Behavioral Ecology

The official journal of the
ISBE
International Society for Behavioral Ecology

Behavioral Ecology (2021), 32(3), 359–373. doi:10.1093/beheco/arab004

Invited Review

How can the MHC mediate social odor via the microbiota community? A deep dive into mechanisms

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Received 25 September 2020; revised 4 January 2021; editorial decision 6 January 2021; accepted 22 January 2021; Advance Access publication 18 May 2021.

Genes of the major histocompatibility complex (MHC) have long been linked to odor signaling and recently researchers' attention has focused on MHC structuring of microbial communities and how this may in turn impact odor. However, understanding of the mechanisms through which the MHC could affect the microbiota to produce a chemical signal that is both reliable and strong enough to ensure unambiguous transmission of behaviorally important information remains poor. This is largely because empirical studies are rare, predictions are unclear, and the underlying immunological mechanisms governing MHC–microbiota interactions are often neglected. Here, we review the immunological processes involving MHC class II (MHC-II) that could affect the commensal community. Focusing on immunological and medical research, we provide background knowledge for nonimmunologists by describing key players within the vertebrate immune system relating to MHC-II molecules (which present extracellular-derived peptides, and thus interact with extracellular commensal microbes). We then systematically review the literature investigating MHC–odor–microbiota interactions in animals and identify areas for future research. These insights will help to design studies that are able to explore the role of MHC-II and the microbiota in the behavior of wild populations in their natural environment and consequently propel this research area forward.

Key words: **immune response, kin recognition, major histocompatibility complex, scent, systematic review, tolerance**

INTRODUCTION

Animals use olfactory cues during social communication, and microbiota have been implicated in governing chemical cues relevant for social communication (Archie and Theis 2011; Maraci et al. 2018). Furthermore, genetic determination of the microbiota's composition (Zoetendal et al. 2001; Stewart et al. 2005) and its shaping by the host immune system, specifically the major histocompatibility complex (MHC) (Toivanen et al. 2001; Kubinak et al. 2015; Wadud Khan et al. 2019), have been hypothesized and investigated. However, the number of empirical studies is limited, and they often neglect the underlying immunological mechanisms linking microbiota and odor, and therefore do not allow the formulation of clear predictions for testing. Thus, the purpose of this review is to summarize the extensive medical and immunological literature linking the key players potentially involved in generating microbial-based odor cues for social communication and to present immunological evidence that could aid in prospective study design and interpretation of results. We first introduce links between the MHC, microbiota, and odor signaling. We then present the state of knowledge of the immunological mechanisms governing host microbial communities. Finally, we systematically review empirical studies investigating MHC–microbiota–odor associations to identify areas in need of future research.

ODOR AND SOCIAL COMMUNICATION

Animals use olfactory cues, such as scent marks or body odor, to broadcast information. In mammals, scent marks include secretions from anal, genital, frontal, or sternal glands, as well as urine and feces (Johnson 1973). Birds can perform "bill-wiping" to mark substrates with secretions from their uropygial gland (Whittaker et al. 2014). Similarly, fecal pellets (Gautier and Miaud 2003) and post-cloacal gland secretions (Simons et al. 1994) in amphibians and femoral gland secretions in reptiles (Mason and Parker 2010) can act as scent marks. These secretions appear to play an important role in social communication (Johnson 1973) and there is

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evidence that scent marks and body odor, which is generated by secretions and metabolites remaining on the body, provide a wealth of information about the dispatcher.

Chemical signals can transfer information about an individual's status (such as sex, age, rank and sexual receptivity (Greene and Drea 2014; Harris et al. 2014; Vaglio et al. 2016; Marneweck et al. 2017; Spence-Aizenberg et al. 2018)) to conspecifics. Similarly, information on general health (Harris et al. 2018), parasite load (Mitchell et al. 2017), or infection and injury (Zala et al. 2004) can be conveyed through scent. This may occur through particular chemicals associated with the infection or the immune response to it (e.g., Arakawa et al. 2010), or through reallocation of resources or the presence of fever affecting the microbial community (Harris et al. 2018). Signature mixtures (variable mixtures of chemicals) can be used for individual and social group recognition (Smith 2006; Scordato et al. 2007; Theis et al. 2012, 2013), and to assess relatedness and genetic compatibility (Charpentier et al. 2008; Stoffel et al. 2015).

Usage of such chemical signals can have important fitness consequences as identifying relatives helps to avoid inbreeding depression (Pusey and Wolf 1996) and enables help to be directed toward close relatives, increasing indirect fitness (Hamilton 1964). Apart from determining relatedness, odor might be used to perceive genetic quality of a potential mate (in terms of "good genes" or genetic diversity), and genetic compatibility, which can be independent of overall relatedness (Lenz et al. 2009). This may in turn increase genetic quality and thus offspring attractiveness or survival, resulting in elevated parental fitness (Møller and Alatalo 1999). Both genetic diversity and similarity might be signaled through odor profiles, but assessing similarity requires a self-referencing mechanism for comparing conspecifics' to an individual's own odor (Hauber and Sherman 2001).

Odors providing information on the genetic make-up of an individual, such as relatedness, quality, and compatibility, are particularly interesting as their nature suggests that they must have a genetic basis. An excellent candidate exhibiting sufficient polymorphism for conveying genetic information while also having an important role in immune response are the genes of the MHC.

A PROMISING CANDIDATE—THE MHC

The MHC encodes membrane glycoproteins essential for the adaptive immune response (Bjorkman et al. 1987) through regulating discrimination between self-derived and foreign peptides, and is present across jawed vertebrates (Kaufman 2018). The MHC molecules bind peptides and present them to professional immune cells, which then either initiate immune response or not (Knapp 2005). MHC molecules are divided into class I and II, with class I molecules (MHC-I) being expressed on nearly all nucleated cells. They present peptides mostly from the cytoplasm to cytotoxic T cells which, once activated, can initiate the death of the MHC-peptide carrying cell (Klein 1986). In contrast, class II (MHC-II) molecules are expressed by professional antigen-presenting cells (APCs) (e.g., macrophages, B cells and dendritic cells, among others), and present engulfed peptides (Neefjes et al. 2011). Therefore, MHC-I mostly presents self-derived peptides and peptides originating from viruses or other pathogens that have entered the cell, while MHC-II molecules predominantly present peptides derived from exogenous sources, such as bacteria or parasites, that have been ingested by the MHC-II carrying cell (Rammensee et al. 2013). Throughout we refer only to classical MHC, distinguished from nonclassical by solely presenting peptides to T cells and having high expression and polymorphism (Braud et al. 1999; Alfonso and Karlsson 2000). Instead, functions of nonclassical MHC are diverse, including antigen processing and immunomodulatory effects in both innate and adaptive response (Braud et al. 1999; Alfonso and Karlsson 2000).

Both classical MHC-I and MHC-II molecules have high polymorphism that is most pronounced in the peptide binding region that contains the peptide binding sites (PBS) interacting directly with the antigen (Bjorkman et al. 1987; Brown et al. 1993). This polymorphism enables presentation of a wide range of peptides, with greater functional difference between alleles, encoding for different PBS, leading to a greater number of peptides bound (Pierini and Lenz 2018). Hence, individuals expressing many different MHC molecules should theoretically be able to detect a higher variety of peptides and thus interact with a greater range of microbes which might in turn be reflected in their odor.

AN ARMY OF SUPPORTERS—THE COMMENSAL MICROBIAL COMMUNITY

Animals host a diverse range of microbial phyla on their surfaces such as the skin, glands, and gut (Ley et al. 2008). Before birth or hatching, mammals, birds, and reptiles reside in environments classically considered sterile, although this view is questioned (Kohl 2012; Perez-Muñoz et al. 2017; Trevelline et al. 2018). After birth or hatching, animals acquire bacteria from their surrounding environment, including the mother's birth canal and genitalia during birth, as well as from parents, litter, or nest mates (Kohl 2012; Sylvain and Derome 2017). Successive colonization events result in composition shifts until a rather stable commensal population has formed (Luckey 1972; Kohl 2012; Oh et al. 2012).

Interestingly, microbiota composition can differ considerably between individuals of the same species (Jami and Mizrahi 2012). These interindividual differences can be related to exogenous factors, such as stochastic microbe population dynamics, diet, and environment (reviewed in Spor et al. 2011; Davenport et al. 2014; Rothschild et al. 2018). Additionally, endogenous factors, such as an animal's stage of life, the body site's microclimate, and the host's genotype can influence an individual's microbiota (Spor et al. 2011). The microbial community appears to display a certain stability and dependence on host genetics, as it can re-establish even after severe perturbation such as antibiotic treatment (e.g., Antonopoulos et al. 2009). However, evidence from human twin studies investigating the microbiota's genetic basis is ambiguous with some claiming genetic determination (e.g., Stewart et al. 2005; Goodrich et al. 2014) while others do not support this dependency (e.g., Turnbaugh et al. 2009).

Hosting microbiota can provide fitness benefits, such as disease resistance (Rosshart et al. 2017) and metabolic efficiency (Tremaroli and Bäckhed 2012), causing the host's immune system to face a conflict: ensuring clearance of harmful pathogens while simultaneously tolerating beneficial commensals. Disruption of this balance can spark dysregulated or overaggressive immune responses toward harmless materials resulting in persistent inflammations or autoimmune diseases (Chung and Kasper 2010). Hosting microbiota may also help signal information used in social communication (Archie and Theis 2011). Gorman (1976) advanced the fermentation hypothesis stating that microbes inhabiting bodily surfaces produce substances detectable by conspecifics. Regulation by immune genes, such as those of the

MHC, may therefore cause microbiota to reflect their host's genetic composition (Khan et al. 2019).

MHC INVOLVEMENT IN ODOR PRODUCTION

The MHC may directly affect odor by either binding nonvolatile peptides acting as a source of odor (peptide hypothesis) (e.g., Milinski et al. 2005, 2013; Spehr et al. 2006; Hinz et al. 2013), or less likely, through MHC molecules themselves breaking down to become odorants (MHC molecule hypothesis) (Boehm and Zufall 2006). MHC molecules might also indirectly affect odor in two ways (Figure 1). First, MHC molecules, as key players in the immune response, have the potential to affect the outcome of infections with viruses or parasites thus affecting the health status of an individual, which can be reflected in volatile composition of odor (Kimball et al. 2013; Grieves et al. 2018). Second, MHC molecules might affect odor through regulating the composition of the commensal flora (microflora hypothesis) (Singh et al. 1990). Specifically, these commensal microbes produce volatiles as products of their metabolism and thus influence odor. Due to the MHC's polymorphism and its central role in the adaptive immune response combined with the diversity of microbial species, regulation of microbially produced odor cues via the MHC has the potential to generate detailed cues for social communication and thus we decided to further elaborate on this interaction.

Control of the microbiota by the MHC might happen via different mechanisms that can also be of direct and indirect mode. The MHC might govern microbiota directly by binding and presenting peptides and thus inducing an immune response aimed at the peptide source (Howard 1977; further details are given in the paragraph below on the activation of T cells). Alternatively, the MHC might shape microbiota indirectly and there are several hypotheses describing the mechanism of such an indirect link. As supposed by the peptide-microbe hypothesis, the MHC allele-specific immune responses might affect what molecules are available to the microbiota to metabolize thus influencing microbiota composition and consequently microbially produced odors. Because immune responses are mounted against microbial peptides matching the PBS of the MHC molecule, MHC allele diversity might determine the repertoire of peptide ligands that is available to the microbial community to metabolize. Furthermore, by immunologically controlling microbiota composition, MHC allele diversity might govern molecules and microbial secondary metabolites available to the microbes, the products of which might affect odor (Penn and Potts 1998a). Alternatively, regulation by the MHC might cause interspecific interactions between microbes and thus indirectly determine microbiota composition by favoring or preventing the establishment of certain species. Additionally, the MHC can influence other adaptive immune mechanisms following peptide detection via the MHC that lead to tolerance toward certain microbiota species (Kubinak et al. 2015; Khan et al. 2019; see also the paragraph on the role of IgA).

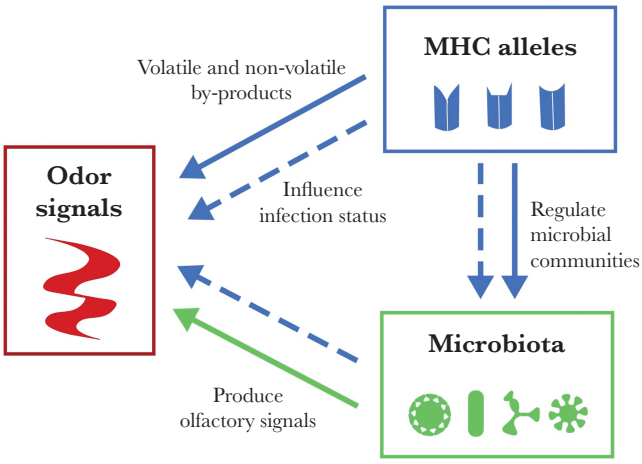

Figure 1
MHC–microbiota interactions in chemical communication. Schematic of the interactions between genes of the MHC and the microbiota and their potential influence on odour. MHC polymorphism (blue arrows) might directly influence odour (solid arrows) through volatile and nonvolatile by-products such as urinary signals or peptide ligands or indirectly (dashed arrows) by influencing infection status or through regulation of the microbiota (green arrow) producing volatiles.

Individuals might discriminate MHC-based microbial odor using a familial imprinting system and thus base their mate choice decisions on learned familiarity cues as observed in mice (Yamazaki et al. 1988). In a more elaborate mechanism called self-referencing, individuals use their own odor as a reference for comparison of conspecific odors to optimize offspring genetics (Reusch et al. 2001; Aeschlimann et al. 2003; Milinski et al. 2005).

The underlying chemical properties of the molecules suspected to carry information via direct or indirect mechanisms of MHC-linked odor signaling differ substantially (see Penn and Potts 1998a; Ruff et al. 2012; and Overath et al. 2014 for critical discussion of the mechanisms). Both the peptides bound by MHC molecules as well as the MHC molecules themselves, which are supposed to serve as odorants, are nonvolatile peptides. Despite their nonvolatility, there is strong evidence for MHC peptide ligands to convey information about the MHC. Female sticklebacks have been shown to use a self-referencing mechanism and count alleles of their potential mates to optimize their offspring's MHC composition (Reusch et al. 2001; Aeschlimann et al. 2003). In a further experiment, Milinski et al. (2005) determined the source of information used by the female sticklebacks by experimentally modifying the odor of males with synthetic MHC peptide ligands. Thus, it is possible for MHC genotype to be detected without the involvement of the microbiome. However, nonvolatile peptides are unlikely to be the only indicators of MHC genotype as the urine of MHC-congenic mice devoid of peptides could still be discriminated (Singer et al. 1993; Kwak et al. 2009). This suggests that volatile molecules produced by the bacterial metabolism might generate MHC-based odors as well. In addition, while MHC-dependent peptide ligands corresponding to different MHC molecules can evoke unique activation patterns reflecting MHC composition (Leinders-Zufall et al. 2004), many MHC molecules can bind the same set of peptides. For example, up to 50% of peptide ligands bind multiple MHC-I molecules in humans (Rao et al. 2011). Overlap in MHC-mediated activation patterns would prevent unambiguous sensory discrimination of MHC composition suggesting that additional information may be required to reliably determine MHC genotype via odor.

POTENTIAL MHC-RELATED MECHANISMS OF MICROBIOTA STRUCTURING

With its immunological function and high polymorphism, the MHC rightly is a promising candidate for governing microbially derived odor cues. However, still many questions remain unanswered.

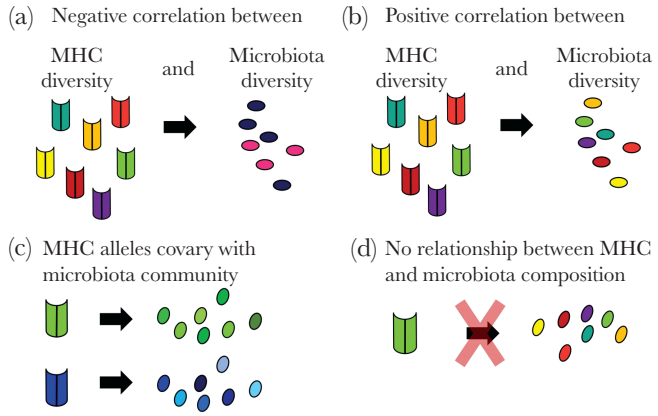

Figure 2
MHC–microbiota interaction. (A) A negative correlation is characterized by high MHC diversity leading to low microbiota diversity. (B) A positive correlation may be caused by high MHC diversity tolerating more diverse microbiota communities. (C) Covariation between MHC genotypes and microbiota community structure may be caused by specific MHC binding motifs selecting for the presence of certain groups of microbes. (D) No detectable relationship between MHC and microbiota community may indicate the MHC is not a major determinant of the microbiota community.

For example: How does a system evolved to eliminate pathogens establish tolerance to microorganisms? How does the MHC orchestrate microbiota composition and maintain its stability? How does MHC diversity affect microbiota composition?

Knowledge of the immunological mechanisms of MHC–microbiota interaction poses the basis for establishing hypotheses and for the interpretation and validation of results, and four conflicting predictions of the relationship between MHC and microbial diversity have been made. One possibility is a negative correlation between MHC diversity and microbiota diversity (Bolnick et al. 2014; Leclaire et al. 2019). Considering the MHC's role in the response to pathogens and that each MHC molecule binds a particular repertoire of peptides, a higher diversity of MHC molecules might lead to a higher diversity of peptides presented and thus a larger number of microbes that can be eliminated, causing lower microbiota diversity (Figure 2A). Second, it is possible that we may observe the reverse relationship, with higher MHC-II diversity causing higher microbiota diversity (Hernández-Gómez et al. 2018). This is possible because the immune system does not only eliminate microbes but also forms symbiotic bonds with commensals, hence a positive correlation may arise if a higher diversity of MHC molecules initiates tolerance to a more diverse range of microbes (Figure 2B). Consequently, both negative and positive relationships signaled via the microbiota should theoretically enable detection of MHC diversity. Third, certain MHC motifs might also interact with specific groups of microbes, leading to covariation of MHC genotypes with specific microbial community structuring (Figure 2C). This association of certain MHC alleles with particular microbes could allow the detection of specific alleles and thus enable choosing a mate with complementary MHC alleles via self-referencing. Finally, MHC and microbiota diversity or composition may not be linked, as genes other than the MHC or environmental influences might determine the commensal community of a host (Figure 2D). Indeed, the specificity between MHC genotype and microbiota community should not be assumed a priori. The great variety of microbial species and microbial peptides derived from each species results in a plethora of different peptides that can act as ligands for MHC molecules. Hence, it is possible that the great diversity in MHC ligands impedes specificity of MHC-II-bound microbes (Rammensee et al. 1999).

MHC–microbiota interactions will also be affected by the diverse habitats that microbes experience on different host surfaces. A recent meta-analysis investigating the association of environmental and host physiological and phylogenetic factors with the microbiome indicates that external microbiomes, such as skin or feather microbiomes, are best explained by environmental factors such as precipitation seasonality and temperature (Woodhams et al. 2020). In contrast, internal microbiomes derived from feces or the gut, were best explained by host associated factors such as immune complexity/phylogeny, trophic level or diet, and climate. Moreover, within the same host or even organ, body site-specific microclimates cause varying local microbial communities (Spor et al. 2011), and tissue-specific immunological adaptations limiting inflammation and increasing tolerance to microbes exist. Nonetheless, different organs such as the skin and the gut also show major histological and immunological commonalities (Artis 2008; Pasparakis et al. 2014). Both organs have an epithelia-cover, rely on immune response initiated by MHC-II-bearing cells and share tolerance-facilitating components (Hepworth et al. 2013; Kobayashi et al. 2019). Hence, the relationship between MHC-II and the microbiota should theoretically apply similarly to different organs. However, understanding of the immunological crosstalk between the microbiota and tissues remains limited.

UNDERSTANDING THE IMMUNOLOGICAL MECHANISMS—WHAT WE KNOW SO FAR

Understanding the causal connections between the MHC and the microbiota might reveal new questions and solve existing challenges in diverse fields. Hence, we now provide an overview of MHC-related mechanisms initiating either an immune response or tolerance of microbiota. Specifically, we review findings from immunology and medical research, particularly in mice and humans, where the interplay between the immune system and commensal bacteria has been extensively researched. However, we do not aim at explaining these immunological processes in their great complexity and detail but rather focus on the mechanisms involving the MHC and the microbiota (for further review, see Marietta et al. 2015; Honda and Littman 2016). We want to provide immunological background knowledge on the interrelation of the MHC and the microbiota potentially important for chemical communication for a nonimmunologist audience to help explain the observed patterns of MHC and microbiota correlation and covariation in empirical studies.

We note that there are reports of the MHC, particularly MHC-I, directly influencing odor either through the MHC molecules itself or its peptide ligands acting as odor cues (e.g., Leinders-Zufall et al. 2004). Nonetheless, as we want to summarize findings that help understand the possible interactions of the MHC with microorganisms as a potential regulator of odor, we focus only on MHC-II because these molecules predominantly present phagocytized antigens originating from extracellular microorganisms, such as commensals.

STARTING THE FIGHT—OR NOT? INITIATING THE ADAPTIVE IMMUNE RESPONSE

APCs, such as B cells or macrophages, phagocytize and process peptides and present them with their MHC-II molecules together with other surface molecules to helper T (Th) cells, a certain type of T (developing in the thymus) cell (Neefjes et al. 2011). The interaction between the APC and the Th cell can either cause an immune response toward the presented antigen (Figure 3A) or no response (Figure 3B) (Jurewicz and Stern 2019). Activation of the Th cell only occurs if it can recognize the antigen and thus T cell responses depend on the repertoire of T cell receptors (TCRs) available, which is determined during T cell development and maturation.

During T cell development, tolerance to certain antigens is initiated in a two-step process, called positive and negative selection, within the thymus (reviewed in detail in Jurewicz and Stern 2019). During positive selection, T cells are selected for their ability to respond to MHC-self-peptide complexes, with those that do not respond being eliminated (Huseby et al. 2005). The second step, negative selection, describes the elimination of T cell receptors showing an excessive response to MHC-self-peptide complexes (Klein et al. 2014). Thereby, T cells potentially causing autoimmune reactions are excluded. Once outside of the thymus, the remaining T cells receive boosting signals from MHC II-bearing cells which stimulates their survival. Consequently, the diversity of the TCR repertoire together with the MHC-II molecules determines the set of peptides against which an adaptive immune response is mounted. Thus, complementary to the mechanisms by which MHC-II diversity might impact microbiota composition (see also the paragraph on MHC-related microbiota structuring), the TCR diversity has the potential to regulate the commensal microbiota.

But how exactly does the MHC's polymorphism influence the TCR repertoire, thus affecting adaptive immune responses and potentially governing microbiota? Theoretical models suggest that MHC diversity can be negatively linked to the TCR repertoire retained after selection in the thymus (Nowak et al. 1992; Woelfing et al. 2009). This relationship depends on the higher diversity of MHC molecules leading to more TCRs being removed during negative selection because of self-reactiveness. Thus, individuals should try to achieve an intermediate number of MHC alleles in their offspring to optimize resistance to parasites (Wegner, Reusch, et al. 2003; Wegner, Kalbe, et al. 2003). An empirical study on bank voles (*Myodes glareolus*) supports this negative relationship between MHC diversity and TCR repertoire, though only for MHC-I and not MHC-II (Migalska et al. 2019). Consequently, the relationship between MHC-II and TCR diversity has not been fully explained.

Apart from the interplay between the TCR and the MHC-II during thymic selection, the type of T cell involved as well as additional signals can influence the outcome of the APC–T cell interaction (Banchereau and Steinman 1998). For naive Th cells that have not encountered the antigen before, activation by the MHC-II-peptide-complex alone does not cause an immune response. Instead, it requires additional costimulation from the APC consisting of an interaction of different receptors present during inflammation to elicit an immune response (the "danger signal"). Lack of this second costimulatory signal can thus prevent immune responses toward antigens of nonpathogenic origin (Figure 3B; Bour-Jordan et al. 2011; Chen and Flies 2013) and facilitate symbiotic relationships with commensals.

Once a Th cell has been activated by an APC through the MHC-II-peptide complex in combination with a costimulatory signal, it can in turn activate other immune cells, such as B cells. This causes B cells to initiate antibody production (Figure 3A). Furthermore, B cells can bind and internalize free antigens via their B-cell receptor, initiating their maturation and antibody production as well. More frequently than that, B cells act as APCs themselves, presenting peptides via their MHC-II molecules to T cells to initiate activation of further immune cells such as other B cells (Sprent 1984). Consequently, as B cells themselves carry MHC-II molecules and T cells depend on MHC-II-carrying APCs for activation, B cell–T cell interaction as well as antibody production by B cells depend on the allelic polymorphism of MHC-II (Hünig and Schimpl 1979; Sprent 1984).

TINY BUT MIGHTY—IGA PERFORMS DIVERSE TASKS

After activation by MHC-II-activated Th cells, B cells can produce antibodies, called immunoglobulins of the class A (IgA). This class of antibodies performs diverse tasks and plays an important role in mediating tolerance to commensals on mucous surfaces such as the gut. IgA not only combats viruses, bacteria, and toxins through neutralization, agglutination, and binding (Pabst 2012), but is also involved in diminishing inflammatory and oxidative responses toward microbiota and reducing their pathogenicity (Peterson et al. 2007; Cullender et al. 2013). This key role in regulating tolerance is demonstrated in patients with low IgA levels who suffer from an overactive or misregulated immune response (Ammann and Hong 1971; Teahon et al. 1994).

The interaction between APC, Th cell, and B cell necessary to initiate antibody production depends on the diversity of MHC-II molecules. A more diverse repertoire of MHC-II molecules on APCs enables detection of a wider range of peptides. Consequently, a wider range of peptides recognized by MHC-II molecules interacts with a more diverse set of Th cells and thus results in a more diverse set of activated B cells producing a more diverse set of IgA. In turn, the resulting larger IgA repertoires facilitate tolerance against a wider range of microbes (Fransen et al. 2015). For example, Fransen et al. (2015) demonstrated a positive relationship between IgA diversity and microbiota diversity in two mice strains differing in several immunological features. As similar levels of IgA diversity could not be achieved by cohousing of mice nor by fecal transplants in one strain, they concluded that contact with microbiota alone might not be sufficient to increase IgA diversity and that there might be a genetic basis to the production of diverse IgA. By influencing the IgA repertoire, MHC-II diversity might hence be positively linked to microbiota diversity through facilitating tolerance responses.

KEEPING THE PEACE—TREG CELLS AND ILCS

Apart from mounting immune responses aimed at eliminating pathogens, the immune system must be capable of tempering inflammation to protect tissues from oxidative damage, to promote tolerance to benign foreign entities, and to enable symbiotic relationships with commensals. Hence the immune system includes anti-inflammatory components such as regulatory T (Treg) cells (Fontenot et al. 2005) and innate lymphoid cells (ILCs), which are

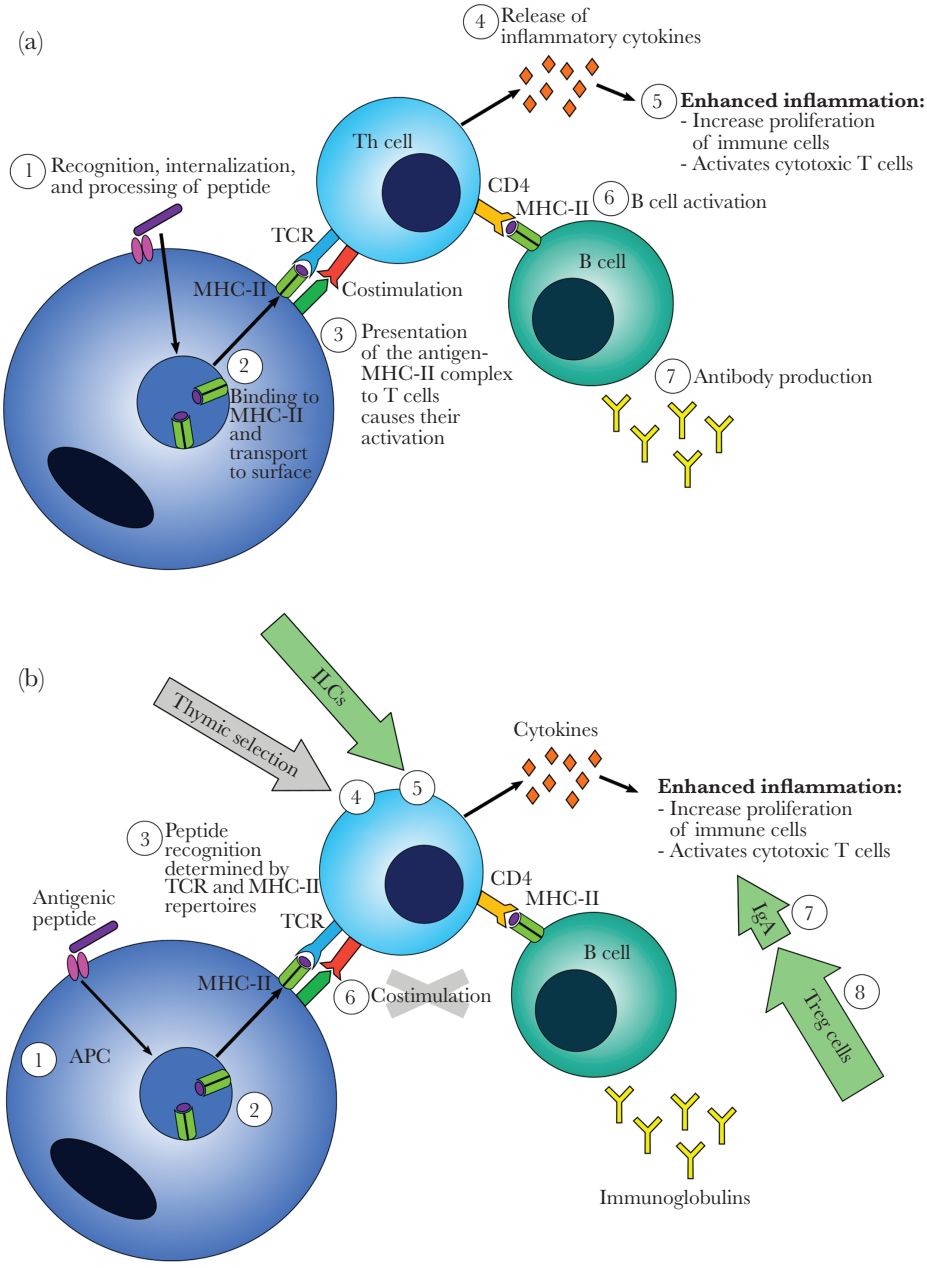

Figure 3

Immune response. Steps of immune response involving MHC-II leading to (A) elimination and (B) tolerance of the pathogen. (A) (1) After recognition by an APC, the peptide is internalized, processed and (2) presented by the MHC-II. (3) Interaction of the MHC-II-peptide-complex with the TCR together with an inflammatory costimulatory signal cause Th cell activation. (4) Inflammation is further exacerbated through cytokine release by Th cells, (5) causing activation of cytotoxic T cells and increased proliferation of immune cells. Activated Th cells (6) activate B cells that (7) produce antibodies. (B) (1) The type of APC as well as (2) the processing of the peptide can influence peptide recognition. (3) MHC-II and TCR strongly affect the set of presented peptides and the type of response. (4) MHC-II diversity is genetically determined, whereas the TCR repertoire is also determined by thymic selection. (5) ILCs can temper inflammation by inducing cell death of T cells acting against commensal bacteria. (6) In case of missing costimulation through an inflammatory signal, Th cell activation is prevented. (7) IgA produced by B cells can facilitate tolerance. (8) Treg cells promote IgA diversity and thus temper inflammation. Arrows displaying processes are colored in grey, cellular or humoral components are colored in green.

involved in maintaining homeostasis toward commensal microbiota (Hepworth et al. 2013, 2015).

Alterations in this anti-inflammatory response can have severe consequences for the immune system and the microbiota. Inhibiting the ability of ILCs to process and present peptides through selective deletion of their surface-bound MHC-II molecules causes a dysregulated immune response toward commensal bacteria and thus facilitates spontaneous intestinal inflammation (Hepworth et al. 2013). These findings indicate an MHC-II-dependent mechanism involving ILCs by which homeostasis is promoted and overreactive immunological responses against commensal microbiota are reduced. Furthermore, ILCs intrinsically expressing MHC-II induce cell death of T cells that act against commensal bacteria, thus providing a potential role for the

MHC-II to act on microbiota composition through enhancing tolerance (Hepworth et al. 2015).

Similar to the inhibition of ILCs, the loss of specific Treg cells can have consequences for gut homeostasis and involves a decline in IgA levels (Cong et al. 2009), which in turn have an important role in shaping the microbiota community (see previous section). These findings were reinforced by discoveries made by Josefowicz et al. (2012) who created mice deficient in a certain type of Treg cell and thereby caused increased levels of cytokines acting against extracellular parasites paired with mucosa-associated inflammation. Since these mice additionally showed an altered microbiota composition, they concluded that these Treg cells play an important role in orchestrating the composition of the microbiota.

For the generation of both Th and Treg cells, microbiota appear to play a crucial role (e.g., Strauch et al. 2005; Atarashi et al. 2008). Kawamoto et al. (2014) even postulated a symbiotic regulatory loop in which Treg cells modulate microbial diversity by tempering inflammation and facilitating higher IgA diversity (Supplementary Figure S5). Likewise, increased microbiota diversity promotes Treg cell diversity and thus IgA diversity. Consequently, as T cell and B cell activation and thus IgA production is linked to MHC-II polymorphism, MHC-II diversity has the potential to influence microbiota composition and diversity via this symbiotic regulatory loop including IgA and Treg cells. MHC-II polymorphism displays potential in attenuating adaptive immune responses and enhancing tolerance toward microbiota. However, despite evidence for the MHC-II initiating and regulating adaptive immune responses aimed at the microbiota, the mechanisms of how exactly MHC-II allelic diversity affects tolerance toward a broader community of microbes has yet to be answered.

SYSTEMATIC REVIEW OF THE EVIDENCE

To investigate the current evidence provided by empirical studies on the mechanisms linking the MHC, microbiota, and odor, we systematically reviewed the literature up to 30th January 2020 in both PubMed and Web of Science. We excluded human studies, as they include cultural, technological, and socioeconomic features unique to humans (reviewed in Winternitz and Abbate 2015), which could influence microbiota, odor, and behavior. Full steps for the systematic review, including search terms, PRISMA flowchart, studies included and excluded, and reasons for exclusions, can be found in the Supplementary Materials (Supplementary Tables S1–S3, Supplementary Figures S1–S4, Supplementary Methods).

Overall, we screened 577 publications (from both search engines combined, no duplicates) and retained 64 publications relevant for our review (listed in Supplementary Tables S1–S3). These were subdivided into those on the relationship between the microbiota and odor (n = 6 studies; Supplementary Table S1), the MHC and odor (n = 51 studies; Supplementary Table S2), and the MHC and the microbiota (n = 7 studies; Supplementary Table S3). We did not find any publication that had investigated the interaction of all three components: MHC, microbiota, and odor.

Through additional searching for relevant publications in recent reviews and publications, we found nine publications (including three studies not indexed) that had not been captured by our systematic search. However, we agree with Nakagawa and Lagisz (2019) that comprehensiveness of a systematic review can be impracticable or even impossible to achieve. Instead, requirements of a good systematic review are unbiasedness and transparency in the search process. This can be achieved by conducting the searches in at least two data bases and predefining search and data extraction strategies (Nakagawa et al. 2017). Since we fulfill these prerequisites of best practice, we contend that our systematic search is of appropriate quality and defend the usage of our search strings (to be comprehensive, the nine relevant but missing studies are included in the Supplementary Methods and labeled as such). Thus, we added nine relevant publications (microbiota and odor: n = 5 publications; MHC and odor: n = 2 publications; MHC and microbiota: n = 3 publications, with one publication (Zomer et al. 2009) found in our search for MHC and odor covering both topics), yielding a total number of 73 relevant publications. In the following sections, we summarize these findings (an extensive list of publications can be found in the Supplementary Materials, Supplementary Tables S1–S3).

MICROBIOTA AND ODOR

The 11 publications that have investigated potential links between microbiota and odor have been conducted solely on wild species (with the exception of one hybrid; a Bengal cat (*Felis catus* × *Prionailurus bengalensis*)) (Supplementary Table S1). Support for a relationship between microbes and volatile chemicals that compose odor profiles comes from studies on spotted hyenas (*Crocuta crocuta*) (Theis et al. 2013), European badgers (Buesching et al. 2016), meerkats (*Suricata suricatta*) (Leclaire et al. 2017), and South American tree frogs (*Boana prasina*) (Brunetti et al. 2019), in which odor and microbiota profiles, obtained from secretions from the subcaudal scent pouch or gland, anal glands, and skin, respectively, showed significant covariation. However, this was not the case in great tits (*Parus major*) (Jacob et al. 2018) and Carolina dark-eyed juncos (*Junco hyemalis carolinensis*) (Whittaker et al. 2016). Despite missing covariation between odor and microbiota profiles in Carolina dark-eyed-juncos, which might be caused by either only a subset of the microbiota contributing to odor or redundancy in the odor-producing members of the microbial community, the ability of members of the microbiota community to produce volatiles found in secretions has been demonstrated in northern dark-eyed juncos (*Junco hyemalis hyemalis*) (Whittaker et al. 2019). Likewise, studies on meerkats (Leclaire et al. 2017) and a Bengal cat (Yamaguchi et al. 2019) found microbes associated with volatile production, suggesting that microbes contribute to odor in these species. Evidence for the involvement of bacteria in odor generation also comes from African elephants (*Loxodonta africana*), where Goodwin et al. (2016) showed that removal of bacteria from exogenously aging urine of African Elephants hindered the formation of odorous compounds.

Evidence for a causal mechanism linking the microbiota community and odor was found in a study conducted by Whittaker et al. (2019) in which antibiotics were used to artificially perturb the microbiota in northern dark-eyed juncos. This treatment affected the volatile odor profile, which had been linked to the presence of particular bacterial species in a previous experiment on Carolina dark-eyed juncos (Whittaker et al. 2016). Support for a direct link between microbiota and odor also comes from a comparable study on European hoopoe nestlings (*Upupa epops*) (Martín-Vivaldi et al. 2010) and from Indian mongooses, in which secretions from antibiotically treated anal pockets were observed to lack chemical compounds that are present in secretions of untreated anal pockets (Gorman et al. 1974).

All eleven studies investigated the effect of microbiota on odor by analyzing odor profiles developed using gas chromatographic

methods such as gas chromatography—mass spectrometry (GC-MS, a technique that separates odor into its chemical subcomponents based on chemical properties and mass), and studies did not investigate whether chemical differences were detected or responded to by conspecifics. Thus, evidence for the ability of animals to detect these differences in the odor profiles for social communication is still lacking.

MHC AND ODOR

The influence of the MHC on odor has been of particular interest in studies of MHC-dependent mate choice as well as kin discrimination. In this regard, the ability of animals to detect MHC-differences in conspecifics' or other animals' odors has been studied extensively (reviewed in Kwak et al. 2010). In early studies, laboratory animals were trained to differentiate between odors of conspecifics or other laboratory species. Results showed that mice could discriminate between odors of strains differing only at the MHC (Bard et al. 2000; Willse et al. 2006), that MHC-linked odor differences are already detectable in pups (Yamazaki et al. 1992), and that fetal MHC-odortype is discriminable in pregnant mice (Beauchamp et al. 1994). However, these pioneering studies often rely on small sample sizes of laboratory strains using mostly Y-maze odor discrimination trials (Supplementary Table S2). A criticism of odor discrimination trials is that the ability to discriminate odors could arise due to training, resulting in laboratory animals discriminating cues that their untrained counterparts cannot distinguish in a natural situation (Penn and Potts 1998b). Our literature search found 19 preference trials testing untrained animals (both wild or wild-caught [n = 14] and laboratory [n = 5]) in flow chambers or y-mazes, and these studies predominantly support an important role for MHC-based cues in mate choice or kin recognition (e.g., Grieves et al. 2019). Importantly, preference trials have since been complimented by habituation/dishabituation trials under naturalistic settings, fortifying evidence for the discriminability of MHC-based odor differences (Brown et al. 1989; Penn and Potts 1998b) with a certain minimum distance at the peptide-binding site (Carroll et al. 2002) and odor formation based on soluble MHC molecules (Pearse-Pratt et al. 1998; Janssen et al. 2001).

Although underrepresented, studies on MHC–odor interaction have also been conducted on animals living in the wild or on wild-type animals held in captivity (n = 18 of 51 studies), and generally show support for a link between MHC and odor. For example, in song sparrows (*Melospiza melodia*), black-legged kittiwakes (*Rissa tridactyla*), and mandrills (*Mandrillus sphinx*) (Setchell et al. 2011; Leclaire et al. 2014; Slade et al. 2016; Grieves et al. 2019), there are positive correlations between MHC genetic distance and chemical distance of the odor profile, the latter being established using GC-MS. Of the two studies on captive ring-tailed lemurs (*Lemur catta*), one found a statistically nonsignificant relationship between the absence of certain MHC sequences and the concentration of volatile compounds in samples obtained from the brachial gland and the tail (Knapp et al. 2006) while the other found that MHC diversity and similarity is signaled via genital secretions in a sex- and season-dependent manner (Grogan et al. 2019).

In addition to support from correlational studies, wild animals have been shown to discriminate MHC-based odor differences in conspecifics. For example, Arctic char (*Salvelinus alpinus*) discriminate between siblings who do and do not share the same MHC-genotype as themselves (Olsén et al. 1998). Similarly juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) spent more time in water conditioned by kin sharing MHC alleles than in water conditioned by kin not sharing MHC alleles when given the choice in a flow chamber (Rajakaruna et al. 2006). Captive ring-tailed lemurs also discriminate MHC-diversity in the genital odors of opposite-sex conspecifics as they spent more time investigating or reacting to genital secretions of MHC-similar compared to MHC-dissimilar scent donors (Grogan et al. 2019).

Despite the MHC's potential importance, external influences such as diet can have stronger impact on odortype (Brown et al. 1996; Kwak et al. 2008) and hinder discrimination of odortypes (Schellinck et al. 1993, 1997). Interestingly, odors lacking MHC-derived peptides have been discriminable (Singer et al. 1993) and carboxylic acids appear to play a role in shaping laboratory mouse odortypes and their discriminability (Singer et al. 1997). The circumstances under which the MHC is important in odor communication are therefore unclear and further research is warranted to detangle genetic from environmental influences on odor.

MHC AND MICROBIOTA

Apart from directly influencing odor through shed MHC molecules or MHC peptide ligands, MHC-II has the potential to indirectly shape odor by governing microbiota (Figure 2). In European plaice (*Pleuronectes platessa*), a weak but significant correlation between MHC-IIB matrices and pathogen abundance matrices of gill microbiota was observed with certain alleles being positively linked to the presence of certain bacterial genera (Wegner et al. 2012). In male Leach's storm petrels (*Oceanodroma leucorhoa*), MHC-II DAB homozygosity explained 72% of variation in the microbiota community structure of the uropygial gland (Pearce et al. 2017). Similarly, Holstein dairy cows expressing two different MHC variants exhibit a different composition of microbiota in their mammary glands on the day of calving but not on following days (Derakhshani et al. 2018). These studies provide evidence for a link between the MHC and the microbiota community, but they do not offer insights into the mechanisms acting in MHC-based microbiota structuring.

Studies on blue petrels (*Halobaena caerulea*) (Leclaire et al. 2019) and sticklebacks (*Gasterosteus aculeatus)* (Bolnick et al. 2014) present evidence for a negative correlation between MHC diversity and microbial diversity (Supplementary Table S3), supporting the hypothesis that a diverse MHC genotype causes detection and elimination of more microbiota species and thus a less diverse microbiota community. However, not all studies found a negative relationship. For instance, in eastern hellbenders (*Cryptobranchus alleganiensis bishopi*), individual MHC amino acid distance was positively linked to microbial community richness (Hernández-Gómez et al. 2018). Furthermore, in laboratory mice, MHC heterozygosity has been shown to enhance functional diversity of the microbiome (Wadud Khan et al. 2019). The primary role of the MHC-II in shaping the microbiota and its role in presenting extracellular rather than intracellular peptides is also supported by Kubinak et al. (2015) who show that MHC-II had a stronger influence on the microbiota than MHC-I.

Although our search strings did not yield publications linking all three components (the MHC, microbiota, and odor), the search aimed at MHC–odor interactions yielded a study investigating the influence of the MHC on both odor and the microbiota (Zomer et al. 2009). It showed that in laboratory

mice the MHC affected both volatile and microbiota profiles, however the effect of the MHC was weaker than the effect of the genetic strain of the study animals. These findings are supported by another study on laboratory mice indicating that both MHC haplotype and background genotype impact odor profiles (Lanyon et al. 2007). However, although the study by Zomer et al. (2009) included all three components, it did not investigate the link between microbiota and odor, so it is unclear to what degree MHC-odor relationships might be impacted by the microbiota. Furthermore, GC-MS was used to investigate the effect of MHC on the odor profiles. While this is an appropriate technique for the question in hand, it leaves unanswered whether animals can make use of these subtle composition differences for social communication. Therefore, evidence of the MHC and the microbiota acting on odor to provide reliable information for social interactions has yet to be demonstrated.

COMPOSITION OF RETRIEVED STUDIES REGARDING STUDY TYPE AND SPECIES

Overall, results of our systematic review show that most studies focus on correlational rather than causal investigation of interactions between MHC and microbiota (n = 6 correlational vs. n = 3 experimental studies). However, this pattern is reversed for studies linking MHC and odor (n = 6 correlational vs. n = 46 experimental studies; plus one observational/methodological publication), caused by the great number of experimental studies on laboratory animals. For publications investigating the relationship between microbiota and odor the proportion is almost equal (n = 5 correlational vs. n = 6 experimental studies). Altogether, publications using laboratory-reared animals, mostly mice and rats, make up a similar portion (37/73) compared to publications investigating wild or wild-type animals (36/73).

The phylogenetic composition of the study species used varies between the three links investigated. Whereas rodents make up the majority of study animals for publications investigating the link between MHC and odor (65%, 35/54, Figure 4) with the remaining portion of study species stemming from eight different taxonomic orders, study species of publications investigating MHC and microbiota and microbiota and odor are more evenly distributed over five (microbiota and odor) and six (MHC and microbiota) different taxonomic orders. The relationship between MHC and microbiota and between MHC and odor has so far not been investigated in carnivores, and for fish evidence for a link between microbiota and odor is missing. Furthermore, there is a gap in publications investigating the link between MHC and microbiota and microbiota and odor in reptiles and the interrelation between the MHC and odor has not yet been investigated in amphibians.

Compiling the empirical evidence for potential mechanisms regulating MHC-based microbiota structuring showed that five publications retrieved in our systematic search found a link between the composition of the MHC and the composition of the microbiota community (Wegner et al. 2012; Kubinak et al. 2015; Pearce et al. 2017; Derakhshani et al. 2018; Wadud Khan et al. 2019). In contrast, there were no publications found that contest the link between MHC and microbiota composition (Figure 5), although publication bias of positive results cannot be ruled out. Publications investigating the effect of MHC diversity on microbiota diversity also miss nonsignificant results, showing support for two opposing hypotheses instead. Two studies provide support for a limiting effect of MHC diversity on microbiota diversity, causing a negative relationship (Bolnick et al. 2014; Leclaire et al. 2019) while evidence for a positive relationship between MHC diversity and microbiota diversity comes from a single study (Hernández-Gómez et al. 2018). Thus, further studies are necessary to clarify whether the MHC has a role in affecting social odors through shaping the microbiota community and to determine the potential mechanisms acting between the MHC and the microbiota.

KNOWLEDGE GAPS AND FUTURE OUTLOOK

Despite 73 publications investigating the interaction of the microbiota and odor, the MHC and odor, or the MHC and microbiota, their

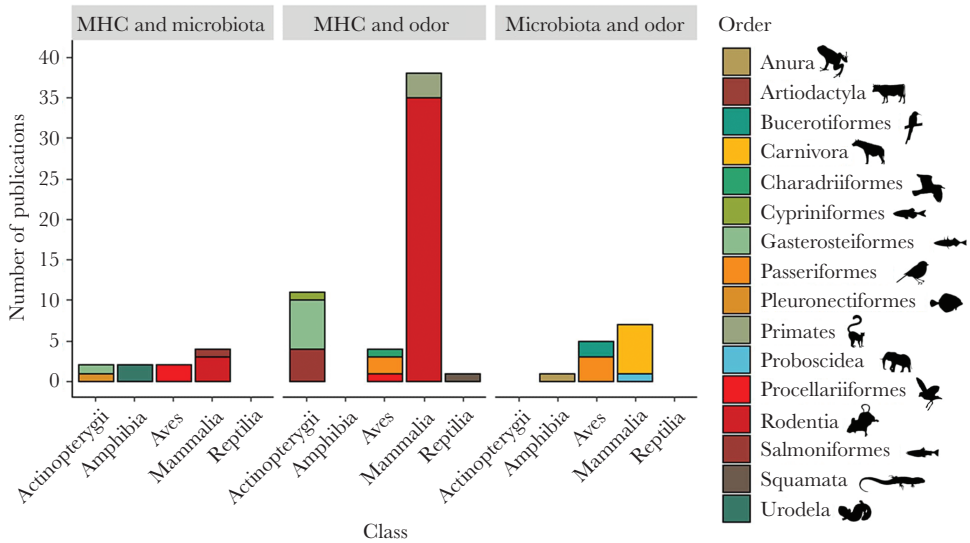

Figure 4
Study species used in studies investigating the links between MHC and microbiota, between MHC and odor, and between microbiota and odor. Number of publications that investigated either the link between MHC and microbiota, the MHC and odor, and the microbiota and odor is represented for the different classes. Within classes, publication numbers are further broken down into taxonomic orders.

results do not yield clear patterns explaining the relations. Thus, we list several suggestions and recommendations for future studies to develop credible evidence for the proposed mechanisms (Figures 1 and 2).

(1) Findings on MHC–microbiota correlation are ambiguous and study numbers are low. For wild mammals, evidence for any of the mechanisms governing these links comes from a single publication only, which did not investigate the relationship between MHC diversity and microbiota structure (Pearce et al. 2017). Our review of the immunological processes points to possibilities for the MHC to both limit and facilitate microbiota diversity (Supplementary Figure S5). Hence we argue researchers should investigate whether patterns of MHC–microbiota diversity are consistent within species with varying levels of MHC-II diversity. Studies involving a diverse range of species and comparing the microbes of different body sites (including scent glands) would be particularly beneficial as they will allow investigation of the circumstances under which positive, negative and no relationships between MHC and microbial diversity are found.

An alternative explanation of the mixed results between MHC and microbial diversity is based on the optimality hypothesis (Nowak et al. 1992; Woelfing et al. 2009). Imagine a U-shaped curve with microbial richness on the y-axis and MHC diversity on the x-axis, where the optimum MHC allelic diversity has the lowest microbial diversity. On the left side of the MHC optimum, the relationship between MHC and microbiota diversity would be negative. On the right of the optimum, the relationship between MHC and microbiota diversity would be positive. Thus, to test the optimality hypothesis multiple data points from the same study species at different MHC variabilities (or different microbiota diversities) are required.

(2) While there is clear evidence for the ability of wild animals to discriminate odor cues based on MHC in an experimental setting, there is a lack of studies demonstrating the application of this MHC-based discrimination of conspecifics for inbreeding avoidance or cooperation in order to increase fitness. We encourage studies on wild animals to verify use of this mechanism in a natural context. This could be performed in wild species for which the ability to discriminate has already been shown or on wild species for which, due to their behavior in mate choice or other social contexts, MHC-based odor discrimination may yield a substantial fitness benefit. MHC genotyping as well as odor and microbiota profiles combined with life history and behavioral data can provide evidence and thus help unravel whether decisions having severe fitness consequences are based on MHC-and microbiota-governed social odor cues in the natural context.

(3) Researchers should base their experiments on sample sizes that allow reliable conclusions. The extreme polymorphism of the MHC makes it a promising target for governing odor cues used in social communication, but simultaneously it causes studies investigating the role of the MHC in shaping odor or the microbiota to require relatively large sample sizes in order to have enough power to detect small effect sizes (Gaigher et al. 2019). Researchers should consider the level of MHC polymorphism found in their study organisms and the likely effect size when designing their studies, for example, by performing power analyses.

(4) Researchers should be aware that both microbiota and odor are affected by genetic loci other than the MHC as well as exogenous factors. Studies have reported that other proteins, such as MUPs, play an important role in odor discrimination in mice (Cheetham et al. 2007) and that the mouse laboratory strain appears to have an even stronger impact on odor than the MHC (Zomer et al. 2009). However, MUPs are not universal to all species and we therefore recommend testing the influence of the MHC while controlling for genetic similarity or relatedness (e.g., using high coverage SNPs, microsatellites or a pedigree) in order to disentangle the effect of the MHC from the influence of other loci.

(5) Our systematic review showed that studies focusing on MHC-microbiota and microbiota–odor interaction in wild animals mostly use correlational approaches and causal evidence is lacking. While experimental investigation of causal mechanisms is particularly

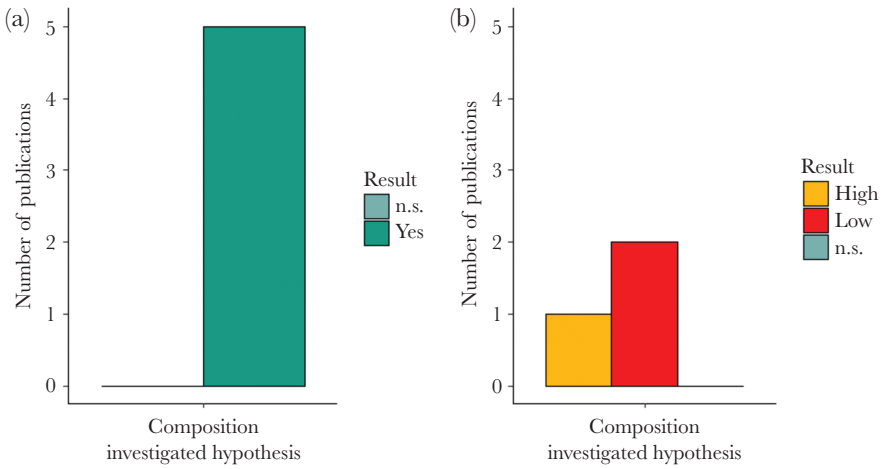

Figure 5
Empirical evidence for the relationship between MHC composition or diversity and the microbiota community. Number of publications investigating the link between MHC diversity or composition and the composition of the microbiota community (A) and MHC diversity or composition and microbiota diversity (B). Publications investigating the relationship between MHC composition or diversity and the composition of the microbial community (A) invariably provide evidence for a link between MHC diversity/composition and the composition of the microbial community ("yes") while no publications have been published that question this link due to nonsignificant results ("n.s."). Publications investigating the relationship between MHC diversity or composition and the diversity of the microbial community (B) either provide evidence for a negative correlation (high MHC diversity causing low microbiota diversity, "low") or for a positive relationship (high MHC diversity causing high microbiota diversity, "high"). There are no publications showing a nonsignificant relationship between MHC and microbiota diversity ("n.s.").

difficult in wild animals, it is nonetheless necessary to demonstrate the usage of MHC- and microbiota-governed odor cues in social communication in a natural context. This could be achieved by artificially altering odor by adding MHC ligands (e.g., Milinski et al. 2005; Spehr et al. 2006; Hinz et al. 2013; Milinski et al. 2013) to the odor profile. Another option might be the modification of microbiota composition either with fecal transplants (reviewed in Lively et al. 2014) or with antibiotics (Gorman et al. 1974; Whittaker et al. 2019). However, antibiotic treatment might have additional confounding effects impacting odor. Furthermore, potential negative effects of antibiotics and the possibility of facilitating resistances in microbes should be considered when designing a study. Another functional approach is testing whether microbiota found in the commensal community of an animal produce odorants present in its volatile profile. Discrimination of odors produced by a host versus those produced by its microbiota is vital to uncover the microbiota's role in chemical communication.

(6) Theories suggest that either MHC molecules themselves, the volatiles the MHC molecules might carry or volatiles developing due to the MHC's role in binding peptides could be potential sources of odor (Penn and Potts 1998a). However, what chemical components apart from MHC peptide ligands can enable or contribute to the discriminability of MHC-based odors has not yet been clearly determined. Most studies investigating MHC-governed odor profiles focus on GC-MS to determine the volatile components of odor. Few studies have investigated the role of proteins in influencing odors governed by the MHC, with some showing that proteins or MHC molecules are not necessary for the discrimination of odor (Brown et al. 1987; Singer et al. 1993), that MHC molecules alone do not ensure odor discriminability, and that MHC cannot be discriminated through serum (Brown et al. 1987). Contrariwise, other studies investigating the role of proteins in the generation of odor show that injection of soluble MHC molecules or soluble MHC peptide ligands alters odor (Pearse-Pratt et al. 1998; Janssen et al. 2001; Milinski et al. 2010). These conflicting findings hint for a role of proteins such as MHC molecules themselves or their ligands influencing odor through binding or regulating volatiles rather than being an odor source themselves. Thus, we suggest that studies, apart from focusing solely on volatiles, should also look at other compounds such as proteins to help unravel the mechanism behind MHC-based odor regulation.

(7) We need studies with a holistic approach combining interactions of all three components, the MHC, the microbiota, and odor, as, to our knowledge, no studies have investigated the links of all components simultaneously. For example, there is evidence that the MHC directly impacts on male Storm Petrels' microbiota composition (Pearce et al. 2017) and that odor profiles reflect genetic distance at the MHC (Leclaire et al. 2014; Slade et al. 2016; Grieves et al. 2019). However, causal links between all three are missing and it is unclear whether MHC, odor and microbiota are directly linked or if the MHC affects odor and the microbiota through separate mechanisms. Investigating the interconnections of all three in focal species could reveal the mechanisms underlying chemical communication and disclose the roles and interrelations of the MHC, the microbiota, and odor.

CONCLUSION

The MHC-II as an essential part of the complex immunological network has the potential to affect the microbiota and consequently odor through various pathways. Findings regarding immunological mechanisms suggest that MHC-II diversity can potentially facilitate microbiota diversity by inducing tolerance rather than solely limit its diversity through elimination. However, the small number of empirical studies conducted thus far has produced mixed results, with some finding negative or no relationship. Insights from immunology provide great potential for unravelling MHC–microbiota–odor interactions by presenting new starting points and hypotheses, and we hope that this review stimulates advances in the investigation and understanding of this potential key pathway for social communication.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

FUNDING

This work was supported by the German Research Foundation (project number 416495992 to N.S., project number 316099922, 396780709 to J.C.W.); Leverhulme International Fellowship (project number IAF-2018-006 to H.J.N.); and the Alexander von Humboldt Foundation (Humboldt Research Fellowship for Experienced Researchers to H.J.N.).

We thank Jim Kaufman, Barbara Caspers, Marc Gilles, and Alfredo Sánchez-Tójar for valuable comments.

Conflict of Interest: We Declare We Have No competing interests.

Author Contributions: N.S. and J.C.W. conceived the review and designed the figures. N.S. conducted the literature review and drafted the manuscript. N.S., J.C.W., and H.J.N. revised the manuscript and approved the final version for publication.

Data Availability: Analyses reported in this article can be reproduced using the data provided by Schubert et al. (2021).

Handling editor: Leigh Simmons

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