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Long Antibody Persistence and Transgenerational Transfer of Immunity in a Long-Lived Vertebrate

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ABSTRACT: Although little studied in natural populations, the persistence of immunoglobulins may dramatically affect the dynamics of immunity and the ecology and evolution of host–pathogen interactions involving vertebrate hosts. By means of a multiple-year vaccination design against Newcastle disease virus, we experimentally addressed whether levels of specific antibodies can persist over several years in females of a long-lived procellariiform seabird—Cory's shearwater—and whether maternal antibodies against that antigen could persist over a long period in offspring several years after the mother was exposed. We found that a single vaccination led to high levels of antibodies for several years and that the females transmitted antibodies to their offspring that persisted for several weeks after hatching even 5 years after a single vaccination. The temporal persistence of maternally transferred antibodies in nestlings was highly dependent on the level at hatching. A second vaccination boosted efficiently the level of antibodies in females and thus their transfer to offspring. Overall, these results stress the need to consider the temporal dynamics of immune responses if we are to understand the evolutionary ecology of host–parasite interactions and trade-offs between immunity and other life-history characteristics, in particular in long-lived species. They also have strong implications for conservation when vaccination may be used in natural populations facing disease threats.

Keywords: humoral response, life-history traits, maternal antibodies, *Calonectris borealis*, Newcastle disease virus, parasite resistance, trade-off, vaccination.

Introduction

Infectious diseases are thought to play a significant role in local population extinctions, losses of biodiversity, population declines, coevolutionary processes, and shifts in community composition (Daszak et al. 2000; Gandon and Day 2007). Wildlife in general and birds in particular can

play a role in the maintenance and ecology of pathogens (e.g., Olsen et al. 2006) but also be exposed to spillovers of infectious agents originating from domesticated animals (Gardner et al. 1997; Cardenas Garcia et al. 2013). In this eco-epidemiological context, basic knowledge on the diverse strategies of resistance to pathogens across animal taxa becomes essential for a global understanding of host–parasite interactions. For instance, life-history theory predicts a trade-off between investing in immunity and in other physiologically demanding life-history components (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Ricklefs and Wikelski 2002; Staszewski and Boulinier 2004; Viney et al. 2005). Different life histories should thus result in a diversity of immunological strategies (Ricklefs and Wikelski 2002). This trade-off is expected to be particularly apparent between the innate and acquired arms of the immune system (Lee 2006; Garnier et al. 2012a). Long-lived species would, for instance, be expected to skew their immune investment toward more specific and persistent responses (Lee 2006; Garnier et al. 2013). In these species, the temporal dynamics of specific antibody levels (notably of immunoglobulin G [IgG] in mammals and immunoglobulin Y [IgY] in birds) throughout an individual's lifetime is thus of particular interest.

Yet very little is known about the dynamics of the immune response in natural populations of wild long-lived vertebrates (Staszewski et al. 2007b; Miller and Olea-Poppelka 2009; Root et al. 2010; Rossi et al. 2011; Bodewes et al. 2013). Most studies so far have focused on humans, livestock and poultry species, model laboratory animals, and relatively fast-living species (Feuer et al. 1999; Frank 2002; Beal et al. 2004; Bunikis et al. 2004; Gibbs et al. 2005; Kuenzi et al. 2005; Henning et al. 2006; Martin et al. 2006; Amanna et al. 2007; Tizard 2013). In addition, the few studies of long-lived species usually lack the longitudinal dimension necessary to assess how immune responses vary over the course of a life span (Sandland and

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Minchella 2003; Chang et al. 2007; Guiserix et al. 2007; Lecomte et al. 2010; Garnier et al. 2012*b*). One mechanism that offers the potential for such studies is the transgenerational transfer of antibodies from mothers to offspring. It represents the very first step of natural selection while facing pathogenic infections, and it is relatively well described (Brambell 1970), even in wild species (Grindstaff et al. 2003; Boulinier and Staszewski 2008; Hasselquist and Nilsson 2009). However, although the dynamics of the transferred antibodies in the offspring is well described in mammals, it remains relatively poorly studied in birds. For instance, a recent study in a long-lived seabird species showed that maternal antibodies can persist several weeks after hatching (Garnier et al. 2012*b*), much longer than previously thought for any bird species (Davison et al. 2008). Because initial levels of antibodies in the newborn depend on maternal levels (Gasparini et al. 2001, 2002) and in turn determine their persistence (Grindstaff 2010), the temporal dynamics of antibody levels in adults represents highly valuable information. In addition to being key for the understanding of the transfer of maternal antibodies, this information is necessary to accurately interpret serological measures of wild-caught individuals (Staszewski et al. 2007*a*) or egg yolks (Pearce-Duvel et al. 2009) and can also provide essential elements for the parameterization of epidemiological models of infectious agents in wild populations (notably in case a vaccine could be used as a management tool; Haydon et al. 2006; Keeling and Rohani 2008). From an evolutionary ecology standpoint, this trait can also be expected to vary among species as a function of their life histories.

Many long-lived bird species suffer from worldwide population declines and are threatened. In addition to mortality generated directly and indirectly by human activities (Lewison et al. 2004; Le Bohec et al. 2008), several disease outbreaks are potentially threatening many species (Rolland et al. 2009; Descamps et al. 2012; Massaro et al. 2012). Long-lived organisms are characterized by delayed maturities, low reproductive rates, and high adult survival. Small reductions in survival caused by disease agents may thus have dramatic consequences on the reproductive value of individuals and, ultimately, on population trends of these long-lived species. Directly transmitted parasites are of particular ecological, evolutionary, and epidemiological interest in species having regular social interactions (Loehle 1995), such as colonially breeding species. Some of these agents—such as Newcastle disease virus (NDV), highly pathogenic avian influenza viruses, the bacteria responsible for avian cholera (*Pasteuralla multocia*), or the bacteria responsible for erysipelas (*Erhysipelotrix rhusopathiae*)—share the particularity of being prevalent in poultry, where they can be responsible for important losses but also for possibly affecting wild birds. Because of their im-

portance for poultry, efficient vaccines are available or can be developed against several of these agents. The temporal dynamics and the efficacy of the humoral immune response of chickens to these vaccines can vary greatly (e.g., Walker et al. 2010), but very little is known about the dynamics of the humoral immune response in long-lived bird species (Bailey et al. 1998; Momayez et al. 2007; Garnier et al. 2012*b*; Tolf et al. 2013). For instance, whether the long persistence of maternal antibodies reported in a long-lived seabird species (Garnier et al. 2012*b*) is associated with the persistence of high antibody levels in adults would be of interest. The immune dynamics of adult females would indeed be expected to affect the initial levels of antibodies passively acquired by chicks and, thus, the duration of protection by maternal antibodies.

In order to address these questions, we investigated the interannual and transgenerational dynamics of specific IgY in females and offspring of a long-lived procellariiform seabird, Cory's shearwater *Calonectris borealis*, using a long-term vaccination experiment against NDV. Specifically, we collected data that allowed us to model (1) the specific humoral response over six entire years following the first antigen exposure, (2) the transgenerational transfer of maternal antibodies to offspring by monitoring the dynamics of specific anti-NDV antibody levels in both chicks and their mothers before laying, and (3) the persistence of maternal antibodies over the chick-rearing period. We also investigated the existence of potential trade-offs between maternal antibody persistence and other physiologically demanding processes, such as chick growth. By addressing these issues, this study highlights the potential importance of considering the temporal dynamics of immune responses within the life span of individuals and across generations if we are to better understand the ecology and evolution of host-parasite interactions in long-lived species.

Methods

Model Species and Study Colony

Cory's shearwater is a medium-sized procellariiform species that has a long life span of more than 30 years. This colonial pelagic petrel breeds annually in remote islets and islands, mainly visiting the nest during the night. As do all procellariiforms, Cory's shearwaters invest highly in reproduction: adults arrive at the colony in late February or early March, females lay a single egg in late May or early June, both mates share incubation duties for long periods (54 days in total on average), and chicks hatch in mid-late July and fledge in late October–early November (Thibault et al. 1997). During this long chick-rearing period (about 90 days), the chick is left alone in its burrow

while both male and female forage at sea, alternating chick provisioning duties every 1–10 nights (depending on the size and age of the chick). Cory's shearwaters, like many colonial seabirds, are highly monogamous and show strong philopatry and interannual breeding burrow fidelity. Mates can therefore often be found in the same burrows year after year (Thibault et al. 1997). This study was conducted in a Cory's shearwater colony in Gran Canaria (15°47'18"N, 27°50'41"E; Canary Archipelago, Spain), and fieldwork experiments took place over six breeding seasons from 2008 to 2013.

Experimental Protocol and Sampling Design

Because only breeding females were of interest for our study, sex was determined using biometric (Navarro et al. 2009) or genetic (primers 2550F and 2718R; as described by Ellegren 1996) criteria before sampling. During the arrival periods of 2008 and 2010 (early March), 90 females were caught, and 1.0 mL of blood was collected from the tarsal vein, using sterile syringes and needles. Blood samples were stored in 1.3-mL microtubes with Li-heparin (Sarstedt, Germany) and kept cool until centrifugation a few hours later. After blood sampling, each female received a subcutaneous injection in the back of the neck of either 0.25 mL of a killed vaccine against NDV (Nobivac Paramyx P201, Intervet, France) or the same amount of a saline solution.

Given that the aim of this study was to explore the temporal dynamics of the immune response while controlling antigen exposure by using a vaccine (Staszewski and Boulinier 2004), we checked that the study population was not naturally exposed to the NDV infectious agent. All values for the control and first-caught females were well below the enzyme-linked immunosorbent assay (ELISA) kit threshold for positivity. Moreover, among all the female Cory's shearwaters sampled before any manipulation, none was above a negative threshold computed using the subset of females that had received the saline injection (mean percentage of inhibition [PI], 10.3%; 95% confidence interval [CI], 8.2–12.4; $n = 105$ females). When possible, experimental females were also blood sampled around the time of egg-laying each year (note that sampling effort was not constant throughout the study period; see capture probability in table A1; tables A1–A3 available online). Additionally, 16 females already vaccinated in 2008 were revaccinated at the beginning of the breeding season of 2010 to test for the effect of boosting the immune response with a second exposure to the antigen. Using capture-mark-recapture techniques (M-Surge ver. 1.8, with a total of 147 female capture-recapture histories over the 2008–2013 period; Choquet et al. 2006), we did not detect any short-term effect of the vaccination

on either bird capturability or their survival (preferred model $\Phi_{(\cdot)} p_{(t)}$, where survival remained constant over the period while capture probability depended on the year of sampling; quasi-likelihood Akaike information criterion [ΔQAICc] ($\Phi_{(\text{group} \times t)} p_{(\text{group} \times t)} - \Phi_{(\cdot)} p_{(t)}$) = 16.24; for details, see table A1).

Daily checks of burrows were performed close to the expected hatching date in the 2010 and 2012 breeding seasons. Chicks were then sampled repeatedly throughout the respective rearing periods, starting at 1 day posthatch for some chicks in late July. Chicks were further sampled at 5, 10, 20, 30, and 40 exact days posthatch, a last sample being obtained between 65 and 70 days for the oldest chicks in early October. Two chicks reared by reexposed females (i.e., revaccinated) were excluded from the analyses of the experiment because the low sample size for this group precluded us from being able to account for a potential effect of female reexposure on their chick decay rate; the decay rate of those two chicks was nevertheless qualitatively similar to that of chicks for the other vaccinated females. At each visit, chicks were weighed; their culmen, bill-head, tarsus, and wing length were measured; and a blood sample was taken from the tarsal vein (ranging from 0.3 to 1.0 mL, according to the age and weight of the chick). This allowed the determination of the dynamics of maternal antibodies in the chick plasma to ultimately investigate the interannual dynamics of the transfer and the persistence of maternal antibodies in the chicks. To explore the potential of parental provisioning as an alternative antibody transfer route (e.g., Prevost 1962; Jacquin et al. 2012), in 2012 we also sampled stomach oil from ten 5-day-old chicks (five chicks from vaccinated mothers in 2010 and five chicks from nonvaccinated mothers), using blunt-ended 5-mm-diameter plastic cannula attached to a suction bulb (Janssens et al. 1999). Additionally, throughout the study period, we opportunistically sampled a few egg yolks from deserted burrows (i.e., nonvisited for 15 days) for which we had already sampled the females (three from vaccinated females and three from control females).

Quantification of NDV Antibodies

Plasma obtained from chick and female blood samples, egg yolks, and stomach oil samples from chicks were kept frozen at -20°C until immunological assays. Antibody extractions from yolks and oil samples were carried out by diluting 1 : 1 and homogenizing samples in phosphate-buffered saline, adding an equivalent volume of chloroform and recovering the supernatant after centrifugation (6 min at 6,000 g; Polson 1990; De Meulenaer and Huyghebaert 2001). Measures of specific anti-NDV antibody levels in female/chick plasma and in egg yolk and stomach oil extracts were performed once for each sample, using

a competitive ELISA test (ID Screen NDV kit, IDVet, Montpellier, France). Results are expressed as PI, calculated using the optical density (OD) of the sample and the mean OD of the negative control (NC) of the kit as follows: $PI = [(OD_{NC} - OD_{sample})/OD_{NC}] \times 100$. We estimated a negativity threshold using the PI values from control/nonvaccinated females (values of control females after the saline solution injection as the mean + 2 × standard deviation = 27.2%, $n = 36$ females). Analyses conducted on subsamples allowed us to check the high repeatability of the measures (within-plate coefficient of variation: $n = 86$, $4.5\% \pm 4.3\%$ [0.0–18.2]; between-plates coefficient of variation: $n = 10$, $11.6\% \pm 7.2\%$ [4.7–22.1]). Both chick and female data are deposited in the Dryad Digital Repository: <http://doi.org/10.5061/dryad.rt214> (Ramos et al. 2014).

Statistical Analyses

We modeled the dynamics of anti-NDV antibody levels in vaccinated, revaccinated, and nonvaccinated females (females⁺, females⁺⁺, and females⁻, respectively), using generalized additive mixed models (GAMMs; see Garnier et al. 2012b). We used the library *mgcv* in R (R Development Core Team 2010), on the basis of penalized regression splines and generalized cross-validation to select the appropriate smoothing parameters (Wood and Augustin 2002). GAMMs combine the utilities of linear mixed models (Pinheiro and Bates 2000) and generalized additive models (Hastie and Tibshirani 1990) so that random factors, fixed factors, and nonlinear predictor variables can all be estimated in the same statistical model. We included the vaccination treatment of females (i.e., females⁺, females⁺⁺, and females⁻) as a fixed factor, the number of days since first manipulation as a smooth term, and female identity as a random term. In addition, to specifically test for the effect of the boost vaccination/revaccination, we compared PI levels in females 2 years after their last exposure to the vaccine, that is, at 2 years in females⁺ and at 4 years in females⁺⁺. Because the data did not deviate from the normal distribution (Shapiro-Wilk normality tests: $W_{females^+} = 0.96$, $P = .541$; $W_{females^{++}} = 0.90$, $P = .239$), a one-way ANOVA approach with repeated measures was used to conduct comparisons (including z -tests for pair comparisons, when appropriate).

We also modeled the dynamics of anti-NDV antibody levels in chicks using GAMMs according to when their respective mothers had been vaccinated, that is, the same year as chick sampling, 2 years before, 4 years before, or from mothers never vaccinated (chicks^{+1y}, chicks^{+3y}, chicks^{+5y}, and chicks⁻, respectively). Then, we included this treatment factor as a fixed effect, the age of the chick (in days) as a smooth term, and the chick identity as a random term. To

compare the dynamics of decay of maternal antibodies accounting for their level at hatching, we also calculated the half-life of these antibodies for chicks^{+1y}, chicks^{+3y}, and chicks^{+5y}. To do so, we first standardized the PI values of the chicks by subtracting the mean PI of the chicks from control mothers +2 SDs (mean = 8.9%; SD = 9.4%; standardization threshold = 27.8%). We then determined the curve of exponential decrease in antibody concentration of every chick individual (measured as the standardized PI value, so that it reaches 0) as a function of the number of days since hatching, using mixed models with chicks as a random effect, and then we calculated the half-life for each chick group, using the equation

$$t_{(1/2)} = \frac{\ln(1/2)}{a},$$

where $\ln(\text{standardized PI}) = a \cdot \text{age} + b$.

We investigated potential differences in chick growth rate among the four chick treatment groups (i.e., chicks^{+1y}, chicks^{+3y}, chicks^{+5y}, and chicks⁻). Procellariiforms chicks grow according to an increase rate belonging to the family of sigmoid growth curves (Huin and Prince 2000; Weimerskirch and Lys 2000) best described by Richards equation (Richards 1959):

$$M = \frac{A}{[1 + \lambda \cdot e^{-K(t-T_i)}]^{(1/\lambda)}},$$

where A is the asymptotic or maximum value, K is the growth rate, and T_i is the time of inflection of the growth curve or the maximum growth rate. Then, we modeled individually the weight gain and the chick growth (the latter estimated using a principal components analysis with culmen, bill-head, tarsus, and wing length as variables; explained variability of the first principal component = 95.0%) along the rearing period, using nonlinear logistic regression (SSlogis function in R), and obtained the value of K (i.e., the slope) for every chick curve.

The effect of the immunological status of chicks (i.e., chicks^{+1y}, chicks^{+3y}, chicks^{+5y}, and chicks⁻) on their PI profile, weight, and growth was evaluated using generalized linear mixed models including the mother identity as a random effect. With a similar approach, we tested whether the immunological status of the seropositive chicks influenced the half-lives of their maternally derived antibodies. Finally, we also explored whether individual antibody half-lives varied as a function of weight and growth parameters.

Results

Interannual Antibody Persistence in Adult Cory's Shearwaters

We obtained a total of 355 blood samples from 105 females throughout the 6 years of this study. All control females

(i.e., females⁻) were negative throughout the period ($n = 46$, mean \pm SD [95% CI] = 8.7 ± 8.1 [7.2–10.2]), while first immunization of females resulted in high levels of anti-NDV antibodies approximately 3 months after challenge (i.e., $\text{PI}(\text{females}^-) = \text{PI}(\text{females}_{1y}^+) \ll \text{PI}(\text{females}_{3y}^+)$; table A2; fig. 1A). After that, specific antibody levels in females⁺ decreased slowly throughout the 6 years (i.e., $\text{PI}(\text{females}_{2y}^+) > \text{PI}(\text{females}_{3y}^+) > \text{PI}(\text{females}_{4y}^+) > \text{PI}(\text{females}_{5y}^+) > \text{PI}(\text{females}_{6y}^+)$), with antibody levels at the end of the 6-year period still above the negative threshold ($\text{PI}(\text{females}_{6y}^+) \gg \text{PI}(\text{females}^-)$) although statistically lower than their respective values the first time after the immunochallenge ($\text{PI}(\text{females}_{1y}^+) \gg \text{PI}(\text{females}_{6y}^+)$; for details, see table A2).

A subgroup of 16 females⁺ already vaccinated 2 years before was reexposed to the NDV vaccine (females⁺⁺) in 2010 (fig. 1A). Anti-NDV antibody levels of those females⁺⁺ increased again during this second challenge, reaching relatively high values that did not differ from those reported after the first challenge (table A2). Antibody levels then slowly decreased over the years (i.e., $\text{PI}(\text{females}_{3y}^{++}) > \text{PI}(\text{females}_{4y}^{++}) > \text{PI}(\text{females}_{5y}^{++}) > \text{PI}(\text{females}_{6y}^{++})$; table A2). Finally, revaccination had only a marginal positive effect on the antibody levels 4 years after injection, as shown by the comparison of the PI levels of females⁺_{4y} with those of females⁺⁺_{4y} (Student's t -test, $t_{27} = -1.81$; $P = .082$).

Maternal Antibody Persistence in Shearwater Chicks

All chicks⁻ from control mothers presented PI values under the negative threshold throughout the rearing period (table A3), while immunization of mothers in different years resulted in detectable levels of anti-NDV antibody in early offspring life (fig. 1B). Antibody levels in females of Cory's shearwaters showed a high and positive correlation with PI levels of their respective chicks soon after hatching (also in the egg yolk; fig. 2A), and these relationships between female and chick antibody levels lasted throughout the chick-rearing period (fig. 2). PI values of the chicks differed among groups of vaccinated mothers (i.e., vaccinated in different years) throughout the chick rearing period (table 1).

Antibody half-lives of chicks did not differ statistically among treatment groups (tables 1, A3). Finally, PI values of stomach oil samples from 5-day-old chicks from vaccinated mothers did not differ from those of chicks from control mothers ($F_{\text{Welch}, 1, 8} = 0.2$, $P = .818$), with all oil samples being below the negative threshold (PI (chicks with vaccinated mothers) = $8.3\% \pm 6.4\%$; PI (chicks with control mothers) = $9.2\% \pm 5.7\%$).

The growth rate of the chicks (estimated as the K parameter in sigmoid growth curves of weight as a function

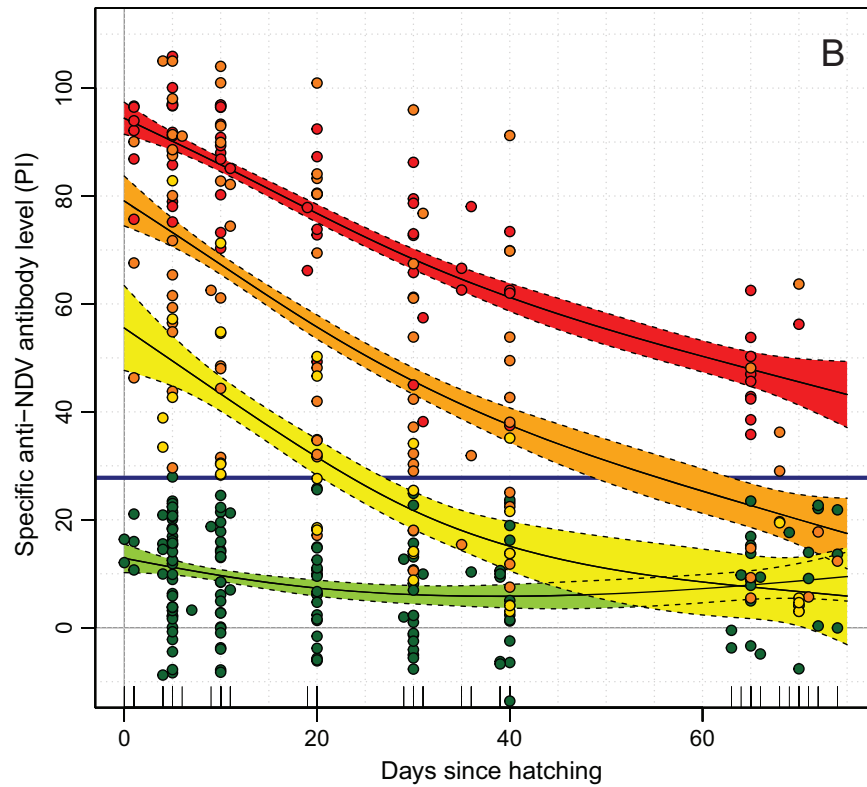
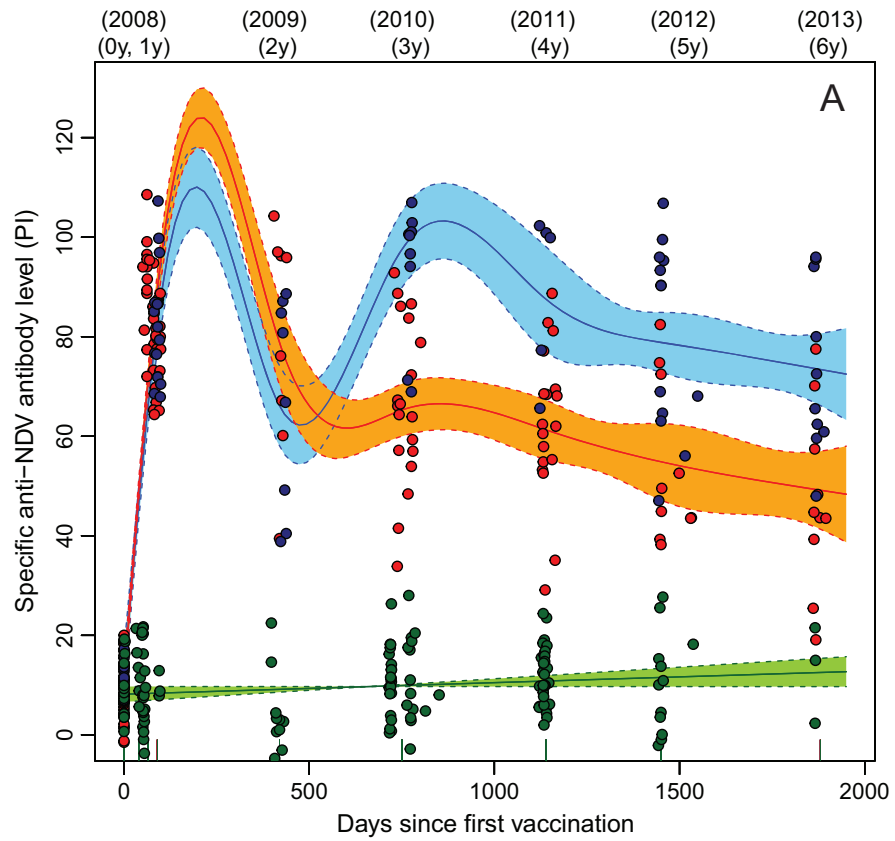
of size) was not different among the four groups (K_{weight} : 10.9 ± 2.2 [$n = 53$]; $F_{3, 49} = 1.0$, $P = .423$; K_{size} : 26.5 ± 2.4 [$n = 53$]; $F_{3, 49} = 0.04$, $P = .965$). Similarly, there was no relationship between the growth rate and individual antibody half-lives (weight: $F_{1, 25} = 0.3$, $P = .593$; size: $F_{1, 25} = 2.3$, $P = .143$; fig. A1, available online).

Discussion

Investigating ecological and evolutionary aspects of antibody persistence in long-lived vertebrates is essential not only to understand and predict the dynamics of their interactions with some infectious agents (Keeling and Rohani 2008) but also to disentangle selection pressures that may have influenced the evolution of diverse life histories (Ricklefs and Wikelski 2002). Here, by implementing a field experiment using an interannual vaccination protocol, we addressed these issues in a natural population of a long-lived colonial procellariiform, Cory's shearwater. This design allowed us to mimic a natural exposure of individuals to a novel parasite in a species with a very slow pace of life. The design and vaccine we used did not allow us to directly evaluate the assumed benefits of having high levels of circulating antibodies against a pathogen circulating in the population, but it permitted us to specifically control antigen exposure and explore the dynamics of specific antibody levels in adults and offspring at unprecedented time frames. Our results show that antibodies persist over an extended period of time in adults of this long-lived procellariiform species, allowing for maternal transfer of these specific antibodies up to at least 5 years after vaccination. We also provide confirmatory evidence that these maternally acquired antibodies are long lived and can persist throughout the slow growth period of nestlings, as we have recently reported (Garnier et al. 2012b). These results emphasize that the study of procellariiforms and their extremely slow pace of life (having the longest life spans among birds and very long chick-rearing periods) might yield key fundamental insights into the evolution of diverse immunological strategies.

Acquired Immune Response in Long-Lived Birds

Because of logistical and study design constraints in most studies involving natural populations of vertebrates, the dynamics of antibody levels over several years remain largely unexplored (Bailey et al. 1998; Nordling et al. 1998; Lloyd and Wernery 2008; Almqvist et al. 2009). The tools to study the immune response in wild animal populations are, however, increasingly available (Garnier and Graham 2014) and are starting to be applied to wild animal populations that are the focus of long-term research programs (Lachish et al. 2009; Graham et al. 2010; Rossi et al. 2011;



Chambert et al. 2012). These include measuring specific subtypes of antibodies (e.g., Nussey et al. 2014) or monitoring antibody levels for conservation purposes (e.g., Chang et al. 2007). Detailed studies on the persistence of humoral immunity are also available for domesticated animals and humans (e.g., Amanna et al. 2007). Measles in humans represents a particularly interesting case because this disease affects young offspring in long-lived social species. In humans, the occurrence of dense social groups may favor the exposure of children to measles (and other infectious diseases). A similar situation may be encountered by long-lived seabirds, potentially with even more dramatic consequences for infectious diseases because juveniles are living at sea for several years after they first leave the colony. Further, as for the procellariiform species we studied, there is a long persistence of measles antibodies in women as well as a long persistence of maternal antibodies in offspring (Cáceres et al. 2000; Leuridan et al. 2010). Reexposure of women to a vaccine or natural antigen can consolidate their immunity, although it does not augment the titer of antibodies in the long term (Leuridan et al. 2010), a finding similar to our result in Cory's shearwater with the NDV vaccine. The transfer of colostrum and milk after birth nevertheless allows the persistence of significant levels of specific antibodies for several months after birth in humans (Mandomando et al. 2008; Leuridan et al. 2010). Our results thus highlight the need for further comparative approaches exploring relationships between life-history traits, patterns of exposure to infectious diseases, and characteristics of transgenerational immunity.

In a natural host-parasite system, a high consistency of individual antibody levels between years could be due to a recurrent exposure to the pathogen (Staszewski et al. 2007b). However, we found no trace of natural infection by NDV in unvaccinated birds over the course of our study. In addition, reexposure of previously vaccinated females would have resulted in an increase of antibodies comparable to that of revaccinated females, and this pattern was not observed either. Overall, this suggests that recurrent exposure to NDV is unlikely to be the explanation for the slow decay of antibody levels observed in adults. The detailed mechanistic reasons for this long-term persistence are nevertheless not clear. In mammals, the

maintenance of specific antibody levels has been linked to both antigen-independent (Bernasconi et al. 2002) and antigen-dependent (Hunter 2002; Zinkernagel and Hengartner 2006) stimulation of memory B-cells. In addition, memory plasma cells with the ability to secrete antibodies over long periods of time have been recognized (Yoshida et al. 2010). Although none of these mechanisms have been specifically identified in birds, the existence of similar pathways could explain our observation. The long half-life of the IgY (as inferred from the long persistence of maternal antibodies in nestlings) is also likely to contribute to the maintenance of high levels of specific IgY antibodies. In any case, specific research is still needed to better understand the underlying mechanistic processes responsible for such long-term immune responses (Garnier et al. 2012b).

When birds were deliberately reexposed to the antigen 2 years after the first challenge, specific antibody levels showed an increase compared with the levels of individuals exposed only once. Secondary immune responses are known to be mounted faster and result in higher levels of specific antibodies compared with primary responses in poultry and short-lived passerine birds (Ardia et al. 2003; Råberg and Stjernman 2003; Beal et al. 2004; Martin II et al. 2006). However, little is known regarding their persistence in time, despite their importance for wild long-lived species (Simonsen et al. 1996). Our study provides confirming evidence that secondary immune responses in long-lived species may result in neither longer nor stronger persistence of specific antibodies (Damme et al. 2003; Leuridan et al. 2010; Ott et al. 2012). These results may have special relevance when vaccination may be considered as part of conservation strategies of endangered species threatened by vaccine-preventable diseases (e.g., Haydon et al. 2006). They indeed suggest that one single injection may be enough every several years in long-lived species, although a case by case study should be carried out (e.g., Li et al. 2001; Livingston et al. 2013).

Transgenerational Immunization of Slow-Growing Offspring

The long-term persistence of antibody levels in both primary and secondary immunizations could have important

Figure 1: Specific anti-Newcastle disease virus (NDV) antibody levels (expressed as percentage of inhibition [PI]) in Cory's shearwater females (A) and their chicks (B). A, Specific anti-NDV antibody levels in females throughout the 6-year experiment are shown for the three treatment groups: control females (females⁻; green), vaccinated females (females⁺; red), and revaccinated females (females⁺⁺; blue). Sampling years are shown in parentheses. B, Decay of specific anti-NDV antibody levels in Cory's shearwater chicks from mothers exposed to NDV vaccine (green, chicks from nonvaccinated females [chicks⁻]; red, chicks from females vaccinated the same year of sampling [chicks^{+1y}]; orange, chicks from females vaccinated 2 entire years before sampling [chicks^{+2y}]; yellow, chicks from females vaccinated 4 years before sampling [chicks^{+4y}]). In both cases, solid lines correspond to the mean for each group estimated using generalized additive mixed models (GAMMs), and colored regions around the means delimited by dashed lines represent the associated 95% confidence interval of the slopes. GAMMs are used to control for individual effect and nonlinear dynamics.

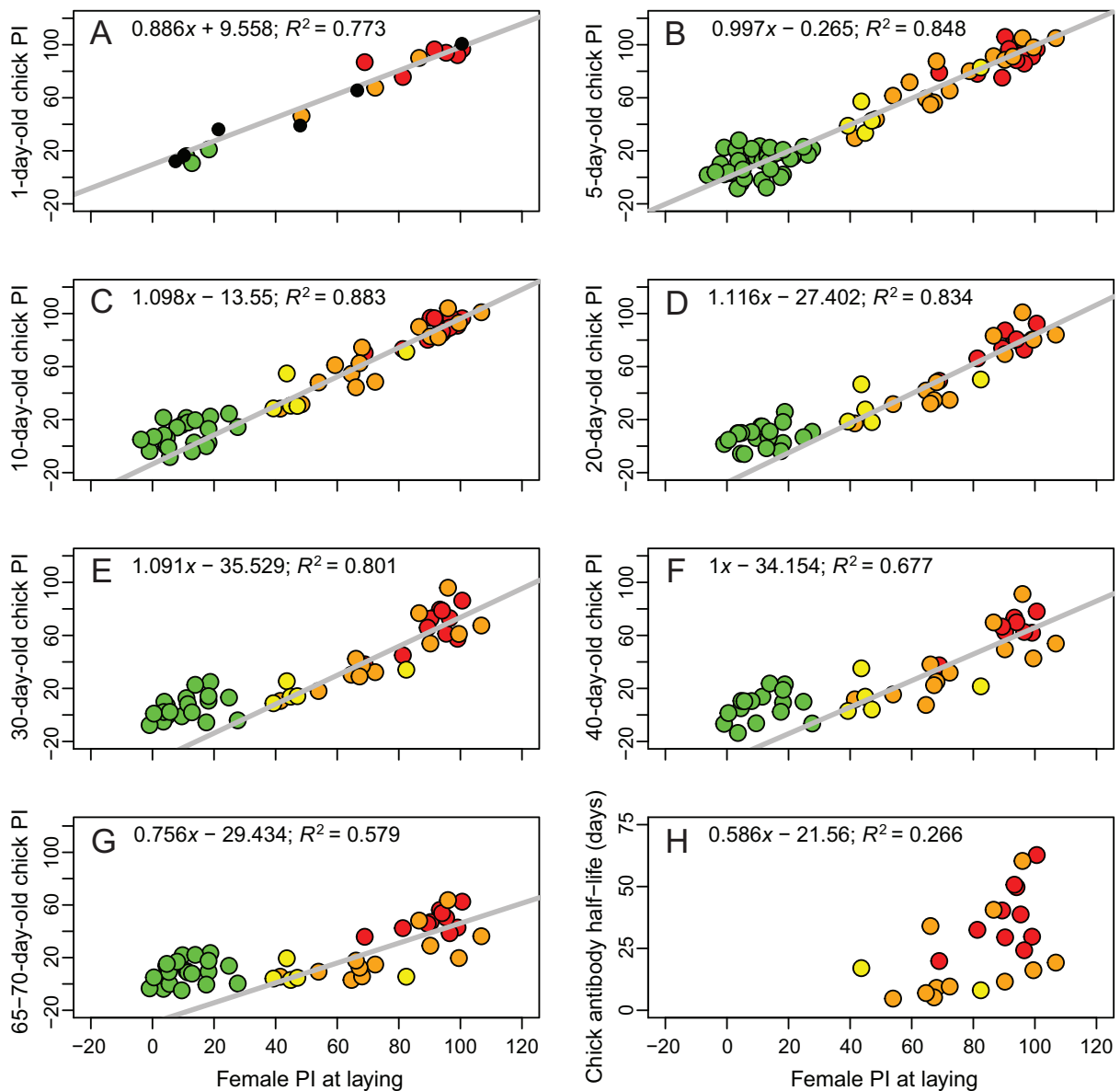


Figure 2: Specific anti–Newcastle disease virus (NDV) antibody levels in Cory’s shearwater chicks sampled at different ages (days posthatch) as a function of their mothers’ anti-NDV antibody levels at the time of egg laying (A–G). Linear relationships (excluding controls) are depicted for each chick age (gray lines), and linear descriptors are shown. Anti-NDV antibody levels of a few egg yolk samples are also plotted in A against their respective mothers’ antibody levels (black). Additionally, estimated antibody half-lives of chicks (days) from vaccinated mothers were related to anti-NDV antibody levels of the respective mothers at the time of egg laying (H). Four treatment groups are differentially depicted: chicks from nonvaccinated (i.e., control) females (chicks⁻; green), chicks from females vaccinated the year of sampling (chicks^{+1y}; red), chicks from females vaccinated 2 entire years before sampling (chicks^{+3y}; orange), and chicks from females vaccinated 4 years before sampling (chicks^{+5y}; yellow).

fitness consequences for long-lived species. In the case of procellariiforms—as well as in most long-lived colonial marine vertebrates, for instance—the maintenance of relatively high antibody levels should help adults deal with reexposure to parasites during subsequent breeding attempts (Staszewski et al. 2007b; Bodewes et al. 2013). Evi-

dence of a protective (beneficial) role of maternal antibodies is clear from studies in humans and domesticated animals (e.g., Reynolds and Maraqa 2000), but much less information is available in natural host-parasite settings (Boulinier and Staszewski 2008; Hasselquist and Nilsson 2009).

Table 1: Parameter estimates (\pm SE) from generalized linear mixed models fitted to seven age groups (days posthatch) of Cory's shearwater chicks

	Age group (days posthatch)							Antibody half-life ^b
	1 ^a	5	10	20	30	40	65–70	
AICc:								
Group (–, +1y, +3y, +5y)	98.6	617.7	475.9	412.3	402.8	362.9	383.9	202.8
Constant	121.4	740.5	558.5	474.7	454.7	402.6	426.2	198.3
Effect:								
Fixed (estimate \pm SE):								
Chicks [–] (intercept)	15.9 \pm 7.0	11.1 \pm 2.2	8.2 \pm 3.4	6.7 \pm 3.6	5.3 \pm 3.7	6.1 \pm 4.0	8.3 \pm 2.7	...
Chicks ^{+1y}	74.4 \pm 8.6	79.9 \pm 4.5	78.7 \pm 5.5	70.2 \pm 6.3	61.1 \pm 6.3	58.0 \pm 7.1	39.2 \pm 4.7	27.0 \pm 3.7
Chicks ^{+3y}	52.1 \pm 9.9	63.2 \pm 4.2	59.2 \pm 5.3	47.9 \pm 6.0	40.9 \pm 6.0	32.2 \pm 6.3	13.8 \pm 4.4	...
Chicks ^{+5y}	...	39.9 \pm 6.7	35.9 \pm 7.8	26.4 \pm 8.2	14.8 \pm 8.1	9.4 \pm 8.4	–1.0 \pm 6.1	...
Random (variance \pm SE):								
Mother	...	1.9 \pm 1.4	85.1 \pm 9.2	57.5 \pm 7.6	39.7 \pm 6.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
Residual	...	197.1 \pm 14.0	169.0 \pm 13.3	211.4 \pm 14.5	222.4 \pm 14.9	275.0 \pm 16.6	148.0 \pm 12.2	317.8 \pm 17.8

Note: In all seven cases, the best-supported model included the experimental group (i.e., immunological status of chicks: chicks[–], chicks⁺¹, chicks^{+3y}, and chicks^{+5y}) as fixed effect. Additionally, parameters for individual antibody half-lives were also estimated (only for chicks from vaccinated mothers; i.e., chicks^{+1y}, chicks^{+3y}, and chicks^{+5y}), and here the model with no group effect was preferred. All evaluated models included the mother as a random effect, except in the first age group (1 day old) model because of insufficient sample sizes. AICc, corrected Akaike information criterion. Boldface indicates the models considered.

^a Not enough data to test the random effect of the mother or the level chicks^{+5y}.

^b Chicks[–] is not included, and chicks^{+1y} was used instead as the intercept in the half-life modeling.

Similarly to what we had already reported (Garnier et al. 2012b), we found that Cory's shearwater chicks still showed detectable levels of maternally derived antibodies several weeks after hatching. Nestlings may be protected by these passively acquired antibodies over a great part of the chick-rearing period, especially if mothers have been recently exposed to a parasite and currently have high levels of circulating antibodies. In addition to a protection against pathogen infection, maternal antibodies could potentially facilitate the growth of the chicks by allowing the chick not to divert excessive energy at mounting immune responses, and one could thus hypothesize that higher persistent levels would allow for faster growth (Hasselquist et al. 2012). A few studies focusing on the constitutive immunity of short-lived passerine species found evidence of a potential trade-off between the development of immunity and growth rate (Soler et al. 2003; Brommer 2004; Mauck et al. 2005). Ultimately, increased maternal (exogenous) contributions could be observed as part of compensating mechanisms (Arriero et al. 2013). We did not find any evidence of an association between the dynamics of decay of the maternal antibodies throughout the chick-rearing period and the individual growth rate of nestlings. It should be noted that we did not expose the chick to an immune challenge, which might have revealed an effect of maternal antibody provisioning on growth. However, this result suggests that the slow decay of maternal antibodies is generally associated with the slow growth observed in our model species and maybe in other procellariiforms.

Finally, young nestlings have limited ability to synthesize antibodies endogenously at the time of hatching. Although

the egg yolk represents the main route of transfer, chicks of some species can receive additional antibodies by being later fed an immunoglobulin-rich substance produced in parental crops (Engberg et al. 1992), as is already known in Columbiformes and Phoenicopteriformes (i.e., pigeons and flamingos). In shearwater, both parents provide their newborn with a crop oil that could play a similar role. However, none of the five early chicks from vaccinated mothers showed any trace of specific anti-NDV IgY in their stomach oil samples (although antibodies in their plasma were detected). Thus, it does not seem that Cory's shearwater—and maybe procellariiforms in general—rely on oral transfer of antibodies to their offspring (Apanius 1998). However, it should be noted that crop milk antibodies detected in pigeons were mostly IgA (Engberg et al. 1992), which we did not measure in our oil samples and are not likely to reach the bloodstream of the chick. Such antibodies could nevertheless provide local protection at the gut level in a way similar to what is suspected in mammals (e.g., Sadeharju et al. 2007) and could also have profound effect on the establishment of the gut microbiota of these chicks (Rogier et al. 2014). Further studies are thus required to fully understand potential postnatal transfer of antibodies in procellariiforms and in birds in general.

Perspectives

Life-history theory predicts a stronger investment in acquired rather than innate immunity in long-lived species (Ricklefs and Wikelski 2002; Lee 2006). This is because

innate immunity is associated with a highly costly response, while acquired responses in vertebrates include memory mechanisms that reduce the costs of multiple encounters of the same parasite, an event more likely to happen in long-lived species (Lee et al. 2008). Although we did not quantify the investment in innate immunity, our results demonstrate a strong investment in the acquired immunity in this type of species.

Certain life-history traits—such as high longevity, colonial breeding, phylopatry, and nidicolous and altricial offspring—are likely shaping host immunity in eco-evolutionary terms because of strong spatiotemporal correlation with infectious diseases. In this context, breeding in aggregates of hundreds to thousands of pairs leads to high contact rates between individuals and ultimately favors the spread of disease agents (Loehle 1995). Returning to the same colony and nest sites year after year also favors reexposure to recurrent diseases, affecting not only adults but also offspring. Moreover, slow pace of life and slow growth of newborns are also likely to increase the exposure to pathogens of both parents and offspring. In such ecological settings, it would be interesting to explore further potential trade-offs in the allocation of limited resources between immunity, survival, and reproductive investment. Deciphering procellariiform immunity may also prove important for the conservation of several endangered species of albatrosses and petrels. For instance, previous work on population modeling and vaccination strategies did not consider the exact dynamics of the immune response in adults in the context of an infectious disease deleterious for young offspring (Garnier et al. 2012b). These specific parameters would be key components when ensuring the efficacy of a vaccination campaign in the wild as well as when modeling population viability in cases where maternal antibody transfer plays a role. In fact, vaccination has been considered a relevant part of conservation programmes of endangered species (Haydon et al. 2006), and our results suggest that considering the prolonged persistence of some protective antibodies in long-lived colonial species could greatly increase the potential usefulness of such specific vaccines as management tools. The use of a vaccine in this context could also allow a powerful experimental test of the expected beneficial effect of maternal antibodies in a seminatural system.

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