
11	LMM	size	within matching/mismatching: sex treatment sex*treatment
12	LMM	mass	within matching/mismatching: sex treatment sex*treatment
13	GLMM	Survival	within matching/mismatching: sex treatment sex*treatment mass date mass*date
14	GLMM	Survival	ROM OXY sex ROM*sex OXY*sex mass date mass*date
15	GLM	Sex ratio	within nest of origin: treatment
16	GLM	Sex ratio	within nest of rearing: treatment

LMM: general linear mixed model
GLMM: generalized linear mixed model
GLM: generalized linear model