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# Association between louse abundance and MHC II supertypes in Galápagos mockingbirds

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## Abstract

Major histocompatibility complex class II (MHC II) is an essential molecule triggering the adaptive immune response by the presentation of pathogens to helper T cells. The association between individual MHC II variants and various parasites has become a frequent finding in studies of vertebrate populations. However, although bird ectoparasites have a significant effect on their host's fitness, and the host's immune system can regulate ectoparasitic infections, no study has yet investigated the association between MHC II polymorphism and ectoparasite infection in the populations of free-living birds. Here, we test whether an association exists between the abundance of a chewing louse (*Myrsidea nesomimi*) and MHC II polymorphism of its hosts, the Galápagos mockingbirds (*Mimus*). We have found that the presence of two MHC II supertypes (functionally differentiated clusters) was significantly associated with louse abundance. This pattern supports the theory that a co-evolutionary interaction stands behind the maintenance of MHC polymorphism. Moreover, we have found a positive correlation between louse abundance and heterophil/lymphocyte ratio (an indicator of immunological stress) that serves as an additional piece of evidence that ectoparasite burden is affected by immunological state of Galápagos mockingbirds.

**Keywords** Arms race · Co-evolution · Immunity · Ectoparasite · Supertype

## Introduction

The immune system is a multilevel defence scheme composed of innate and adaptive immune pathways that interact towards the common goal of controlling pathogens (Medzhitov 2007; Murphy and Janeway 2008). Reciprocal interaction between pathogens attempting to maximize their fitness at the expense of hosts, and hosts controlling pathogens by their immune system, is a key force driving the evolution of the immune system (Schneider and Ayres 2008). All immune pathways are initiated by a pathogen recognition that is provided by various kinds of host receptors. Among the most important recognition receptors are the molecules of the major histocompatibility complex (MHC) which represent an essential part of the adaptive immune system (Rock et al. 2016). More specifically, MHC class II is a complex of proteins that serves as the detector of extracellular pathogens. Several MHC II proteins form a trans-membrane molecule that binds an antigen in a peptide-binding groove and presents it to helper T cells (CD4+). Helper T cells subsequently initiate a pathway of adaptive immune responses against the presented antigen with the ultimate goal of clearing the extracellular pathogen (Murphy and Janeway 2008; Rock et al. 2016). The binding of the antigen in the MHC peptide-binding groove (PBG) is the crucial moment for the subsequent immune response.

The biochemical properties of the PBG, primarily defined by the amino acid sequence, determine the capacity to bind a specific antigen. Some MHC variants may overlap in their binding capacity due to their amino acid chains possessing similar biochemical properties (Sette and Sidney 1998). For this reason, MHC supertypes reflecting PBG binding properties have been used instead of individual variants in the majority of association studies (Trachtenberg et al. 2003; Schwensow et al. 2007; Sepil et al. 2013; Pilosof et al. 2014; Buczek et al. 2016). Supertypes are defined by clustering of the MHC variants with similar binding properties based

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on the physicochemical properties of individual amino acid sites that are in contact with antigens (Doytchinova and Flower 2005). Supertypes thus represent a cluster of variants that are very likely to recognize a similar set of antigens and allow a substantial reduction of the number of predictors in association models in situations where there are too many alleles, as in the case of songbirds (Bollmer et al. 2010).

The high levels of intra-specific and trans-species polymorphism of MHC indicate adaptive importance of the locus (Klein et al. 2007). More specifically, the polymorphism is maintained by balancing selection propelled either by sexual or by natural selection, or most likely by a combination of both (Apanius et al. 1997). Considerable attention has been focused on the effects of natural selection mediated by pathogens. Several specific hypotheses have been developed to understand the exact evolutionary mechanisms that maintain such polymorphism (Spurgin and Richardson 2010). The heterozygote advantage (overdominance) hypothesis predicts that individuals heterozygous at the MHC will have higher fitness and therefore more variants will persist in a population and their frequencies will not vary considerably in time (Doherty and Zinkernagel 1975). On the other hand, the negative frequency-dependent selection (rare allele advantage) hypothesis depends on the co-evolutionary arms race between hosts and pathogens. It assumes that one MHC variant increases in frequency due to its ability to recognize a particular antigen until the antigen mutates and subsequently a different MHC variant comes under positive selection due to its ability to recognize the new antigen. These fluctuations continue ad infinitum, and a loss of MHC alleles is precluded by the episodic directional selection drives of the rare alleles (Slade and McCallum 1992). Both hypotheses have found support in a wide range of reports. Evidence for overdominance comes from studies where heterozygous individuals showed higher pathogen resistance compared with homozygous individuals (Oliver et al. 2009; Worley et al. 2010; Savage and Zamudio 2011; Bolnick et al. 2014), whereas associations between specific MHC variants and pathogen resistance or fitness in birds (Westerdahl et al. 2013; Dunn et al. 2013; Sepil et al. 2013; Bateson et al. 2016) and mammals (Oppelt et al. 2010; Pilosof et al. 2014; Buczek et al. 2016), together with a simulation study (Ejsmond and Radwan 2015), support rare allele advantage as the prevalent mode of co-evolution between MHC and individual pathogens. However, it must be noted that the two modes of balancing selection are non-exclusive with the polymorphism of MHC being a result of both forces, especially when multiple pathogens are taken into account (Apanius et al. 1997; Oliver et al. 2009).

Compared with the examples linking PBG polymorphism with severe pathogens or endoparasites, we have focused on the association between PBG polymorphism and bird louse infections. Although ectoparasites live on a host's body surface, their effect on the host's fitness is considerable (Lehmann 1993; Richner et al. 1993; Clayton et al. 2015). A large body of evidence exists on the interaction between ectoparasites and immune system in birds and other vertebrates (Wikel 1982; James 1999; evidence in birds reviewed in Owen et al. 2010). Ectoparasites can trigger an immune response (e.g. northern fowl mite (*Ornithonyssus sylviarum* Canestrini and Fanzago, 1877); King et al. 2011), and a bird's immune response can affect the survival of ectoparasites (e.g. great tit (*Parus major* Linnaeus, 1758) vs. hen flea (*Ceratophyllus gallinae* Schrank, 1803); Walker et al. 2003). Not only the strictly bloodsucking ectoparasites (e.g. ticks or Anoplura lice) but also chewing lice from the order Amblycera can interact with the host's immune system as they feed on the skin and come into direct contact with the blood by chewing growing pin feathers (Marshall 1981). Thus, chewing lice were found to be associated with a high production of eosinophils in the ring-billed gull (*Larus delawarensis* Ord, 1815) (Fairn et al. 2012), and the biodiversity of Amblyceran lice was associated with the intensity of immune response (Møller and Rózsa 2005).

Despite a large body of evidence for the interaction between ectoparasites and immune response (see Clayton et al. 2015 chap. 3 for a review), only one study has analysed the effect of MHC polymorphism on the resistance to ectoparasites in birds. Owen et al. (2008) found that domestic chicken (*Gallus gallus* Linnaeus, 1758) carrying a specific MHC variant showed a reduced abundance of northern fowl mite due to their stronger inflammation of the skin compared with hens carrying other MHC variants (Owen et al. 2009). In mammals, the evidence is more abundant from both domestic and free-living species (Untalan et al. 2007; Oliver et al. 2009; Schad et al. 2012). Finally, an association between an ectoparasite and MHC variant has been found also in poikilotherms, e.g. in sand lizards (*Lacerta agilis* Linnaeus, 1758) (Olsson et al. 2005). But, to our knowledge, no study has yet focused on the effect of MHC polymorphism on ectoparasites in free-ranging populations of birds.

Here, we aim to assess whether MHC II polymorphism affects the abundance of louse *Myrsidea nesomimi* Palma and Price, 2010 in the populations of Galápagos mockingbirds (*Mimus*). We also include heterophil/lymphocyte (H:L) ratio, a measure of stress and inflammation (Davis et al. 2008), to control for bird stress condition. More specifically we aim to test the following questions:

1. Does louse abundance correlate with the stress index represented by H:L ratio and (or) other host-determined environmental factors (sex and weight)?

2. Does louse abundance correlate with the number of MHC II alleles, the number of supertypes in an individual or microsatellite heterozygosity?
3. Does louse abundance correlate with the presence or absence of individual MHC II supertypes?
4. Does H:L ratio correlate with the presence/absence of a supertype?

In order to answer the questions, we have focused on the populations of four species of Galápagos mockingbirds (GM): *Mimus macdonaldi* Ridgway, 1890, *Mimus melanotis*, *Mimus parvulus* and *Mimus trifasciatus* Gould, 1837. Allopatric populations occupy islands of different sizes and there is a limited gene flow between them (Hoeck et al. 2010). GM were initially used to test how restricted population size affects genetic diversity and health status (Hoeck et al. 2010; Hoeck and Keller 2012). Although neutral genetic diversity was largely shaped by population size, the health status represented by H:L ratio, lysis, agglutination and number of ectoparasites was correlated neither with population size nor with the level of inbreeding. It has been argued that the lack of correlation was caused by the fact that the neutral genetic variation does not reflect variation in the genes that code for immune traits. In a follow-up study (Vlček et al. 2016), we have described the diversity in MHCIIβ subunit and showed that MHC diversity, represented by the number of alleles in an individual and by the number of supertypes, was not affected by population size to the same degree as neutral microsatellite diversity. We have also found supporting evidence for balancing selection in the excess of non-synonymous mutations in antigen-binding sites and in extensive trans-species polymorphism. Observed patterns implied that genetic drift was partially outweighed by balancing selection; however, the effect of MHC on fitness or parasite load was not analysed. Here, we fuse previously published data on MHC with data on ectoparasite abundance to understand the effect of MHC polymorphism on ectoparasite load.

## Material and methods

In order to analyse the effect of MHC polymorphism, we tested for statistical associations between ectoparasite load, MHCIIβ polymorphism, neutral genetic diversity and H:L ratio in 121 individuals of GM. The samples from 10 different populations and 4 species of GM were collected between 2006 and 2008. Microsatellite data (Hoeck et al. 2009; Hoeck and Keller 2012; Vlček et al. 2016) were used to calculate mean observed heterozygosity for each individual as an index of neutral genetic diversity. Ectoparasite load was assessed by dust-ruffling, a method where bird feathers are treated with an insecticide and ectoparasites that fall out within a predefined treatment duration (5 min) are collected and counted (Walther and Clayton 1997; Hoeck and Keller 2012). This method has proved generally reliable in estimating chewing lice numbers (Koop and Clayton 2013). In GM, we found three species of ectoparasites, i.e. *Analgid* mites Linnaeus, 1758; *Brueelia galapagensis* Kellogg and Kuawana, 1902; and *Myrsidea nesomimi* lice (Štefka et al. 2011), but for the purpose of our study, we used only *Myrsidea* louse due to its widespread occurrence and high variance in abundance. Moreover, this species displays the closest contact with the bird's skin, and due to its blood-feeding habit, it is more likely to interact with the bird's immune system than *Brueelia* louse or *Analgid* mite (Møller and Rózsa 2005; Møller et al. 2010). *Myrsidea* abundance (sum of the number of nymphs and adults) was assessed for 121 samples from our dataset with only 6 birds being completely louse free.

The numbers of MHCIIβ alleles per individual were used as an index of MHC genetic diversity. MHCIIβ genotypes per individual were adopted from Vlček et al. (2016). The dataset contained 120 MHCIIβ alleles in total with 1–12 alleles per individual. The reason behind such a wide variation in the number of alleles per individual is the co-occurrence of at least six MHCIIβ paralogs and the substantial copy number variation that is a common feature of MHCII in songbirds (Bollmer et al. 2010). Therefore, to express MHC genetic diversity in our models, we used the total number of alleles per individual. This index combines both aspects of MHC variation—heterozygosity of individual loci and their total number.

Supertypes, functionally distinct MHCIIβ clusters, were used to represent individual functional variants. The supertypes (adopted from Vlček et al. 2016) were identified by clustering all MHCIIβ peptide variants based on physicochemical properties of nine amino acid sites in PBG according to the approach developed by Doytchinova and Flower (2005). We used only nine amino acid sites that were found under balancing selection, thus indicating importance of these sites in direct interaction with antigens (details in Vlček et al. 2016). For the purpose of this study, we excluded one of the eight originally found supertypes because of its extremely low frequency.

Heterophil/lymphocyte (H:L) ratio was used as an index of a health status of individual birds. An increased number of heterophils relative to lymphocytes indicates stress and inflammation in birds (Tompkins et al. 2006; Davis et al. 2008). We assumed that H:L ratio could be linked with louse abundance (e.g. more stressed birds being more louse susceptible), but also, we considered a situation in which H:L ratio was affected by MHCIIβ supertypes. Therefore, we included H:L ratio in the whole model with ectoparasite load as a response (question 1), but we also tested whether H:L ratio can be explained by MHCIIβ supertype presence in a separate linear model (question 4). H:L values, based on white blood cell counts, were adopted from a previous study (Hoeck and

Keller 2012). One extreme outlier of H:L ratio was removed from the dataset, and the H:L ratio was normalized by natural logarithm.

To test if louse abundance is affected by H:L ratio, or host-determined environmental factors (question 1), or overall MHC diversity (number of MHC supertypes and alleles) (question 2), we constructed a single generalized linear mixed model with package lme4 (Bates et al. 2015). We used louse abundance on individual birds as the response variable explained by sex of the host, its body weight (log-scaled) and scaled body weight (ratio of body weight to tarsus length reflecting condition), H:L ratio, number of MHC alleles per individual ($A_i$), number of MHC supertypes and microsatellite heterozygosity. We used negative binomial error distribution in the model because the response variable showed a non-random aggregation of high values in some individuals causing a high level of overdispersion, which is typical for parasite abundance data (Clayton et al. 2015, chap. 2). To account for differences caused by the sampling from 10 different populations, we supplied population identity as a factor with random effect. Species identity was not used because it largely overlapped with the population identity; moreover, it did not explain any extra variation and it did not change the general outcome when added as another random factor. Furthermore, a previous study (Štefka et al. 2011) showed that populations of both the birds and their parasites from individual islands function as highly related but genetically separated units, whether at an inter- or intra-specific level. In order to determine relevant predictors, we used a backward stepwise model selection, in which predictors were eliminated based on the changes in Akaike's information criterion (ΔAIC). If the elimination of a predictor caused an increase of AIC by more than 2, the predictor was retained in the model; otherwise, the predictor was removed. Two pairs of the predictors showed an elevated level of cross-correlation (number of MHC alleles and number of supertypes, and weight and scaled weight variance inflation factor (VIF) = 1.65, 9.5, respectively), but the number of alleles and the scaled weight were eliminated early in the process of model selection so the cross-correlation of explanatory variables did not bias the final model. Predictors that showed statistical significance in this exploratory analysis were further used in the statistical model for question 3.

To test question 3, we constructed a generalized linear model with louse abundance as a response term and the presence or absence of each individual supertype as a predictor. Moreover, we added H:L ratio as an explanatory variable because it was the only significant predictor from the previous model. As above, the model was constructed with a negative binomial error distribution and population identity as an explanatory factor with random effect. The best predictors were selected by backward selection using ΔAIC (see above). Because of the need for higher stringency in gene disease association studies (Manly 2005), several follow-up tests were run on the final model predictor selection. First, we evaluated association as robust if 95% confidence intervals of coefficient estimates did not approach zero. Second, we estimated effect sizes of the final predictors by Cohen's $D$ method (Cohen 1988). Finally, we also tested a scenario in which supertypes confer a dissimilar effect on louse abundance in individual populations. Such a scenario would be expected under negative frequency dependent selection, where independent associations are assumed to evolve. This hypothesis was tested by a comparison of the AIC of two models. In the first model, louse abundance was the response variable and the presence of an individual supertype was the explanatory variable with fixed effect. Population identity was supplied as a random effect of the louse abundance. In the second model, we added the random effect of the interaction between supertype and population. If the second model explained more variance, it would have suggested that the supertype confers a dissimilar effect in different populations.

The fourth question dealing with the association of MHC supertype and H:L ratio was tested in a separate linear mixed model. In this model, H:L ratio was explained by the presence or absence of the supertypes, with population identity used as a factor with a random effect.

All statistical analyses were performed in R 3.2.3 (R Core Team 2015).

## Results

The first model, testing associations between louse abundance, H:L ratio, host-determined environmental factors (question 1) and MHCIIβ and microsatellite diversity (question 2), revealed that H:L ratio is the only statistically significant predictor of louse abundance (Table 1). The relationship was positive indicating that birds with a higher H:L ratio also showed higher louse abundance. No significant relationship was evident between louse abundance and the number of MHC alleles, number of supertypes and microsatellite heterozygosity.

The second model, testing association between louse abundance and individual MHC supertypes (question 3), revealed that the incidence of supertype 2 and supertype 4, and H:L ratio were statistically significant predictors of louse abundance (Table 2). Moreover, each association of the supertypes showed a significant effect size (Cohen's $D_2 = 0.415$, $D_4 = 0.381$) and the confidence intervals of the estimates did not reach zero (Table 2). Individuals with supertype 2 had a significantly lower number of lice (5.58 lice on average) compared with individuals without the supertype (8.08 lice on average). On the other hand, individuals with supertype 4 had a significantly higher number of lice (8.93 on average) compared with individuals without the supertype (6.61 on average) (Fig. 1). Nevertheless, we have to consider that supertype 4 was present only in 15 individuals out

**Table 1** Results of the first statistical model testing associations between louse abundance, MHC diversity (no. of MHC alleles, no. of MHC supertypes), microsatellite diversity (Microsat. Ho), heterophil/lymphocyte (H:L) ratio and other factors determining louse niche parameters (scaled weight, weight and host sex). Predictors are ordered according to their elimination from the generalized linear model. Coefficient estimate of each predictor is given together with standard error (SE) of the estimate, *Z* test statistics, probability of *Z* test being significant (*P* value) and the difference in Akaike's information criterion between the model without and with the predictor (ΔAIC). Statistically significant predictors are in italic.

| Predictor | Coefficient | SE | *Z* value | *P* value | ΔAIC |
|---|---|---|---|---|---|
| No. of MHC alleles | 0.001 | 0.039 | 0.021 | 0.983 | − 2.000 |
| Scaled weight | 1.087 | 1.436 | 0.757 | 0.449 | − 1.428 |
| Sex | − 0.229 | 0.175 | − 1.308 | 0.191 | − 0.286 |
| No. of MHC supertypes | − 0.026 | 0.066 | − 0.399 | 0.690 | − 1.839 |
| Microsat. Ho | − 1.083 | 0.484 | − 2.237 | 0.025 | 1.783 |
| Weight | − 0.693 | 0.572 | − 1.213 | 0.225 | − 0.586 |
| H:L ratio | *0.209* | *0.088* | *2.357* | *0.018* | *3.378* |

of 121; thus, one can consider this result less reliable due to unequal group size compared with supertype 2, which was present in 57 individuals. Furthermore, we found no dissimilar effect of the two supertypes among GM populations as the models with a random effect of supertype and population interaction explained no more variance than the models without this random effect (ΔAIC < 2). H:L ratio as the index of stress remained the only significant predictor of louse abundance apart from the supertypes. Finally, the third model (question 4) showed that the presence or absence of any supertype did not significantly affect the H:L ratio (removal of any of the predictors did not show ΔAIC > 2).

## Discussion

Replicated populations of the Galápagos mockingbird study system allowed us to test the effects of various genetic indices on the abundance of a widespread ectoparasite species. We observed no effect of neutral microsatellite heterozygosity or number of MHC alleles and supertypes (Table 1), which suggests that heterozygote advantage does not play any important role in the coevolution between *Myrsidea nesomimi* and MHCIIβ in GM. However, we found a statistically significant association between the incidence of two MHCIIβ supertypes and the abundance of *Myrsidea* louse (Table 2). The presence of supertype 2 was associated with reduced louse abundance, while the presence of supertype 4 was associated with higher louse abundance. Associations between individual MHC variants, either positive or negative, and parasites have recently become a common finding (e.g. Sepil et al. 2013; Pilosof et al. 2014); however, this study is the first to document an MHC–ectoparasite association in free-living populations of birds.

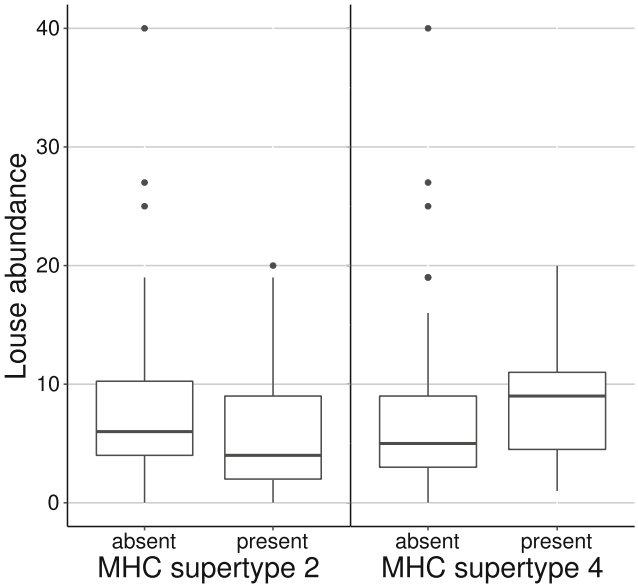


**Fig. 1** Abundance of *Myrsidea* louse on Galápagos mockingbirds is lower in individuals carrying MHCIIβ supertype 2 and higher in individuals carrying supertype 4. Distribution of the abundance is expressed by box-and-whisker plots that show the median (horizontal line), upper and lower quartiles (box) and maximum and minimum values (excluding outliers represented by a dot)

**Table 2** Results of the second statistical model testing association between louse abundance, individual MHC supertypes and heterophil/lymphocyte (H:L) ratio. Predictors are ordered according to their elimination from the generalized linear model. Coefficient estimate of each predictor is given together with standard error (SE) of the estimate, 95% confidence interval (Confint), *Z* test statistics, probability of *Z* test being significant (*P* value) and the difference in Akaike's information criterion between the model without and with the predictor (ΔAIC). Statistically significant predictors are in italic.

| Predictor | Coefficient | SE | Confint | *Z* value | *P* value | ΔAIC |
|---|---|---|---|---|---|---|
| Supertype 3 | − 0.009 | 0.212 | – | − 0.043 | 0.966 | − 1.998 |
| Supertype 8 | 0.080 | 0.216 | – | 0.371 | 0.711 | − 1.860 |
| Supertype 1 | − 0.134 | 0.245 | – | − 0.546 | 0.585 | − 1.698 |
| Supertype 5 | − 0.123 | 0.189 | – | − 0.654 | 0.513 | − 1.563 |
| H:L ratio | *0.214* | *0.084* | *0.050–0.384* | *2.551* | *0.011* | *4.529* |
| Supertype 4 | *0.636* | *0.247* | *0.166–1.159* | *2.579* | *0.010* | *5.140* |
| Supertype 2 | *− 0.441* | *0.168* | *− 0.771–− 0.096* | *− 2.626* | *0.009* | *4.142* |

The observed associations are based on a correlation; thus, it should be noted that correlation does not necessarily mean causality. Other factors (such as other pathogens or environmental conditions) could drive similar patterns of louse abundance, with MHC not being directly involved. On the other hand, we controlled for many major factors that can determine louse abundance like host body weight, sex and population identity. Moreover, correlations with incidence of supertypes showed significant effect size and confidence intervals that did not reach zero. This provides our results relevant credibility of correlative associations that are worth discussing. Nevertheless, only a manipulative experiment would provide the power to unambiguously disentangle a causal relationship.

It might seem unexpected for a variation in a signalling molecule of the adaptive immune system to be linked with the abundance of an ectoparasite, yet there are several plausible pathways that can explain our result. There is some evidence that MHC haplotypes affect ectoparasite community. The majority of the evidence comes from the studies of mammals and their bloodsucking ectoparasites (Untalan et al. 2007; Schad et al. 2012), or fish and their gill ectoparasites (Seifertová et al. 2016). To our knowledge, only one very compelling study exists in birds. Owen et al. (2008) showed that domestic chickens carrying a specific MHC haplotype harbour less northern fowl mites. Apart from the scarce MHC association studies, evidence of a general interaction between a host immune system and bloodsucking ectoparasites is well documented in birds (Owen et al. 2010). The louse species in our study belongs to the Amblyceran taxon of lice that usually live near their host's skin and feed on blood only occasionally (Marshall 1981). Therefore, the contact with the host will definitely be less intimate than between domestic chicken and northern fowl mites, mainly due to the lack of a piercing mouth apparatus. Nevertheless, it has been observed that even non-bloodsucking lice can stimulate an immune response, although this result comes from sheep and their lice (James 1999). Furthermore, Amblyceran lice were found to be capable of transmitting microfilarial worms via blood (Cohen et al. 1991). This evidence would allow us to explain the effect of the MHC supertype by a direct interaction between the louse and the immune system. In such cases, individuals with supertype 2 would be able to recognize louse antigens penetrating via bites, initiating a proper immune response that would hinder louse fitness by skin inflammation. On the other hand, individuals with supertype 4 would be more susceptible to louse infection because of the supertype inability to recognize louse antigens and initiate an appropriate reaction.

The indirect effect of MHCIIβ on louse abundance is another plausible explanation of the observed pattern. MHCIIβ can affect composition of uropygial gland wax or skin and feather microbial communities that can subsequently affect louse abundance. The uropygial gland produces various waxes and other chemical compounds that keep a bird's plumage in good condition (Jacob and Ziswiler 1982; Haribal et al. 2005). It affects microbial communities (Soler et al. 2012; Jacob et al. 2014), and to some degree also ectoparasites (Moreno-Rueda 2010). MHC variants were found to alter preen wax composition and related odours in seabirds (Leclaire et al. 2015) and songbirds (Slade et al. 2016). Leclaire et al. (2019) also found that the distribution of MHCIIβ functional diversity affected feather microbial community in blue petrel (*Halobaena caerulea* Gmelin, 1789). Furthermore, Leclaire et al. showed a strong association with a bacterium from the genus *Arsenophonus* that is known as a symbiont of avian ectoparasites (Nováková et al. 2009). Such indirect evidence allows us to argue that supertypes 2 and 4 can affect either the composition of preen gland waxes and/or bacterial communities, which subsequently affects *Myrsidea* abundance.

Theoretically, the indirect effect of MHCIIβ on louse abundance through other pathogens is also possible. If a supertype conferred resistance to a virulent pathogen, resistant individuals would have more energy to combat ectoparasites than susceptible individuals. But this situation is unlikely in GM where screening of a wide range of infectious disease agents in one of the GM species by Deem et al. (2011) showed absence of virulent endoparasites (e.g. haemoparasites or infectious bacteria) that could impair a bird's health.

On a more general level, we have tried to investigate the link between MHCIIβ, louse abundance and heterophil/lymphocyte (H:L) ratio, a measure indicating infection and stress in birds (Davis et al. 2004, 2008). We have found a significant positive correlation between H:L ratio and *Myrsidea* abundance, but no link between MHCIIβ supertypes and H:L ratio. Two alternative hypotheses can explain the observed pattern. Either the presence of a higher number of lice causes more stress and an increase in H:L ratio, or the birds are stressed by something else (e.g. other pathogen, ecological factors), allowing the lice to proliferate on such impaired individuals. We cannot discern between these two options based on our correlative approach. Several studies showed a link between white blood cell–derived indices and ectoparasite abundance. Fairn et al. (2012) found that louse abundance in ring-billed gulls does not correlate with H:L ratio but only with the proportion of eosinophils in the blood. In the study of Eurasian bee-eaters (*Merops apiaster* Linnaeus, 1758), manipulation with louse abundance affected bird weight as well as blood sedimentation and haematocrit (Hoi et al. 2012). Notwithstanding the causality, both studies, together with our results, indicate the existence of a link between immunity and louse abundance in wild populations of birds. However, the exact pathways for this phenomenon remain unresolved. An experimental approach considering all possible factors, from immunogenetic diversity or host morphology and exhaustive information on major pathogens to

environmental factors, should be used to evaluate this interaction.

In Galápagos hawks (*Buteo galapagoensis* Gould, 1837), a negative correlation was found between the level of genetic diversity, louse abundance and the levels of natural antibodies, showing that a reduction in population size negatively affected immunocompetence (Whiteman et al. 2006). Such patterns were not observed in GM, where indices of immune response and ectoparasite abundance were independent of neutral genetic diversity and population size (Hoeck and Keller 2012). The current study has a potential to explain the lack of the population size–dependent pattern in Galapagos mockingbirds as we found that the louse abundance is affected by incidence of individual MHCIIβ variants rather than by the total amount of neutral or MHCIIβ genetic diversity.

In the previous study (Vlček et al. 2016), we have also found that MHCIIβ supertype diversity is partially resistant to genetic drift, possibly due to the effect of balancing selection. One of the patterns typically generated by balancing selection is trans-species polymorphism (Spurgin and Richardson 2010; Lighten et al. 2017). Trans-species polymorphism is maintained if a common pathogen is present in different species and shared MHC alleles confer resistance to that common pathogen. In the previous study (Vlček et al. 2016), we have observed a lot of shared polymorphisms between different species and populations, but we did not know whether common supertypes confer some effect. Current observation of individual supertypes associated with *Myrsidea* abundance notwithstanding population identity serves as evidence that observed trans-species polymorphism is functional; thus, balancing selection can be considered an evolutionary force maintaining diversity of MHCIIβ supertypes.

Considering the type of balancing selection, our association results correspond to frequency-dependent selection rather than heterozygote advantage. In the case of heterozygote advantage, we should observe a negative relationship between the number of MHC alleles and abundance of a parasite (Doherty and Zinkernagel 1975), whereas frequency-dependent selection usually results in either positive or negative associations with individual variants (Westerdahl et al. 2012). Frequency-dependent selection is also more prevalent when considering the arms race between a single pathogen and its host, whereas MHC diversity becomes proportionally more important when multiple pathogens are considered (Apanius et al. 1997; Oliver et al. 2009). Therefore, because we have not sampled all possible pathogens, we cannot tell the exact type of balancing selection that maintains MHCIIβ diversity in Galapagos mockingbirds.

In conclusion, our study provides the first evidence for an association between MHC variants and ectoparasite abundance in the populations of free-living birds. On top of the louse abundance being associated with variation in the signalling molecule of the adaptive immune system, we have found a positive correlation with the ratio of heterophils/lymphocytes. These observations indicate that louse infection can be linked with the state of the host's immune system. Additionally, the GM study system comprises populations highly restricted in size, where genetic drift may have the potential to counterbalance the forces of selection. However, in agreement with several other studies (Aguilar et al. 2004; Vlček et al. 2016; Marmesat et al. 2017), the results presented here provide another piece of evidence for the capability of balancing selection to maintain functional diversity in MHC despite reduced population size.

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**Data availability** The dataset generated during the current study is available in the Mendeley Data repository, https://data.mendeley.com/datasets/75rn24pbvz/1

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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