

Original Article

Interactions between prenatal maternal effects and posthatching conditions in a wild bird population

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Received 13 December 2013; revised 4 July 2014; accepted 15 July 2014; Advance Access publication 26 August 2014.

Resources and cues provided by the mother before birth are important mediators of developmental plasticity. It has been suggested that the adaptive value of such prenatal maternal effects may depend on the environment encountered by the offspring after birth, and that offspring may perform better when environmental conditions encountered by the mother and the offspring match, than when a mismatch occurs. Here, we test how prenatal maternal effects and postnatal conditions interact in influencing offspring growth and development in wild-living great tits (*Parus major*) by manipulating food availability experienced by the mother before egg laying, partially cross-fostering nestlings between nests, and manipulating food availability after hatching. We observed significant interaction effects between pre- and postnatal food conditions. Nonsupplemented nestlings reached a similar fledging mass, a trait closely linked to postfledging survival, as food-supplemented nestlings when their biological mother had received extra food during egg laying. It shows that prenatal maternal investment can compensate for growth-limiting conditions after hatching. This effect was sex specific, with daughters benefiting more than sons. Furthermore, food-supplemented nestlings grew largest when their biological mother had not received extra food during egg laying, suggesting that offspring were primed prenatally, possibly through differential egg composition, to use resources more efficiently. However, we found no evidence that offspring performed generally better when pre- and postnatal food conditions matched than when a mismatch occurred. Our results demonstrate the importance of considering the postnatal environment when testing for the ecological and evolutionary consequences of prenatal maternal effects in natural populations.

Key words: anticipatory maternal effects, environmental change, environmental predictability, mismatch hypothesis, prenatal maternal effects, silver spoon, yolk androgens.

INTRODUCTION

In most species, it is the mother who provides the first environment an individual encounters in its life, even before it is born. This prenatal environment can have significant and long-lasting effects on an individual's morphology, physiology, and behavior, and is thereby an important determinant of individual variation in fitness (Mousseau and Fox 1998). Key mediators of such prenatal maternal effects are resources (e.g., nutrients, Georges et al. 1995; Christians 2002) and developmental cues (e.g., hormones, Groothuis et al. 2005) mothers pass on to their offspring during early development. The quality and quantity of maternal components transferred to the offspring depends, at least partly, on the environment mothers encounter before and during reproduction

(e.g., Gil et al. 1999; Tschirren et al. 2004; Fontaine and Martin 2006; Crean and Marshall 2008). Thereby, they allow mothers to convey information about local environmental condition to the developing young.

Such environmentally induced maternal effects are assumed to have evolved as an adaptation to heterogeneous, but predictable environments (Mousseau and Fox 1998; Agrawal et al. 1999; Galloway and Etterson 2007). In many species, young (and especially unborn) individuals have a far more limited ability to assess current and predict future environmental conditions than their mothers. Maternal cues that adaptively guide offspring developmental trajectories, and thereby help the offspring to cope better with the environment they will encounter, will therefore be favored by natural selection ("anticipatory maternal effects," Marshall and Uller 2007). However, phenotypic plasticity is costly and not unlimited (DeWitt et al. 1998), and organizational effects during early development are often irreversible (Hales and Barker 2001).

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Consequently, transgenerational programming can be selectively neutral, or may even become maladaptive, if environmental conditions change, and a mismatch between maternal and offspring environments occurs (Hales and Barker 2001; Rickard and Lummaa 2007; Wells 2007; Monaghan 2008).

In line with the hypothesis that the adaptive value of maternal effects depends on the stability or predictability of the environment, experimental studies demonstrated that a mismatch between the conditions experienced by mothers during reproduction and the conditions experienced by the offspring after birth can affect offspring performance. In American bellflower (*Campanula americana*), for example, offspring achieve higher fitness if they are grown in the same light environment as their mother, compared with plants grown in a mismatched environment (Galloway and Etterson 2007). Similarly, in sheep (*Ovis aries*) a mismatch between pre- and postnatal nutritional conditions leads to health problems in offspring, whereas no such effects are observed when pre- and postnatal food conditions match (Cleal et al. 2007). Finally, in canaries (*Serinus canaria domestica*), mothers modify their offspring's posthatching food demand, likely through differential allocation of androgens to the eggs, to match their own provisioning capacity (Hinde et al. 2009). However, despite these examples, a recent meta-analysis revealed that evidence for anticipatory maternal effects remains surprisingly weak, and that few studies have experimentally tested the "matching environment hypothesis" in natural vertebrate populations (Uller et al. 2013).

Whereas under the "matching environment hypothesis" we would predict that offspring perform better when pre- and postnatal conditions match than when a mismatch occurs, under alternative scenarios prenatal condition might affect offspring performance independent of the environment encountered after hatching (Marshall and Uller 2007). For example, beneficial conditions experienced by mothers before and during reproduction might allow them to transfer a higher quality or larger quantity of limited resources (e.g., nutrients) to the developing young. This may boost offspring performance independent of the environment encountered after birth ("silver spoon effect," Grafen 1988; Lindström 1999). Furthermore, in cooperatively breeding birds, it has been found that females encountering favorable conditions during reproduction may reduce (rather than increase) their reproductive investment in anticipation of the possibility for compensation by other family members during the posthatching period (Russell et al. 2007). Under these 2 alternative scenarios, prenatal maternal effects may have long-lasting effects on offspring performance, but these effects will not depend on a match or mismatch between pre- and postnatal conditions.

Here, we investigated how food-mediated prenatal maternal effects and postnatal conditions interact in shaping offspring growth and development in wild-living great tits (*Parus major*). We focused on food availability, rather than other ecological factors, because previous work on captive animals (Cleal et al. 2007; Hinde et al. 2009; van der Waaij et al. 2011) and humans (Hales and Barker 2001; Gluckman et al. 2008) suggested a particularly important role of interactions between food-mediated prenatal maternal effects and postnatal nutritional conditions in creating mismatch effects. Birds are particularly suited to investigate effects of the prenatal environment and its interaction with postnatal conditions on offspring performance in the wild because the embryo development takes place outside of the mother's body, facilitating the measurement of prenatal factors (e.g., Grootuis et al. 2005). Furthermore, the prenatal environment (i.e., egg size and composition) can easily

be separated from postnatal conditions by cross-fostering nestlings between nests. Yet, work with wild-living birds also puts some limitations on the traits that can be measured. For example, we here focused on fledging mass and size as fitness proxies (Tinbergen and Boerlijst 1990; Both et al. 1999; Naef-Daenzer et al. 2001), but could not measure physiological traits that were found to be affected by nutritional mismatches in humans studies (Hales and Barker 2001; Rickard and Lummaa 2007; Wells 2007).

In our study, we experimentally manipulated food availability encountered by mothers during egg laying and food availability during the rearing period in a 2×2 design. We partially cross-fostered nestlings between nests, which allowed us to disentangle effects of the pre- and postnatal treatments. The cross-fostering also ensured that half of the biological siblings and half of the nestmates of each rearing nest experienced a match between prenatal and posthatching conditions, whereas the other half experienced a mismatch. We tested 1) if food-mediated prenatal maternal effects have consequences for offspring growth and development after hatching, 2) if and how prenatal and posthatching conditions interact, and 3) if offspring performs better, in terms of early growth, if pre- and postnatal food conditions match than when a mismatch occurs. Finally, we measured egg size and yolk androgen concentrations to test if they mediate potential food-mediated prenatal maternal effects on offspring traits (as found in Christians 2002; Verboven et al. 2003; Gasparini et al. 2007; Hinde et al. 2009).

MATERIALS AND METHODS

Study species and experimental protocol

The study was conducted between April and June 2011 in a nest-box-breeding population of great tits (*P. major*) on the island of Gotland, Sweden. Nest-boxes were checked regularly to monitor the progress of nest building. After the birds had started to build their nest, we experimentally manipulated the food availability experienced by the female before and during egg laying by providing extra food in half of the nest-boxes. To this end, we attached a small plastic cup on the inside wall of all nest-boxes and, after the birds had started to build their nest, alternately assigned nests to the prelaying food supplementation (pre-F) or the control group (pre-NF). Pre-F nests received a food supplementation of 15 g of maggots (*Sarcophaga* spp.) placed in the plastic cup every other day until the clutch was completed. The pre-NF nests were visited and treated as the pre-F nests, but no food was added to their plastic cup. This prelaying food treatment was effective in influencing maternal egg provisioning, as evidenced by its significant effect on nestling mass and size (see Results for details). Because nestlings were cross-fostered (see following section), we can exclude the possibility that these prelaying effects were due to carry-over effects on adult food provisioning after hatching. The treatment started 4.6 ± 0.4 days before the first egg was laid. After the clutch was completed, the food supplementation stopped and females incubated their eggs without receiving extra food.

To create a match or mismatch between conditions experienced during egg laying and conditions experienced during the rearing period, and to control for potential carry-over effects of the prelaying treatment on parental provisioning after hatching, we carried out a partial cross-fostering 1 day after hatching (day 1) between a pre-F and a pre-NF nest with the same hatching date ($N = 52$ dyads). For the cross-fostering, nestlings were weighed and ranked according to their mass in their original nest. The heaviest

nestling was randomly assigned to stay in the nest of origin or to be moved to the foster nest. Cross-foster treatment (stay or go) was then alternated through the mass-based rank list. This procedure ensured that there were no initial weight differences between the 2 broods of a cross-foster dyad after cross-fostering, or between cross-fostered and noncross-fostered siblings raised in the original or a foster nest (see Results for details). For identification, nestlings were marked individually by clipping down feathers. During the transport between nests (mean transport time ± 1 standard deviation [SD]: 14 ± 7 min), nestlings were kept warm in a padded box to minimize potential stress. Nestlings that remained in the nest of origin were handled in the same way and removed from their nest-box for a similar duration as cross-fostered siblings to ensure that the treatment of the 2 cross-foster groups was as similar as possible.

After cross-fostering, 1 brood of each cross-foster pair was assigned to the posthatching food supplementation group (post-F, $N = 52$ broods), whereas the other received no extra food during the rearing period (post-NF, $N = 52$ broods). We alternated if the pre-F nest of a cross-foster dyad was assigned to the post-F or post-NF group. As for the prelaying treatment, post-F nests received 15 g of maggots (*Sarcophaga* spp.) placed in the plastic cup inside the nest-box every other day from cross-fostering (day 1) until day 13 posthatching. The post-NF nests were visited and treated as the post-F nests, but no food was added to their plastic cup.

Food is a limited resource for great tits during reproduction (van Noordwijk et al. 1995; Naef-Daenzer et al. 2001; Thomas et al. 2001). We can, therefore, assume that nonsupplemented broods experienced harsher conditions than food-supplemented broods. The faster growth and higher body mass of nestlings raised in food-supplemented nests in this (see Results for details) and a previous study (Tschirren et al. 2007a) is in line with this assumption. Video observation during this previous study confirmed that parents feed the provided maggots to the nestlings (Tschirren B, personal observation). However, we cannot exclude the possibility that parents ate a part of the maggots themselves, and that nestlings benefited indirectly, for example, through a higher provisioning of other than the supplemented food by well-fed parents.

Nestling measures

We measured nestling body mass before cross-fostering (day 1, $N = 790$ nestlings) and on day 14, shortly before fledging ($N = 584$ nestlings). Additionally, we measured nestling body mass twice during the period of linear body mass gain (day 5 and day 9) to assess the growth rate during the main growth period. Growth rate was calculated as (body mass 9 – body mass 5)/4. On day 14, we measured metatarsus length, a proxy of body size in birds, to the nearest 0.1 mm. When nestlings were 9 days old, they were ringed with a numbered aluminum ring and a small blood sample ($<20 \mu\text{L}$) was collected from the tarsal vein for molecular sex determination (as described in Tschirren et al. 2003). Nestling mortality between hatching and fledging was recorded. All procedures were conducted under licenses from the Swedish National Board for Laboratory Animals (S-54-11) and the Bird Ringing Centre of the Swedish Museum of Natural History (Stockholm, Sweden).

Egg composition

Because effects of the prelaying food supplementation on offspring morphology and survival are most likely mediated through differential egg composition, we analyzed 3 egg components that have previously been shown to be important mediators of prenatal

maternal effects in birds, namely, the total nutritional content of an egg (egg weight, Christians 2002; Krist 2011), and the concentrations of maternally derived yolk androstenedione (yolk A4) and yolk testosterone (yolk T) (Schwabl 1993; Verboven et al. 2003; Groothuis et al. 2005; Gasparini et al. 2007; Hinde et al. 2009).

For each clutch, we collected the fourth egg on the day it was laid. The mean clutch size (± 1 SD) in the study population was $8.5 (\pm 1.4)$ eggs. The fourth egg is thus one of the middle eggs in the laying sequence. On the same day, we weighed the egg, separated the yolk from the albumen, weighed the yolk, and froze it at -20°C until hormone analysis. Egg weight and yolk weight were highly correlated ($r = 0.723$, $P < 0.001$, $N = 97$), and we, therefore, only considered egg weight in the analyses. Including yolk weight instead of egg weight did not change the results of the analyses qualitatively.

In great tits, variation in egg mass, yolk A4, and yolk T concentrations is much smaller within than among clutches (among clutch variation in egg mass: 71% [Tschirren B, unpublished data; Christians 2002], yolk A4 concentrations: 62%, and yolk T concentrations: 64% [Postma et al. 2014]), and the change in yolk A4 and yolk T with laying sequence is small (Tschirren et al. 2004). The fourth egg's weight, yolk A4, and yolk T concentration is, therefore, representative for the prenatal conditions experienced by its siblings during embryonic development.

We analyzed the concentrations of yolk A4 and yolk T by radioimmunoassay as described in Tschirren et al. (2009). In short, the yolks were thawed and homogenized with $400 \mu\text{L}$ of distilled water. Aliquots of this yolk/water emulsion (~ 100 mg) were taken, weighed (to the nearest 0.1 mg), and mixed with $150 \mu\text{L}$ of distilled water and $50 \mu\text{L}$ of ^3H Tracer (~ 2000 counts/min) to assess extraction efficiency. The samples were extracted twice with 2.5 mL of 70% diethyl ether/30% petroleum ether (vol:vol) and dried under a stream of nitrogen. These extracts were then dissolved in 1 mL of 70% methanol, centrifuged, and decanted. The supernatant was dried under a stream of nitrogen and redissolved in phosphate-buffered saline. Yolk A4 and T were measured using Diagnostic System Laboratories (Webster, TX) radioimmunoassay kits following the manufacturer's protocol. The average recovery rate was 86% (range: 77–93%) for yolk A4 and 86% (range: 77–93%) for yolk T. We corrected measured yolk A4 and yolk T concentrations (pg/mg yolk) for extraction efficiency (i.e., concentration * 100/recovery rate). Dilution curves confirmed reliability of extraction and assay protocols. Yolks were analyzed in a single assay. Intra-assay coefficient of variation was 3.4% for yolk A4 and 2.6% for yolk T. Yolk A4 and yolk T concentrations were log-transformed for the statistical analyses.

Statistical analyses

We tested whether nestling traits (growth rate, body mass, tarsus length) were affected by environmental conditions before egg laying, by the conditions experienced after hatching, and by the interaction between prenatal and posthatching conditions using general linear mixed-effect models. Prelaying treatment (pre-F or pre-NF), posthatching treatment (post-F or post-NF), nestling sex, and all 2-way interactions were included as fixed factors. We also included the cross-foster state of a nestling (cross-fostered or raised in its original nest) to test for consequences of mismatches between prenatal and posthatching environments other than those induced by the food treatment (e.g., parasite-induced maternal effects, Tschirren et al. 2004, 2007b). Clutch size, hatching date, feather length, egg weight, and yolk androgen concentrations were included as covariates to

test for potential effects of nestling competition, seasonal variation in food availability, differences in developmental stage, or egg size- and yolk androgen-mediated maternal effects on nestling traits.

Nest of origin (nested in the prelaying treatment) and nest of rearing (nested in the posthatching treatment) were included as random effects to account for the nonindependence of siblings and birds raised in the same nest. If significant interaction effects between the prelaying and posthatching food treatment were observed (see Results for details), we performed post-hoc contrasts based on least squares means to test which treatment groups differed significantly from one another. The match or mismatch of food conditions experienced by the rearing mother (rather than the nestlings) before egg laying and during nestling rearing did not affect nestling growth or development (results not shown) and was therefore not further considered in the analyses.

We used general linear models to test for effects of the prelaying food treatment on clutch size, egg weight, and yolk androgen concentrations. Laying date, clutch size, and the time between treatment start and laying date (for egg weight and yolk androgens), and egg weight (for yolk androgens) were included as covariates.

For the analysis of survival from hatching until fledging, we ran a generalized linear mixed model with a binomial error structure, and the same factors and covariates as described previously using the `glmer` function, part of the `lme4` package (Bates et al. 2011).

For all tests, final models were obtained by removing factors and covariates with a $P > 0.1$, starting with the least significant term. Random effects as well as the prelaying and posthatching food treatments were always retained in the models. Results of the final models are presented in the Results. If nonsignificant results are presented for a factor or covariate of interest, F and P values before dropping the term from the model are shown. A Kenward–Roger correction was used to calculate the degrees of freedom in mixed models. Residuals of the models were checked for heteroscedasticity and normality. All tests were 2-tailed with a significance level set at $P \leq 0.05$. Sample sizes differ among tests because of nestling mortality or missing data. Statistical analyses were performed in JMP 10 (SAS Institute Inc., Cary, NC, 1989–2007) and R 2.14.1 (R Development Core Team 2011). Means \pm 1 SD are presented.

RESULTS

Effects of the prelaying and posthatching food manipulation on nestling growth and development

Hatching mass

There was no significant difference in body mass 1 day after hatching between nestlings originating from a pre-F or pre-NF nest ($F_{1, 72.76} = 0.026$, $P = 0.873$) or between post-F and post-NF broods ($F_{1, 24.02} = 0.001$, $P = 0.988$). The interaction effect between the prelaying and posthatching treatment on hatching mass was nonsignificant ($F_{1, 77.89} = 0.103$, $P = 0.749$).

Growth

There was no interaction effect between the prelaying and posthatching food treatment on the rate of body mass gain during the main growth period ($F_{1, 560.2} = 0.108$, $P = 0.743$, Figure 1A). Furthermore, there was no main effect of the prelaying food supplementation on offspring mass gain ($F_{1, 54.35} = 2.771$, $P = 0.102$). However, nestlings that received extra food during the posthatching period grew significantly faster than nestlings from nonsupplemented broods ($F_{1, 58.35} = 6.670$, $P = 0.012$, Figure 1A).

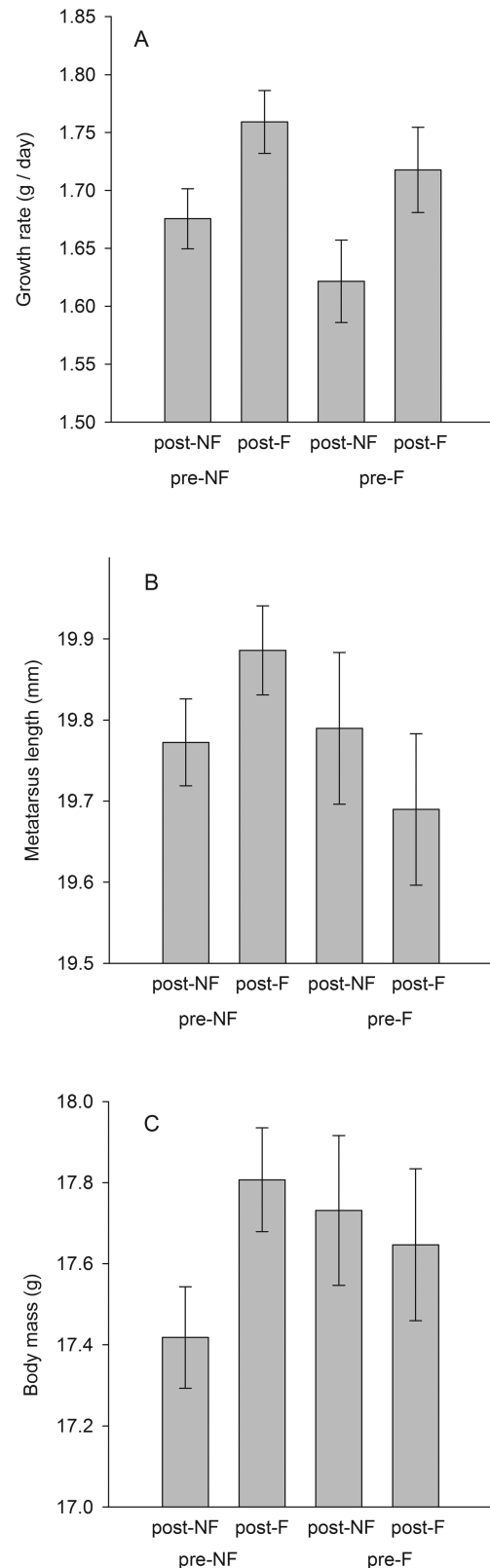


Figure 1

Effects of the prelaying (pre-NF/pre-F) and posthatching (post-NF/post-F) food treatment on nestling growth rate (A), nestling body size on day 14 posthatching (B), and nestling body mass on day 14 posthatching (C). Least squares means \pm 1 standard error (SE) are shown.

Fledging mass and size

At the end of the nestling period, we observed a significant interaction effect between the prelaying and the posthatching food supplementation on tarsus length ($F_{1, 296.3} = 5.911$, $P = 0.016$, Figure 1B) and body mass ($F_{1, 468.7} = 6.155$, $P = 0.014$, Figure 1C).

To better understand the observed interaction effects between the prelaying and posthatching treatment, and the relative importance of prenatal maternal effects and posthatching conditions on offspring development, we performed post-hoc contrasts between treatment groups. We found that if the biological mother had not received extra food during the egg laying period, food-supplemented nestlings grew larger than nonsupplemented nestlings (post-hoc contrast pre-NF/post-NF vs. pre-NF/post-F: $F_{1, 45.73} = 6.563$, $P = 0.014$, Figure 1B). The difference in body size between supplemented and nonsupplemented nestlings was not significant if their biological mother had received extra food during egg laying (post-hoc contrast pre-F/post-NF vs. pre-F/post-F: $F_{1, 149.3} = 1.636$, $P = 0.203$, Figure 1B). Interestingly, food-supplemented nestlings tended to be larger when their mother had not received extra food during the egg laying period than when their mother had received extra food (post-hoc contrast pre-NF/post-F vs. pre-F/post-F: $F_{1, 110.8} = 3.327$, $P = 0.071$, Figure 1B).

Similarly, nestlings of mothers that had not received extra food during the egg laying period were significantly heavier if they received extra food during the nestling period (post-hoc contrast pre-NF/post-NF vs. pre-NF/post-F: $F_{1, 55.23} = 7.262$, $P = 0.009$, Figure 1C). Again, this difference was not significant in the pre-F group (post-hoc contrast pre-F/post-NF vs. pre-F/post-F: $F_{1, 135.4} = 0.192$, $P = 0.662$, Figure 1C).

Nestlings of supplemented mothers raised in a nonsupplemented brood reached a similar body mass as nestlings that received extra food during the nestling period (post-hoc contrast pre-F/post-NF vs. both post-F: $F_{1, 129} = 0.000$, $P = 0.982$, Figure 1C), showing a long-lasting, compensatory effect of favorable prenatal conditions on offspring mass.

Survival

Complete nest failure was more common later in the season ($\chi^2_1 = 5.362$, $P = 0.021$), but it was not significantly influenced by the posthatching food treatment ($\chi^2_1 = 2.377$, $P = 0.123$). In broods where at least 1 nestling fledged, there was no significant effect of the prelaying treatment ($\chi^2_1 = 0.474$, $P = 0.491$) or the posthatching treatment ($\chi^2_1 = 0.285$, $P = 0.594$) on nestling mortality, and there was no significant interaction effect between the treatments ($\chi^2_1 = 0.651$, $P = 0.420$).

Sex difference in growth and development

Male nestlings were significantly heavier than female nestlings 1-day posthatching ($F_{1, 379} = 6.712$, $P = 0.010$). They also grew at a faster rate ($F_{1, 503.9} = 52.409$, $P < 0.001$) and reached a larger body size ($F_{1, 510.3} = 261.017$, $P < 0.001$) and a higher body mass ($F_{1, 456.4} = 96.023$, $P < 0.001$) at the end of the nestling period. Female nestlings tended to be heavier when their mother had received extra food during the egg laying period, whereas the prelaying treatment did not affect body mass of male nestlings (interaction sex \times prelaying treatment: $F_{1, 454.2} = 3.496$, $P = 0.062$, Figure 2).

Effect of the prelaying treatment on clutch size and egg composition

We tested if the prelaying food treatment affected clutch size and egg composition, and if these components explained a significant amount of variation in nestling growth and development.

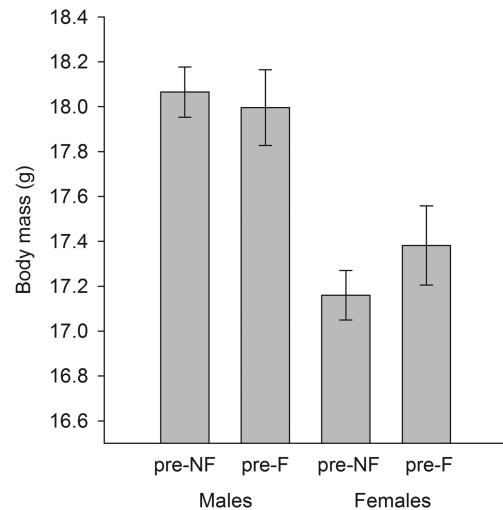


Figure 2

Effects of the prelaying food treatment (pre-NF/pre-F) on body mass of male and female nestlings on day 14 posthatching. Least squares means \pm 1 SE are shown.

The prelaying food treatment did not significantly affect clutch size (pre-F: 8.1 ± 0.77 eggs, pre-NF: 8.6 ± 1.46 eggs; $F_{1, 102} = 0.482$, $P = 0.489$), egg weight (pre-F: 1.71 ± 0.15 g, pre-NF: 1.69 ± 0.12 g; $F_{1, 95} = 0.556$, $P = 0.458$), yolk A4 concentration (pre-F: 115.85 ± 33.24 pg/mg yolk, pre-NF: 106.93 ± 22.03 pg/mg yolk; $F_{1, 79} = 0.983$, $P = 0.324$), or yolk T concentration (pre-F: 77.20 ± 31.54 pg/mg yolk, pre-NF: 69.63 ± 18.44 pg/mg yolk; $F_{1, 79} = 0.756$, $P = 0.387$).

Nestlings originating from broods with larger eggs ($F_{1, 71.96} = 7.257$, $P = 0.009$) and nestlings originating from broods with lower yolk A4 concentrations ($F_{1, 69.65} = 10.175$, $P = 0.002$) were heavier 1-day posthatching.

Fledging mass was higher in smaller broods ($F_{1, 85.39} = 4.418$, $P = 0.039$). The weight, yolk A4, or yolk T concentration of the fourth egg of a clutch did not explain a significant amount of variation in fledging mass (egg weight: $F_{1, 70.1} = 0.534$, $P = 0.468$; yolk A4: $F_{1, 67.1} = 0.513$, $P = 0.477$; yolk T: $F_{1, 59.9} = 0.187$, $P = 0.667$) or fledging size (egg weight: $F_{1, 73.9} = 0.649$, $P = 0.423$; yolk A4: $F_{1, 66.42} = 0.055$, $P = 0.816$; yolk T: $F_{1, 65.2} = 1.183$, $P = 0.281$).

Neither the weight ($\chi^2_1 = 0.166$, $P = 0.684$) nor the yolk T concentration ($\chi^2_1 = 0.500$, $P = 0.480$) of the fourth egg of a clutch were significantly associated with nestling survival. However, nestlings originating from broods with higher yolk A4 concentrations were significantly more likely to survive ($\chi^2_1 = 22.403$, $P < 0.001$).

Effects of cross-fostering on offspring growth and development

To test if other, nonfood-mediated mismatches between pre- and posthatching conditions influence offspring development, we compared the early growth, fledging mass, and size of nestlings that were raised in their original nest (noncross-fostered) and nestlings that were raised in a foster nest (cross-fostered).

There was no significant difference in hatching mass between cross-fostered and noncross-fostered nestlings ($F_{1, 408.7} = 0.664$, $P = 0.416$). However, nestlings that were raised in their original nest grew faster than nestlings that were raised in a foster nest ($F_{1, 495.9} = 4.951$, $P = 0.027$, Figure 3A). This difference was not explained by the time it took to move nestlings from the

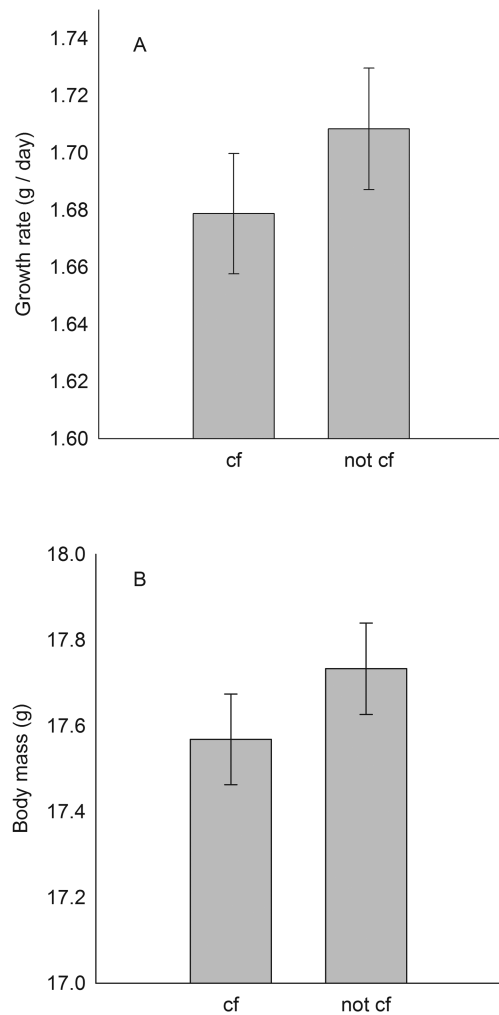


Figure 3

Growth rate (A) and nestling body mass on day 14 posthatching (B) of nestlings that were raised in a foster nest (cf, cross-fostered) and nestlings that were raised in their original nest (not cf, not cross-fostered). Least squares means \pm 1 SE are shown.

original nest to the foster nest during cross-fostering ($F_{1,477} = 0.640$, $P = 0.424$).

At the end of the nestling period, nestlings that were raised in their original nest reached a higher body mass ($F_{1,461.4} = 6.118$, $P = 0.014$, Figure 3B). Again, the time required to move nestlings from the original nest to the foster nest during cross-fostering did not explain variation in fledging mass ($F_{1,464} = 0.050$, $P = 0.823$). No difference in fledging size between cross-fostered and noncross-fostered nestling was observed ($F_{1,493.8} = 0.066$, $P = 0.797$).

DISCUSSION

We experimentally tested how food-induced prenatal and postnatal effects interact in influencing offspring growth and development in a wild bird population. Growth rate during the period of linear mass gain (between day 5 and day 9 posthatching) was strongly influenced by the posthatching food treatment, demonstrating that the amount of extra food provided to the supplemented broods was sufficient to affect nestling development. Food-supplemented nestlings grew faster than controls, and this effect was independent of

the prelaying food treatment. Interestingly, however, body mass at the end of the nestling period did not differ between food-supplemented nestlings and nonsupplemented nestlings whose mother had received extra food during the egg laying period. It was, however, significantly lower in nonsupplemented nestlings whose mothers had not received extra food. It demonstrates that prenatal maternal effects can negate growth-limiting conditions after hatching, and, given that fledging mass is strongly linked to first-year survival in small passerines (Tinbergen and Boerlijst 1990; Both et al. 1999; Naef-Daenzer et al. 2001), that conditions experienced before birth can affect traits closely linked to fitness.

A similar interaction effect between the pre- and posthatching food treatment was observed on offspring tarsus length, a proxy for body size. Offspring grew largest when their mother had not received extra food during egg laying, but food was supplemented after hatching (pre-NF/post-F). Interestingly, these nestlings were even larger than food-supplemented nestlings whose mother had received extra food during the egg laying period (pre-F/post-F). This finding is in line with the results of studies in humans (Hales and Barker 2001) and domesticated animals (George et al. 2012), and suggests that food-mediated prenatal maternal effects influence how efficiently offspring use available resources later in life.

Although we observed significant interaction effects between prelaying and posthatching food conditions on both fledging mass and size, we found no evidence that nestlings performed better when they experienced the same conditions before and after birth (i.e., a match between prelaying and posthatching nutritional conditions) than when a mismatch occurred. Thus, unlike in domesticated animals (Cleal et al. 2007; Hinde et al. 2009; van der Waaij et al. 2011) and humans (Hales and Barker 2001; Gluckman et al. 2008), short-term fluctuations in nutritional conditions do not appear to lead to detrimental mismatch effects in the offspring. However, it is important to note that we only measured short-term effects on fledging mass and size which are, although strong predictors of first-year survival (Tinbergen and Boerlijst 1990; Both et al. 1999; Naef-Daenzer et al. 2001), only one aspect of performance. It would be interesting, although practically challenging given the low local recruitment rate in our population, to follow birds that experienced a match or mismatch between prenatal and posthatching conditions throughout their life to detect potential long-term costs on fitness. Furthermore, it would be interesting to measure physiological responses, which have been shown to be most strongly affected by mismatch effects in humans and domesticated animals (Hales and Barker 2001; Cleal et al. 2007; Gluckman et al. 2008).

The observed prenatal maternal effects on fledging mass and size were not mediated by egg weight (see also Nager et al. 1997; Christians 2002; but see Bolton et al. 1992), although we only measured the weight of the fourth egg and individual variation in egg weight within broods may still play a role. It suggests that changes in the composition of the eggs or differential incubation behavior by the female in response to the food treatment caused this effect. Previous work has shown that maternal food supplementation before and during egg laying influences egg composition, and in particular the transfer of maternal yolk androgens. Food-supplemented lesser black-backed gull females (*Larus fuscus*), for example, transferred lower androgen concentrations in their eggs compared with controls (Verboven et al. 2003). Similar effects were observed in replacement clutches of black-legged kittiwake (*Rissa tridactyla*, Gasparini et al. 2007). Furthermore, several studies have shown that exposure to high yolk androgen concentrations during embryonic development promotes posthatching growth (e.g.,

Schwabl 1996; Groothuis et al. 2005; Tschirren et al. 2005; but see Sockman and Schwabl 2000). Here, we found no indication that the prelaying food treatment influenced maternal A4 or T transfer to the eggs, or that yolk A4 or T concentrations were associated with nestling growth, mass, or size. However, nestlings originating from a brood with higher yolk A4 concentrations were significantly less likely to die during the nestling period. It indicates that the observed prenatal effects on nestling body mass were mediated by other, unmeasured components of the egg, such as carotenoids (Romano et al. 2008), immunoglobulins (Hasselquist and Nilsson 2009), or stress hormones (Meylan and Clobert 2005; Henriksen et al. 2011; Sheriff and Love 2013), or by differential incubation behavior of the female.

Interestingly, daughters tended to benefit more from food-mediated prenatal maternal effects than sons. Such sex-specific consequences of prenatal maternal effects have been described previously (e.g., Gorman and Nager 2004; Helle et al. 2013), but there is no consensus on which sex benefits from the “silver spoon.” For example, an experimental increase of yolk androgen concentrations in Collared flycatcher (*Ficedula albicollis*) eggs increased the growth of female, but reduced the growth of male nestlings (Pitala et al. 2009), whereas the exact opposite effect was observed in Barn swallows (*Hirundo rustica*, Saino et al. 2006). Understanding why such sex-specific responses to the prenatal environment occur and what factors determine which sex benefits will be the focus of future work.

Although we here focused on the consequences of a match and mismatch between pre- and postnatal nutritional conditions, maternal effects are likely to arise in response to a wide range of additional environmental factors not directly measured or manipulated in this study. For example, it has been shown that nest-based ectoparasites mediate prenatal maternal effects that promote offspring defense (Heeb et al. 1998; Tschirren et al. 2007b). Because investment in immune defense is costly and only pays when infection occurs (Tschirren and Richner 2006), a mismatch between predicted and actual parasite load would have negative consequences for the offspring. Similarly, fitness costs might occur if nestlings are maladapted to their local microhabitat or climate (Lloyd and Martin 2004; Goodenough et al. 2008) or to the microbial assemblage in the nest (Goodenough and Stallwood 2012). Although it would be difficult to identify and manipulate all the environmental factors than potentially induce maternal effects, cross-fostering nestlings between nests provides an indirect way of creating an overall mismatch between the conditions mothers experienced during egg laying and the conditions nestlings are encountering after hatching. Interestingly, we found that nestlings grew faster and were heavier at the end of the nestling period when they were raised in their original nest (see also Berthouly et al. 2007 for similar cross-fostering effects on immune response). This effect is unlikely due to the cross-fostering procedure itself because all nestlings were removed from the box and handled during cross-fostering. Furthermore, the time nestlings spent outside the nest during cross-fostering did not explain significant amount of variation in growth or body mass. It thereby provides indirect evidence that mismatches between the anticipated and actual environment nestlings encounter can have negative consequences and that environmental factors other than food availability may be the main drivers of such mismatch effects.

In conclusion, we show that food-mediated prenatal maternal effects can have important consequences for offspring traits closely linked to fitness. In particular, our results suggest that prenatal maternal cues can influence how efficiently offspring use available

resources after hatching, and that a favorable prenatal environment can compensate for growth-limiting conditions after hatching. Moreover, we observed significant interaction effects between prenatal maternal effects and postnatal conditions on offspring development. Such interaction effects may at least partly explain discrepancies in the findings of maternal effect studies in natural populations and highlight the role of directional or stochastic environmental change in mediating the consequences of maternal effects in the wild.

FUNDING

This work was supported by the Swiss National Science Foundation (PP00P3_128386 to B.T.).

We thank L. Gustafsson for giving us the opportunity to use the Gotland study population for this project; J. Huber, N. Fresneau, and G. Baudouin for field assistance; I. Weites, B. de Vries, and D. Waldvogel for help in the lab; and E. Postma, P. Smiseth, and an anonymous reviewer for valuable comments on the manuscript.

Handling editor: Nick Royle

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