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# Sex and hatching order modulate the association between MHC-II diversity and fitness in early-life stages of a wild seabird

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## Abstract

Genes of the major histocompatibility complex (MHC) play a pivotal role in parasite resistance, and their allelic diversity has been associated with fitness variations in several taxa. However, studies report inconsistencies in the direction of this association, with either positive, quadratic or no association being described. These discrepancies may arise because the fitness costs and benefits of MHC diversity differ among individuals depending on their exposure and immune responses to parasites. Here, we investigated in black-legged kittiwake (*Rissa tridactyla*) chicks whether associations between MHC class-II diversity and fitness vary with sex and hatching order. MHC-II diversity was positively associated with growth and tick clearance in female chicks, but not in male chicks. Our data also revealed a positive association between MHC-II diversity and survival in second-hatched female chicks (two eggs being the typical clutch size). These findings may result from condition-dependent parasite infections differentially impacting sexes in relation to hatching order. We thus suggest that it may be important to account for individual heterogeneities in traits that potentially exert selective pressures on MHC diversity in order to properly predict MHC–fitness associations.

**KEYWORDS**

divergent allele advantage, fitness, heterozygote advantage, immunity, *Ixodes uriae*, parasite-mediated selection

## 1 | INTRODUCTION

Identifying the genetic bases of fitness differences among individuals is a long-standing goal in evolutionary biology (Chapman, Nakagawa, Coltman, Slate, & Sheldon, 2009; Ellegren & Sheldon, 2008; Merila & Sheldon, 1999). In this context, immune-related genes have drawn substantial attention (Bateson et al., 2016; Froeschke & Sommer, 2005; Oliver, Telfer, & Piertney, 2009; Sepil, Lachish, Hinks, & Sheldon, 2013) because parasite resistance often covaries with fitness in wild populations (Asghar et al., 2015; Hamilton & Zuk, 1982; Moller, Arriero, Lobato, & Merino, 2009; Willink & Svensson, 2017). The major histocompatibility complex (MHC) is a cluster of genes coding for cell surface proteins that are essential for the adaptive immune system. The MHC plays a critical role in modulating self/nonself-discrimination and in activating an immune response against parasites (Murphy & Weaver, 2017). In recent

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decades, several studies have reported associations between MHC allelic diversity and fitness, but with some major inconsistencies. For instance, depending on the considered species or population, either maximal or intermediate MHC diversity was found to maximize fitness (Bonneaud, Mazuc, Chastel, Westerdahl, & Sorci, 2004; Kalbe et al., 2009; Lenz, Mueller, Trillmich, & Wolf, 2013; Thoss, Ilmonen, Musolf, & Penn, 2011; Wegner, Reusch, & Kalbe, 2003).

The reported inconsistencies in the fitness consequences of MHC diversity probably stem from the variations in the trade-off between the different functions of the MHC. The fact that each MHC protein can bind a limited set of antigens leads to the straightforward expectation that an increase in MHC diversity increases the number of antigens recognized, thereby providing resistance to a wider range of parasites (reviewed by Milinski, 2006). Higher MHC diversity may also lead to more efficient immune responses during infection (Behnke & Wahid, 1991; Doherty & Zinkernagel, 1975; McClelland, Penn, & Potts, 2003). However, theoretical models have shown that high MHC diversity can also incur costs by limiting the potential for inducing an immune reaction (Nowak, Tarczyhornoch, & Austyn, 1992; Woelfing, Traulsen, Milinski, & Boehm, 2009; see also Migalska, Sebastian, & Radwan, 2019) or by increasing the potential for autoimmune disorders (Borghans & De Boer, 2001).

Variation in the level of MHC diversity maximizing fitness should be shaped by any selective pressure associated with changes in exposure to parasites, immune response strength or susceptibility to autoimmunity. Several studies have shown that MHC diversity varies among species or populations according to key life-history traits. For instance, increased mean MHC diversity has been associated with migratory behaviour and female promiscuity, two traits that may increase exposure and/or reduce immune response to parasites (Gohli et al., 2013; Minias, Pikus, Whittingham, & Dunn, 2019; Whittingham, Dunn, Freeman-Gallant, Taff, & Johnson, 2018; Winternitz et al., 2013). In contrast, studies investigating interindividual variation in the fitness consequences of MHC diversity within a population are scarce, although there is extensive evidence for interindividual variations in these potential selective pressures (i.e., exposure to parasites, immune response strength or susceptibility to autoimmunity). For instance, it has been recently hypothesized that sex differences in the effects of immunosuppressive sex hormones on the strength of the immune response and on the susceptibility to autoimmunity should result in different optima of MHC diversity between males and females (Roved, Westerdahl, & Hasselquist, 2017). Specifically, the authors argued that males should benefit from higher levels of MHC diversity than females because sex hormones reduce immune activation in males and increase susceptibility to autoimmunity in females. Accordingly, the association between MHC diversity and reproductive success was positive in adult males but not in adult females in great reed warblers (*Acrocephalus arundinaceus*) (Roved, Hansson, Tarka, Hasselquist, & Westerdahl, 2018). This hypothesis may explain similar findings in other species (Huchard, Knapp, Wang, Raymond, & Cowlishaw, 2010; Sauermann et al., 2001; Schaschl et al., 2012). There may be other explanations for why males benefit from higher levels of MHC diversity than females. For instance, male–male contests increase males' risk of wounds and thus infections (Huchard et al., 2010, and references therein), and deplete males' energetic reserves, thereby possibly leading to less energy available for allocation to immune functions (Schaschl et al., 2012 and references therein). While a few other studies investigated sex-specific associations between MHC diversity and fitness (Hablutzel et al., 2014; Jager et al., 2007; Lenz, Eizaguirre, Scharsack, Kalbe, & Milinski, 2009), no studies have investigated whether other individual traits may modulate these associations. Sex is not the only trait modulating exposure to parasites or immune response strength. For instance, social status and personalities have been associated with infection risks by influencing frequency and duration of interactions with conspecifics (Boyer, Reale, Marmet, Pisanu, & Chapuis, 2010; Drewe, 2010; Habig & Archie, 2015).

Here, we investigated whether differences in sex and hatching order are associated with variation in the fitness consequences of MHC class-II diversity during the nestling stage in a wild population of the monogamous black-legged kittiwakes (*Rissa tridactyla*). In kittiwakes, female and second-hatched chicks are smaller, grow slower (Merkling et al., 2012; Vincenzi, Hatch, Mangel, & Kitaysky, 2013; Vincenzi, Hatch, Merkling, & Kitaysky, 2015) and suffer more from sibling aggressions (Delaunay, 2018; White, Leclaire, et al., 2010) than other chicks, suggesting that they are less competitive for food and in poorer condition. In several other species, including birds, food shortage and reduced condition have been linked to reduced immune responses via energy trade-offs (Beldomenico & Begon, 2010; Brzek & Konarzewski, 2007) or chronic stress (Glaser & Kiecolt-Glaser, 2005). In addition, in kittiwakes, second-hatched chicks hatch from eggs containing increased levels of androgens compared to first-hatched chicks (Benowitz-Fredericks, Kitaysky, Welcker, & Hatch, 2013; Gasparini et al., 2007). This may lead to reduced immune responses as these sex hormones are known to be immunosuppressive in other species (Klein & Flanagan, 2016; Smyth, Caruso, Davies, Clutton-Brock, & Drea, 2018). Thus, females and second-hatched chicks are predicted to be immunologically disadvantaged compared to males and first-hatched chicks. Because individuals that have lower efficient immune responses are hypothesized to be particularly advantaged by high MHC diversity (Roved et al., 2017), we expect the fitness of females and second-hatched chicks to be more dependent on MHC diversity than the fitness of males and first-hatched chicks. We thus investigated whether the association between fitness-related traits and MHC class-II diversity varied with sex and hatching order. We tested several fitness-related traits, namely survival in the nest, growth rate and tick infection during the nestling stage. Growth rate is an important component of fitness in kittiwakes because faster growing chicks are more likely to recruit as breeders (Vincenzi et al., 2015). Ticks can have strong deleterious effects on kittiwake chicks by reducing growth rate when food is scarce (McCoy, Boulinier, Schjorring, & Michalakis, 2002), potentially leading to death in the case of hyperinfestation (Chastel, Monnat, Lelay, & Balouet, 1987). Antigens contained in tick saliva are recognized by MHC class II molecules, which present them to T lymphocytes, thereby activating an immune

response that can reduce tick-feeding efficiency and eventually lead to tick detachment (Andrade, Texeira, Barral, & Barral-Netto, 2005; Oliver et al., 2009; Owen, Nelson, & Clayton, 2010).

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

The study was conducted during the 2007–2013 and 2016–2017 breeding seasons (May–August) on a colony of black-legged kittiwakes nesting on an abandoned US Air Force radar tower on Middleton Island (59°26′N, 146°20′W), Gulf of Alaska. The 400 nest sites created on the upper walls of the tower can be observed from inside the building through sliding one-way mirrors and birds are individually identified using colour and metal bands (Gill & Hatch, 2002). All nest sites were checked twice daily (9:00 a.m. and 6:00 p.m.) to record laying, hatching and death events. We focused on two-egg clutches, which is the typical clutch size in this kittiwake population (range 1–3; Gill & Hatch, 2002). There is generally a 2-day interval between laying of the first and second egg, which usually results in an asynchronous hatching by 1 or 2 days (Coulson, 2011). On the day of laying, A- and B-eggs (first- and second-laid eggs, respectively) were labelled individually with a nontoxic marker. Chicks were marked on the head with similar markers for identification shortly after hatching.

### 2.2 | DNA collection and sexing

Chicks were sexed molecularly using DNA extracted from eggshells, tissues from embryos (in cases of prehatching death) or blood collected from the metatarsal vein (for a detailed sexing protocol see Merkling et al., 2012). A few chicks ($n$ = 18) were sexed a posteriori when they came back to the colony as adults (identified with a numbered metal ring). We used DNA extracted from a blood sample collected on adults with a syringe or capillaries from the brachial vein to determine sex using the same molecular method as for chicks (Merkling et al., 2012). The sex of a few adults ($n$ = 4) was determined using sex-specific behaviour (i.e., copulation and courtship feeding during the prelaying period; Danchin, 1991; Jodice, Lanctot, Gill, Roby, & Hatch, 2000).

### 2.3 | Molecular analysis of major histocompatibility complex

#### 2.3.1 | MHC genotyping

The DNA samples were used to amplify 258-bp fragments (218 bp excluding primers) of exon 2 of the black-legged kittiwake MHC class-IIB. We used the MHC class-IIB specific primers (forward: 5′-GCACGAGCAGGGTATTTCCA and reverse: 5′-GTTCTGCCACACACTCACC) designed by Leclaire et al. (2014), which amplify at least two MHC class-IIB loci. These loci are presumed to be functional as shown by signs of positive selection, by the absence of stop codon or frame shift mutations in the translated alleles and by at least three alleles being transcribed in each of the two individuals studied (Leclaire et al., 2014). To discriminate samples after sequencing, the 5′ end of both forward and reverse primers included a combination of two different 8-bp tags. The PCR (polymerase chain reaction) amplification was performed in 20 µl mixtures containing 2 µl of extracted DNA, 0.5 µm of each primer, 1 × AmpliTaq Gold 360 Master Mix (Applied Biosystems) and 3.2 µg bovine serum albumin (Roche Diagnostics). The PCR programme consisted of 10 min initial denaturation at 95°C, followed by 30 cycles of 30 s denaturation at 95°C, 30 s annealing at 57°C and 90 s extension at 72°C. A final elongation step was run at 72°C for 7 min. Amplicons were then purified using the MinElute PCR Purification protocol (Qiagen). Amplicons were sequenced in two runs with an Illumina MiSeq platform, using the 2 × 300-bp protocol (Fasteris SA). We included PCR blank controls, as well as unused tag combinations, in the sequenced multiplex to detect and withdraw potential mistagging biases (Esling, Lejzerowicz, & Pawlowski, 2015).

Amplicon sequences were analysed with AMPLISAS, a three-step pipeline that consists of read demultiplexing, unique sequence clustering and erroneous sequence filtering (Sebastian, Herdegen, Migalska, & Radwan, 2016). First, the Illumina data were filtered to remove low-quality sequences (sequences with Phred scores < 50). Sequences were then clustered using the default AMPLISAS parameters for Illumina sequences (substitution errors: 1%, indel errors: 0.001%, minimum frequency with respect to dominant: 25%), and sequences that were potential chimeras or that had less than 3% frequency were discarded. We discarded samples with a depth inferior to 500 reads. Considering the larger set of samples analysed in the two MiSeq runs ($n$ = 2,064 samples, including 699 samples that were part of this study), the reproducibility of genotype between the two runs ($n$ = 25 DNA samples that were split and processed in independent PCRs) was 100%. After processing, we had an average (±*SD*) of 4,735 ± 846 reads per individual (range: 509–5,000 reads). We obtained 83 different MHC class-II alleles. All detected alleles were of the same length. The number of alleles per individual was 3.38 ± 0.83 (mean ± *SD*; range: 1–7), suggesting the presence of at least four functional loci.

#### 2.3.2 | Chick MHC-II diversity

Several measures of MHC diversity have been used across studies (Lenz et al., 2013; Radwan et al., 2012; Sepil, Lachish, & Sheldon, 2013). The most widely used proxy of MHC diversity is the number of different MHC alleles possessed by an individual (e.g., Huchard et al., 2010; Kalbe et al., 2009; Roved et al., 2018). This measure relates to the "heterozygote advantage" (or overdominance) hypothesis. This hypothesis posits that heterozygous individuals should have a selective advantage over homozygous

individuals, because they have a higher number of different MHC molecules, and thus can bind a higher number of antigens (Doherty & Zinkernagel, 1975). Wakeland et al. (1990) later proposed the "divergent allele advantage" hypothesis, which posits that, in heterozygous individuals, those with a higher degree of functional divergence between alleles should have a selective advantage because their MHC molecules can bind a broader range of antigens. Several studies on a single MHC locus have thus used the functional divergence between two alleles to estimate MHC diversity (Pierini & Lenz, 2018). Studies considering several MHC loci and using functional divergence to estimate MHC diversity are scarce. A few of them used the average functional divergence between all alleles and considered that individuals with higher mean divergence should be advantaged (Lenz, Wells, Pfeiffer, & Sommer, 2009; Schwensow, Eberle, & Sommer, 2010). However, the "divergent allele advantage" is expected to work in concert with the "heterozygote advantage" (Wakeland et al., 1990) and, when considering several loci, this estimate of MHC diversity is therefore unlikely to be strongly correlated with the range of antigens bound by all alleles together. A few studies have thus used the degree of divergence over all loci to estimate MHC diversity (Grieves, Gloor, Bernards, & MacDougall-Shackleton, 2019; Huchard, Baniel, Schliehe-Diecks, & Kappeler, 2013; Leclaire et al., 2019; Radwan et al., 2012). This measure is expected to estimate the range of antigens recognized by MHC molecules, regardless of whether a high range is due to the possession of many alleles that are somewhat divergent or to the possession of a few alleles that are very divergent. In our study, we therefore decided to estimate MHC-II diversity as the degree of divergence between alleles over all loci.

We used Faith's diversity index to estimate MHC-II diversity as the degree of divergence of alleles across all loci (as in Grieves et al., 2019 and Leclaire et al., 2019). We first translated MHC-II DNA sequences into amino acid sequences, and considered DNA sequences as functionally identical if they had the same amino acids in the peptide-binding regions (PBRs; inferred from Leclaire et al., 2014). Non-PBR sites were characterized by a low nucleotide diversity and codons with no significant excess of nonsynonymous substitutions (Leclaire et al., 2014). We obtained a total of 68 functional alleles. The mean number of functional alleles per individual was 3.31 ± 0.78 (mean ± *SD*; range: 1–7; Figure S1) and did not significantly vary among years (Kruskal–Wallis, $U = 4.89$, $df = 8$, $p = .77$; Figure S2). Then, following the approach of Schwensow, Fietz, Dausmann, and Sommer (2007), we described the chemical binding properties of each amino acid in the PBRs with Sandberg's five physicochemical descriptors (z-descriptors; Sandberg, Eriksson, Jonsson, Sjostrom, & Wold, 1998). Following an approach adapted from Strandh et al. (2012), we used this Sandberg matrix to construct an alternative maximum-likelihood phylogenetic tree with "Rcontml" in the R package RPHYLIP (Revell & Chamberlain, 2014). This tree represents clusters of functionally similar MHC-II sequences (see Figure S3) and was used as a reference to calculate the functional diversity of an individual as the minimum total length of all the branches required to span its MHC-II alleles (i.e., Faith's phylogenetic diversity; Faith, 1992) with the R function "pd" in the PICANTE R package (Kembel et al., 2010). In other words, for each additional allele, only the part of the peptide-binding characteristics that is not shared with other alleles is summed. Chick Faith's MHC-II diversity varied from 0.89 to 9.81 (mean ± *SD*: 6.01 ± 1.19; Figure S4) and did not significantly vary among years (Kruskal–Wallis, $U = 4.24$, $df = 8$, $p = .83$; Figure S5).

For comparison with Faith's MHC-II diversity, we also carried out analyses on the association between fitness traits and the number of functional MHC-II alleles or the mean MHC-II divergence. We calculated the number of functional MHC-II alleles as the number of amino acid PBR sequences per individual. To calculate mean MHC-II divergence, we used the Sandberg matrix to compute the Euclidean distance between all possible pairs of functional alleles (Lenz, Wells, et al., 2009) with the R function "distance" in the PHILENTROPY R package (Drost, 2018). Then for each individual, we calculated MHC-II divergence as the sum of Euclidean distances between each pair of alleles possessed by this individual, divided by the number of allele pairs. It was thus not possible to calculate MHC-II divergence for chicks carrying only one MHC-II allele ($n = 2$ individuals). Chick functional MHC-II divergence varied from 6.57 to 21.93 (mean ± *SD*: 17.32 ± 1.79; Figure S6) and did not significantly vary among years (Kruskal–Wallis, $U = 10.22$, $df = 8$, $p = .24$; Figure S7).

## 2.4 | Chick fitness parameters

### 2.4.1 | Survival

To record disappearance and death, all nests were checked twice daily (9:00 a.m. and 6:00 p.m.) throughout the season until we left the study site (August 15).

### 2.4.2 | Morphological measurements and growth

Chicks were measured every 5 days from hatching to the age of 35 days. We measured body mass to the nearest 0.1 g using an electronic scale, tarsus length to the nearest 0.1 mm with a caliper and wing length to the nearest 1 mm with a wing ruler.

We estimated body mass and size growth rates over 35 days by calculating the maximum slope of a logistic growth curve between morphological measures and age (Merkling et al., 2012) using the GROFIT package in R (Kahm, Hasenbrink, Lichtenberg-Frate, Ludwig, & Kschischo, 2010). Chick size was estimated by taking the scores of the first principal component analysis on wing and tarsus length at 0, 5, 10, 15, 20, 25, 30 and 35 days together. Because such measurement necessarily excludes chicks that were not measured up to 35 days old, we also estimated growth rates over the first 10 days by calculating the slope of the linear regression between the morphological measures and age (Merkling et al., 2014, 2016).

### 2.4.3 | Tick infection

For each chick hatched in the 2008, 2009 and 2010 breeding seasons, we recorded the number of attached ticks (*Ixodes uriae*) every 5 days from 5 to 30 days through visual examination and palpation (Danchin, 1992). *I. uriae* is the only tick species known to infect kittiwakes on Middleton Island (B. M. Williams, personal communication). Ticks generally start to feed on 5-day-old chicks and parasitism may continue until fledging (Boulinier & Danchin, 1996).

## 2.5 | Statistical analysis

### 2.5.1 | Sample size

In all statistical analyses, eggs were excluded when their handling for other experimental purposes could have affected fitness. Because our study aimed at investigating the effect of chick (or embryo) sex on MHC-II–fitness relationships, we excluded unsexed chicks (or embryos) from the analyses ($n$ = 94 individuals). These filters, together with the fact that tick infections were checked only over 3 years, led to different sample sizes for each fitness parameter: 17 nonhatched embryos and 429 chicks were used for survival analyses, 680 chicks for the analyses on condition and size at hatching, 292 chicks for growth rate analyses over the first 10 days, 209 chicks for growth rate analyses over 35 days and 138 chicks for tick infection analyses.

### 2.5.2 | Model selection

We used a corrected Akaike information criterion (AICc)-based information-theoretic approach to test how Faith's MHC-II diversity was associated with fitness-related traits (Burnham & Anderson, 2004; Burnham, Anderson, & Huyvaert, 2011). For each fitness-related trait, we built a set of candidate models corresponding to biologically plausible hypotheses explaining the response variable. Each set of candidate models also included a null model (intercept only) and when a model included an interaction, we always considered an additive model (i.e., without the interaction). We selected the best models based on their ΔAICc (i.e., the difference between the AICc of a given model and the AICc of the best model) by keeping every model with ΔAICc ≤4. This cut-off can be considered as conservative and retains the true best model with an approximate 95% confidence (Richards, 2005). Using these best models, we computed natural model-averaged parameter estimates, standard errors and 95% confidence intervals without shrinkage; that is, parameter estimates of each variable were averaged using only the models with ΔAICc <4 in which they appear (Nakagawa & Freckleton, 2011). Model selection and averaging were conducted using the MUMIN package (Bartoń, 2018) and based on maximum likelihood estimation (see the Supporting Information for an outline of all models). All statistical analyses were performed with R 3.5.2 (R Core Team, 2018).

For each fitness-related trait, we built a set of models that included Faith's MHC-II diversity, the square of Faith's MHC-II diversity, sex, hatching order, and two- and three-way interactions between sex, hatching order and Faith's MHC-II diversity. We standardized continuous variables in all analyses and checked for collinearity issues. We detected a significant association between sex and Faith's MHC-II diversity at hatching (see Results), and thus ran separate models for male and female chicks for all analyses. We included clutch identity (ID), pair ID and year as random effects in the models and checked for a normal distribution of random effects using the best model in model selection. However, the pair ID random effect was removed from models because associated variance estimates were virtually zero.

### 2.5.3 | Survival at the nest

We tested for the effect of Faith's MHC-II diversity on chick survival at the nest using Cox proportional hazard mixed-effect models in the R package COXME (Therneau, 2018). We considered chick survival between 0 and 35 days (i.e., before fledging; Coulson & White, 1958; Maunder & Threlfall, 1972). Sixteen chicks younger than 35 days (mean ± *SD*: 30.6 ± 4.3 days old) were still alive when we left the study site. To be confident that survivors included in analyses fledged after our departure, we excluded those 16 chicks because chicks' likelihood of fledging is very high once they reach 35 days old (Barrett & Runde, 1980; Merkling et al., 2014).

### 2.5.4 | Body condition and size at hatching and growth

We used linear mixed models (LMMs) in the LME4 R package (Bates, Machler, Bolker, & Walker, 2015) to test for the effects of Faith's MHC-II diversity on body condition and size at hatching and on body mass and size growth rates. Chick size at hatching was included in the model built for body mass at hatching, which can thus be interpreted as size-adjusted body mass, or body condition (Garcia-Berthou, 2001). We checked for normality and homoscedasticity of residuals using the best model in model selection.

### 2.5.5 | Tick infection

We investigated the association between Faith's MHC-II diversity and three variables linked to tick infection. First, we determined whether Faith's MHC-II diversity was associated with the probability and the timing of first infection by ticks during the nestling stage (between 5 and 30 days old; $n$ = 138 chicks) by fitting Cox proportional hazard mixed-effect models in the R package COXME (Therneau, 2018). Chicks that were not infected by ticks during this period were right-censored ($n$ = 28). Second, we focused on quantitative resistance by testing whether Faith's MHC-II diversity was

associated with the maximum number of ticks that chicks had during the nestling period ($n$ = 110 chicks, excluding those without ticks) using zero-truncated models with a Poisson distribution using the GLMMTMB R package (Brooks et al., 2017). If models with a zero-truncated Poisson distribution were overdispersed, we compared their fit to the fit of models with zero-truncated negative binomial 1 and zero-truncated negative binomial two distributions. We pooled the values over 15 to "15+" to reduce overdispersion (i.e., four chicks had more than 15 ticks). Finally, we determined whether Faith's MHC-II diversity was associated with the probability and the timing of tick clearance during the nestling stage ($n$ = 86 chicks) by fitting Cox proportional hazard mixed-effect models in the R package COXME (Therneau, 2018). Chicks without ticks ($n$ = 28) or discontinuously infected by ticks ($n$ = 24) were excluded from this analysis. Chicks that still had ticks at the end of the observation period were right-censored. We removed the clutch ID random effect from models because associated variance estimates were virtually zero.

#### 2.5.6 | Comparison of MHC measures

To test the association between fitness-related traits and the number of functional MHC-II alleles or MHC-II divergence, we used the same statistical approach as for Faith's MHC-II diversity. MHC divergence is theoretically not related to the number of alleles but, in our data set, there was a negative correlation between these measures (Pearson correlation, $t$ = −5.67; $r$ = 0.21; $p$ < .001; $n$ = 697, as in Roved, 2019). Including both measures into the same models leads to variance inflation factor (VIF) values >5, indicating collinearity issues (Zuur, Ieno, & Elphick, 2010). We therefore carried out separate analyses for the number of functional MHC-II alleles and MHC-II divergence. Because most chicks had three or four alleles in the restricted data set used for tick analyses, we transformed the "number of MHC-II alleles" in a binary variable with number of MHC-II alleles ≤3 or ≥4 for analyses on ticks. The square of the number of MHC-II alleles and the interactions that included this variable were thus removed from the corresponding models. Full models including either Faith's MHC-II diversity, MHC-II divergence or number of MHC-II alleles were compared according to AICc to explore the relative importance of these three different MHC measures.

## 3 | RESULTS

### 3.1 | Sex-difference in Faith's MHC-II diversity

There was a significant association between sex and Faith's MHC-II diversity at hatching ($t$ test: $t$ = −2.75; $p$ = .006; $n$ = 680 chicks) and at 35 days old ($t$ test: $t$ = −2.10; $p$ = .037; $n$ = 209 chicks). At hatching and at 35 days old, male chicks showed lower MHC-II diversity than female chicks (mean ± $SD$: males at hatching: 5.90 ± 1.48; males at 35 days old: 5.98 ± 1.20; females at hatching: 6.14 ± 1.31; and females at 35 days old: 6.30 ± 1.15).

### 3.2 | Survival at the nest

Among 430 monitored eggs, 142 chicks (33%) died before reaching 35 days of age. In single-sex models, the interaction between Faith's MHC-II diversity and hatching order was significant only in females (estimate ± $SE$ = −0.58 ± 0.28, 95% confidence interval [CI]: −1.13, −0.04; Tables S1 and S2). Mortality significantly decreased with increasing Faith's MHC-II diversity in female B-chicks, but not in female A-chicks, while we detected no significant effect of Faith's MHC-II diversity on mortality in males (Figure 1; Figure S8).

### 3.3 | Body condition and size at hatching

Body condition at hatching was not significantly associated with Faith's MHC-II diversity (Tables S3 and S4). Although there was a negative trend in males, size at hatching was not significantly associated with Faith's MHC-II diversity (females: estimate ± $SE$ = 0.01 ± 0.06, 95% CI: −0.10, 0.12; males: estimate ± $SE$ = −0.10 ± 0.05, 95% CI: −0.20, 0.01; Tables S5 and S6).

### 3.4 | Growth

In females only, body mass growth rate (females: estimate ± $SE$ = 0.26 ± 0.09, 95% CI: 0.08, 0.43; males: estimate ± $SE$ = −0.001 ± 0.08, 95% CI: −0.15, 0.15; Tables S7 and S8; Figure 2a) and body size growth rate over the first 10 days (females: estimate ± $SE$ = 0.24 ± 0.09, 95% CI: 0.06, 0.42; males: estimate ± $SE$ = 0.06 ± 0.07, 95% CI: −0.08, 0.20; Tables S9 and S10; Figure 2b) were significantly and positively associated with Faith's MHC-II diversity. There was also a significant effect of the interaction between the square of Faith's MHC-II diversity and hatching order on body size growth rate in females (estimate ± $SE$ = 0.26 ± 0.11, 95% CI: 0.05, 0.47; Table S9). However, this interaction became non-significant after removing one A-female with high Faith's MHC-II diversity and moderate size growth rate (estimate ± $SE$ = 0.20 ± 0.13, 95% CI: −0.06, 0.46; Table S11). Similar results were obtained for growth rates over 35 days (Tables S12–S15; Figure S9).

### 3.5 | Tick infection

In single-sex models, there was no significant association between Faith's MHC-II diversity and the age of first infection by ticks (females: estimate ± $SE$ = −0.15 ± 0.14, 95% CI: −0.43, 0.12; males: estimate ± $SE$ = −0.10 ± 0.14, 95% CI: −0.37, 0.17; Tables S16 and S17) or the maximum number of ticks (females: estimate ± $SE$ = −0.29 ± 0.22, 95% CI: −0.73, 0.15; males: estimate ± $SE$ = −0.01 ± 0.26, 95% CI: −0.51, 0.51; Tables S18 and S19). However, in females only, tick clearance was positively and significantly associated with Faith's MHC-II diversity (females: estimate ± $SE$ = 0.54 ± 0.20, 95% CI: 0.14, 0.94; males: estimate ± $SE$ = −0.07 ± 0.21, 95% CI: −0.49, 0.34; Tables S20

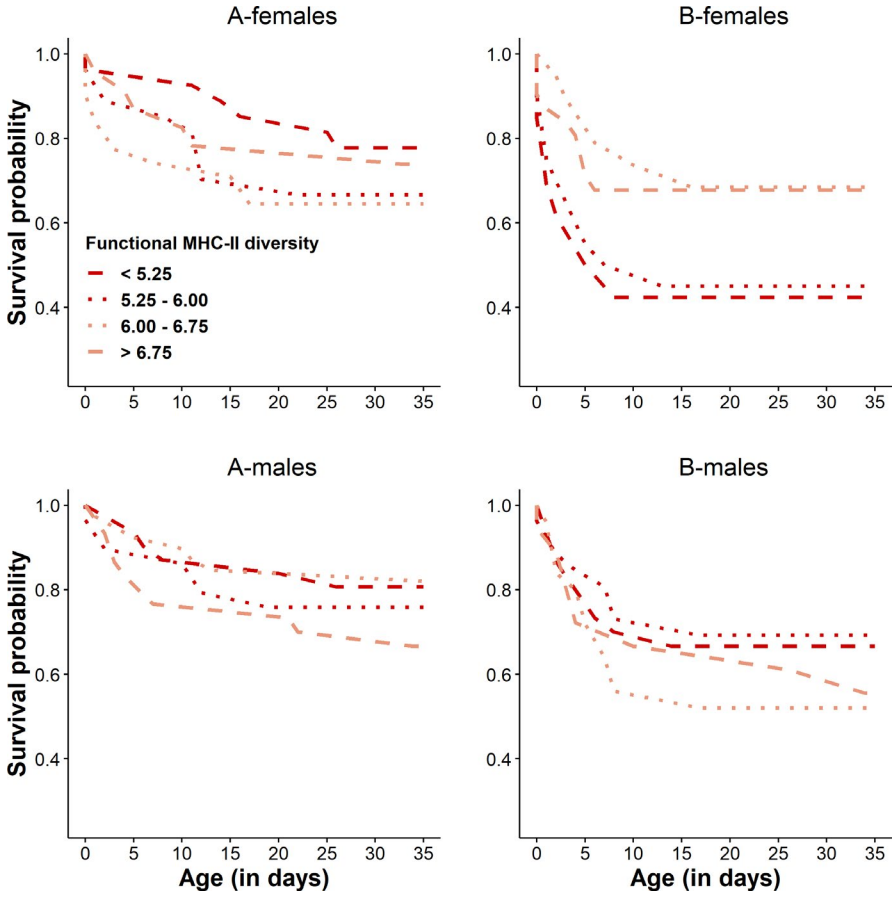


**FIGURE 1** Chick survival probability during the nestling stage according to functional MHC-II diversity for female A- ($n = 107$), female B- ($n = 96$), male A- ($n = 128$) and male B-chicks ($n = 99$). Although functional MHC-II diversity was analysed as a continuous variable (see text), it is displayed here as a categorical variable for illustrative purposes. We categorized MHC-II diversity into four groups using quartiles of the whole data set used for survival analyses ($n = 430$). The colours and line types represent MHC-II diversity, with red corresponding to the first (dashed line) and second (dotted line) quartiles (i.e., low MHC-II diversity) and pink to the third (dotted line) and fourth (dashed line) quartiles (i.e., high MHC-II diversity). See Figure S10 for a different display (heatmap) [Colour figure can be viewed at wileyonlinelibrary.com]

and S21). With increasing female MHC-II diversity, the more likely and the faster they were to clear ticks (Figures 3 and 4). There was no significant effect of any other parameter on these three response variables (Tables S16–S21).

### 3.6 | Comparison of MHC measures

Faith's MHC-II diversity was significantly and positively correlated with both the number of functional MHC-II alleles (Pearson correlation, $t = 8.90$; $r = 0.32$; $p < .001$; $n = 699$) and MHC-II divergence (Pearson correlation, $t = 2.89$; $r = 0.11$; $p = .004$; $n = 697$). No effects of functional MHC-II allele number or mean MHC-II divergence on fitness traits were detected except for a sex-specific effect of allele number on tick infection, with females, but not males, possessing three MHC-II alleles or fewer being more likely to become infected than females with four MHC-II alleles or more (see Supporting Information for more details). There was also a weak effect of the square of MHC-II divergence on body mass growth rate, with chicks harbouring intermediate MHC-II divergence growing faster than those with low or high MHC-II divergence (see Supporting Information for more details). For fitness traits associated with Faith's MHC-II diversity, full models including Faith's MHC-II diversity had lower AICc than similar models including MHC-II divergence or MHC-II allele number (Table S22). This suggests that Faith's MHC-II diversity is a better predictor of these different fitness traits than MHC-II divergence or the number of MHC-II alleles in kittiwake chicks.

## 4 | DISCUSSION

Although fitness advantages have been associated with different levels of MHC-II diversity in a wide range of species and populations (Bonneaud et al., 2004; Brouwer et al., 2010; Lenz et al., 2013; Thoss et al., 2011; Wegner et al., 2003), only a handful of studies have investigated the possibility that individuals within a population might benefit from different levels of MHC-II diversity. Yet, individuals can differ greatly in exposure and immune responses to parasites, leading to the expectation that they might not benefit from MHC-II diversity in the same way. In this study, we investigated whether, in kittiwake chicks, MHC-II–fitness associations depended upon sex and hatching order, two factors expected to modulate exposure and immune response to parasites. As expected, we detected positive associations between MHC-II diversity and female chick survival, but only in second-hatched female chicks. In contrast, no association between MHC-II diversity and survival was detected in male chicks. High MHC-II diversity was also associated with faster growth and tick clearance in female chicks only. Our results therefore suggest that female chicks, especially those hatched in second position, benefit from maximal levels of MHC-II diversity while male chicks do not.

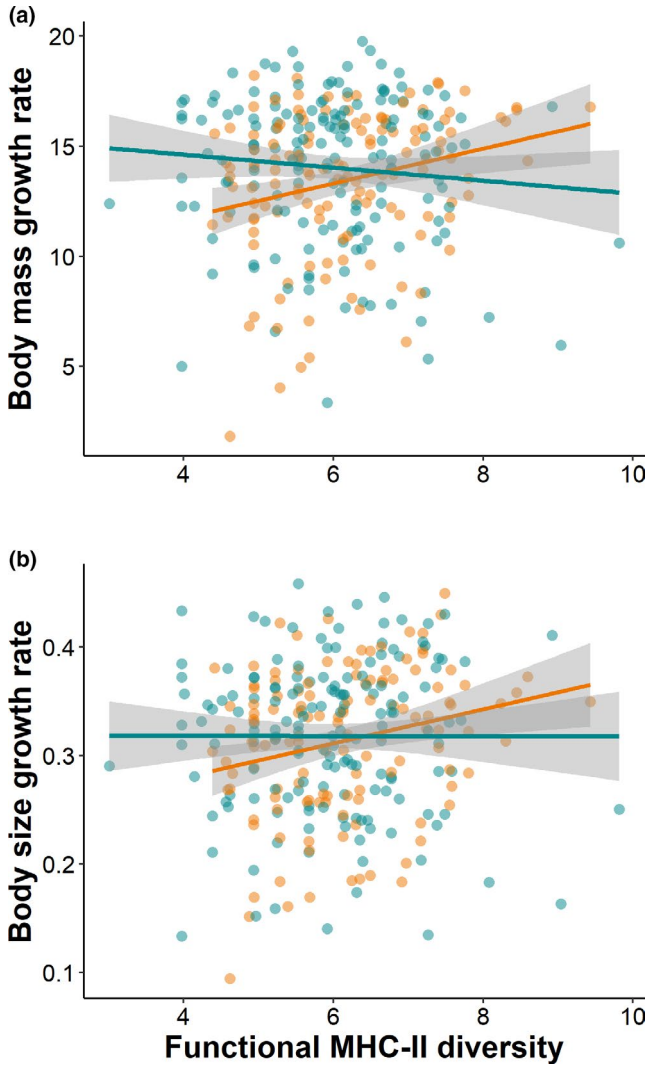


**FIGURE 2** Growth rate of (a) chick body mass and (b) chick body size over the first 10 days according to functional MHC-II diversity in females ($n = 131$, in orange) and males ($n = 160$, in blue). Chick size was estimated by taking the scores of the first principal component analysis on wing and tarsus length. Growth rate was calculated as the slope of a linear regression between morphological measures and age (see Methods for more details). One male with a very low MHC-II diversity (i.e., 0.88) has been removed to improve the clarity of the figure (see Figure S12 for a figure including this male). Removing this male from analyses did not change the results. Regression lines were derived from single-sex models including functional MHC-II diversity of chicks as a fixed effect. Random effects (year and clutch ID) were not considered in the models used for graphic representations. Shaded areas represent confidence intervals [Colour figure can be viewed at wileyonlinelibrary.com]

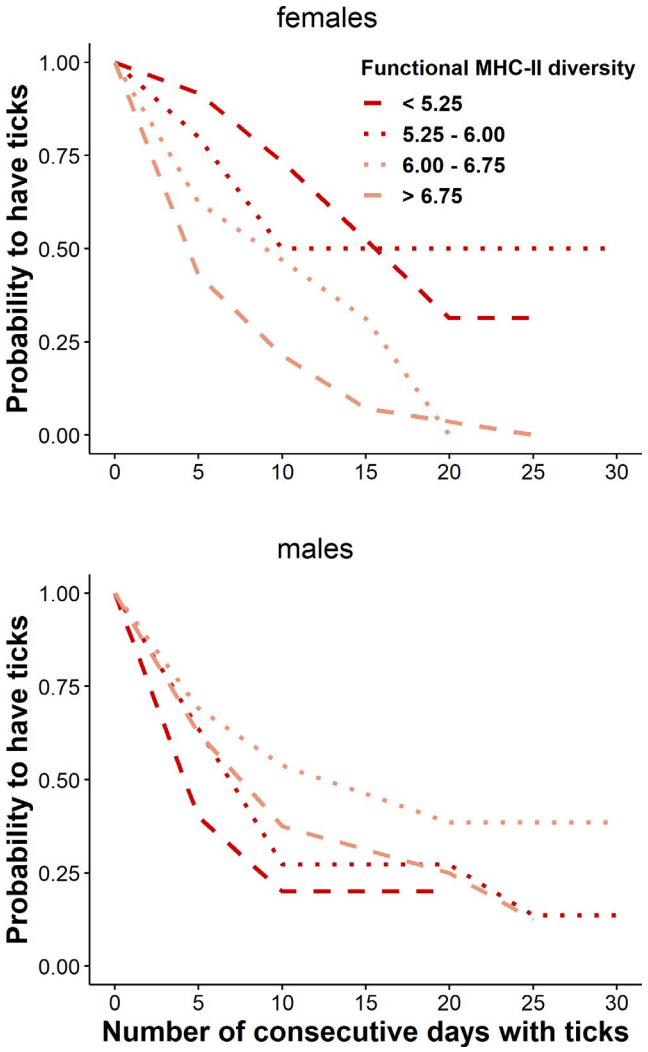


**FIGURE 3** Probability of chicks being infected according to infection duration and functional MHC-II diversity for females ($n = 44$) and males ($n = 42$). Although functional MHC-II diversity was analysed as a continuous variable (see text), it is displayed here as a categorical variable for illustrative purposes. We categorized MHC-II diversity in four groups using quartiles of the whole data set used for tick analyses ($n = 86$). The colours and line types represent MHC-II diversity, with red corresponding to the first (dashed line) and second (dotted line) quartiles (i.e., low MHC-II diversity) and pink to the third (dotted line) and fourth (dashed line) quartiles (i.e., high MHC-II diversity) [Colour figure can be viewed at wileyonlinelibrary.com]

Several underlying mechanisms may explain the sex- and hatching rank-dependent effects of MHC-II diversity on fitness in kittiwake chicks. Because high MHC-II diversity provides resistance to a wider range of parasites and is associated with a more efficient immune response, the fitness benefits associated with high MHC-II diversity in female chicks, particularly when hatched in second position, might be explained by weaker immune responses and/or higher exposure to parasites compared to males and A-chicks. A decreased immune response may result from higher levels of immunosuppressive sex hormones, such as androgens (Klein & Flanagan, 2016). Although higher levels of androgens have been found in the yolk of B-eggs compared to A-eggs in kittiwakes (Benowitz-Fredericks et al., 2013; Gasparini et al., 2007), sex-differences in circulating levels of androgens have not been investigated in kittiwake chicks. However, female chicks have been found to have higher levels of androgens than male chicks in several other species (Fargallo, Martinez-Padilla, Toledano-Diaz, Santiago-Moreno, & Davila, 2007, and references therein). Nonexclusively, weaker immune responses might also result from a trade-off between

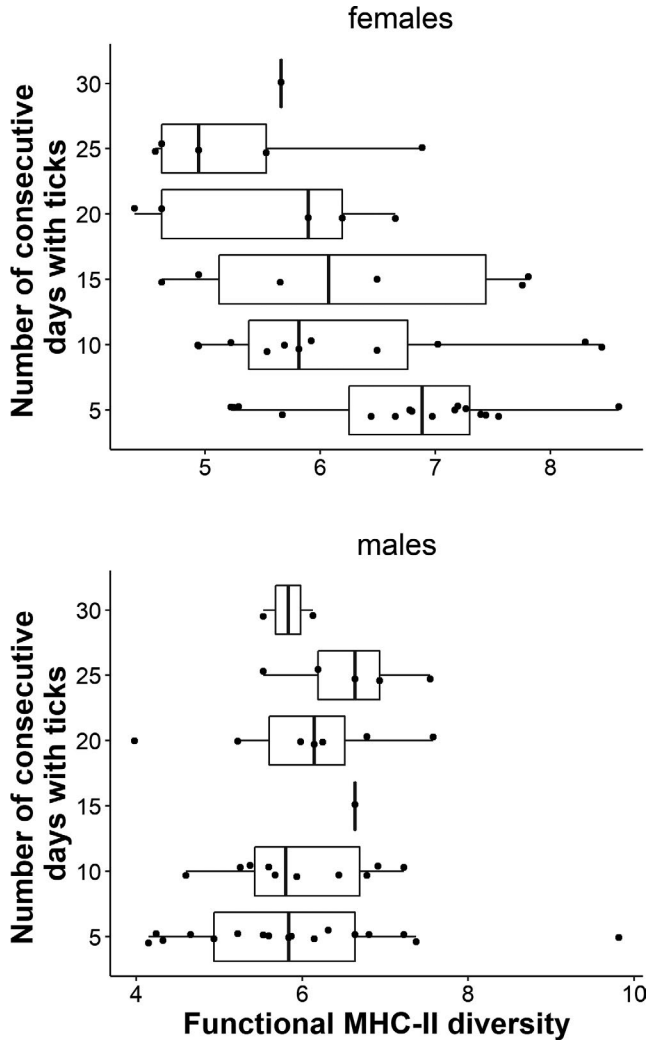


**FIGURE 4** Boxplots of functional MHC-II diversity according to the number of consecutive days chicks were observed with ticks for females ($n = 44$) and males ($n = 42$)

immunity and other competing functions that require metabolic resources (Zuk & Stoehr, 2002). In kittiwakes, female B-chicks are smaller (Merkling et al., 2012), in poorer condition (this study; see Tables S3, S4, S23 and S24; Figure S10) and suffer more from sibling aggression than other chicks (Delaunay, 2018; White, Leclaire, et al., 2010), suggesting that they might be less competitive for food, and thus have a lower amount of resources to allocate to immune functions (Beldomenico & Begon, 2010; Beldomenico et al., 2008). Interestingly, when restricting our MHC-II-fitness analyses to broods where one egg did not hatch, thereby excluding the role of competition and aggression between siblings in driving MHC-II-fitness associations, we did not find any significant effect of MHC-II diversity on survival in female B-chicks ($n = 49$ females) or on growth rate ($n = 34$ females) or tick clearance ($n = 14$ females) in female chicks (Tables S25–S28). Although these results must be taken with caution because of the reduced sample size, they suggest that sibling interactions are a potential driver of sex- and rank-specific effects of MHC-II diversity on fitness in this species.

A surprising finding of our study was that female chicks had, on average, a higher MHC-II diversity than male chicks at hatching (mean ± *SD*: 6.14 ± 1.31 vs. 5.90 ± 1.48). Yet, females and males should exhibit the same level of MHC diversity as they share the genetic architecture of the MHC (i.e., MHC genes are located on autosomes; Murphy & Weaver, 2017). A sex-difference in mean MHC diversity has already been reported in humans, rats and mice, with increased MHC diversity in newborn males compared to newborn females (Dorak, Lawson, Machulla, Mills, & Burnett, 2002, and references therein). Proposed mechanisms for this difference include selective fertilization, egg resorption and embryo loss (Dorak et al., 2002). Selective fertilization and embryo loss have been associated with both the degree of MHC-similarity between parents (Lenz, Hafer, Samonte, Yeates, & Milinski, 2018; Ober, Hyslop, Elias, Weitkamp, & Hauck, 1998; Wedekind, Chapuisat, Macas, & Rulicke, 1996; Zhu, Wan, Zhang, & Fang, 2019) and the sex of the embryo (or the sex chromosome of the gametes; Navara, 2018). However, whether they can be affected by these two parameters in interaction remains largely unknown. In our data set, female hatchlings still had greater MHC-II diversity than males when we considered only clutches with no egg loss (mean ± *SD*: 6.20 ± 1.12 vs. 5.93 ± 1.15; *t* test: $t = -2.55$; $p = .01$; $n = 448$ chicks), suggesting that the sex-difference in hatchling MHC-II diversity is not triggered by a sex-specific effect of MHC-II diversity on post-laying embryo mortality. Regardless of the underlying mechanism, parents of similar MHC-II diversity, which are more likely to produce chicks with low MHC-II diversity (Setchell, Abbott, Gonzalez, & Knapp, 2013), might benefit from avoiding the fitness costs associated with the production of daughters with low MHC-II diversity.

While our results are consistent with a direct effect of MHC-II on fitness-related traits, some methodological limitations and alternative explanations must be acknowledged. First, we amplified exon 2 of the MHC-II because it codes for the majority of amino acids that form the peptide-binding groove in model species such as humans (Brown et al., 1993; Saper, Bjorkman, & Wiley, 1991) and because it has been the focus of most MHC research in nonmodel avian species (Minias, Pikus, Whittingham, & Dunn, 2018). However, both exon 2 and exon 3 encode the peptide binding grooves on MHC-II molecules. Second, we cannot rule out the possibility that variation at other genes may partly explain our results. For instance, MHC-II alleles might be in linkage disequilibrium with other MHC genes (e.g., MHC class I genes), owing to the compact architecture of the avian MHC (Hess & Edwards, 2002). Third, our results may possibly be explained by a broader effect of inbreeding on fitness, as variations at MHC genes can be correlated with genome-wide genetic variation depending on the life history, dispersal ability and breeding system of the study species (Sommer, 2005). Several studies have reported sex-specific effects of inbreeding on fitness-related traits (Billing et al., 2012; T. Coulson, Albon, Slate, & Pemberton, 1999; Rioux-Paquette, Festa-Bianchet, & Coltman, 2011). In hihis (*Notiomystis cincta*), the loss of inbred female embryos at a very early stage was proposed to explain increased heterozygosity in females later in development (Brekke, Bennett, Wang, Pettorelli, & Ewen, 2010). When testing for an association between MHC-II diversity and an estimate of genome-wide diversity (i.e., standardized heterozygosity;

Coltman, Pilkington, Smith, & Pemberton, 1999) in a collection of 614 adults for which we had both MHC-II and microsatellite data (nine microsatellite loci; see Pineaux et al., 2019 for details), however, we found no significant correlation (Pearson correlation: $t = 0.27$; $r = 0.01$; $p = .79$; Figure S11). In future, these potential effects on fitness could be disentangled by studies including multiple MHC genes and other immune genes alongside a better measure of overall inbreeding status.

This study underlines the importance of considering traits that are expected to shape an individual's exposure and immune responses to parasites when predicting the association between MHC diversity and fitness. Sex-specific effects of MHC diversity on fitness have been mostly studied in polygynous species, with a positive association between MHC diversity and survival or reproductive success found in adult males (Huchard et al., 2010; Roved et al., 2018; Sauermann et al., 2001; Schaschl et al., 2012). Here, we provide evidence for sex-specific associations between MHC-II diversity and fitness in the early life of a monogamous species, thus calling for further research in species or populations with differing life-history strategies. A recent study in adult Leach's storm-petrels (*Oceanodroma leucorhoa*), a monogamous seabird, found a positive association between MHC diversity and reproductive success in adult females but not in males (Hoover et al., 2018). The underlying explanation was that males avoided females of low MHC diversity during mate choice but it was unclear whether reduced reproductive success of low MHC diversity in females also partly resulted from a direct, detrimental effect of reduced female quality on offspring viability. For instance, female petrels may be more likely to suffer from sexually transmitted infections than males, as shown in kittiwakes (Van Dongen et al., 2019; White, Mirleau, et al., 2010), thereby explaining sex-specific effects of MHC diversity on fitness. Future investigations of sex-specific associations between MHC-II diversity and post-fledging survival or reproductive success in kittiwakes represent an interesting avenue to explore whether the benefits of high MHC-II diversity found in female chicks persist in adult females. Importantly, ignoring early-life stages may yield only a partial picture of how MHC affects fitness if selection removes the less fit genotypes early in life, leaving only relatively high-quality individuals that survived long enough to be sampled. Such a biased picture may in turn lead to a misunderstanding of the evolution of reproductive strategies.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

M.P., P.B. and S.L. conceived and designed the study. E.D., M.P., P.B., S.L. and T.M. collected the data. M.P., S.L. and T.M. carried out the molecular analyses. S.A.H. is responsible of the long-term monitoring on Middleton Island and gave access to the study area. M.P. and T.M. performed the statistical analyses. M.P. wrote the manuscript and all authors contributed comments. All authors gave final approval for publication.

## DATA AVAILABILITY STATEMENT

Data are available on Open Science Framework. Pineaux, M., et al. (2020). MHC-II fitness associations in kittiwakes. Open Science Framework. https://doi.org/10.17605/OSF.IO/DN5Y8. (https://osf.io/dn5y8/?view_only=97ddd8f65a264c62bd540b1e4e9148a5).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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