Ecological and life-history factors influencing the evolution of maternal antibody allocation: a phylogenetic comparison

BriAnne Addison[1,*], **Kirk C. Klasing**[2], **W. Douglas Robinson**[3], **Suzanne H. Austin**[3] **and Robert E. Ricklefs**[1]

[1]*Biology Department, University of Missouri-St Louis, 1 University Boulevard, St Louis, MO 63130, USA*
[2]*Department of Animal Science, University of California Davis, 1 Shields Avenue, Davis, CA 95616, USA*
[3]*Oak Creek Lab of Biology, Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, USA*

Maternally derived yolk antibodies provide neonates with immune protection in early life at negligible cost to mothers. However, developmental effects on the neonate's future immunity are potentially costly and thus could limit yolk antibody deposition. The benefits to neonatal immunity must be balanced against costs, which may depend on neonate vulnerability to pathogens, developmental trajectories and the immunological strategies best suited to a species' pace of life. We measured yolk antibodies and life-history features of 23 species of small Neotropical birds and assessed the evidence for each of several hypotheses for life history and ecological effects on the evolution of yolk antibody levels. Developmental period and yolk antibodies are negatively related, which possibly reflect the importance of humoral immune priming through antigen exposure, and selection to avoid autoimmunity, in species with a slower pace of life. There is also a strong relationship between body size and yolk antibody concentration, suggesting that larger species are architecturally equipped to produce and transfer higher concentrations of antibodies. These results suggest that developmental effects of maternally derived antibodies, such as imprinting effects on B-cell diversity or autoimmune effects, are important and deserve more consideration in future research.

Keywords: disease ecology; immune development; maternal effects; life-history physiology; parasites; yolk antibodies

1. INTRODUCTION

Maternal influences on offspring phenotype, termed maternal effects (Mousseau & Fox 1998), have received special attention as an indirect genetic effect influencing an individual's life-history traits. The recent literature has focused on maternal allocation of hormones that direct resources to influence offspring behaviour, growth and development (Schwabl 1996; Gil 2003). However, few studies to date have investigated maternal provisioning of antibodies, which protect offspring from pathogens, influencing resource allocation and development of immunity (Grindstaff *et al.* 2003; Boulinier & Staszewski 2008; Hasselquist & Nilsson 2009).

Maternally derived yolk antibodies protect neonates against environmental pathogens during early life (Graczyk *et al.* 1994; Baintner 2007; Pravieux *et al.* 2007). Yolk antibodies are deposited during yolk formation by the active transport of circulating immunoglobulins across the follicle (Linden & Roth 1978; West

et al. 2004). The primary antibody present in avian eggs is immunoglobulin Y (IgY is for yolk, Rose *et al.* 1974; Tizard 2002), which is usually secreted by B-cells in direct response to an immune challenge. IgA and IgM appear at much lower concentrations and are primarily in the albumen of the egg. Chicken embryos undergo a period of rapid IgY uptake from their residual yolk, starting approximately 3 days prior to hatch and continuing for approximately 2 days post-hatch (Rose & Orlans 1981). Because most IgY is produced in a specific response to an immune challenge, neonates receive protection against pathogens likely to be relevant within their lifetime, and thus their passive immunity is shaped by their mothers' previous disease exposure. Maternally derived antibodies benefit chicks through two documented routes; first, they directly interfere with pathogenicity by coating pathogens (Carlier & Truyens 1995; Leuridan & Van Damme 2007; Nemeth *et al.* 2008), and second, they reduce the demands on the neonate immune system, and thus free resources to be directed towards other growth or maintenance needs (Pihlaja *et al.* 2006; Gallizzi *et al.* 2008; Grindstaff 2008). There has also been a proposed imprinting effect of maternal antibodies on immune development of offspring

* Author for correspondence (brianne.addison@gmail.com).

(Lemke & Lange 1999), where the B-cell repertoire is proposed to be modelled after the antibodies present during development. This is usually considered a potential benefit as there is some evidence that 'naive' offspring of non-naive mothers have higher responses to an antigen long after maternal antibodies have dissipated (Reid *et al.* 2006), though it can also have negative effects if mothers transfer autoantibodies (Greeley *et al.* 2002) or if imprinting mechanisms interfere with other immune system arms (Lemke & Lange 1999).

Despite their protective function, yolk antibodies make up only a small proportion of the total protein in the yolk. Presumably, maternal provisioning of antibodies also imposes costs that limit the amount allocated to eggs. These costs could be imposed on the hen during egg formation, or they may be imposed on the developing chick. The direct cost to the hen of producing antibodies is relatively low and is not likely to be higher than producing other egg proteins (Klasing 1998). Physiological factors might also constrain antibody synthesis by egg producing females. During reproduction, the immune system may be generally suppressed or reproductive resource use prioritized. For instance, during egg production in waterfowl, antibody synthesis changes to an alternate isotype (called IgYΔFc), which reduces the inflammatory response but is also not transferred to yolks (Humphrey *et al.* 2004). Two distinct developmental costs to chicks of maternal antibodies have been proposed. During B-cell development, lineages of pre-B-cells are positively selected if they are functional and bind to environmental antigens presented in the bursa or bind very loosely to self-antigens that might have molecular patterns similar to pathogenic organisms (Baumgarth *et al.* 2005). Pre-B-cells that are not functional or that bind tightly to self-antigens are negatively selected. The resulting set of B-cells recognizes a diverse set of antigens, but should not inflict damage via autoimmunity. However, large volumes of maternally derived antibodies could coat, and effectively hide, antigens that are important to B-cell development (Carlier & Truyens 1995). There is evidence that maternal antibodies coat antigens, inhibiting the juvenile immune response (Lung *et al.* 1996; Staszewski *et al.* 2007). If environmental and self-antigens were hidden from the B-cell selection process, antibody diversity in later life might be compromised by a failure of positive selection at the pre-B-cell stage. The hiding of self-antigens could also cause autoimmune disorders through a failure of the negative selection process. The influence of maternal antibodies on diversification of the B-cell repertoire (Fink *et al.* 2008) or autoimmune disease has a relatively short history in the medical literature (Zinkernagel 2001; Von Herrath & Bach 2002; Agarwal & Agarwal 2006; Victora *et al.* 2007). Although the costs remain poorly understood, we can assume that these costs do influence yolk antibody allocation decisions.

Many ecological and life-history factors influence total and specific maternal antibody allocation to eggs (Grindstaff *et al.* 2003). Antibody allocation should match the exposure of offspring to pathogens, which will be determined by characteristics of the rearing habitat, the nest type and the amount of time spent in the nest exposed to parasites. The developmental strategy will not only influence exposure period, but could also alter the relative importance of allocation of resources to immune responses or maturation (Lee 2006). We investigated ecological and life-history patterns of maternal antibody allocation to eggs in a variety of lowland tropical bird species. If mothers adaptively allocated antibodies in response to ecological or life-history factors, we would expect support for two non-mutually exclusive hypotheses: (i) developmental life-history hypothesis: a fast life-history strategy, where growth is rapid and adaptive immunity is less important (Lee 2006), will select for higher yolk antibody levels because this will help divert resources towards growth, despite costs to adaptive immune development; and (ii) nestling ecology hypothesis: open nests and long nestling periods, where offspring are relatively exposed to environmental pathogens and vectors of disease, will select for higher yolk antibody levels relative to enclosed nests or short nestling periods. Alternatively, enclosed nests have been suggested to harbour more ectoparasites and so may select for higher yolk antibodies compared with open nests.

2. MATERIAL AND METHODS

We located nests of passerine birds in and around Gamboa, Panama, from 15 March to 30 June 2006 and from 27 February to 25 May 2007. Gamboa is adjacent to Neotropical lowland dry forest in the Panama Canal Zone. The study period from February to May covers the dry-to-wet season transition (usually around mid-April) during the onset of the breeding season for most species in the area. Nests located during the building stage, or with only one egg, were used in the present study. Nests were checked daily, and first eggs were collected on the day the second egg was laid to prevent nest abandonment (except in clay-coloured robins *Turdus grayi*, where whole clutches were collected for another study). Collected eggs were stored at 4°C for up to 24 h until IgY extraction.

(a) *Life-history and ecological traits*

We included the following life history and ecological variables in the analyses: clutch size; incubation and nestling periods; nest type (open or enclosed) and adult body mass. We collected life-history data by locating nests and weighing adult birds captured during the breeding season. We found nests of most species during the nest construction and egg-laying phases of the breeding cycle. Nests were visited daily during egg laying and near expected hatch and fledging dates. Eggs were numbered individually as they were laid, and chicks were marked with non-toxic markers to facilitate individual identification. In this study, clutch size is the mean number of eggs per nest. Incubation period is the time from onset of incubation to hatching of the first egg; onset of incubation in most species was on the evening the penultimate egg of a clutch was laid. Nestling period is the time between hatching of the first egg and fledging of the first hatched chick. We categorized nests as open when they were open cups, platforms or scrapes on the ground. If nests were burrows, tree cavities, gourd- or pendant-shaped nests or cavities in termitaria, we categorized them as enclosed. Body masses were obtained from mist-netted birds at and near Gamboa.

(b) *Yolk immunoglobulin Y extraction and measurement*

Eggs were weighed whole and then broken open, and the yolks separated and weighed to the nearest 0.001 g. For

extraction, we aliquoted 0.1 g of yolk into a 1.5 ml microcentrifuge tube and removed the lipid by vortexing with 400 μl of 3.5 per cent PEG-6000 in 0.9 per cent NaCl, incubating overnight at 4°C and centrifuging for 30 min at 8000*g*, 4°C. The yolk pellet was discarded, and the supernatant reserved at 4°C for analysis. For yolk samples processed fresh, extraction efficiency is close to 100 per cent, as validated with a stripped yolk pool spiked with known quantities of chicken IgY (B. Addison 2007, unpublished data).

Yolk IgY was quantified by SDS–PAGE on 5 per cent gels run at 90 V for 30 min. Chicken IgY (purified polyclonal, Sigma-Aldrich, St Louis, MO, USA) standard dilutions were run simultaneously to generate a standard curve. Protein was stained with gelcode blue (Coomassie) stain (Pierce Biotechnology, Rockford, IL, USA) for 1 h and washed in distilled water overnight. Gels were then photographed on a white light table, and the images were analysed for band density using the gel tool in ImageJ (NIH, figure S1, electronic supplementary material). Integrated density values were converted to concentration, in mg ml^{-1}, using the chicken IgY standard curve, and the values were multiplied by 4 to account for dilution during extraction. We used yolk IgY concentration in the analysis because our species varied greatly in egg size.

Martinez *et al.* (2003) raise concerns about IgY overlap with other serum proteins, making SDS–PAGE methods for comparing among species difficult. However, to some degree, these concerns do not apply to our methods. First, we measure antibodies in yolk, which has significantly fewer proteins than plasma, and so concerns about protein overlap are to a large degree alleviated by this. Moreover, we use an extraction technique that separates protein from the plasma component of yolk and probably also selects to some degree for antibody-like proteins. This excludes a significant number of proteins from the SDS–PAGE analysis. To more thoroughly address these concerns, we calculated the amount of protein overlapping the IgY band of yolk plasma extracts from chicken yolk based on the table in Mann & Mann (2008). The emPAI (a measure of protein concentration) of IgY in the plasma portion only was reported as 1394. We calculated the emPAI values for all yolk plasma proteins overlapping the IgY band to total 53.3 (an overestimate, since some of these proteins were spread over regions beyond the IgY band). While the degree to which these proteins overlap and the proportions of total yolk protein are likely to vary among species, we expect this would only introduce noise or dampen relationships.

(c) Statistical analysis

For all species, we calculated the mean value of IgY concentration for our comparative analysis (response variable). We selected nine statistical models from the parameters we measured, including a null model and a global model for AICc model ranking and multimodel inference (Burnham & Anderson 2002). Our candidate model set included a global model with all parameters, a nestling ecology model, a development model, the bivariate models for each of the individual parameters and a null model (table 1).

We used a tree pruned from Cohen *et al.* (2008) to test for the effect of phylogeny. Using the regressionv2.m package (Lavin *et al.* 2008) in MATLAB (R2007a), we first fitted the best model of evolution for our traits of interest and then selected a set of candidate models for explaining life history and ecology effects on yolk IgY, accounting for phylogeny

Table 1. List of models included in the AICc analysis of life-history effects on yolk IgY deposition. The parameters included are clutch size (species mean and clutch), incubation period (species mean in days, incp), nestling period (species mean in days, nestlp), adult body mass (mean in g, mass), nest type (**o**pen or **c**losed, nesttype) and an evolutionary model (evolmodel).

ID	model
global 1	clutch, incp, nestlp, mass, nesttype, nestlp*nesttype, incp*nestlp, evolmodel
nestling ecology 2	clutch, nestlp, mass, nesttype, nestlp*nesttype, evolmodel
development 3	incp, nestlp, mass, incp*nestlp, evolmodel
4	clutch, evolmodel
5	incp, evolmodel
6	nestlp, evolmodel
7	mass, evolmodel
8	nesttype, evolmodel
null	evolmodel

and our model of evolution. The regressionv2.m package selects the best model of evolution for the traits of interest by fitting the saturated model for each of several models of evolution, including species values (ordinary least squares, no phylogenetic signal), Brownian motion (phylogenetic generalized least squares, equivalent to Felsenstein's contrasts), Ornstein–Uhlenbeck process (OU, drift about a fitness peak) or with branch lengths transformed using Pagel's λ parameter. The OU and Pagel's λ fitting procedures calculate branch length transformation parameters, *d* for the OU process that is a function of time and λ for Pagel's transformation, which is constant across the tree, by restricted maximum likelihood (REML). Values close to 1 indicate that evolution is close to Brownian motion, whereas values close to 0 approximate a star phylogeny.

3. RESULTS

We obtained life history and yolk antibody values for 23 species including dove, suboscine and oscine species (figure S2, electronic supplementary material). All species included have an altricial developmental mode, with incubation periods ranging from 12 to 23 days, nestling periods from 9 to 28 days, clutch size from two to four eggs, mass from 7.3 to 68 g and yolk antibody concentrations from 0.10 to 0.75 mg ml^{-1}. The within-species variability in IgY varied greatly by species.

In AICc evaluation of the evolutionary models for phylogenetic signal, the OLS star phylogeny was the best fit to the data for the global model with an AICc weight of 0.9 (table 2). To be conservative, we also evaluated evolutionary models for the null model. Both the OU and OLS trait evolution models had ΔAICc values under 2 and can be considered to have support. Furthermore, the REML estimates of phylogenetic signal were λ = 0.75 (Pagel's lambda evolution model) and *d* = 0.35 (OU evolution model) for the null model and λ = 0.39 and *d* = 0.15 for the global model, such that the λ- and *d*-transformed trees had shorter interior branches for the traits of interest. We report AICc scores for all life-history ecology models of yolk IgY for both OLS and OU evolutionary models.

Table 2. Evaluation of the best-fit evolutionary model for the global model and the null model. Non-phylogenetic analysis (OLS) was best fit for the global model, but second to models of a weak phylogenetic signal (OU) in the null model.

model	K	n	LgL	AICc	ΔAICc	$L\Delta$AICc	AICcW	evidence ratio	r^2
global = clutch, incp, nestlp, mass, nesttype, nestlp*nesttype, incp*nestlp, evolmodel									
OLS	9	23	12.358	7.130	0.000	1.000	0.920	1.000	0.707
OU	10	23	12.500	13.333	6.203	0.045	0.041	22.229	0.667
λ	10	23	12.358	13.617	6.487	0.039	0.036	25.626	0.669
PGLS	9	23	6.4207	19.005	11.875	0.003	0.002	379.001	0.573
null = evolmodel									
OU	2	23	-0.872	9.008	0.000	1.000	0.440	1.000	0
OLS	2	23	-2.584	9.768	0.761	0.684	0.301	1.463	0
λ	3	23	-1.842	10.947	1.939	0.379	0.167	2.637	0
PGLS	3	23	-3.768	12.136	3.128	0.209	0.092	4.779	0

The strongest model for both the OLS and OU analyses was the development model of yolk IgY (AICc weights of 0.95 and 0.81, respectively, table 3). The models that followed in support were the bivariate models of the parameters contained within the development model. Because the results for the OLS and OU analyses were qualitatively similar and because the AICc model weight for the top OLS model was greater than 0.95, we report only the OLS parameter estimates for the top model (table 4). The relationships between yolk IgY and both incubation and nestling periods were negative, but the interaction term was positive, indicating that the slopes are not equal. For species with longer incubation periods, the relationship with nestling period was weaker (figure 1). These patterns hold even with the exclusion of the common tody-flycatcher (*Todirostrum cinereum*) and grey-breasted martin (*Progne chalybea*), the species with the longest incubation and nestling periods, respectively, in our dataset. There was also a strong positive relationship between yolk IgY and adult body mass (figure 1).

We found no support for the effects of clutch size or nest type on yolk IgY, and the relationship with nestling period was opposite to the direction expected for the nestling ecology hypothesis.

4. DISCUSSION
We evaluated models representing two hypotheses for the evolution of yolk IgY deposition strategies. Both in AICc model evaluation and in multimodel inference, factors related to life history and development weighted substantially more important than factors related to nestling ecology. Life history, specifically development period, has a strong effect on yolk IgY allocation in the resident Neotropical birds sampled in our study. Moreover, the effect of phylogenetic history on this trait was weak, suggesting that it is highly labile. The effect of development on yolk IgY could be operating through several pathways, which are not mutually exclusive.

Larger adults deposit higher concentrations of yolk IgY in their eggs, which could reflect a greater capacity for antibody synthesis in larger animals (Davis *et al.* 1999; Magnadóttir *et al.* 1999; Lee *et al.* 2008) or some other allometric reason (Hasselquist 2007). Alternatively, the offspring of larger species may have a longer development period (Gillooly *et al.* 2008); however, there was no relationship between body mass and developmental

period in our species set. The effect of body size appears to reflect the architectural costs of yolk antibody deposition to the hen. It would be interesting to investigate whether these differences are indeed driven largely by plasma cell density and productivity or whether there are also differences in IgY receptor expression on the yolk follicle.

The strong negative relationship with incubation period most likely derives from incubation period serving as an index of development rate and/or pace of life. Since yolk IgY remains relatively inert until uptake by the embryo a short time before hatching (Tressler & Roth 1987; Kaspers *et al.* 1991), incubation period *per se* should not be related to yolk IgY deposition. If degradation of IgY over time prior to embryonic uptake were of concern, we would predict a positive relationship between incubation period and yolk IgY, which is the opposite of what we found. Thus, the relationship between IgY and this index of pace of life fits well with a developmental cost to maternal antibody allocation (discussed subsequently). There is little information on comparative rates of immune development in avian species, and what is known is largely restricted to precocial species (Apanius 1998).

Although species with longer nestling periods also deposit less IgY into their eggs, the relationship was weaker for species with long incubation periods, generating a positive interaction between incubation and nestling periods in their effect on yolk IgY. This could reflect a compromise between long development periods and long exposure periods, suggesting weak effects of nestling ecology. For instance, species with long incubation periods tend to have correspondingly long nestling periods (Lack 1968), which make the nestling more vulnerable to parasites normally found in high densities in the nest, such as philornid flies and mites. Nestling period is a poor index of the developmental period in the species in our dataset because many tropical species leave the nest at an early developmental state (Ricklefs 1976; Remes & Martin 2002). However, it might be a good indication of developmental priorities in very early life, as species leaving the nest early probably expend more resources prior to nest departure on traits such as feather growth and muscle maturation, required to achieve that early nest departure. As such, the benefits of maternal antibodies could be of greater importance, while the potential developmental costs are diminished, for these species.

Table 3. AICc ranking, delta values, likelihoods and weights and model r^2 for models of life-history effects on yolk IgY deposition. Analysis was done assuming a star phylogeny (OLS) and assuming stabilizing selection with drift (OU) using a tree compiled from the literature and presented in Cohen *et al.* (2008).

model	K	n	LgL	AICc	ΔAICc	LΔAICc	AICcW	evidence ratio	r^2
evolmodel = OLS									
3	6	23	10.517	−3.784	0.000	1.000	0.953	1.000	0.656
7	3	23	1.648	3.968	7.752	0.021	0.020	48.220	0.297
5	3	23	0.933	5.398	9.181	0.010	0.010	98.558	0.254
6	3	23	0.623	6.018	9.801	0.007	0.007	134.380	0.187
1	9	23	12.358	7.130	10.913	0.004	0.004	234.317	0.707
8	3	23	−0.255	7.773	11.557	0.003	0.003	323.223	0.176
2	7	23	6.326	8.814	12.598	0.002	0.002	543.915	0.505
null	2	23	−2.584	9.768	13.552	0.001	0.001	876.573	0.000
4	3	23	−2.043	11.350	15.133	0.001	0.000	1932.556	0.044
evolmodel = OU									
3	7	23	10.658	0.151	0.000	1.000	0.814	1.000	0.624
7	4	23	3.093	4.037	3.886	0.143	0.117	6.980	0.297
5	4	23	1.406	7.411	7.261	0.027	0.022	37.723	0.171
6	4	23	1.503	7.216	7.066	0.029	0.024	34.220	0.131
null	3	23	−0.872	9.008	8.857	0.012	0.010	83.799	0.000
8	4	23	0.171	9.880	9.729	0.008	0.006	129.632	0.078
4	4	23	−0.651	11.524	11.373	0.003	0.003	294.864	0.019
2	8	23	7.645	10.996	10.846	0.004	0.004	226.507	0.492
1	10	23	12.500	13.333	13.182	0.001	0.001	728.460	0.667

Table 4. Parameter estimates of effect on yolk IgY deposition based on the top OLS model (assuming star phylogeny) that had an AICc weight greater than 0.95.

	estimate	(±s.e.)
intercept	3.723478	1.298166
incp	−0.2828359	0.08787441
nestlp	−0.2780414	−0.08941521
mass	0.008275306	0.002668124
incp*nestlp	0.0171757	0.005923048

The idea that maternally derived antibodies have a blocking effect on offspring immunity has been important for the development of vaccination schedules in human and veterinary medicine (Block *et al.* 2007; Siegrist 2007). Although the effect of maternal antibodies on immune responses is well documented, it is not clear whether this blocking effect is exclusive to T-cell-mediated responses or whether it might also affect lymphocyte development (Carlier & Truyens 1995). The recognition of antigen is an important component of B-cell development (Tizard 2002; Baumgarth *et al.* 2005), presenting a potential mechanism for a negative effect of maternal antibodies on immune system development. However, some evidence also supports a positive effect via immunological imprinting (Gasparini *et al.* 2006; Grindstaff *et al.* 2006; Reid *et al.* 2006). The exact mechanism is unclear, but the B-cell repertoire may somehow model itself after antibodies in circulation (Fink *et al.* 2008).

The relationship between maternal antibodies, development time and immunity is probably complex and dependent on the arm of the immune system of interest and possibly even the route of response to a specific antigen. The evidence for an effect of developmental period, or pace of life in general, on immunity is mixed, and much more needs to be done to understand what predictions should be made for different arms of the immune system. Tella *et al.* (2002) found that slow pace of life species have higher cell-mediated immunity assayed by phytohaemagglutinin (PHA) wing web swelling, whereas Palacios & Martin (2006) found the opposite relationship using the same measure of immunity. Tieleman *et al.* (2005) found that slow pace of life species had higher innate immunity measured by bactericidal activity of blood. Lee *et al.* (2008) found that slow pace of life species had higher natural antibodies (relationship with incubation period) and lower complement activity (relationship with clutch size) in a set of species that includes many of the same species as this study. Different arms of the immune system could evolve independently, and the exact directional predictions probably depend a great deal on what aspect of immunity is measured (Blount *et al.* 2003). Perhaps, one of the best illustrations of this is a study of immune function in high (fast pace of life) and low latitude (slow pace of life) populations of house sparrows (*Passer domesticus*, Martin *et al.* 2006), which found that the slow pace of life population had a higher speed of humoral response measured as secondary antibody response to wing web injections of keyhole limpet haemocyanin and a higher magnitude of cell-mediated response measured by PHA wing web injection, but a lower magnitude peak of humoral response and lower T-cell response to KLH challenge compared with the fast pace of life population. It has been suggested that long developmental periods, and a slow pace of life, permit and perhaps necessitate the development of a stronger adaptive immune system (Lee *et al.* 2008). Species with long incubation periods have previously been shown to have lower parasite-induced nestling mortality (Møller 2005) and lower adult stage hematozoan infection prevalence (Ricklefs 1992), suggesting
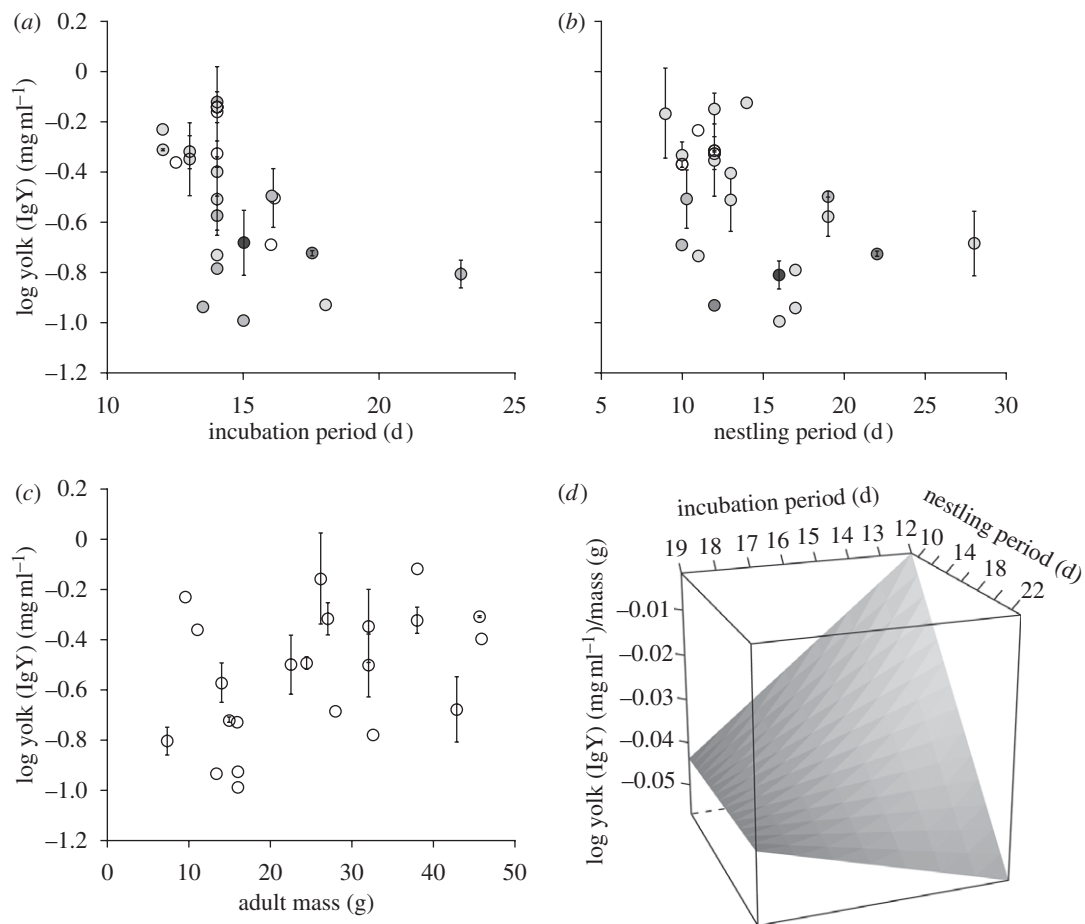
Figure 1. Life-history predictors of yolk IgY deposition. Points are species means ± s.e. (*a*) Incubation period is strongly negatively related to yolk IgY deposition, even if the common tody-flycatcher (*T. cinereum*, incubation period of 23 days) is excluded. Marker shade is coded by the nestling period, darker colours correspond to longer nestling periods. (*b*) Nestling period is also negatively related to yolk IgY deposition, again, even with the exclusion of the extreme value of the grey-breasted martin (*P. chalybea*, nestling period of 28 days). Marker shade corresponds to incubation period. (*c*) Adult body mass is positively related to yolk IgY deposition. (*d*) Predictive surface showing the relationship between incubation period, nestling period and mass corrected yolk [IgY], generated without the two extreme value species.

potentially important effects of pace of life on immune development.

Yolk IgY concentrations measured in this study were lower than levels measured in chickens (1.15–2.26 mg ml^{-1}, Hamal *et al.* 2006) or quail *Coturnix japonica* (3.27–4.35 mg ml^{-1}, Grindstaff *et al.* 2005) and comparable to estimates reported in *Ficedula albicollis* (0.63 mg ml^{-1} relative to chicken standard, Hargitai *et al.* 2006). Several factors could contribute to the low levels measured in wild altricial birds. Domesticated fowls have better nutrition and thus more easily produce antibodies for allocation to eggs. Alternatively, the species studied in the wild are generally of small body size, and this appears to be a very important factor. Also, the altricial developmental mode, where much of the immune development probably occurs after hatch when maternal antibodies are active, may contribute to lower levels. Many of the studies of maternal antibodies in wild species have not attempted to quantify actual concentrations and have instead used relative values measured in immunoassays. This inhibits further comparative analysis, and researchers are encouraged to quantify actual values alongside relative values whenever possible.

Previous studies have found that hen plasma antibody levels and egg yolk antibodies are correlated

(Hamal *et al.* 2006). For specific antigens, the slope of the correlation between hen plasma and yolk antibodies differs for different idiotypes (Tizard 2002), which could indicate a degree of selectivity for the movement of antibodies across the follicle membrane. There are two possible ways for adjustment of yolk antibody levels. Adjustment of circulating plasma antibodies allows control both of the total antibodies deposited in yolks as the molecules will tend to move down a concentration gradient and of the proportion of different antibody idiotypes in the yolk. The second way to modify yolk antibodies is by adjustment of yolk follicle receptor density. It is not clear to what degree different species employ these two possible strategies.

Much of the ecoimmunology literature has addressed almost exclusively benefits of maternal antibody allocation (Boulinier & Staszewski 2008; Hasselquist & Nilsson 2009), but the low levels of yolk IgY in wild species strongly indicate important costs. We suggest three physiological avenues of investigation that remain unexplored in a comparative context. First, we need to understand the role of maternally derived antibodies in B-cell development, in particular during pre-B-cell selection in the bursa, and in T-cell development. Studies should consider the different modes of immune response,

T-cell dependent versus independent, for different antigen patterns. Second, we need more information on the time course of immune development for different arms of the immune system and among different life-history strategies. For instance, species investing little in humoral immunity should prioritize development of the innate arms of the immune system, with B-cell development occurring later and more rapidly. Third, we need to understand the long-term consequences of maternal antibodies for B-cell and T-cell repertoires, immunologic responsiveness and memory, immunosenescence, and autoimmunity.

Overall, our analysis suggests a developmental cost limiting yolk IgY deposition, at least in small Neotropical birds. Although life history appears to be an important determinant of the evolution of this maternal effect, outstanding questions remain about the causes of inter-individual variability of this trait and the physiological activity of maternal antibodies, especially with respect to costs.

REFERENCES

Agarwal, K. & Agarwal, R. 2006 Effects of maternal auto-immune diseases on fetus and neonates. *J. Neonatol.* **20**, 172–175.

Apanius, V. 1998 The immune system. In *Avian growth and development: evolution within the altricial–precocial spectrum* (eds J. M. Starck & R. E. Ricklefs), pp. 203–222. Oxford, UK: Oxford University Press.

Baintner, K. 2007 Transmission of antibodies from mother to young: evolutionary strategies in a proteolytic environment. *Vet. Immunol. Immunopathol.* **117**, 153–161. (doi:10.1016/j.vetimm.2007.03.001)

Baumgarth, N., Tung, J. W. & Herzenberg, L. A. 2005 Inherent specificities in natural antibodies: a key to immune defense against pathogen invasion. *Semin. Immunopathol.* **26**, 347–362. (doi:10.1007/s00281-004-0182-2)

Block, H., Meyer-Block, K., Rebeski, D. E., Scharr, H., de Wit, S., Rohn, K. & Rautenschlein, S. 2007 A field study on the significance of vaccination against infectious bursal disease virus (IBDV) at the optimal time point in broiler flocks with maternally derived IBDV anti-bodies. *Avian Pathol.* **36**, 401–485. (doi:10.1080/03079450701589175)

Blount, J. D., Houston, D. C., Møller, A. P. & Wright, J. 2003 Do individual branches of immune defence correlate? A comparative case study of scavenging and non-scavenging birds. *Oikos* **102**, 340–350. (doi:10.1034/j.1600-0706.2003.12413.x)

Boulinier, T. & Staszewski, V. 2008 Maternal transfer of antibodies: raising immuno-ecology issues. *Trends Ecol. Evol.* **23**, 282–288. (doi:10.1016/j.tree.2007.12.006)

Burnham, K. P. & Anderson, D. R. 2002 *Model selection and multimodel inference: a practical information-theoretic approach.* New York, NY: Springer-Verlag.

Carlier, Y. & Truyens, C. 1995 Influence of maternal infection on offspring resistance towards parasites. *Parasitol. Today* **11**, 94–99. (doi:10.1016/0169-4758(95)80165-0)

Cohen, A. A., McGraw, K. J., Wiersma, P., Williams, J. B., Robinson, W. D., Robinson, T. R., Brawn, J. D. & Ricklefs, R. E. 2008 Interspecific associations between circulating antioxidant levels and life-history variation in birds. *Am. Nat.* **172**, 178–193.

Davis, C. R., Marty, G. D., Adkison, M. A., Freiberg, E. F. & Hedrick, R. P. 1999 Association of plasma IgM with body size, histopathologic changes, and plasma chemistries in adult Pacific herring. *Clupea pallasi. Dis. Aquat. Organ.* **38**, 125–133. (doi:10.3354/dao038125)

Fink, K., Zellweger, R., Weber, J., Manjarrez-Orduno, N., Holdener, M., Senn, B. M., Hengartner, H., Zinkernagel, R. M. & Macpherson, A. J. 2008 Long-term maternal imprinting of the specific B cell repertoire by maternal antibodies. *Eur. J. Immunol.* **38**, 90–101. (doi:10.1002/eji.200737872)

Gallizzi, K., Guenon, B. & Richner, H. 2008 Maternally transmitted parasite defence can be beneficial in the absence of parasites. *Oikos* **117**, 223–230. (doi:10.1111/j.2007.0030-1299.16172.x)

Gasparini, J., McCoy, K. D., Staszewski, V., Haussy, C. & Boulinier, T. 2006 Dynamics of anti-Borrelia antibodies in blacklegged kittiwake (*Rissa tridactyla*) chicks suggest a maternal educational effect. *Can. J. Zool.-Revue Canadienne De Zoologie* **84**, 623–627. (doi:10.1139/Z06-024)

Gil, D. 2003 Golden eggs: maternal manipulation of off-spring phenotype by egg androgen in birds. *Ardeola* **50**, 281–294.

Gillooly, J. F., Londono, G. A. & Allen, A. P. 2008 Energetic constraints on an early developmental stage: a comparative view. *Biol. Lett.* **4**, 123–126. (doi:10.1098/rsbl.2007.0460)

Graczyk, T. K., Cranfield, M. R., Shaw, M. L. & Craig, L. E. 1994 Maternal antibodies against *Plasmodium* spp. in African black-footed penguin (*Spheniscus demersus*) chicks. *J. Wildl. Dis.* **30**, 365–371.

Greeley, S. A. W., Katsumata, M., Yu, L., Eisenbarth, G. S., Moore, D. J., Goodarzi, H., Barker, C. F., Naji, A. & Noorchashm, H. 2002 Elimination of maternally transmitted autoantibodies prevents diabetes in nonobese diabetic mice. *Nat. Med.* **8**, 399–402. (doi:10.1038/nm0402-399)

Grindstaff, J. L. 2008 Maternal antibodies reduce costs of an immune response during development. *J. Exp. Biol.* **211**, 654–660. (doi:10.1242/jeb.012344)

Grindstaff, J. L., Brodie, E. D. & Ketterson, E. D. 2003 Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proc. R. Soc. Lond. B* **270**, 2309–2319. (doi:10.1098/rspb.2003.2485)

Grindstaff, J. L., Demas, G. E. & Ketterson, E. D. 2005 Diet quality affects egg size and number but does not reduce maternal antibody transmission in Japanese quail *Coturnix japonica. J. Anim. Ecol.* **74**, 1051–1058. (doi:10.1111/j.1365-2656.2005.01002.x)

Grindstaff, J. L., Hasselquist, D., Nilsson, J. A., Sandell, M., Smith, H. G. & Stjernman, M. 2006 Transgenerational

priming of immunity: maternal exposure to a bacterial antigen enhances offspring humoral immunity. *Proc. R. Soc. B* **273**, 2551–2557. (doi:10.1098/rspb. 2006.3608)

Hamal, K. R., Burgess, S. C., Pevzner, I. Y. & Erf, G. F. 2006 Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poult. Sci.* **85**, 1364–1372.

Hargitai, R., Prechl, J. & Torok, J. 2006 Maternal immuno-globulin concentration in collared flycatcher (*Ficedula albicollis*) eggs in relation to parental quality and laying order. *Funct. Ecol.* **20**, 829–838. (doi:10.1111/j.1365-2435.2006.01171.x)

Hasselquist, D. 2007 Comparative immunoecology in birds: hypotheses and tests. *J. Ornithol.* **148**, 571–582. (doi:10. 1007/s10336-007-0201-x)

Hasselquist, D. & Nilsson, J. A. 2009 Maternal transfer of antibodies in vertebrates: trans-generational effects on off-spring immunity. *Phil. Trans. R. Soc. B* **364**, 51–60. (doi:10.1098/rstb.2008.0137)

Humphrey, B. D., Calvert, C. C. & Klasing, K. C. 2004 The ratio of full length IgY to truncated IgY in immune com-plexes affects macrophage phagocytosis and the acute phase response of mallard ducks (*Anas platyrhynchos*). *Dev. Comp. Immunol.* **28**, 665–672. (doi:10.1016/j.dci. 2003.11.003)

Kaspers, B., Schranner, I. & Losch, U. 1991 Distribution of immunoglobulins during embryogenesis in the chicken. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **38**, 73–79.

Klasing, K. C. 1998 Nutritional modulation of resistance to infectious diseases. *Poult. Sci.* **77**, 1119–1125.

Lack, D. L. 1968 *Ecological adaptations for breeding in birds.* London, UK: Methuen.

Lavin, S. R., Karasov, W. H., Ives, A. R., Middleton, K. M. & Garland, T. 2008 Morphometrics of the avian small intestine compared with that of nonflying mammals: a phylogenetic approach. *Physiol. Biochem. Zool.* **81**, 526–550. (doi:10.1086/590395)

Lee, K. A. 2006 Linking immune defenses and life history at the levels of the individual and the species. *Integr. Comp. Biol.* **46**, 1000–1015. (doi:10.1093/icb/icl049)

Lee, K. A., Wikelski, M., Robinson, W. D., Robinson, T. R. & Klasing, K. C. 2008 Constitutive immune defences correlate with life-history variables in tropical birds. *J. Anim. Ecol.* **77**, 356–363. (doi:10.1111/j.1365-2656. 2007.01347.x)

Lemke, H. & Lange, H. 1999 Is there a maternally induced immunological imprinting phase a la Konrad Lorenz? *Scand. J. Immunol.* **50**, 348–354. (doi:10.1046/j.1365-3083.1999.00620.x)

Leuridan, E. & Van Damme, P. 2007 Passive transmission and persistence of naturally acquired or vaccine-induced maternal antibodies against measles in newborns. *Vaccine* **25**, 6296–6304. (doi:10.1016/j.vaccine.2007.06.020)

Linden, C. D. & Roth, T. F. 1978 IgG receptors on foetal chick yolk sac. *J. Cell Sci.* **33**, 317–328.

Lung, N. P., Thompson, J. P., Kollias, G. V., Olsen, J. H., Zdziarski, J. M. & Klein, P. A. 1996 Maternal immunoglobulin G antibody transfer and development of immunoglobulin G antibody responses in blue and gold macaw (*Ara ararauna*) chicks. *Am. J. Vet. Res.* **57**, 1162–1167.

Magnadóttir, B., Jónsdóttir, H., Helgason, S., Björnsson, B., Jøgensen, T. Ø. & Pilström, L. 1999 Humoral immune parameters in Atlantic cod (*Gadus morhua* L.). II. The effects of size and gender under different environmental conditions. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **122**, 181–188. (doi:10.1016/S0305-0491(98) 10157-8)

Mann, K. & Mann, M. 2008 The chicken egg yolk plasma and granule proteomes. *Proteomics* **8**, 178–191. (doi:10. 1002/pmic.200700790)

Martin II, L. B., Hasselquist, D. & Wikelski, M. 2006 Invest-ment in immune defense is linked to pace of life in house sparrows. *Oecologia* **147**, 565–575. (doi:10.1007/s00442-005-0314-y)

Martinez, J., Tomas, G., Merino, S., Arriero, E. & Moreno, J. 2003 Detection of serum immunoglobulins in wild birds by direct ELISA: a methodological study to validate the technique in different species using antichicken antibodies. *Funct. Ecol.* **17**, 700–706. (doi:10.1046/j. 1365-2435.2003.00771.x)

Møller, A. P. 2005 Parasites, predators and the duration of developmental periods. *Oikos* **111**, 291–301. (doi:10. 1111/j.0030-1299.2005.14005.x)

Mousseau, T. A. & Fox, C. W. 1998 The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**, 403–407. (doi:10.1016/S0169-5347(98)01472-4)

Nemeth, N. M., Oesterle, P. T. & Bowen, R. A. 2008 Passive immunity to West Nile virus provides limited protection in a common passerine species. *Am. J. Trop. Med. Hyg.* **79**, 283–290.

Palacios, M. G. & Martin, T. E. 2006 Incubation period and immune function: a comparative field study among coex-isting birds. *Oecologia* **146**, 505–512. (doi:10.1007/ s00442-005-0220-3)

Pihlaja, M., Siitari, H. & Alatalo, R. V. 2006 Maternal antibodies in a wild altricial bird: effects on offspring immunity, growth and survival. *J. Anim. Ecol.* **75**, 1154–1164. (doi:10.1111/j.1365-2656.2006.01136.x)

Pravieux, J. J., Poulet, H., Charreyre, C. & Juillard, V. 2007 Protection of newborn animals through maternal immu-nization. *J. Comp. Pathol.* **137**, S32–S34. (doi:10.1016/j. jcpa.2007.04.009)

Reid, J. M., Arcese, P., Keller, L. F. & Hasselquist, D. 2006 Long-term maternal effect on offspring immune response in song sparrows *Melospiza melodia*. *Biol. Lett.* **2**, 573–576. (doi:10.1098/rsbl.2006.0544)

Remes, V. & Martin, T. E. 2002 Environmental influences on the evolution of growth and developmental rates in passer-ines. *Evolution* **56**, 2505–2518.

Ricklefs, R. E. 1976 Growth rate of birds in the humid New World tropics. *Ibis* **118**, 176–207. (doi:10.1111/j. 1474-919X.1976.tb03065.x)

Ricklefs, R. E. 1992 Embryonic development period and the prevalence of avian blood parasites. *Proc. Natl Acad. Sci. USA* **89**, 4722–4725. (doi:10.1073/pnas.89.10.4722)

Rose, M. E. & Orlans, E. 1981 Immunoglobulins in the egg, embryo and young chick. *Dev. Comp. Immunol.* **5**, 15–20. (doi:10.1016/S0145-305X(81)80003-1)

Rose, M. E., Orlans, E. & Buttress, N. 1974 Immunoglobu-lin classes in hens egg—their segregation in yolk and white. *Eur. J. Immunol.* **4**, 521–523. (doi:10.1002/eji. 1830040715)

Schwabl, H. 1996 Maternal testosterone in the avian egg enhances postnatal growth. *Comp. Biochem. Physiol. A Physiol.* **114**, 271–276. (doi:10.1016/0300-9629(96)00009-6)

Siegrist, C. A. 2007 The challenges of vaccine responses in early life: selected examples. *J. Comp. Pathol.* **137**, S4–S9. (doi:10.1016/j.jcpa.2007.04.004)

Staszewski, V., Gasparini, J., McCoy, K. D., Tveraa, T. & Boulinier, T. 2007 Evidence of an interannual effect of maternal immunization on the immune response of juveniles in a long-lived colonial bird. *J. Anim. Ecol.* **76**, 1215–1223. (doi:10.1111/j.1365-2656.2007. 01293.x)

Tella, J. L., Scheuerlein, A. & Ricklefs, R. E. 2002 Is cell-mediated immunity related to the evolution of life-history

strategies in birds? *Proc. R. Soc. Lond. B* **269**, 1059–1066. (doi:10.1098/rspb.2001.1951)

Tieleman, B. I., Williams, J. B., Ricklefs, R. E. & Klasing, K. C. 2005 Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. *Proc. R. Soc. B* **272**, 1715–1720. (doi:10.1098/rspb.2005.3155)

Tizard, I. 2002 The avian antibody response. *Semin. Avian Exot. Pet Med.* **11**, 2–14. (doi:10.1053/saep.2002.28216)

Tressler, R. L. & Roth, T. F. 1987 IgG receptors on the embryonic chick yolk-sac. *J. Biol. Chem.* **262**, 15 406–15 412.

Victora, G. D., Bilate, A. M. B., Socorro-Silva, A., Caldas, C., Lima, R. C., Kalil, J., Coelho, V. & Victora, C. G. 2007 Mother–child immunological interactions in early life affect long-term humoral autoreactivity to heat shock protein 60 at age 18 years. *J. Autoimmun.* **29**, 38–43. (doi:10.1016/j.jaut.2007.02.018)

Von Herrath, M. & Bach, J. F. 2002 Juvenile autoimmune diabetes: a pathogenic role for maternal antibodies? *Nat. Med.* **8**, 331–333. (doi:10.1038/nm0402-331)

West, A. P., Herr, A. B. & Bjorkman, P. J. 2004 The chicken yolk sac IgY receptor, a functional equivalent of the mammalian MHC-related Fc receptor, is a phospholipase A(2) receptor homolog. *Immunity* **20**, 601–610.

Zinkernagel, R. M. 2001 Maternal antibodies, childhood infections, and autoimmune diseases. *N. Engl. J. Med.* **345**, 1331–1335. (doi:10.1056/NEJMra012493)