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# Major Histocompatibility Complex, demographic, and environmental predictors of antibody presence in a free-ranging mammal

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ABSTRACT

Major Histocompatibility Complex (MHC) variability plays a key role in pathogen resistance, but its relative importance compared to environmental and demographic factors that also influence resistance is unknown. We analyzed the MHC II DRB exon 2 for 165 raccoons (*Procyon lotor*) in Missouri (USA). For each animal we also determined the presence of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to two highly virulent pathogens, canine distemper virus (CDV) and parvovirus. We investigated the role of MHC polymorphism and other demographic and environmental factors previously associated with predicting seroconversion. In addition, using an experimental approach, we studied the relative importance of resource availability and contact rates. We found important associations between IgG antibody presence and several MHC alleles and supertypes but not between IgM antibody presence and MHC. No effect of individual MHC diversity was found. For CDV, supertype S8, one allele within S8 (Prlo-DRB*222), and a second allele (Prlo-DRB*204) were positively associated with being IgG+, while supertype S4 and one allele within the supertype (Prlo-DRB*210) were negatively associated with being IgG+. Age, year, and increased food availability were also positively associated with being IgG+, but allele Prlo-DRB*222 was a stronger predictor. For parvovirus, only one MHC allele was negatively associated with being IgG+ and age and site were stronger predictors of seroconversion. Our results show that negative-frequency dependent selection is likely acting on the raccoon MHC and that while the role of MHC in relation to other factors depends on the pathogen of interest, it may be one of the most important factors predicting successful immune response.



## 1. Introduction

Among mammalian carnivores, canine distemper virus (CDV) and parvoviruses are important, highly virulent pathogens that are typically enzootic as well as frequently associated with epizootics (Harder and Osterhaus, 1997; Allison et al., 2013). These viruses are present throughout domestic and wild carnivore populations worldwide, and antibody prevalence among susceptible species often exceeds 50% and may approach ubiquity for the oldest age classes (e.g. Gompper et al., 2011). Both pathogens are likely significant causes of mortality in free-ranging populations (Williams et al., 1988; Kennedy et al., 2000; Barker and Parrish, 2001). To better forecast the impact of these pathogens, it is necessary to identify factors that predict host exposure and the subsequent risk of mortality.

Recent research has focused on the genetic basis of host susceptibility, and in particular, the role of genes involved in the immune response. Ultimately, these genes contribute to a host's ability to overcome infection. Many studies of host survival following pathogen exposure have focused on the Major Histocompatibility Complex (MHC), a multi-gene family that encodes more than one hundred proteins involved in the adaptive immune response (Klein et al., 2007). Classical MHC class I and class II genes code for proteins that present pathogen-derived antigens to T-cells and subsequently trigger an adaptive immune response (Hughes and Yeager, 1998). MHC class I and class II genes are among the most polymorphic within the vertebrate genome (Sommer,

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2005). Polymorphism primarily occurs in the antigen binding sites, also referred to as the peptide binding region (PBR), which are directly involved in antigen recognition (Hughes and Nei, 1988). High levels of polymorphism are mainly maintained by pathogen-mediated selection (Bernatchez and Landry, 2003; Piertney and Oliver, 2006) and variation at MHC has been linked to susceptibility (the risk of infection) and resistance (the ability to survive after exposure) for a wide range of pathogenic and host taxa (Hill, 1991; Grimholt et al., 2003; Harf and Sommer, 2005; Kloch et al., 2010; Savage and Zamudio, 2011; Srithayakumar et al., 2011).

Prior studies in carnivores suggest that MHC variation plays an important role in overcoming infection by CDV and parvoviruses (Hedrick et al., 2003; Castro-Prieto et al., 2012). However, the role of the MHC relative to other factors that also determine host response to pathogen exposure is unknown. For example, seasonality, habitat quality, and age and sex of an individual have been shown to contribute to, or correlate with, high seroprevalence or epizootics of CDV and parvoviruses in several carnivore species (Roscoe, 1993; Barker and Parrish, 2001; Rossiter et al., 2001; Biek et al., 2006; Åkerstedt et al., 2010; Gompper et al., 2011).

Therefore, understanding the role of MHC variation in predicting host immune response requires concurrent examination of non-genetic factors intrinsic and extrinsic to the host. Such approaches facilitate assessing the relative importance of genetic, demographic, and environmental factors underpinning successful immunologic response to challenges. Here we use a multivariate approach to investigate associations between the MHC and the likelihood of host seroconversion (the presence of CDV and parvovirus antibodies as a result of infection) and their importance when placed in the context of demographic and environmental factors. We focus on free-ranging populations of raccoons (*Procyon lotor*) in Missouri, USA, where virtually all individuals (>80% of adults) are eventually exposed to both CDV and parvovirus (Gompper et al., 2011). We analyzed the MHC II DRB exon 2 because it is highly polymorphic, is subject to natural selection (Castillo et al., 2010), and has been linked to rabies resistance and susceptibility in raccoons (Srithayakumar et al., 2011) and to CDV and canine parvovirus resistance in wolves (Hedrick et al., 2003). We tested whether the probability of individuals mounting a successful immune response (i.e. seroconversion) was associated with either carrying a particular MHC II DRB exon 2 allele or individual MHC diversity measured as the number of alleles. In addition, because the DRB exon 2 in raccoons is highly polymorphic, we clustered alleles with similar amino acid properties that can potentially bind similar antigens into functional supertypes (Doytchinova and Flower, 2005), and tested whether the likelihood of seroconversion was associated with carrying specific MHC supertypes or individual MHC supertype diversity (number of supertypes).

Since other (non-MHC) genetic and non-genetic factors are likely to influence the likelihood of exposure and successful seroconversion, we incorporated several additional factors into our analyses. We manipulated several of our populations to alter contact rates and food availability, as both factors have shown an important association with macroparasite infection in raccoons (Monello and Gompper, 2010, 2011) and are likely important in the spread of CDV and parvovirus through populations. In addition, in our models we included factors linked to CDV and parvovirus exposure and epizootics in other populations of raccoons, including the timing of exposure, the age and sex of the hosts, and the neutral genetic diversity of the hosts (Barker and Parrish, 2001; Rossiter et al., 2001; Gompper et al., 2011). Given the strong selective pressure that these viruses may have on carnivore populations, we expected to find important associations between seroconversion and MHC (either specific alleles or MHC diversity). However, we predicted that its relative importance would be small compared to other environmental and demographic factors as the predictive strength of these latter factors is often relatively robust (Gompper et al., 2011).

## 2. Methods

### 2.1. Study populations

Raccoons were sampled from 2005 to 2007 at 12 forested sites located at 6 different conservation areas within 60 km of Columbia, Missouri, USA (Ruiz-López et al., 2012). All sites had similar ecological characteristics and raccoon population densities. Details on sites, raccoon populations, associated parasite communities, and host and parasite sampling protocols are reported elsewhere (Monello and Gompper, 2007, 2009, 2010, 2011; Gompper et al., 2011). Briefly, raccoons were live-trapped, anesthetized, ear-tagged, weighed, sexed, measured, and aged (Grau et al., 1970; Monello and Gompper, 2007) as class I (<14 months), II (15–38 months), III (39–57 months) or IV (>58 months) individuals. Blood samples were collected for genetic and immunologic analyses. During years 2006 and 2007 contact rates and resource availability were manipulated by providing supplemental food in either a clumped or dispersed fashion. Sites within the six study areas were randomly assigned to a treatment category. The three treatments were: (1) receipt of a permanent feeding station with 35 kg/wk of dried dog food at a single location to aggregate raccoons ($n = 5$ sites; high contact rates, food supplementation); (2) the same quantity of food, placed at highly dispersed and temporally variable locations to control for the effects of food additions without aggregating hosts ($n = 3$ sites; low contact rates, food supplementation), or (3) no food supplementation ($n = 4$ sites; no aggregation, no food supplementation).

Research was carried out under Missouri Department of Conservation permit #12869, and University of Missouri Animal Care and Use Protocol #3927.

### 2.2. Viral seroconversion

Exposure to CDV and parvovirus was quantified using the Biogal Immunocomb 'dot'-Elisa kits for both immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies (Biogal-Galad Labs, Kibbutz Galad, Israel) (Gompper et al. 2011). The titer of each individual was quantified in S unit (range: S0–S6) following manufacturer's instructions and after which IgG and IgM antibodies were classified as present or absent for each host based on a threshold value of S2 (ca 1:32 IgG or IgM titer using a haemagglutination inhibition test or a serum neutralization test). For individuals that were captured and tested in multiple years, we randomly chose one test result.

The presence of IgM antibodies (IgM+) signals a current infection or recent re-exposure. During the course of the infection, IgG levels rise and IgM levels fall. An individual that had IgG antibodies but lacked IgM antibodies (IgM−/IgG+) was assumed to have overcome viral exposure. Individuals who were IgM−/IgG− were assumed not to have been exposed to the virus, as anti-CDV and anti-parvovirus IgG antibodies persist throughout life (Barker and Parrish, 2001; Williams, 2001).

### 2.3. Genetic analyses

Total genomic DNA was extracted from blood samples using DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The genetic analyses included MHC genotyping, as well as microsatellite genotyping to assess neutral genetic diversity.