Received: 21 June 2021 | Accepted: 18 November 2021

DOI: 10.1111/1365-2656.13642

Journal of Animal Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Immunosenescence in the wild? A longitudinal study in a long-lived seabird

Coraline Bichet[1,2] | Maria Moiron[1,3] | Kevin D. Matson[4] | Oscar Vedder[1] | Sandra Bouwhuis[1]

[1]Institute of Avian Research, Wilhelmshaven, Germany

[2]Centre d'Etudes Biologiques de Chizé, CNRS-La Rochelle Université, UMR-7372, Villiers-en-Bois, France

[3]CEFE, Université de Montpellier, CNRS, EPHE, IRD, Université Paul Valéry Montpellier 3, Montpellier, France

[4]Wildlife Ecology and Conservation, Environmental Sciences Group, Wageningen University, Wageningen, The Netherlands

Correspondence
Coraline Bichet
Email: coraline.bichet@univ-lr.fr

Funding information
Marie Curie Individual Fellowship, Grant/Award Number: 793550

Handling Editor: Ben Dantzer

Abstract

1. Longitudinal studies of various vertebrate populations have demonstrated senescent declines in reproductive performance and survival probability to be almost ubiquitous. Longitudinal studies of potential underlying proximate mechanisms, however, are still scarce.
2. Due to its critical function in the maintenance of health and viability, the immune system is among the potential (mediators of) proximate mechanisms that could underlie senescence.
3. Here, we studied three innate immune parameters—haemagglutination titre, haemolysis titre and haptoglobin concentration—in a population of common terns (*Sterna hirundo*) known to undergo actuarial senescence. We repeatedly sampled birds of known sex and age across 11 years and used random regression models to (a) quantify how immune parameters vary among individuals and (b) describe within-individual age-specific changes in, and potential trade-offs between, immune parameters.
4. Our models revealed no differences between males and females in haemagglutination titre and haptoglobin concentration, and very low among-individual variation in these parameters in general. Within individuals, haemagglutination titre increased with age, while haptoglobin concentration did not change. We found no indication for selective (dis)appearance in relation to haemagglutination titre or haptoglobin concentration, nor for the existence of a trade-off between them. Haemolysis was absent in the majority (76%) of samples.
5. Common terns do not exhibit clear senescence in haemagglutination titre and haptoglobin concentration and show very little among-individual variation in these parameters in general. This may be explained by canalisation of the immune parameters or by the colonial breeding behaviour of our study species, but more longitudinal studies are needed to facilitate investigation of links between species' characteristics and immunosenescence in wild animals.

KEYWORDS
ageing, immunity, immunology, innate immune system, natural antibodies, ontogeny

1 | INTRODUCTION

Most organisms exhibit senescence, an irreversible decline in performance with age (Shefferson et al., 2017). In natural populations, senescence has mainly been studied from an evolutionary perspective, focusing on the two key fitness components: viability (*actuarial senescence*) and fertility (*reproductive senescence*; Lemaître & Gaillard, 2017; Monaghan et al., 2008). Proximate mechanisms underlying the observed senescent declines, however, have received less empirical attention (Bouwhuis & Vedder, 2017; Peters et al., 2019). Investigating these mechanisms may increase our understanding of observed interspecific (e.g. Bouwhuis et al., 2012) or intraspecific (e.g. Bouwhuis et al., 2010; Holand et al., 2016; Lemaitre et al., 2013) variation in senescence patterns, which, at present, is still in its infancy.

Due to its critical function in the maintenance of health and viability, the immune system is among the potential (mediators of) proximate mechanisms that could underlie senescence. In vertebrates, the immune system comprises an innate arm and an acquired arm (Hoebe et al., 2004). Each arm defends an individual via a range of mechanisms involving cellular and soluble (humoral) components (Akira et al., 2006; Bonilla & Oettgen, 2010; Cooper & Alder, 2006; Nathan, 2006). Furthermore, a functioning immune system involves many interactions between the two arms (Iwasaki & Medzhitov, 2010, 2015).

Developing, maintaining and using an immune system incurs energetic and other nutritional costs (Graham et al., 2005; Lochmiller & Deerenberg, 2000; Maizels & Nussey, 2013). Allocation to immune defence therefore is likely to be traded off against allocation to other life-history traits, such as growth, repair and reproduction (Eraud et al., 2009; Graham et al., 2010; Hanssen et al., 2004; Lemaitre et al., 2015; Viney et al., 2005). The disposable soma theory, one of the general theories of senescence based on the idea of trade-offs, asserts that the optimal level of allocation to any form of defence or repair will be less than what would be required for the body to maintain its state indefinitely (Kirkwood, 1977, 2017). As a consequence, the theory predicts that an individual's soma will deteriorate over its lifetime. This deterioration likely includes decreased immune system performance with age, that is, immunosenescence (reviewed in Lavoie, 2006; Pawelec, 2018; Shanley et al., 2009; Simon et al., 2015).

Immunosenescence has mostly been studied in humans and laboratory animals. Generally, acquired immunity has been found to decline with age, while innate immunity has been found to remain unchanged (Frasca et al., 2011; Panda et al., 2009; Shaw et al., 2013; Simon et al., 2015) or to increase, leading to increasing levels of persistent inflammation (*inflammaging*; Franceschi & Campisi, 2014; Franceschi et al., 2007, 2018; Goto, 2008; Pawelec, 2018). Although some of these findings indeed reflect immunosenescence, age-related immune changes are not necessarily all detrimental and rebalancing allocation to different immune defences, the so-called *immune remodelling*, could help protect ageing individuals from changing immune challenges (Fülöp et al., 2018; Mueller et al., 2013; Nikolich-Zugich, 2018).

Adding to the complexity, age-related immunological variation or age-related (resolution of) trade-offs may differ among individuals, for example in relation to sex. Interest in sex-specific immunosenescence has grown out of observed sex differences in the rate of ageing (Lemaître et al., 2020; Tidière et al., 2015; Xirocostas et al., 2020) and in immune function, perhaps arising from effects of sex hormones or sex-specific behaviour (Klein & Flanagan, 2016; Ortona et al., 2019; Restif & Amos, 2010). So far, results of studies of sex-specific patterns of immunosenescence are contradictory: some suggest sex differences (e.g. Gubbels Bupp et al., 2018; van Lieshout et al., 2020; Tidière et al., 2020), while others do not (e.g. Brooks & Garratt, 2017; Cheynel et al., 2017; Kelly et al., 2018; Peters et al., 2019). The reasons for these contrasting results are unknown.

Investigations of (sex-specific) changes in immune function with age are relatively rare in natural populations, but seem to confirm the age-related immune patterns observed in humans and laboratory animals: declines in acquired immunity and increases or no change in innate immunity (Peters et al., 2019). One caveat is that most of this work is based on cross-sectional analyses. Population-level patterns, however, do not necessarily provide accurate insight into within-individual processes such as senescence, as they may also reflect changes in the phenotypic composition of a population (e.g. van de Pol & Verhulst, 2006). With respect to immune parameters, this may be especially likely. If individuals with poor immune defences selectively disappear from a population, then individuals with high levels of defences will be overrepresented among old-aged individuals (e.g. Graham et al., 2010), thereby potentially masking within-individual age-specific immunological changes. As such, longitudinal analyses are needed to demonstrate immunosenescence, to help interpret findings from cross-sectional studies and to assess evolutionary consequences (e.g. Nussey et al., 2008).

We investigated within- and among-individual variation in three innate immune parameters—haemagglutination titre, haemolysis titre and haptoglobin concentration—in an extensively studied population of common terns *Sterna hirundo* with the aim of offering new insights into immunosenescence using a longitudinal analytical approach. We repeatedly sampled individuals of known sex and age across 11 years and compiled a dataset comprising 1,023 measurements from 396 individuals, sampled between 1 and 10 times (mean = 2.67, Appendix 1, Figure S1), at ages ranging between 3 and 27 years (mean = 10.2, Appendix 2, Figure S2). We analysed this dataset using random regression models to (a) quantify how immune

parameters vary among individuals, both overall and in relation to sex and (b) describe within-individual age-specific changes in, and potential trade-offs between, immune parameters.

Because common terns show actuarial senescence (Zhang et al., 2015), we expected to find immunosenescence. However, because common terns are only slightly sexually dimorphic (Becker & Wink, 2003) and share parental care (Riechert & Becker, 2017), and because males and females undergo actuarial senescence at similar rates (Zhang et al., 2015), we did not expect to find sex-specific immunosenescence. Based on a previous, longitudinal study of the innate immune parameters studied here, conducted in great tits (*Parus major*), we expected immunosenescence to be manifested in two ways. First, we expected an age-specific decline in haemagglutination, which would reflect reduced natural antibody concentrations available for eliminating antigens as birds age. Second, we expected an age-specific increase in haptoglobin concentration, which would reflect increased inflammation as birds age (Vermeulen et al., 2017). We expected little age-specific change in haemolysis, reflecting relatively consistent abilities to lyse foreign cells (Vermeulen et al., 2017).

2 | MATERIALS AND METHODS

This study was performed under ethical approval and licenses from the city of Wilhelmshaven and the Lower Saxony State Office for Consumer Protection and Food Safety (licence numbers 3319-42502-04-16/2113 and 33.19-42502-04-19/3068), Germany.

2.1 | Species and study population

Common terns are socially monogamous migratory seabirds. The samples and data used for this study were collected as part of a long-term study of a breeding colony located at the Banter See in Wilhelmshaven, on the German North Sea coast (53°30′40″N, 08°06′20″E). Here, individual-based monitoring facilitated by an antenna system started in 1992, when 101 breeders (Becker & Wendeln, 1997) and all subsequent fledglings (Becker et al., 2001) were individually marked with subcutaneous transponders. Since 1998, the sex of all locally hatched breeders has been molecularly determined from a feather sample collected shortly before fledging; prior to that, sex was determined by behavioural observations, mostly of copulations (Becker & Wink, 2003).

2.2 | Blood sampling and plasma collection

Blood samples (100–300 μl) were collected between 2008 and 2019, with the exception of 2015, using larval instars of the blood-sucking bug *Dipetalogaster maximus*. To collect a sample, a bug was placed into a hollow dummy egg that was temporarily placed in the nest of a target bird during incubation (see Arnold et al., 2008). After ca. 20 min, the dummy egg was retrieved, and blood was extracted from the bug by pricking its abdomen with a syringe, and then stored in a box with ice packs. After maximum of 3 hr, blood samples were centrifuged (3000 *g*, 7 min), and plasma fractions were collected and stored at −20°C until analysis. This method allows for the collection of blood samples without capture stress, and the fact that bug retrieval after 20 min is sufficient to prevent bug physiological processes to lead to changes in tern physiology assessed using blood samples has been validated for several blood parameters (e.g. hormones: Riechert et al., 2012; metabolites: Bauch et al., 2010; and telomeres: Bauch et al., 2013). To validate it for our immunological parameters, we caught 10 birds that were sampled using bugs, to collect a second blood sample from their brachial veins using needles and heparinised capillary tubes (see below and Appendix 3, Table S1).

2.3 | Immunological assays

To assess the innate immune system functioning of the terns, we measured constitutive levels of three parameters: haemagglutination titre, haemolysis titre and haptoglobin concentration. All three have the advantages of requiring only small plasma volumes (max. 40 μl) and of not requiring recapture (unlike immune challenges) or expensive equipment; therefore, these parameters have been assessed in many field studies (e.g. Aastrup & Hegemann, 2021; Hegemann et al., 2013; Hegemann et al., 2013; Vermeulen et al., 2015; Versteegh et al., 2014). All samples were assayed between September and February in the years 2017–2020 by a single researcher (C.B.). For all tests, samples were randomly distributed across assay plates.

2.3.1 | Haemagglutination and haemolysis

Haemagglutination is the product of natural antibodies (NAbs), whereas haemolysis is the product of an interaction between NAbs and the complement system, an enzyme cascade that neutralises pathogens and induces inflammatory responses (Janeway et al., 2001). Both are involved in the innate immune system.

Multifunctional NAbs circulate in animals even without prior exposure to a particular antigen (Boes, 2000; Matson et al., 2005; Ochsenbein & Zinkernagel, 2000). NAb production is thought to be under stronger genetic control than other immune parameters (Versteegh et al., 2014), less sensitive to environmental conditions, nutritional status or stress levels (Deerenberg et al., 1997), and mostly unaffected by acute infection (Baumgarth et al., 1999; Hegemann, Matson, Versteegh, et al., 2013; Matson et al., 2005).

NAb-mediated haemagglutination and complement-mediated haemolysis titres were measured using a haemagglutination–haemolysis assay, slightly modified from Matson et al. (2005). Each sample was assayed across two round-bottom 96-well plates. To control for initial haemolysis, which can occur during sample

collection, all plasma samples were additionally scored for redness, from 1 to 8, and this initial lysis score was added as a covariate in all statistical models (see below). Twenty microliters of seven plasma samples (rows B–H) and a positive control (plasma collected from a single chicken in 2017 in row A) were added to columns 1 and 2 of the first plate. From columns 2 to 11 of the first plate, samples were serially diluted using 20 μl of PBS (phosphate-buffered saline 1X Dulbecco's). Each serial dilution was then continued from columns 1 to 11 of the second plate. Column 12 of both plates contained PBS only (negative control). 20 μl of a 1% blood cell suspension, made from a rabbit blood pool no older than 7 days, was added to all wells. Plates were incubated in a water bath at 37°C for 90 min. After incubation, the long axis of the plates was tilted at a 45° angle for 20 min to enhance the haemagglutination visualisation. All plates were photographed on a light table to record haemagglutination, and then kept at room temperature for an additional 70 min before being photographed again to record haemolysis. From each picture, the eight rows were randomised and scored blindly from 1 to 22 by a single researcher (C.B.). Higher scores equated to higher haemagglutination or haemolysis titres.

Due to limited plasma volume, we were not able to run samples in duplicate for the haemagglutination–haemolysis assay, such that intra-plate variation cannot be assessed. The positive controls ($n = 158$), however, revealed an inter-plate coefficient of variation (CV) of 12% for haemagglutination and 10% for haemolysis. We found no detectable difference in the haemagglutination nor haemolysis titres between blood samples from the same bird collected using bugs or using needles after catching (paired *t*-test for haemagglutination: $t = -0.32$, $n = 10$, $p = 0.76$, $r = 0.63$; paired *t*-test for haemolysis: $t = 1.96$, $n = 10$, $p = 0.08$, $r = 0.92$). Because haemolysis was absent in 76% of samples (mean haemolysis titre = 0.28 ± 0.02, median = 0.00, $n = 1{,}029$), we excluded it from all subsequent analyses. Haemagglutination titre was measured in 1,023 samples collected from 396 individuals (204 males and 192 females sampled between 1 and 8 times, mean = 2.6, Appendix 1, Figure S1a).

2.3.2 | Haptoglobin

Haptoglobin is a multifunctional acute phase protein that binds free haemoglobin and thereby mitigates damage caused by reactive oxygen components released during inflammation (Andersen et al., 2016; Quaye, 2008). Haptoglobin normally circulates in the blood at low concentrations, but increases rapidly in response to infection, inflammation or trauma (Cray et al., 2009; Matson et al., 2012; Millet et al., 2007; Quaye, 2008). Haptoglobin concentration is thought to reflect health status and physiological condition (Hõrak et al., 2002, 2003) and to predict immune responsiveness (Matson et al., 2012).

We quantified haptoglobin (or a functional equivalent) in 7.5 μl plasma samples using a commercially available assay (TP801, Tri-Delta Diagnostics), which colorimetrically measures the heme-binding capacity of the plasma. We followed the instructions provided by the manufacturer with slight modifications following Matson et al. (2012). The standards, which were included in duplicate in each plate, ranged from 2.5 to 0.039 mg/ml to allow for calculation of low concentrations. A negative control (7.5 μl of diluent) and a positive (7.5 μl of the 2017 chicken plasma) control were included in duplicate in each plate ($n = 9$). We measured absorbance (spectrophotometer: Anthos 2010, Biochrom) at three wavelengths (405, 450 and 620 nm) before adding the final reagent inducing the colorimetric reaction. Final absorbance values at 620 nm were corrected by the pre-scan absorbance values at 620 nm, which allowed us to control for among-sample differences in colour and cloudiness (Matson et al., 2012). The pre-scan absorbance values at 405 nm (positively correlated with those at 450 nm; $R = 0.89$) were used to statistically correct for blood sample haemolysis in all analyses of haptoglobin (see below), since values obtained at 405 nm explained more variation than those obtained at 450 nm (also see Aastrup & Hegemann, 2021; Matson et al., 2012; Wemer et al., 2021).

Using the positive controls, we calculated the intra- and inter-plate CVs to be 20% and 16%, respectively. We found no detectable difference in haptoglobin concentration between blood samples from the same bird collected using bugs or using needles after catching (paired *t*-tests: $t = 1.07$, $n = 10$, $p = 0.31$, $r = 0.45$). Haptoglobin concentration was measured in 590 samples collected from 221 individuals (113 males and 108 females sampled between 1 and 10 times, mean = 2.7, Appendix 1, Figure S1b).

2.4 | Statistical analyses

2.4.1 | Age-specific immune defence

To test whether haemagglutination titre and haptoglobin concentration varied with age, we ran a series of linear mixed models with either haemagglutination titre or log-transformed haptoglobin concentration as the dependent variable, both modelled assuming a Gaussian error distribution. An individual's actual age was partitioned into an 'average age' and 'delta age' component (following van de Pol & Wright, 2009). An individual's average age was calculated as the average of all ages at which we measured an individual's immune parameter, while delta age was calculated as the difference between an individual's actual age at measurement and its average age (i.e. delta age = age − average age). In these models, average age represents the among-individual age effect, delta age the within-individual age effect on immune parameters (van de Pol & Wright, 2009). If the among- and within-individual age effects were to be significantly different from each other, this would indicate that the effect of age among individuals cannot be explained by changes within individuals, meaning that there is age-specific selective (dis) appearance of individuals with certain trait values from the sampled pool of individuals (van de Pol & Wright, 2009).

All models additionally included (a) the interaction between 'average age' and 'delta age' to test for nonlinear age effects, as well as (b) sex and the interaction between 'delta age' and sex to test

for sex differences in immune parameters and immunosenescence, respectively, (c) the time between blood collection and laboratory analysis ('storage time') to account for potential plasma deterioration over time (continuous covariate in years), and (d) year of the assay ('assay batch') to account for potential differences between rabbit blood batches or kits (four classes for haemagglutination and three for haptoglobin). Models of haemagglutination titre also contained initial lysis as a covariate; models of haptoglobin concentration the absorbance values of the pre-scans at 405 nm ('pre-scan absorbance'). Year of sampling and bird identity were modelled as random intercepts.

Final models were obtained by stepwise removal of non-significant interaction terms, which would affect interpretation of the underlying single covariates. Single covariates themselves, however, were not removed in a model-selection approach. In the case of the 'methodological covariates', this made sure we were correcting for potential methodological artefacts, while in the case of the effects of sex and age components this allowed us to answer our main research questions within a single framework.

Models that include average and delta age components provide an insight into whether between- and within-individual age effects differ, and can therefore indicate the general presence or absence of selective (dis)appearance, but cannot separate between selective appearance and disappearance. To do this, one should ideally formulate models that include age, as well as the biological terms 'age at first reproduction' to assess selective appearance and 'age at last observation' to assess selective disappearance (van de Pol & Verhulst, 2006). In our case, because only 141 out of 396 birds can be assumed to have died (because they were not observed in the colony for >1 year; Bouwhuis et al., 2015), we used an alternative approach and further checked the conclusions drawn from the final models including the average and delta age components by formulating models that included age, age at first measurement (AFM) and age at last measurement (ALM) of immune parameters as explanatory variables. These models test for selective appearance in, and selective disappearance from, the immunological dataset, respectively, while also testing the average within-individual age effect (van de Pol & Verhulst, 2006), which was our main objective. The results of these models are presented in Appendix 4 (Table S2).

To investigate whether the within-individual age-trajectories of immune parameters differed among individuals, we re-ran the final age-partitioning models as described above, but including random intercepts and slopes for individual identity. We fitted delta age as the gradient for the slopes rather than actual age, because individuals were measured at different age ranges, and we found no evidence for nonlinear within-individual age effects on immune parameters (see Section 3). For the models with random intercepts and slopes (as well as for the models with only random intercepts), we included all available data, even if individuals had only a single measurement. We did so to improve the statistical power of our analyses (Martin et al., 2011). We assessed whether models with both random intercepts and random slopes better fitted the data than models with random intercepts only using the Akaike information criterion (AIC; Burnham & Anderson, 2002).

All univariate analyses were performed in R 3.6.1 (R Core Team, 2014) using the function 'lmer', implemented in the package LME4 (Bates et al., 2015) with restricted maximum likelihood estimates of the parameters. Assessment of model assumptions was performed by checking the residuals' independence, homogeneity and normality, and testing for temporal autocorrelation. We used the 'sim' function from the R-package ARM to simulate values from the posterior distributions of the model parameters (Gelman & Su, 2020). Based on 5,000 simulations, we extracted 95% credible intervals (CI) around the mean. Assessment of statistical support was obtained from the posterior distribution of each parameter. We also used *p*-values to assess the significance of the fixed effects, with a level of significance (α) of 0.05.

2.4.2 | Among- and within-individual correlations between immune parameters

We could assay 550 samples from 212 individuals for both haemagglutination titre and haptoglobin concentration. A bivariate mixed-effects model was used to investigate whether and how haemagglutination titre and haptoglobin concentration covaried (Dingemanse & Dochtermann, 2013; Hadfield, 2010). It allowed us to simultaneously model both immune parameters as response variables, and to decompose the phenotypic correlation between them into among- and within-individual correlations. To do so, we fitted mean-centred and variance-standardised haemagglutination titre and log-transformed haptoglobin concentration as response variables assuming a Gaussian error distribution. As fixed effects, we included trait-specific effects of age and sex, as well as storage time and 'assay batch'. We also modelled initial lysis and pre-scan absorbance as fixed effects associated with haemagglutination titre and haptoglobin concentration, respectively. We initially fitted an unstructured (co)variance matrix for the three random effect components: bird identity, year of sampling and residual components. However, because a model with the covariance between immune parameters across years did not fit the data better (based on Deviance Information Criterion (DIC) model comparison: DIC model with among-year covariance = 2,294.89, DIC model without among-year covariance = 2,294.73), we proceeded without modelling such covariance.

Bivariate models were fitted using a Bayesian framework with the R-package MCMCGLMM (Hadfield, 2010) in R 3.6.1. For all models, we used parameter-expanded priors (Hadfield, 2010). The number of iterations and thinning interval were chosen to ensure that the minimum MCMC effective sample sizes for all parameters equalled 1,000. Burn-in was set to a minimum of 5,000 iterations. The retained effective sample sizes yielded absolute autocorrelation values lower than 0.1 and satisfied convergence criteria based on the Heidelberger and Welch convergence diagnostic (Heidelberger & Welch, 1981). We drew inferences from the

posterior means and 95% credible intervals (CI). We also report the *pMCMC* values, representing the probabilities that posterior estimates include zero, to test for the significance of the fixed effects.

3 | RESULTS

3.1 | Sex- and age-specific immune defence

The interactions between 'average age' and 'delta age' and between 'delta age' and sex were not significant and removed from the models, indicating that there is no evidence for nonlinear age effects and for males and females to differ in the age trajectories of their immune parameters, respectively. The resulting final models provided no evidence for sex differences in the average levels of the immune parameters either.

We found a within-individual increase in haemagglutination titre with age ($\beta = 0.09$, 95% CI = 0.00–0.17, Table 1; Figure 1a), but no detectable within-individual effect of age on haptoglobin concentration ($\beta = -8.36 \times 10^{-3}$, 95% CI = −0.02 to 0.01, Table 1; Figure 1b). For both immunological parameters, the 95% credible intervals of the within- and among-individual age effects overlapped each other (difference between within- and among-individual effects for haemagglutination: $\beta = 8.69 \times 10^{-2}$, 95% CI = -2.84×10^{-3}, 1.78×10^{-1}; difference between within- and among-individual effects for haptoglobin: $\beta = -7.62 \times 10^{-3}$, 95% CI = -2.31×10^{-2}, 8.43×10^{-3}; Table 1), such that there is no evidence for selective (dis)appearance in relation to either trait. These conclusions were supported by models including age, age at first and age at last immune measurement (Appendix 4, Table S2).

AIC model comparison showed that the models with random intercepts and slopes did not outperform those with random intercepts only (for haemagglutination titre: $AIC_{\text{with random slopes}} = 4534.04$, $AIC_{\text{without random slopes}} = 4530.04$, $\Delta AIC = 4.00$, for haptoglobin concentration: $AIC_{\text{with random slopes}} = -199.85$, $AIC_{\text{without random slopes}} = -202.46$, $\Delta AIC = 2.61$). As such, although haemagglutination titre changes with age, individuals do not detectably differ in the rate at which this change occurs (also see Appendix 5, Figure S3).

3.2 | Among- and within-individual correlations between immune parameters

Our bivariate model revealed that there was no correlation between haemagglutination titre and haptoglobin concentration at the among-individual level ($R_{\text{among}} = -0.02$, 95% CI = −0.78 to 0.72; Appendix 6, Table S3), although we may lack the statistical power to detect it because of having to use a reduced dataset, and because both immune parameters harbour very low among-individual variance, hampering the possibilities for a covariance between the two traits. At the within-individual level, however, the correlation between haemagglutination titre and haptoglobin concentration was positive ($R_{\text{within}} = 0.13$, 95% CI = 0.04 to 0.24, Figure 2).

TABLE 1 Results from models testing whether variation in haemagglutination titre and haptoglobin concentration (log-transformed) is explained by sex or age. Provided are fixed and random effect parameter estimates and associated 95% credible intervals (95% CI). Significant effects (p-value < 0.05 and 95% CI which do not overlap with zero) are presented in bold, '—' means a parameter was not fitted to the model

	Haemagglutination titre (n = 1,023 measurements from 396 individuals)			Haptoglobin concentration (in log mg/ml, n = 590 measurements from 221 individuals)		
Fixed effect parameter	**Estimate**	**95% CI**	***p*-value**	**Estimate**	**95% CI**	***p*-value**
Intercept	1.16×10^{-1}	10.54, 12.67	<0.001	1.91×10^{-1}	0.08, 0.30	0.003
Average age	2.05×10^{-3}	−0.03, 0.04	0.903	-6.35×10^{-4}	−0.01, 0.00	0.766
Delta age	**8.89×10^{-2}**	**0.00, 0.17**	**0.034**	-8.36×10^{-3}	−0.02, 0.01	0.219
Sex (female)	2.70×10^{-3}	−0.26, 0.26	0.984	-9.91×10^{-3}	−0.04, 0.02	0.528
Storage time	-1.77×10^{-1}	−0.37, 0.01	0.096	**-2.31×10^{-2}**	**−0.04, −0.00**	**0.044**
Assay batch (2018)	-7.98×10^{-2}	−0.65, 0.48	0.786	-1.13×10^{-3}	−0.05, 0.05	0.966
Assay batch (2019)	-7.15×10^{-1}	−1.57, 0.13	0.100	—	—	—
Assay batch (2020)	**−4.09**	**−4.98, −3.20**	**<0.001**	7.70×10^{-2}	−0.02, 0.17	0.113
Initial lysis	**2.90×10^{-1}**	**0.22, 0.35**	**<0.001**	—	—	—
Pre-scan absorbance	—	—	—	**1.88×10^{-1}**	**0.18, 0.20**	**<0.001**
Random effect parameter	**Estimate**	**95% CI**		**Estimate**	**95% CI**	
Bird identity	0	0, 0		0	0, 0	
Year	1.09	0.66, 1.68		9.79×10^{-3}	0.01, 0.02	
Residual	4.56	4.18, 5.00		3.46×10^{-2}	0.03, 0.04	

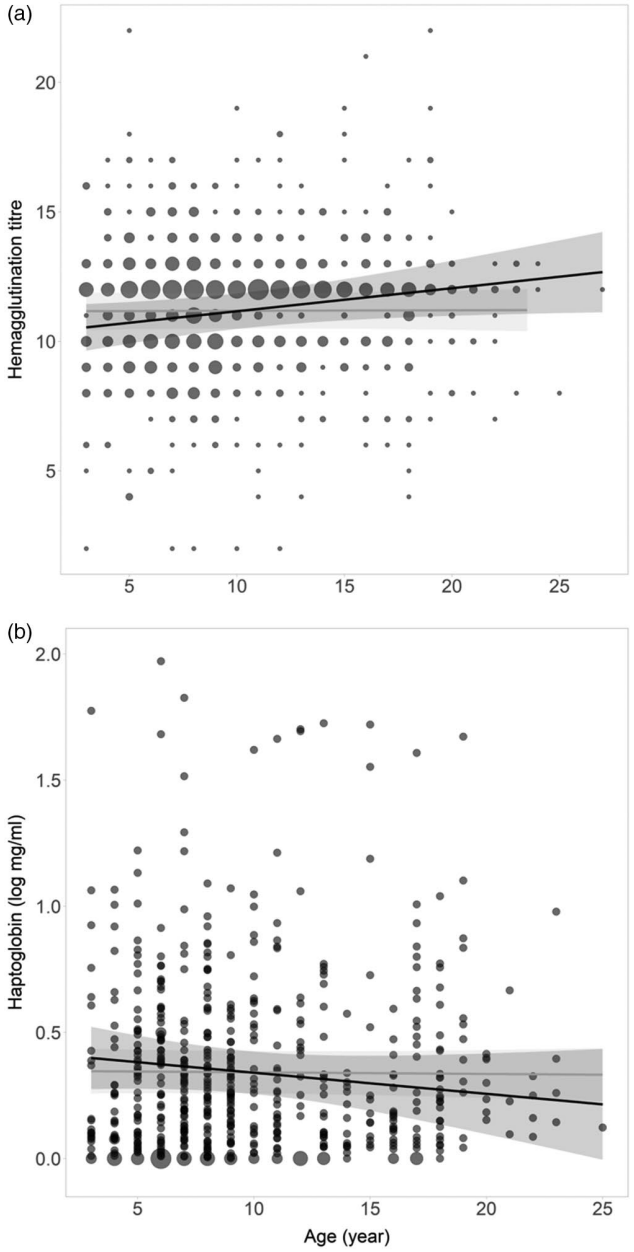

FIGURE 1 Age-specific variation in (a) haemagglutination titre and (b) haptoglobin concentration. Circles represent the raw data with a size proportional to the sample size. Solid lines represent model predictions with their standard errors (grey areas), with the grey line representing the among-individual age effect, the black line the within-individual age effect (centred around the average age based on all samples)

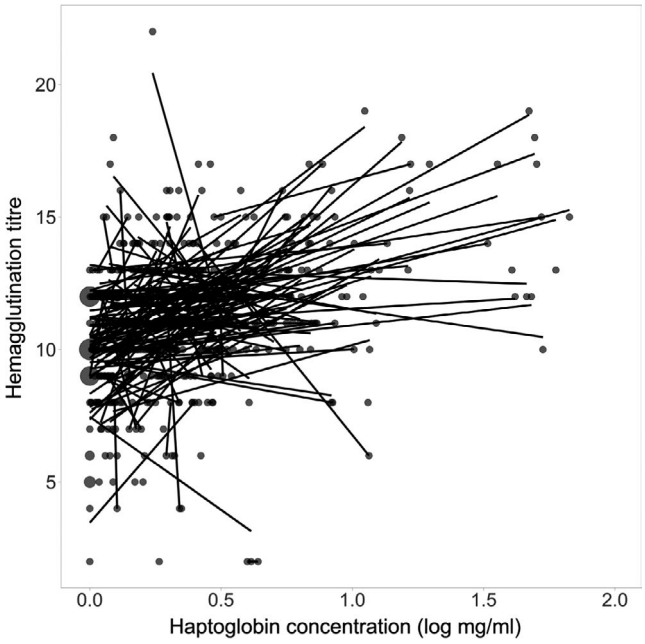

FIGURE 2 Haemagglutination titre and haptoglobin concentration are positively correlated at the within-individual level. Circles represent the raw data with a size proportional to the sample size, solid lines represent individual slopes

4 | DISCUSSION

Longitudinal studies of immune function in natural populations are scarce and their results are variable. In great tits, for instance, Vermeulen et al. (2017) observed an increase in natural antibodies with age in early life, followed by a decrease in late life, while haptoglobin linearly increased with age, both suggesting immunosenescence. Another study, conducted on the greater sac-winged bat *Saccopteryx bilineata*, however, found both a decrease and increase with age in two different immune parameters involved in acquired immunity, while bacterial killing capacity, another innate immunity parameter, did not change with age (Schneeberger et al., 2014). Here, using longitudinal data on common terns sampled between 3 and 27 years of age, we found that haemagglutination, which reflects circulating levels of natural antibodies, increased slightly with age. The rates of change were generally similar among individuals. We also found that haptoglobin, a marker of inflammation, did not change with age.

Animals are exposed to a diverse 'antigenic universe', including antigens associated with parasites and pathogens, as well as ones from commensal micro-organisms, food and their own bodies (Horrocks et al., 2011). As such, exposure history necessarily lengthens with age. Natural antibodies are an early and general innate defence and presumably relatively cheap to produce (Klasing, 2004). The age-associated increase in haemagglutination we observed in the terns therefore could potentially reflect an anti-senescence or anti-inflammatory defence, since natural antibodies help eliminate diverse antigens, including self-derived autoantigens (Reyneveld et al., 2020). The production of natural antibodies, however, does not require prior exposure (Boes, 2000; Matson et al., 2005; Ochsenbein & Zinkernagel, 2000) and is mostly unaffected by acute infection (Baumgarth et al., 1999; Hegemann, Matson, Versteegh, et al., 2013; Matson et al., 2005) such that evidence of natural antibodies varying with antigenic exposure is scarce at best (Horrocks et al., 2015; Panda & Ding, 2015). Alternatively, increased allocation towards the production of cheaper components of the immune system (i.e. natural

antibodies, Klasing, 2004) comes at the expense of that towards more costly components during an age-associated immunological remodelling processes. If this were to be the case, haptoglobin, a defence against, and marker of, inflammation, is unlikely to be involved in such remodelling, since (a) haptoglobin did not decrease with age and (b) haemagglutination and haptoglobin correlated positively, not negatively, within individuals. This latter result also casts doubt on potential anti-inflammatory properties of natural antibodies, pointing instead to their potential 'pathogenic features' (Reyneveld et al., 2020). Further studies would benefit from assessing more facets of the immune system to be able to assess intra-immune-system trade-offs in more detail. Acquired immune components would be particularly interesting to assess in this respect, since these are thought to be more expensive to maintain (e.g. Lee, 2006) and therefore more likely to decline with age (Peters et al., 2019). Furthermore, comparing the age-specific immune defence of animals facing different inflammatory challenges or distinct (perhaps experimentally manipulated) 'antigenic universes' (e.g. van Veelen et al., 2020) could offer new insights into the functional significance of age-related immunological changes.

In many species, males and females differ immunologically (Klein & Flanagan, 2016; Ortona et al., 2019; Restif & Amos, 2010), and these differences can be exacerbated with age (e.g. Gubbels Bupp et al., 2018; van Lieshout et al., 2020). Common terns only show slight sexual dimorphism (Becker & Wink, 2003), share parental care (Riechert & Becker, 2017) and exhibit similar rates of actuarial senescence in males and females (Zhang et al., 2015). We therefore did not expect strong sex differences in innate immune parameters. In addition to indeed not finding sex-specificity of the immune parameters we studied, we found very little among-individual variation in the average levels of these parameters in general: individual identity, added as a random effect to our models, did not explain detectable levels of variation in haemagglutination or haptoglobin (Table 1). One explanation for these overall low levels of variation could be that selection pressures have led to canalisation of innate immune parameters within relatively small boundaries. The large estimates for the random effect of year (14.19%–19.34% for haemagglutination and between 20.00% and 28.95% for haptoglobin), however, suggest otherwise. Birds appeared to have encountered something that influenced their immune systems differently in the different years. This source of interannual variation (which we assume to be ecological and not methodological in nature), seemingly affected all birds similarly, perhaps as a result of their colonial breeding behaviour. Colonial birds are predicted to face greater exposure to pathogens and parasites, and therefore to invest more in their immune system than solitary birds (Moller et al., 2001). In common terns, some immune indices indeed correlate positively with colony size (Drzewińska-Chańko et al., 2021), reinforcing the idea of links between immune function and sociality. Deepening our understanding of whether colonial breeding behaviour is not just linked with increased immune defences, but also with reduced among-individual variation in these defences is an essential next step in linking immunology and life history.

In common terns, reproductive senescence is limited to small reductions in breeding probability (Zhang et al., 2015b) and transgenerational effects (Bouwhuis et al., 2015), whereas fledgling production increases as birds get older (Nisbet et al., 2020; Zhang et al., 2015b). Actuarial senescence, that is, an age-specific reduction in survival probability, is better documented (Vedder et al., 2021; Zhang et al., 2015a). This reduction in survival is known to co-occur with the shortening of telomeres (Bichet et al., 2020). Telomere shortening with age, however, does not vary strongly among individuals, and the association found between telomere length and life span is the result of a positive genetic correlation between these two traits (Vedder, Moiron, et al., 2021). If telomeres and innate immune parameters were similarly related to survival, then individuals' average levels of immune parameters would predict survival. However, we found no support for this prediction: there was no evidence for selective disappearance of terns in relation to average immune parameters. We therefore require a better understanding of the causes of death of these birds to understand whether immune parameters other than the ones we measured could reflect somatic state or individual quality or whether other selection pressures are more important.

5 | CONCLUSIONS

Using longitudinally collected samples from a large number of free-living common terns, we observed very little heterogeneity among individuals with respect to average levels of innate immune parameters and no signs of selective (dis)appearance. There were also no sex differences, and within-individual changes with age were limited to a slight increase in haemagglutination, which may reflect the successful dealing with a longer exposure history of older birds to their 'antigenic universe' rather than senescence.

Given the scarcity of longitudinal studies investigating variation in immune function (Beirne et al., 2016; Froy et al., 2019; Graham et al., 2010; Schneeberger et al., 2014; Vermeulen et al., 2017), it is hard to determine whether our results relate to the colonial breeding behaviour of our study species or to the limited selective pressure exerted by pathogens and parasites. More longitudinal studies are required to gain greater insights into the links between species' traits, including sociality, and variation in immune functions with age in wild animals, thereby revealing possible general patterns across taxa, which so far remain elusive (Peters et al., 2019).

ACKNOWLEDGEMENTS

We are indebted to Peter H. Becker for setting up the long-term common tern study and warmly thank Juliane Riechert, Nathalie Kürten and Götz Wagenknecht for assisting with the management of fieldwork and maintenance of the long-term dataset, as well as all field workers and students who helped with data collection. We also thank Jean-Michel Gaillard, as well as the associate editor and two anonymous reviewers for constructive discussion of our work. M.M.

was funded by a Marie Curie Individual Fellowship (PLASTIC TERN, Grant Agreement Number 793550).

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

S.B., C.B. and K.D.M. designed the study; S.B., O.V. and C.B. collected the blood samples; C.B. did the laboratory work with advice from K.D.M.; C.B. and M.M. analysed the data; C.B. and S.B. wrote the paper with contributions from all authors.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.63xsj3v3q (Bichet et al., 2021).

ORCID

Coraline Bichet https://orcid.org/0000-0003-0255-4966
Maria Moiron https://orcid.org/0000-0003-0991-1460
Kevin D. Matson https://orcid.org/0000-0002-4373-5926
Oscar Vedder https://orcid.org/0000-0003-4689-8568
Sandra Bouwhuis https://orcid.org/0000-0003-4023-1578

REFERENCES

Aastrup, C., & Hegemann, A. (2021). Jackdaw nestlings rapidly increase innate immune function during the nestling phase but no evidence for a trade-off with growth. *Developmental and Comparative Immunology*, *117*. https://doi.org/10.1016/j.dci.2020.103967

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell*, *124*(4), 783–801. https://doi.org/10.1016/j.cell.2006.02.015

Andersen, C. B. F., Stødkilde, K., Sæderup, K. L., Kuhlee, A., Raunser, S., Graversen, J. H., & Moestrup, S. K. (2016). Haptoglobin. *Antioxidants & Redox Signaling*, *26*(14), 814–831. https://doi.org/10.1089/ars.2016.6793

Arnold, J. M., Oswald, S. A., Voigt, C. C., Palme, R., Braasch, A., Bauch, C., & Becker, P. H. (2008). Taking the stress out of blood collection: Comparison of field blood-sampling techniques for analysis of baseline corticosterone. *Journal of Avian Biology*, *39*(5), 588–592. https://doi.org/10.1111/j.0908-8857.2008.04265.x

Bates, D., Maechler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Bauch, C., Becker, P. H., & Verhulst, S. (2013). Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1752), 20122540. https://doi.org/10.1098/rspb.2012.2540

Bauch, C., Kreutzer, S., & Becker, P. H. (2010). Breeding experience affects condition: Blood metabolite levels over the course of incubation in a seabird. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, *180*(6), 835–845. https://doi.org/10.1007/s00360-010-0453-2

Baumgarth, N., Herman, O. C., Jager, G. C., Brown, L., Herzenberg, L. A., & Herzenberg, L. A. (1999). Innate and acquired humoral immunities to influenza virus are mediated by distinct arms of the immune system. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(5), 2250–2255. https://doi.org/10.1073/pnas.96.5.2250

Becker, P. H., & Wendeln, H. (1997). A new application for transponders in population ecology of the common tern. *Condor*, *99*(2), 534–538. https://doi.org/10.2307/1369963

Becker, P. H., Wendeln, H., & Gonzalez-Solis, J. (2001). Population dynamics, recruitment, individual quality and reproductive strategies in Common Terns *Sterna hirundo* marked with transponders. *Ardea*, *89*(1), 241–252.

Becker, P. H., & Wink, M. (2003). Influences of sex, sex composition of brood and hatching order on mass growth in common terns *Sterna hirundo*. *Behavioral Ecology and Sociobiology*, *54*(2), 136–146. https://doi.org/10.1007/s00265-003-0605-4

Beirne, C., Waring, L., McDonald, R. A., Delahay, R., & Young, A. (2016). Age-related declines in immune response in a wild mammal are unrelated to immune cell telomere length. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1825), 20152949. https://doi.org/10.1098/rspb.2015.2949

Bichet, C., Moiron, M., Matson, K. D., Vedder, O., & Bouwhuis, S. (2021). Immunosenescence in the wild? A longitudinal study in a long-lived seabird. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.63xsj3v3q

Boes, M. (2000). Role of natural and immune IgM antibodies in immune responses. *Molecular Immunology*, *37*(18), 1141–1149. https://doi.org/10.1016/s0161-5890(01)00025-6

Bonilla, F. A., & Oettgen, H. C. Adaptive immunity. *Journal of Allergy and Clinical Immunology*, *125*(2), S33–S40. https://doi.org/10.1016/j.jaci.2009.09.017

Bouwhuis, S., Charmantier, A., Verhulst, S., & Sheldon, B. C. (2010). Individual variation in rates of senescence: Natal origin effects and disposable soma in a wild bird population. *Journal of Animal Ecology*, *79*(6), 1251–1261. https://doi.org/10.1111/j.1365-2656.2010.01730.x

Bouwhuis, S., Choquet, R., Sheldon, B. C., & Verhulst, S. (2012). The forms and fitness cost of senescence: Age-specific recapture, survival, reproduction, and reproductive value in a wild bird population. *American Naturalist*, *179*(1), E15–E27. https://doi.org/10.1086/663194

Bouwhuis, S., & Vedder, O. (2017). Avian escape artists? In R. P. Shefferson, O. P. Jones, & R. Salguero-Gómez (Eds.), *The evolution of senescence in the tree of life*. Cambridge University Press. https://doi.org/10.1017/9781139939867.008

Bouwhuis, S., Vedder, O., & Becker, P. H. (2015). Sex-specific pathways of parental age effects on offspring lifetime reproductive success in a long-lived seabird. *Evolution*, *69*(7), 1760–1771. https://doi.org/10.1111/evo.12692

Brooks, R. C., & Garratt, M. G. (2017). Life history evolution, reproduction, and the origins of sex-dependent aging and longevity. *Annals of the New York Academy of Sciences*, *1389*(1), 92–107. https://doi.org/10.1111/nyas.13302

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). https://link.springer.com/book/ https://doi.org/10.1007/b97636 Springer-Verlag.

Cheynel, L., Lemaitre, J.-F., Gaillard, J.-M., Rey, B., Bourgoin, G., Ferte, H., Jego, M., Debias, F., Pellerin, M., Jacob, L., & Gilot-Fromont, E. (2017). Immunosenescence patterns differ between populations but not between sexes in a long-lived mammal. *Scientific Reports*, *7*, 13700. https://doi.org/10.1038/s41598-017-13686-5

Cooper, M. D., & Alder, M. N. (2006). The evolution of adaptive immune systems. *Cell*, *124*(4), 815–822. https://doi.org/10.1016/j.cell.2006.02.001

Cray, C., Zaias, J., & Altman, N. H. (2009). Acute phase response in animals: A review. *Comparative Medicine*, *59*(6), 517–526.

Deerenberg, C., Arpanius, V., Daan, S., & Bos, N. (1997). Reproductive effort decreases antibody responsiveness. *Proceedings of the Royal Society B: Biological Sciences*, *264*(1384), 1021–1029. https://doi.org/10.1098/rspb.1997.0141

Dingemanse, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: Mixed-effect modelling approaches. *Journal of Animal Ecology*, *82*(1), 39–54. https://doi.org/10.1111/1365-2656.12013

Drzewińska-Chańko, J., Włodarczyk, R., Gajewski, A., Rudnicka, K., Dunn, P. O., & Minias, P. (2021). Immunocompetent birds choose larger breeding colonies. *Journal of Animal Ecology*. https://doi.org/10.1111/1365-2656.13540

Eraud, C., Jacquet, A., & Faivre, B. (2009). Survival cost of an early immune soliciting in nature. *Evolution*, *63*(4), 1036–1043. https://doi.org/10.1111/j.1558-5646.2008.00540.x

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *69*(Suppl 1), S4–9. https://doi.org/10.1093/gerona/glu057

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M. P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G. C., & Salvioli, S. (2007). Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of Ageing and Development*, *128*(1), 92–105. https://doi.org/10.1016/j.mad.2006.11.016

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., & Santoro, A. (2018). Inflammaging: A new immune-metabolic viewpoint for age-related diseases. *Nature Reviews Endocrinology*, *14*(10), 576–590. https://doi.org/10.1038/s41574-018-0059-4

Frasca, D., Diaz, A., Romero, M., Landin, A. M., & Blomberg, B. B. (2011). Age effects on B cells and humoral immunity in humans. *Ageing Research Reviews*, *10*(3), 330–335. https://doi.org/10.1016/j.arr.2010.08.004

Froy, H., Sparks, A. M., Watt, K., Sinclair, R., Bach, F., Pilkington, J. G., Pemberton, J. M., McNeilly, T. N., & Nussey, D. H. (2019). Senescence in immunity against helminth parasites predicts adult mortality in a wild mammal. *Science*, *365*(6459), 1296–1298. https://doi.org/10.1126/science.aaw5822

Fülöp, T., Larbi, A., Dupuis, G., Le Page, A., Frost, E. H., Cohen, A. A., Witkowski, J. M., & Franceschi, C. (2018). Immunosenescence and inflamm-aging as two sides of the same coin: Friends or foes? *Frontiers in Immunology*, *8*, 1960. https://doi.org/10.3389/fimmu.2017.01960

Gelman, A., & Su, Y.-S. (2020). *arm: Data analysis using regression and multilevel/hierarchical models* (1.11-2) [Computer software]. Retrieved from https://CRAN.R-project.org/package=arm

Goto, M. (2008). Inflammaging (inflammation plus aging): A driving force for human aging based on an evolutionarily antagonistic pleiotropy theory? *Bioscience Trends*, *2*(6), 218–230.

Graham, A. L., Allen, J. E., & Read, A. F. (2005). Evolutionary causes and consequences of immunopathology. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 373–397. https://doi.org/10.1146/annurev.ecolsys.36.102003.152622

Graham, A. L., Hayward, A. D., Watt, K. A., Pilkington, J. G., Pemberton, J. M., & Nussey, D. H. (2010). Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. *Science*, *330*(6004), 662–665. https://doi.org/10.1126/science.1194878

Gubbels Bupp, M. R., Potluri, T., Fink, A. L., & Klein, S. L. (2018). The confluence of sex hormones and aging on immunity. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.01269

Hadfield, J. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software*, *33*(2), 1–22.

Hanssen, S. A., Hasselquist, D., Folstad, I., & Erikstad, K. E. (2004). Costs of immunity: Immune responsiveness reduces survival in a vertebrate. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1542), 925–930. https://doi.org/10.1098/rspb.2004.2678

Hegemann, A., Matson, K. D., Flinks, H., & Tieleman, B. I. (2013). Offspring pay sooner, parents pay later: Experimental manipulation of body mass reveals trade-offs between immune function, reproduction and survival. *Frontiers in Zoology*, *10*, 77. https://doi.org/10.1186/1742-9994-10-77

Hegemann, A., Matson, K. D., Versteegh, M. A., Villegas, A., & Tieleman, B. I. (2013). Immune response to an endotoxin challenge involves multiple immune parameters and is consistent among the annual-cycle stages of a free-living temperate zone bird. *Journal of Experimental Biology*, *216*(14), 2573–2580. https://doi.org/10.1242/jeb.083147

Heidelberger, P., & Welch, P. D. (1981). A spectral method for confidence interval generation and run length control in simulations. *Communications of the ACM*, *24*(4), 233–245. https://doi.org/10.1145/358598.358630

Hoebe, K., Janssen, E., & Beutler, B. (2004). The interface between innate and adaptive immunity. *Nature Immunology*, *5*(10), 971–974. https://doi.org/10.1038/ni1004-971

Holand, H., Kvalnes, T., Gamelon, M., Tufto, J., Jensen, H., Pärn, H., Ringsby, T. H., & Sæther, B.-E. (2016). Spatial variation in senescence rates in a bird metapopulation. *Oecologia*, *181*(3), 865–871. https://doi.org/10.1007/s00442-016-3615-4

Hõrak, P., Saks, L., Ots, I., & Kollist, H. (2002). Repeatability of condition indices in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology*, *80*(4), 636–643. https://doi.org/10.1139/z02-038

Hõrak, P., Saks, L., Ots, I., Kullissaar, T., Kollist, H., & Zilmer, M. (2003). Physiological effects of immune challenge in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology*, *81*(3), 371–379. https://doi.org/10.1139/z03-020

Horrocks, N. P. C., Hegemann, A., Ostrowski, S., Ndithia, H., Shobrak, M., Williams, J. B., Matson, K. D., & Tieleman, B. I. (2015). Environmental proxies of antigen exposure explain variation in immune investment better than indices of pace of life. *Oecologia*, *177*(1), 281–290. https://doi.org/10.1007/s00442-014-3136-y

Horrocks, N. P. C., Matson, K. D., & Tieleman, B. I. (2011). Pathogen pressure puts immune defense into perspective. *Integrative and Comparative Biology*, *51*(4), 563–576. https://doi.org/10.1093/icb/icr011

Iwasaki, A., & Medzhitov, R. (2010). Regulation of adaptive immunity by the innate immune system. *Science*, *327*(5963), 291–295. https://doi.org/10.1126/science.1183021

Iwasaki, A., & Medzhitov, R. (2015). Control of adaptive immunity by the innate immune system. *Nature Immunology*, *16*(4), 343–353. https://doi.org/10.1038/ni.3123

Janeway, C. A., Travers, P., Walport, M., & Shlomchik, M. J. (2001). The complement system and innate immunity. *Immunobiology: The immune system in health and disease* (5th ed.). Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK27100/

Kelly, C. D., Stoehr, A. M., Nunn, C., Smyth, K. N., & Prokop, Z. M. (2018). *Sexual dimorphism in immunity across animals: A meta-analysis*. Retrieved from https://pubag.nal.usda.gov/catalog/6206256

Kirkwood, T. B. L. (1977). Evolution of ageing. *Nature*, *270*(5635), 301–304. https://doi.org/10.1038/270301a0

Kirkwood, T. B. L. (2017). The disposable soma theory. In R. P. Shefferson, O. R. Jones, & R. Salguero-Gomez (Eds.), *Evolution of senescence in the tree of life* (pp. 23–39). Cambridge Univeristy Press.

Klasing, K. C. (2004). The costs of immunity. *Dong Wu Xue Bao. [acta Zoologica Sinica]*, *50*(6), 961–969.

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews Immunology*, *16*(10), 626–638. https://doi.org/10.1038/nri.2016.90

Lavoie, E. T. (2006). Avian immunosenescence. *AGE*, *27*(4), 281–285. https://doi.org/10.1007/s11357-005-4561-y

Lee, K. A. (2006). Linking immune defenses and life history at the levels of the individual and the species. *Integrative and Comparative Biology*, *46*(6), 1000–1015. https://doi.org/10.1093/icb/icl049

Lemaitre, J.-F., Berger, V., Bonenfant, C., Douhard, M., Gamelon, M., Plard, F., & Gaillard, J.-M. (2015). Early-late life trade-offs and the evolution of ageing in the wild. *Proceedings of the Royal Society*

B: Biological Sciences, *282*(1806), UNSP 20150209. https://doi.org/10.1098/rspb.2015.0209

Lemaître, J.-F., & Gaillard, J.-M. (2017). Reproductive senescence: New perspectives in the wild. *Biological Reviews*, *92*(4), 2182–2199. https://doi.org/10.1111/brv.12328

Lemaitre, J.-F., Gaillard, J.-M., Lackey, L. B., Clauss, M., & Mueller, D. W. H. (2013). Comparing free-ranging and captive populations reveals intra-specific variation in aging rates in large herbivores. *Experimental Gerontology*, *48*(2), 162–167. https://doi.org/10.1016/j.exger.2012.12.004

Lemaître, J.-F., Ronget, V., Tidière, M., Allainé, D., Berger, V., Cohas, A., Colchero, F., Conde, D. A., Garratt, M., Liker, A., Marais, G. A. B., Scheuerlein, A., Székely, T., & Gaillard, J.-M. (2020). Sex differences in adult lifespan and aging rates of mortality across wild mammals. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(15), 8546–8553. https://doi.org/10.1073/pnas.1911999117

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos*, *88*(1), 87–98. https://doi.org/10.1034/j.1600-0706.2000.880110.x

Maizels, R. M., & Nussey, D. H. (2013). Into the wild: Digging at immunology's evolutionary roots. *Nature Immunology*, *14*, 879–883. https://doi.org/10.1038/ni.2643

Martin, J. G. A., Nussey, D. H., Wilson, A. J., & Réale, D. (2011). Measuring individual differences in reaction norms in field and experimental studies: A power analysis of random regression models. *Methods in Ecology and Evolution*, *2*(4), 362–374. https://doi.org/10.1111/j.2041-210X.2010.00084.x

Matson, K. D., Horrocks, N. P. C., Versteegh, M. A., & Tieleman, B. I. (2012). Baseline haptoglobin concentrations are repeatable and predictive of certain aspects of a subsequent experimentally-induced inflammatory response. *Comparative Biochemistry and Physiology A: Molecular & Integrative Physiology*, *162*(1), 7–15. https://doi.org/10.1016/j.cbpa.2012.01.010

Matson, K. D., Ricklefs, R. E., & Klasing, K. C. (2005). A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental & Comparative Immunology*, *29*(3), 275–286. https://doi.org/10.1016/j.dci.2004.07.006

Millet, S., Bennett, J., Lee, K. A., Hau, M., & Klasing, K. C. (2007). Quantifying and comparing constitutive immunity across avian species. *Developmental and Comparative Immunology*, *31*(2), 188–201. https://doi.org/10.1016/j.dci.2006.05.013

Moller, A. P., Merino, S., Brown, C. R., & Robertson, R. J. (2001). Immune defense and host sociality: A comparative study of swallows and martins. *The American Naturalist*, *158*(2), 136–145. https://doi.org/10.1086/321308

Monaghan, P., Charmantier, A., Nussey, D. H., & Ricklefs, R. E. (2008). The evolutionary ecology of senescence. *Functional Ecology*, *22*(3), 371–378. https://doi.org/10.1111/j.1365-2435.2008.01418.x

Mueller, L., Fülöp, T., & Pawelec, G. (2013). Immunosenescence in vertebrates and invertebrates. *Immunity & Ageing*, *10*, 12. https://doi.org/10.1186/1742-4933-10-12

Nathan, C. (2006). Neutrophils and immunity: Challenges and opportunities. *Nature Reviews. Immunology*, *6*(3), 173–182. https://doi.org/10.1038/nri1785

Nikolich-Zugich, J. (2018). The twilight of immunity: Emerging concepts in aging of the immune system. *Nature Immunology*, *19*(1), 10–19. https://doi.org/10.1038/s41590-017-0006-x

Nisbet, I. C. T., Iles, D., Kaneb, A., Mostello, C. S., & Jenouvrier, S. (2020). Breeding performance of Common Terns (*Sterna hirundo*) does not decline among older age classes. *The Auk*, *137*(ukaa022). https://doi.org/10.1093/auk/ukaa022

Nussey, D. H., Coulson, T., Festa-Bianchet, M., & Gaillard, J.-M. (2008). Measuring senescence in wild animal populations: Towards a longitudinal approach. *Functional Ecology*, *22*(3), 393–406. https://doi.org/10.1111/j.1365-2435.2008.01408.x

Ochsenbein, A. F., & Zinkernagel, R. M. (2000). Natural antibodies and complement link innate and acquired immunity. *Immunology Today*, *21*(12), 624–630. https://doi.org/10.1016/s0167-5699(00)01754-0

Ortona, E., Pierdominici, M., & Rider, V. (2019). Editorial: Sex hormones and gender differences in immune responses. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.01076

Panda, A., Arjona, A., Sapey, E., Bai, F., Fikrig, E., Montgomery, R. R., Lord, J. M., & Shaw, A. C. (2009). Human innate immunosenescence: Causes and consequences for immunity in old age. *Trends in Immunology*, *30*(7), 325–333. https://doi.org/10.1016/j.it.2009.05.004

Panda, A., & Ding, J. L. (2015). Natural antibodies bridge innate and adaptive immunity. *Journal of Immunology*, *194*(1), 13–20. https://doi.org/10.4049/jimmunol.1400844

Pawelec, G. (2018). Age and immunity: What is 'immunosenescence'? *Experimental Gerontology*, *105*, 4–9. https://doi.org/10.1016/j.exger.2017.10.024

Peters, A., Delhey, K., Nakagawa, S., Aulsebrook, A., & Verhulst, S. (2019). Immunosenescence in wild animals: Meta-analysis and outlook. *Ecology Letters*, *22*(10), 1709–1722. https://doi.org/10.1111/ele.13343

Quaye, I. K. (2008). Haptoglobin, inflammation and disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *102*(8), 735–742. https://doi.org/10.1016/j.trstmh.2008.04.010

R Core Team (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Restif, O., & Amos, W. (2010). The evolution of sex-specific immune defences. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1691), 2247–2255. https://doi.org/10.1098/rspb.2010.0188

Reyneveld, G. I., Savelkoul, H. F. J., & Parmentier, H. K. (2020). Current understanding of natural antibodies and exploring the possibilities of modulation using veterinary models. A review. *Frontiers in Immunology*, *11*, 2139. https://doi.org/10.3389/fimmu.2020.02139

Riechert, J., & Becker, P. H. (2017). What makes a good parent? Sex- specific relationships between nest attendance, hormone levels, and breeding success in a long- lived seabird. *The Auk*, *134*(3), 644–658. https://doi.org/10.1642/AUK-17-13.1

Riechert, J., Chastel, O., & Becker, P. H. (2012). Why do experienced birds reproduce better? Possible endocrine mechanisms in a long-lived seabird, the common tern. *General and Comparative Endocrinology*, *178*(2), 391–399. https://doi.org/10.1016/j.ygcen.2012.06.022

Schneeberger, K., Courtiol, A., Czirjak, G. A., & Voigt, C. C. (2014). Immune profile predicts survival and reflects senescence in a small, long-lived mammal, the greater sac-winged bat (*Saccopteryx bilineata*). *PLoS ONE*, *9*(9), e108268. https://doi.org/10.1371/journal.pone.0108268

Shanley, D. P., Aw, D., Manley, N. R., & Palmer, D. B. (2009). An evolutionary perspective on the mechanisms of immunosenescence. *Trends in Immunology*, *30*(7), 374–381. https://doi.org/10.1016/j.it.2009.05.001

Shaw, A. C., Goldstein, D. R., & Montgomery, R. R. (2013). Age-dependent dysregulation of innate immunity. *Nature Reviews Immunology*, *13*(12), 875–887. https://doi.org/10.1038/nri3547

Shefferson, R. P., Jones, O. R., & Salguero-Gómez, R. (Eds.). (2017). *The evolution of senescence in the tree of life* (1st ed.). Cambridge University Press.

Simon, A. K., Hollander, G. A., & McMichael, A. (2015). Evolution of the immune system in humans from infancy to old age. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1821), 20143085. https://doi.org/10.1098/rspb.2014.3085

Tidière, M., Badruna, A., Fouchet, D., Gaillard, J.-M., Lemaître, J.-F., & Pontier, D. (2020). Pathogens shape sex differences in mammalian aging. *Trends in Parasitology*, *36*(8), 668–676. https://doi.org/10.1016/j.pt.2020.05.004

Tidière, M., Gaillard, J.-M., Müller, D. W. H., Lackey, L. B., Gimenez, O., Clauss, M., & Lemaître, J.-F. (2015). Does sexual selection shape sex differences in longevity and senescence patterns across vertebrates? A review and new insights from captive ruminants. *Evolution*, *69*(12), 3123–3140. https://doi.org/10.1111/evo.12801

van de Pol, M., & Verhulst, S. (2006). Age-dependent traits: A new statistical model to separate within- and between-individual effects. *The American Naturalist*, *167*(5), 766–773. https://doi.org/10.1086/503331

van de Pol, M., & Wright, J. (2009). A simple method for distinguishing within- versus between-subject effects using mixed models. *Animal Behaviour*, *77*(3), 753–758. https://doi.org/10.1016/j.anbehav.2008.11.006

van Lieshout, S. H. J., Badás, E. P., Mason, M. W. T., Newman, C., Buesching, C. D., Macdonald, D. W., & Dugdale, H. L. (2020). Social effects on age-related and sex-specific immune cell profiles in a wild mammal. *Biology Letters*, *16*(7), 20200234. https://doi.org/10.1098/rsbl.2020.0234

van Veelen, H. P. J., Falcao Salles, J., Matson, K. D., van der Velde, M., & Tieleman, B. I. (2020). Microbial environment shapes immune function and cloacal microbiota dynamics in zebra finches *Teaniopygia guttata*. *Animal Microbiome*, *2*, 21. https://doi.org/10.1186/s42523-020-00039-3

Vedder, O., Moiron, M., Bichet, C., Bauch, C., Verhulst, S., Becker, P. H., & Bouwhuis, S. (2021). Telomere length is heritable and genetically correlated with lifespan in a wild bird. *Molecular Ecology*. https://doi.org/10.1111/mec.15807

Vedder, O., Pen, I., & Bouwhuis, S. (2021). How fitness consequences of early-life conditions vary with age in a long-lived seabird: A Bayesian multivariate analysis of age-specific reproductive values. *Journal of Animal Ecology*. https://doi.org/10.1111/1365-2656.13471

Vermeulen, A., Eens, M., Van Dongen, S., & Muller, W. (2017). Does baseline innate immunity change with age? A multi-year study in great tits. *Experimental Gerontology*, *92*, 67–73. https://doi.org/10.1016/j.exger.2017.03.011

Vermeulen, A., Muller, W., Matson, K. D., Tieleman, B. I., Bervoets, L., & Eens, M. (2015). Sources of variation in innate immunity in great tit nestlings living along a metal pollution gradient: An individual-based approach. *Science of the Total Environment*, *508*, 297–306. https://doi.org/10.1016/j.scitotenv.2014.11.095

Versteegh, M. A., Helm, B., Kleynhans, E. J., Gwinner, E., & Tieleman, B. I. (2014). Genetic and phenotypically flexible components of seasonal variation in immune function. *Journal of Experimental Biology*, *217*(9), 1510–1518. https://doi.org/10.1242/jeb.097105

Viney, M. E., Riley, E. M., & Buchanan, K. L. (2005). Optimal immune responses: Immunocompetence revisited. *Trends in Ecology & Evolution*, *20*(12), 665–669. https://doi.org/10.1016/j.tree.2005.10.003

Wemer, L., Hegemann, A., Isaksson, C., Nebel, C., Kleindorfer, S., Gamauf, A., Adrion, M., & Sumasgutner, P. (2021). Reduced ectoparasite load, body mass and blood haemolysis in Eurasian kestrels (*Falco tinnunculus*) alog an urban-rural gradient. *The Science of Nature*, *108*(5), 42. https://doi.org/10.1007/s00114-021-01745-x

Xirocostas, Z. A., Everingham, S. E., & Moles, A. T. (2020). The sex with the reduced sex chromosome dies earlier: A comparison across the tree of life. *Biology Letters*, *16*(3), 20190867. https://doi.org/10.1098/rsbl.2019.0867

Zhang, H., Vedder, O., Becker, P. H., & Bouwhuis, S. (2015a). Contrasting between- and within-individual trait effects on mortality risk in a long-lived seabird. *Ecology*, *96*(1), 71–79. https://doi.org/10.1890/14-0064.1

Zhang, H., Vedder, O., Becker, P. H., & Bouwhuis, S. (2015b). Age-dependent trait variation: The relative contribution of within-individual change, selective appearance and disappearance in a long-lived seabird. *Journal of Animal Ecology*, *84*(3), 797–807. https://doi.org/10.1111/1365-2656.12321

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Bichet, C., Moiron, M., Matson, K. D., Vedder, O., & Bouwhuis, S. (2022). Immunosenescence in the wild? A longitudinal study in a long-lived seabird. *Journal of Animal Ecology*, 91, 458–469. https://doi.org/10.1111/1365-2656.13642