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Transgenerational effects enhance specific immune response in a wild passerine

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Vertebrate mothers transfer diverse compounds to developing embryos that can affect their development and final phenotype (i.e. maternal effects). However, the way such effects modulate offspring phenotype, in particular their immunity remains unclear. To test the impact of maternal effects on offspring development we treated with Newcastle disease virus (NDV) vaccine wild breeding house sparrow (Passer domesticus) females in Sevilla, SE Spain. Female parents were vaccinated when caring first broods, and their offspring from their following brood were evaluated for their immune response to the same vaccine and to the PHA inflammatory test. Vaccinated chicks from vaccinated mothers developed a stronger specific response that was related to maternal NDV antibody concentration. Chick’s carotenoid concentration and total antioxidant capacity in blood were negatively related to NDV antibody concentration, whereas no relation with PHA response was found. Specific NDV antibodies could not be detected on 10 day old control chicks from vaccinated mothers, implying that maternally transmitted antibodies promote offspring specific immunity through a priming effect, while other immunity components remain unaffected. Maternally transmitted antibodies are short-lived, depend on maternal circulation levels and may be adaptive when chicks are frequently exposed to the same pathogens as their mothers.
TRANSGENERATIONAL EFFECTS ENHANCE SPECIFIC IMMUNE RESPONSE IN
A WILD PASSERINE

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Compliance with Ethical Standards: The authors declare no conflicts of interest, and that none of the material presented in this manuscript has been published or is under consideration elsewhere. This submission for publication has been approved by all relevant authors and institutions; and all persons entitled to authorship have been so named. All authors have seen and agreed to the submitted version of the manuscript. All procedures followed the guidelines of the Estación Biológica de Doñana, CSIC ethical committee and the regional government for the manipulation of birds and collection of blood samples. All research presented in the manuscript was conducted in accordance with all applicable laws and rules set forth by their governments and institutions.
ABSTRACT

Vertebrate mothers transfer diverse compounds to developing embryos that can affect their development and final phenotype (i.e. maternal effects). However, the way such effects modulate offspring phenotype, in particular their immunity remains unclear. To test the impact of maternal effects on offspring development we treated with Newcastle disease virus (NDV) vaccine wild breeding house sparrow (*Passer domesticus*) females in Sevilla, SE Spain. Female parents were vaccinated when caring first broods, and their offspring from their following brood were evaluated for their immune response to the same vaccine and to the PHA inflammatory test. Vaccinated chicks from vaccinated mothers developed a stronger specific response that was related to maternal NDV antibody concentration. Chick’s carotenoid concentration and total antioxidant capacity in blood were negatively related to NDV antibody concentration, whereas no relation with PHA response was found. Specific NDV antibodies could not be detected on 10 day old control chicks from vaccinated mothers, implying that maternally transmitted antibodies promote offspring specific immunity through a priming effect, while other immunity components remain unaffected. Maternally transmitted antibodies are short-lived, depend on maternal circulation levels and may be adaptive when chicks are frequently exposed to the same pathogens as their mothers.
INTRODUCTION

Parents influence the phenotype of their offspring in other ways besides genetic inheritance. Vertebrate mothers may provide a favourable growing environment and care, but additionally can transmit diverse components such as hormones (Groothuis and Schwabl 2008), antioxidants (e.g. Royle et al 2003) or immunoglobulins (Hasselquist and Nilsson 2009) that can have relevant phenotypic consequences on the developing embryo (Mousseau et al 2009). Such maternal effects constitute a major source of transgenerational phenotypic plasticity that can vary according to environmental heterogeneity (e.g. Morosinotto et al 2013) and maternal condition (Boulinier and Staszewski 2008). However, the way these transferred components interact with each other, and modulate the development of the offspring phenotype remains poorly understood (Mousseau et al 2009).

The vertebrate humoral immune response targets specific infectious agents by means of antibodies (Ab), and although it takes long to be effective upon the first encounter with an antigen, persists as immune memory and rapidly reacts upon re-exposure to the same antigen. Newborn individuals require some time to fully develop such capabilities, and may rely on maternally transferred Ab while they develop their own endogenous response (Hasselquist et al 2012). The influence of maternal Ab on the fitness and disease epidemiology of neonates in humans and domestic mammals is well documented, providing the offspring with transient immunity against the microbial infections that the mother has encountered (Hasselquist and Nilsson 2009). In avian species yolk-transmitted maternal Ab are generally short lived, and detectability ranges from days to few months after hatching (reviewed in Hasselquist et al 2012).
Furthermore, whether the effects of maternally transmitted Ab are enhancing the chick’s subsequent response by a priming mechanism (e.g. Grindstaff et al 2006; Reid et al 2006), or blocking the chick’s endogenous response (e.g. Staszewski et al., 2007; Elazab et al 2009; Staszewski and Siitari 2010) remains controversial. Studies on the influence of maternal Ab on host fitness are scarce (Boulinier and Staszewski 2008), despite the important effect that pathogens may exert on wild organism populations (e.g. Hochachka and Dhondt 2000).

Individuals exhibit substantial differences in their immune responses and rarely develop it maximally, which suggests there are some costs associated that individuals manage differently (van der Most et al 2011). Such individual variation in immune investment may arise from a variety of factors, not only adaptive adjustments but also constraints resulting from allocation conflicts with other physiological and life-history traits (Ardia et al 2011). Furthermore, individuals may invest differentially in various components of the immune system such as the non-specific innate (inflammatory) and the acquired (humoral) immune response (Lazzaro and Little 2009). It has been suggested that different investment in such different axes of the immune system may depend on the pathogen identity (Adamo 2004) or constraining nutritional or energetic factors (Hõrak et al 2006). However, other authors have found little evidence for these potential interactions (Ardia 2005; Ahmed et al 2007; Forsman et al 2008).

Oxidative balance and carotenoids have been acknowledged among such factors mediating variation in the immune responses, either by directly competing for resources, or as a currency in trade-offs between immunity and other traits (Hasselquist and Nilsson, 2012). The intensity of immune response is generally coupled with a shift in the oxidative balance and a decrease in carotenoid concentration that are considered detrimental for the individual (Ardia et al 2011).

Oxidative stress is generated as a metabolic by-product resulting in damage to cell
macromolecules (Dowling and Simmons 2009). Organisms balance their oxidative stress by acquiring and producing antioxidants, and although most antioxidative activity is enzymatic, non-enzymatic antioxidants also play a relevant role in maintaining oxidative balance, particularly in blood (Cohen and McGraw 2009). Immunity costs in terms of oxidative balance have been argued to underlie trade-offs between immunity and life-history traits, either by directly increasing oxidative stress (Costantini and Møller 2009) or by competing for antioxidants (Monaghan et al 2009), but see (Speakman and Garratt 2014).

Carotenoids are a diverse group of lipophilic molecules important for immunity and individual fitness, and since they cannot be synthesized de-novo by animals, necessarily need to be ingested or acquired during embryogenesis through maternal transfer (Pérez-Rodríguez 2009). Mothers provide their offspring with carotenoids through diet, which enhance diverse components of their immunity (Blount et al 2003; McGraw and Ardia 2003), and interact with yolk transmitted carotenoids synergistically (Bédécarrats and Leeson 2006; Koutsos et al 2007). Avian mothers transmit carotenoids and antioxidants in addition to Ab that likely stimulate the development of the offspring’s immune phenotype (Simons et al 2012). Therefore after an immune challenge a negative relationship between the intensity of an immune response and such components may be expected. However, little is known on how such maternal effects may interact with each other, and what could be their combined effect, especially on wild and non-model species (Hasselquist et al 2012).

In this study we explored whether maternal effects modulate offspring specific immune response in a wild breeding house sparrow (*Passer domesticus*, Linnaeus 1758) population. Using a vaccine to elicit an immune response to a viral infection we analysed how maternal exposure during first brood affects transmission of antibodies to the following brood, their offspring
development and immune response when exposed to the same antigen. We measured other aspects of the immunity i.e. PHA-induced inflammatory response, to find out whether mother condition affected their offspring specific humoral or general immunity. Furthermore, we controlled for other maternally-transmitted components (antioxidative and carotenoids) which are likely to affect the development of the offspring immune phenotype.
MATERIALS AND METHODS

Study area and model species

The house sparrow is a small-sized (20gr) granivorous passerine that breeds in close association with humans. House sparrows are commonly exposed to a variety of pathogens in the wild, including several epizootic pathogens resulting from the close association with humans and livestock (Anderson 2006). The Newcastle Disease Virus (NDV) is a worldwide distributed avian paramyxovirus that causes highly contagious disease, representing a severe problem for the poultry industry and also wild fauna (Alexander 2009). The virus is circulating in the study area as NDV antibodies were detected previously in 11 out of 81 individuals analyzed (Broggi et al 2013).

The study area is located in a private land surrounded by farmland and mixed forest, la Cañada de los Pájaros (37°14’N, 6°07’W) in Sevilla, SE Spain. The study population is about 100 pairs breeding naturally in wooden nestboxes at an average height of 2 m and within an area of 10 ha. Females lay up to 4 broods per year with an average clutch of 4.5 eggs, and breed repeatedly in the same nestbox if undisturbed. Breeding season starts in early April and lasts until the end of August (own unpublished data).

Experimental approach

From April 1st (the beginning of the breeding season) until August 2010, nestboxes were checked every second day to record breeding parameters. Breeding females were captured at the nest when chicks were older than 8 days to prevent nest desertion. Newly captured females were randomly assigned to the treatment (subcutaneous injection of 0.2 ml of a commercial...
inactivated NDV vaccine HIPRAVIAR® BPL2) or a control group (injection with 0.2 ml of PBS), following the results of a pilot study in the same population (Broggi et al., 2013). Before treatment, blood was sampled from the jugular vein (0.2 ml) and kept cool (~ 4°C) for less than 12h before centrifugation (20 min at 4000 rpm). Cellular phase and sera were stored separately at -20°C for later analyses (see below), and wing, tarsi and body mass were measured. Females were allowed to complete the first breeding attempt without further manipulation to minimise disturbance. During the next breeding attempt the chicks were weighed (to 0.1 g) on their 4th day of age and were inoculated subcutaneously with either NDV vaccine (0.1 ml) or a control treatment (PBS). Chicks within each brood were inoculated alternatively with vaccine or control treatment according to their body mass, and the order was switched in each different brood. Chicks were recognized by innocuous paint in their claw, and were repainted until they were marked with aluminium rings when ~6 days of age. On 10th day of age, chicks were weighed, and their tarsi and wing length measured (to 0.1 mm). Blood samples were taken from the chick’s jugular vein (0.1 ml) and processed as with adult female samples. Finally, chicks were subjected to a phytohaemagglutinin (hereafter PHA) immune challenge before being released in their nestbox. On the following day, chicks were re-measured (see below for details on the PHA immune challenge). Sex of the chicks was determined by molecular techniques based on DNA obtained from blood samples (Fridolfsson and Ellegren 1999). Females were recaptured on the second breeding attempt, and blood was sampled to measure blood metabolites and NDV-Ab concentration (see below). Each experimental female was included once in the dataset, and whenever captured in more than two consecutive nesting events (2 cases), only last breeding episode was included in order to increase variance in female vaccination response. On average, experimental females were challenged 2 to 7 weeks before egg-laying of the following clutch,
well-within the antibody circulation peak after vaccination (Broggi et al 2013). Altogether the dataset consisted of 88 chicks from 25 different females.

Immunological measurements

We used haemagglutination inhibition test (HI) to assess NDV-Ab concentration in sera. The test sera were sequentially diluted in PBS from 1/2 to 1/640 and 4HA units of antigen HIPRAVIAR®-CLON E.Newcastle, clon CL/79 were added to each dilution. The mixture was added to 50 µl of chicken RBC’s and after 30 minutes at room temperature checked for agglutination. NDV-Ab concentration was scored as the highest dilution where agglutination was observed. We used commercial positive and negative controls (VLDIA053 HAR-NDL, NDV strain La Sota and VLDIA030 SPF-CH-Chicken negative), further details can be found in Broggi et al (2013). None of the females at first capture nor control females at second capture presented NDV titers higher than 1/8, the lower limit to consider a serum positive to NDV according to standard laboratory practices (OIE 2015).

At 10th day of age, chicks were challenged with PHA in the patagia. PHA is a mitogen of vegetal origin that when injected intradermally induces an immune response mainly reflecting individual pro-inflammatory potential (Vinkler et al., 2010). Birds were injected in the right wing web with 50 µl of 5:2 PHA-P (L-8754 Sigma-Aldrich) in PBS following Smits et al (1999). Patagium width was measured at the point of injection (to the nearest 0.01 mm) just prior to and after 24 h from challenge, using a pressure sensitive micrometer (Baxlo Precision S.L.). Time between measurements averaged 40.20±0.81 hours, range=25-45 after challenge. PHA-challenge elicits an inflammatory response that reaches its maximum after ~6h from injection, and can last up to
72 hours (Navarro et al. 2003). Three measures were taken and the average was used in the analyses (intra-class correlation R>0.98).

Measurement of blood metabolites

Carotenoid concentration in sera was measured by means of N-1000 NanoDrop spectrophotometer at 450 nm, at the maximum reflectance point for lutein, which is the main circulating carotenoid found in passerines (Pérez-Rodríguez 2009). Total antioxidant capacity (TAC) in sera was measured as described in (Erel 2004), adapted for the Cobas INTEGRA Chemistry autoanalyser. Recent studies point out that TAC is mostly representative of the water soluble components of the antioxidative balance, and in combination with other fat-soluble antioxidants provides a more complete image of the antioxidant system (Cohen and McGraw 2009). Uric acid and total protein were measured according to standard methods implemented on a Cobas Integra 400 plus autoanalyzer with Roche reagents. Uric acid is a common circulating antioxidant generated as a by-product of metabolism that accounts for an important portion of the antioxidant capacity in blood, whereas total protein in sera is a standard diagnostic measure of nutritional condition. Repeatability for carotenoids, uric acid, total protein and TAC in sera, as assessed by the intraclass correlation of repeated blind measures of the same samples was higher than 0.97 for all parameters.

Statistical methods

NDV-Ab concentration (expressed as the base 2 logarithm of the inverted dilution factor) from 10 day old chicks was analyzed as dependent variable in a generalized linear mixed model (GLMM), with normally distributed error and identity link. Mother and chick’s treatments were
included as main effects and nestbox as a random factor in all models. Mother’s final NDV-Ab concentration (when caring the second brood), and time between treatment and sampling were considered as primary covariates in both chick (7.72±0.19 days, range 5-12) and female (37.64±0.72 days, range=24-65) measurements, as differences arose due to hatching asynchrony and capture success respectively. Likewise, in addition to the main effects and random factor, time between measurements was included as primary covariate in the models for PHA response and mass change from challenge to sampling, and otherwise models were identical. Chick survival from 4th day of age until fledging was analysed with a GLMM with binomially distributed error and logit link. Chick and mother treatments were included as fixed factors, together with body mass at the time of chick’s treatment as primary covariate and nest as a random factor. Once these models with main effects and primary covariates were obtained for NDV-Ab concentration in chicks and mothers; and PHA response, mass change and chick survival (i.e. final models), several parameters were included as secondary covariates i.e. breeding parameters (hatching date and clutch size), sex, biometric data (body mass and tarsus length) and blood metabolites (TAC, Uric acid, Total protein and carotenoids). Only breeding parameters were included as secondary covariates in the models on chick survival as no sex, biometric nor blood metabolite information were available from non-surviving chicks. Due to limited sample size, covariates and their interactions with the main effects were included sequentially, and retained whenever significant or as judged by AIC or Generalized Chi² to avoid model over-parametrization. Initial and final models are presented with the corresponding fit statistics and variable parameters (Estimate, F, DF and P values), and non significant predictors are shown with their corresponding values when removed from the final model (Supplementary material).
Least squared means are provided for categorical predictors and slope estimates for covariates ± standard error. Sample size varied among tests due to differential survival and available sera for laboratory analyses. Degrees of freedom for fixed effects were adjusted by the between-within approximation that accounts for within-subject changes of any fixed effect and divides the residual degrees accordingly (Schluchter and Elashoff 1990). Blood metabolite variables were normalized by means of log (x+1) transformation, and residuals from all models on NDV-Ab concentration, PHA response and mass change followed normality. All analyses were performed with procedure GLIMMIX SAS 9.2. (SAS Institute Inc. 2009).

All procedures were approved by the ethical committee (N/RefS.:G YB/AFR/CMM) and complied with current Spanish laws.
Results

Female NDV-Ab concentration before challenge were similar between treatments ($F_{1,14}=0.86; P=0.37$), and no differences between experimental and control groups were found before challenge for any of the physiological (Table 1) nor reproductive parameters studied. After challenge, female NDV-Ab concentration raised significantly in vaccinated individuals with respect to control ones (Control: $0.00\pm0.38$ vs. Vaccinated: $4.70\pm0.41$; $F_{1,20}=70.67; P<0.0001$) (Figure 1). Neither pre-challenge NDV-Ab concentration ($F_{1,12}=0.39; P=0.54$), days between vaccination and re-sampling ($F_{1,13}=0.07; P=0.80$), the physiological nor the reproductive parameters considered (all $P>0.5$) were related to post-challenge NDV-Ab concentration.

Chick NDV-Ab concentration at 10 days of age was independent of maternal treatment ($F_{1,17}=0.06; P=0.81$). Likewise, chick NDV-Ab concentration was independent from chick treatment ($F_{1,19}=3.83; P=0.07$), and the interaction with maternal treatment ($F_{1,19}=0.52; P=0.48$). However, when considering female NDV-Ab concentration as a covariate, the interaction with chick’s treatment appeared significant ($F_{1,53}=6.61; P=0.01$), together with days between chick and mother sampling (Estimate $0.324\pm0.121$; $F_{1,17}=7.20; P=0.02$). NDV-Ab concentration of vaccinated chicks tended to increase with female NDV-Ab concentration, whereas this relation was not significant in control chicks (Control: $-0.11\pm0.14$; $t_{18}=-0.81; P=0.43$ vs. Vaccinated: $0.23\pm0.13$; $t_{18}=1.75; P=0.09$). Neither sex, biometric measurements nor the breeding parameters were related to NDV-Ab concentration in chicks (all $P>0.2$). Vaccinated chicks from vaccinated mothers developed higher specific humoral response, particularly when originating from mothers exhibiting high NDV-Ab titres (Figure 1).
On the other hand, when including blood metabolites as covariates on the previous model, only carotenoids and TAC were related to chick NDV-Ab. Carotenoids decreased with increasing NDV-Ab concentration in chicks, independently of the treatment (slope: $-1.35\pm0.47$; $F_{1,39}=8.11$; $P<0.01$). However, the relation between NDV-Ab and TAC changed slightly according to the chick treatment, the interaction being significant ($F_{1,50}=5.95$; $P=0.02$). NDV-Ab concentration of vaccinated chicks decreased with TAC, whereas the relation was not significant in control chicks (Control: $-0.34\pm0.59$; $t_{50}=-0.59$; $P=0.56$ vs. Vaccinated: $-2.08\pm0.45$; $t_{50}=-4.66$; $P<0.01$). Chick’s concentration of NDV-Ab was negatively related to carotenoids in blood, and in the case of vaccinated chicks also to TAC in blood (Figure 2. in Supplementary material). Uric acid or total protein were unrelated to chick’s NDV-Ab concentration (all $P>0.1$).

PHA in chicks was unrelated to maternal ($F_{1,9}=0.01$; $P=0.93$) or chick ($F_{1,21}=0.02$; $P=0.88$) treatments, even when accounting for chick body mass or change in body mass between challenge and measurement (hereafter body mass change), and time between such measurements (all $P>0.2$). Furthermore, none of the breeding parameters, biometric measurements nor blood metabolites considered were significantly related to PHA response (all $P>0.09$). PHA variation in chicks was only significantly explained by body mass (slope: $-0.06\pm0.02$; $F_{1,29}=5.05$; $P=0.03$), as PHA response was lower the heavier the chick.

Body mass change in chicks from vaccination to sampling date (day 4th to 10th of age) was independent of mother’s and chick’s NDV-Ab titres (all $P>0.30$). Likewise, neither treatment, sex nor the blood metabolite variables were related to body mass change (all $P>0.2$). Only elapsed days between vaccination and sampling (slope: $1.20\pm0.28$; $F_{1,20}=18.23$; $P<0.01$) and tarsus length had a significant influence on body mass change (slope: $0.60\pm0.27$; $F_{1,63}=4.96$;
P=0.03) (Table 1). House sparrow chicks with larger tarsi grew heavier independently of their mother’s or their own level of NDV-Ab, once the period between measurements was accounted for.

Chick’s survival from 4th day of age until fledging was unrelated to neither the maternal (F1,22=0.76;P=0.39), nor the chick’s treatments (F1,23=0.31;P=0.59), and only body mass at the time of vaccination was related to fledging success (slope: 0.49±0.11; F1,89=18.29; P<0.01).
House sparrow mothers developed a significant specific humoral response when challenged with NDV vaccine before egg-laying, and as a result their offspring exhibited a stronger specific humoral response when challenged with the same antigen. However, inter-individual variation in maternal NDV-Ab was the main determinant of chick specific immune response to NDV, as chicks from mothers with high NDV Ab concentration were most likely to develop a significant increase in their response to NDV vaccine (Figure 1).

Maternal transfer may reflect a dynamic balance between the benefits of providing an early specific protection and the costs of blocking the nestling’s immune development (Garnier et al 2011). In our study, maternal Ab could not be detected on 10 day old chicks by means of standard diagnostic techniques, as no differences could be appreciated between control chicks from vaccinated and control mothers. The results suggest that maternal Ab transmission has a priming beneficial effect on the specific response of 10 day old chicks, by stimulating the endogenous production of Ab, rather than by directly transmitting effective Ab.

On the other hand, mothers influence offspring immunity in other ways than transmission of specific Ab, by providing other components through yolk of by modulating parental investment. Therefore it is possible that the enhanced specific immune response in offspring could result from other maternal effects than the transmission of specific Ab, or a combination of them. However, the fact that chicks did not experience any change in PHA-response, which is a non-specific innate immune response suggests that maternal effects were antigen-specific.

The fact that the maternal treatment per-se was ineffective in explaining offspring specific response, and only when considering inter-individual variation in specific Ab levels such
relationship was apparent, can be explained by several non exclusive reasons. First, the response variation among breeding females to the experimental challenge, which led to a significant inter-individual variation in circulating Ab level, suggests that some breeding females may have been previously exposed to the virus in the wild (see Broggi et al 2013). Such variation most likely determined differential levels of circulating Ab levels and thus passive Ab transmission. In fact, a few control chicks originating from control mothers presented NDV-Ab, implying a natural exposure to the antigen (Figure 1). Second, it is possible that laying females effectively transfer Ab only when their own systemic levels are above certain threshold (Grindstaff 2010).

Alternatively, it could be that maternally transmitted Ab do not persist long in the nestling blood, and after 10 days of age they are not detectable anymore (Grindstaff et al 2003). The fact that breeding females were evaluated few weeks after challenge, while their chicks after few days may rend our results conservative as it may be that maternal effects are most effectively transmitted earlier after-challenge, or their effects last longer than 10 days of age, but see (Garnier et al 2011). Our results suggest that laying females with high circulating Ab levels confer a priming effect to their offspring which is beneficial at least at 10 days of age.

So far, the few studies on wild avian species suggest that effects of maternally transmitted Ab on offspring specific immunity is minimal and rarely positive (King et al 2010). On the one hand, short-term enhancing effects of maternal transfer of Ab on the unspecific humoral response in developing chicks have been reported (Grindstaff et al 2006; Pihlaja et al 2006). However, whether such priming effect has long-term consequences for the immunocompetence of the chick remains elusive. Other studies have revealed a blocking effect on tawny owls (Strix aluco, Linnaeus 1758) exposed to a synthetic vaccine against four different pathogens i.e. Tetravac
(Gasparini et al 2009), zebra finches (Taeniopygia guttata, Reichenbach 1862) exposed to KLH or LPS (Merrill and Grindstaff 2014), and kittiwakes (Rissa tridactyla, Linnaeus 1758) and quails (Coturnix coturnix, Linnaeus 1758) to NDV vaccine (Staszewski et al 2007; Staszewski and Siitari 2010). Interestingly, the same host species exposed to different antigens exhibited positive effects on their specific immune response (Gasparini et al 2006; Addison et al 2010) (kittiwakes and quails with Lyme disease and KLH respectively). Finally, other studies have found maternal transmission of Ab to buffer costs of an immune response (Grindstaff 2008), whereas the effects of maternal Ab appeared irrelevant in house sparrow chicks exposed to KLH and WNV (Nemeth et al 2008; King et al 2010). Long-term effects of maternal Ab-transmission on the specific immune response of chicks to antigens administrated on the previous breeding season to pre-laying mothers have been reported. Challenged breeding female kittiwakes raised offspring with impaired specific immune response to NDV vaccine (Staszewski et al 2007), whereas song sparrows (Melospiza melodia, Wilson 1810) challenged with tetanus vaccine raised offspring with improved specific response to tetanus (Reid et al 2006), being so far the only study to detect a positive effect of maternally transmitted Ab on a specific immune response. We found house sparrow mothers to enhance their offspring specific humoral response during the pre-fledging period, but not other aspects of their immunity, implying that specific Ab are transmitted without conditioning the other components of the immune system involved in the response to the PHA test.

There is substantial variation among species in both maternal transmission of Ab, and their effect on the development of the offspring immune capacity (Addison et al 2009; Arriero et al 2013). Interspecific comparisons suggest that larger and longer lived species, which also experience slower developmental times, may rely more strongly on the maternal effects that may be
transmitted in larger amounts and persist for longer periods (Garnier et al 2011, Ramos et al 2014). In line with this suggestion and in contrast with our results, it has been argued that maternal transfer of Ab in fast-developing altricial species (e.g. house sparrow) may not have a relevant role, as endogenous Ab production is achieved soon after hatching (King et al 2010; Nemeth et al 2008). Furthermore, methodological differences among studies may partly explain the varying results on the effects of the maternal transmission of Ab on offspring specific immunity. Although most studies tested the effects by studying both mothers and offspring exposed to the same antigen, this was not always the case (e.g. Gasparini et al 2006; Pihlaja et al 2006; King et al 2010). Our results suggest that maternal transfer of antibodies in the house sparrow is important enough to prime chick specific immunity, despite not being detectable by usual immunological techniques. Additionally, it is possible that there is a (host-pathogen)-specific response to maternally transmitted specific Ab. Furthermore, the effect of maternally transmitted Ab on the offspring specific immune response may be of a hormetic nature (Costantini et al 2010), irrelevant under certain threshold and priming or blocking according to the different concentration of Ab transmitted, the developmental stage or condition of the individual.

The ontogeny of the constitutive immunity is complex, influenced by both maternal and endogenous factors, with long term implications for the individual development and overall immunity (Butler and McGraw 2011; van der Most et al 2011; Arriero et al 2013). Maternal transfer of specific Ab could benefit offspring by enhancing the specific humoral response while permitting growth rate to be maintained (Grindstaff 2008). This was the case of our study, since developing an immune response did not affect chick growth rate. However, chicks developing
stronger specific humoral response experienced decreased carotenoid levels and antioxidative capacity in blood. In addition to Ab, parent females transfer carotenoids and antioxidants to their eggs according to diverse endogenous and environmental factors such as mate attractiveness (Saino et al 2002), own condition (Hammouda et al 2012), pathogen abundance (Gasparini et al 2001) or predation risk (Morosinotto et al 2013). We found no differences in blood carotenoids or TAC measured in females either at challenge or post-challenge sampling times in relation to any of the treatments or NDV-Ab concentration, suggesting that variation in chick’s levels arose from chick’s own physiological adjustments rather than differential maternal transmission. Several studies have shown costly aspects of immunity in terms of antioxidative balance and carotenoid levels. Interestingly, other studies found carotenoid levels to be positively related to humoral response but unrelated to PHA response (Bédécarrats and Leeson 2006). Carotenoids appear to compensate costly immune responses to pathogens (Ewen et al 2009), or become depleted the stronger an immune response (Saino et al 2003). In our study, house sparrow offspring fledged independently of their treatment or NDV-Ab concentration, suggesting that supposed advantages of enhanced specific humoral response, or costs of decreased antioxidative capacity and carotenoids in blood are to be experienced on a longer-term. However, the final cost-benefits balance derived from maternal transmission of antibodies will depend on the rate of exposure to pathogens, and the fitness costs derived from pathogen exposure in chicks with and without maternal antibodies.

In summary, we found that the specific humoral response to NDV in house sparrow chicks is enhanced by maternal exposure to NDV, most likely through passive transmission of specific Ab. However, nestlings investing in specific Ab production exhibit a decreased blood carotenoid
concentration and impaired antioxidative capacity, suggesting that maternal priming of specific humoral response is beneficial but may come to a developmental cost.

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Data Accessibility

Data deposited in the Dryad repository: XXX
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Table 1.

Body mass increase of house sparrow chicks from vaccination at four days of age to blood sampling at ten days of age, and body mass of house sparrow mothers according to the different treatments (C=control, V=Vaccinated). Change is mass is expressed as the least squared means from a GLM with treatments and the number of days elapsed as a covariate. Different treatments correspond to the combination of mother (Mo) and chicks’ (Ch) treatment: control chicks from control mothers (MoC ChC); control chicks from vaccinated mothers (MoV ChC); vaccinated chicks from control mothers (MoC ChV); and vaccinated chicks from vaccinated mothers (MoV ChV). Mean values with the corresponding standard error (SE) are provided for the different blood metabolite parameters (Total antioxidant capacity (TAC); Carotenoids (CAR); Total protein (TPR); Uric Acid (UAC)), for chicks and mothers on different treatments. Sample sizes are given within parentheses.
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<th>Mass change ± SE (g)</th>
<th>TAC ± SE (µmol/L)</th>
<th>CAR ± SE (mg/L)</th>
<th>TPR ± SE (mg/dL)</th>
<th>UAC ± SE (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoC ChC</td>
<td>6.98±0.70 (21)</td>
<td>931.34±111.05 (21)</td>
<td>15.62±2.26 (16)</td>
<td>2.23±0.16 (21)</td>
<td>7.03±0.62 (21)</td>
</tr>
<tr>
<td>MoC ChV</td>
<td>7.14±0.63 (25)</td>
<td>721.07±103.88 (24)</td>
<td>14.70±2.02 (20)</td>
<td>2.14±0.15 (24)</td>
<td>6.82±0.58 (24)</td>
</tr>
<tr>
<td>MoV ChC</td>
<td>5.52±0.73 (19)</td>
<td>890.97±116.75 (19)</td>
<td>10.38±2.26 (16)</td>
<td>2.10±0.17 (19)</td>
<td>6.81±0.65 (19)</td>
</tr>
<tr>
<td>MoV ChV</td>
<td>4.99±0.67 (23)</td>
<td>888.00±111.05 (21)</td>
<td>12.19±2.08 (19)</td>
<td>2.36±0.17 (21)</td>
<td>6.58±0.62 (21)</td>
</tr>
<tr>
<td>Mothers</td>
<td>Mass ± SE (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo C</td>
<td>26.25±0.46 (13)</td>
<td>1101.48±139.12 (12)</td>
<td>8.46±3.19 (12)</td>
<td>3.09±0.22 (12)</td>
<td>14.15±1.78 (12)</td>
</tr>
<tr>
<td>Mo V</td>
<td>26.11±0.48 (12)</td>
<td>963.58±145.31 (11)</td>
<td>10.16±2.88 (12)</td>
<td>3.02±0.23 (11)</td>
<td>14.84±1.85 (11)</td>
</tr>
</tbody>
</table>
Newcastle disease virus (NDV) antibody titres for house sparrow chicks and their mothers, in relation to the different experimental treatments (N=1-20). Sizes of the circles correspond to sample size. Antibody titres are expressed as the log of the inverse of the dilution factor.