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

Abbreviated title: MHC- and microbiota-mediated social odors

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Competing interests

We declare we have no competing interests.

Authors' contributions

NS and JW conceived the review and designed the figures. NS conducted the literature review and drafted the manuscript. NS, JW and HN revised the manuscript and approved the final version for publication.

Lay abstract

Determining relatedness in members of the same species through their smell can help animals cooperate with close relatives or avoid inbreeding. How genetic information is encoded in odor, and what role immune genes (MHC) and microbes play in generating odor, as well as how they interact is unclear. We outline the immune system's involvement in odor-production, highlight gaps in our knowledge regarding immune gene and microbe-mediated social communication, and suggest ways to advance our understanding.

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Abstract

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 non-immunologists 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.

KEYWORDS: Major histocompatibility complex, scent, tolerance, kin recognition, immune response, systematic review

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 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 (for example 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; Theis et al. 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 towards 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 -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 inter-individual 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 (for example Antonopoulos et al. 2009). However, evidence from human twin studies investigating the microbiota's genetic basis is ambiguous with some claiming genetic determination (for example Stewart et al. 2005; Goodrich et al. 2014) while others do not support this dependency (for example 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 towards 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). Albone and Perry (1974) proposed 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 non-volatile peptides acting as a source of odor (peptide hypothesis) (for example Milinski et al. 2005; Spehr et al. 2006; Hinz et al. 2013; Milinski 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 (Fig. 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 inter-specific 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 towards certain microbiota species (Kubinak et al. 2015; Khan et al. 2019; see also the paragraph on the role of IgA).

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 non-volatile peptides. Despite their non-volatility, 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, non-volatile 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.

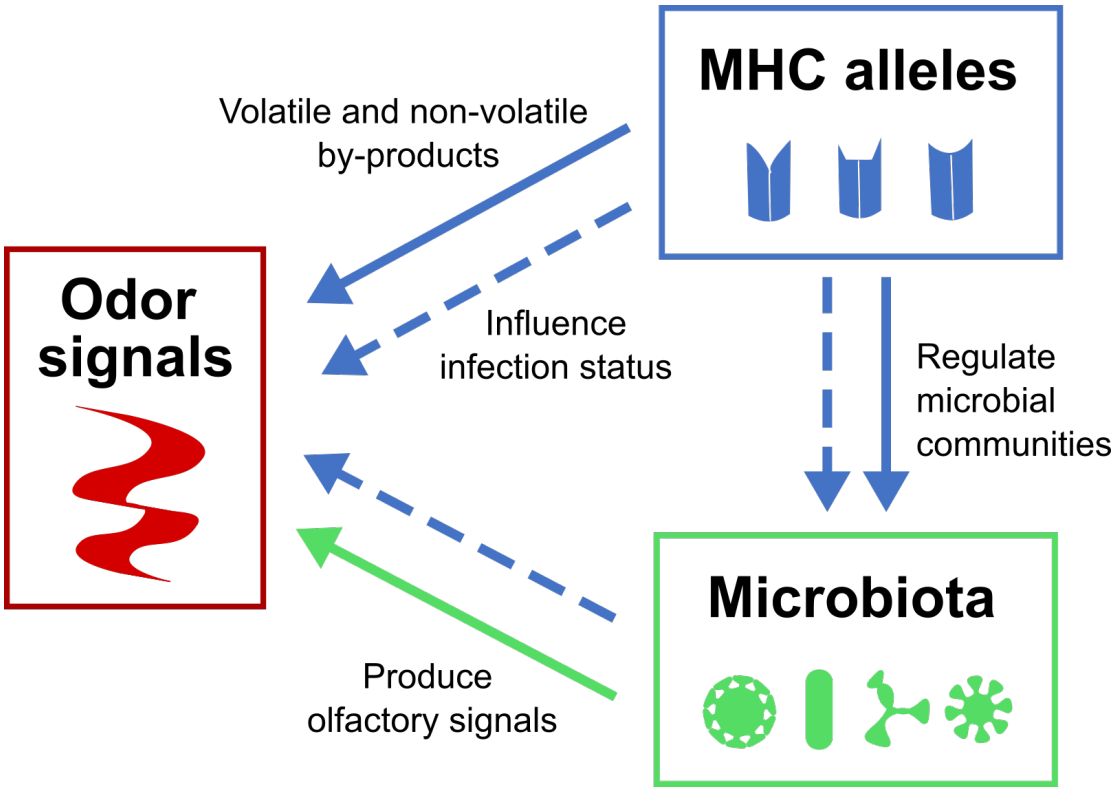


Figure 1. MHC-microbiota interactions in chemical communication. Schematic of the interactions between genes of the MHC and the microbiota and their potential influence on odor. MHC polymorphism (blue arrows) might directly influence odor (solid arrows) through volatile and non-volatile by-products such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

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. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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).

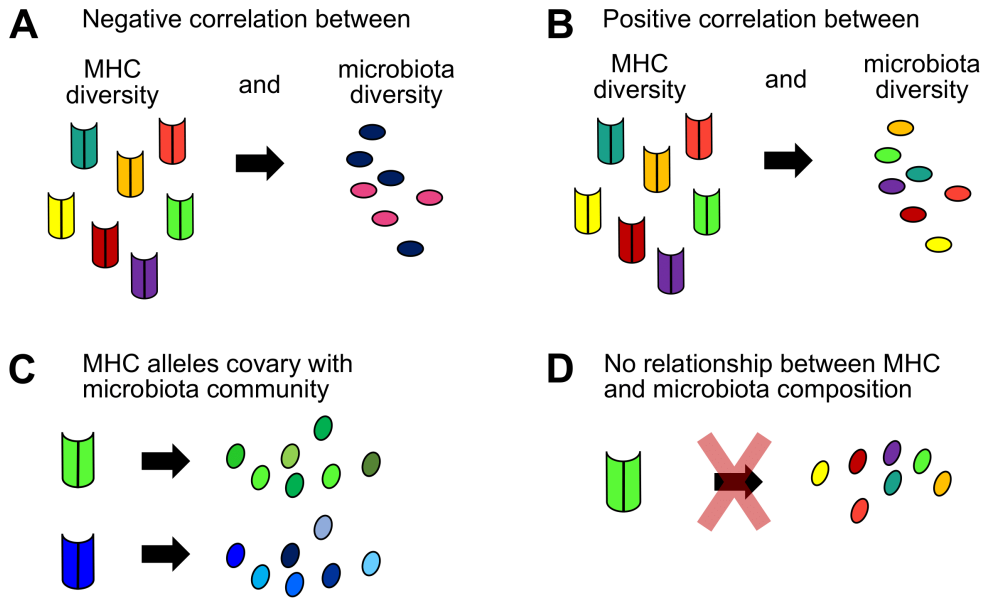


Figure 2. MHC-microbiota interaction. (A) A negative correlation is characterized by high MHC diversity leading to low microbiota diversity. (B) A positive correlation is caused by high MHC diversity tolerating more diverse microbiota communities. (C) Covariation between MHC genotypes and microbiota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

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

389 researched. However, we do not aim at explaining these immunological processes in
390 their great complexity and detail but rather focus on the mechanisms involving the
391 MHC and the microbiota (for further review, see Marietta et al. 2015; Honda and
392 Littman 2016). We want to provide immunological background knowledge on the
393 interrelation of the MHC and the microbiota potentially important for chemical
394 communication for a non-immunologist audience to help explain the observed
395 patterns of MHC and microbiota correlation and covariation in empirical studies.

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

403

404 **Starting the fight – or not? Initiating the adaptive immune response**

405 Antigen-presenting cells (APCs), such as B cells or macrophages, phagocytize and
406 process peptides and present them with their MHC-II molecules together with other
407 surface molecules to helper T (Th) cells, a certain type of T (developing in the
408 thymus) cell (Neefjes et al. 2011). The interaction between the APC and the Th cell
409 can either cause an immune response towards the presented antigen (Fig. 3A) or no
410 response (Fig. 3B) (Jurewicz and Stern 2019). Activation of the Th cell only occurs if
411 it can recognize the antigen and thus T cell responses depend on the repertoire of T
412 cell receptors (TCRs) available, which is determined during T cell development and
413 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 (Benchareau 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 towards antigens of non-pathogenic origin (Fig. 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 (Fig. 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 (Hiinig 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 towards 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

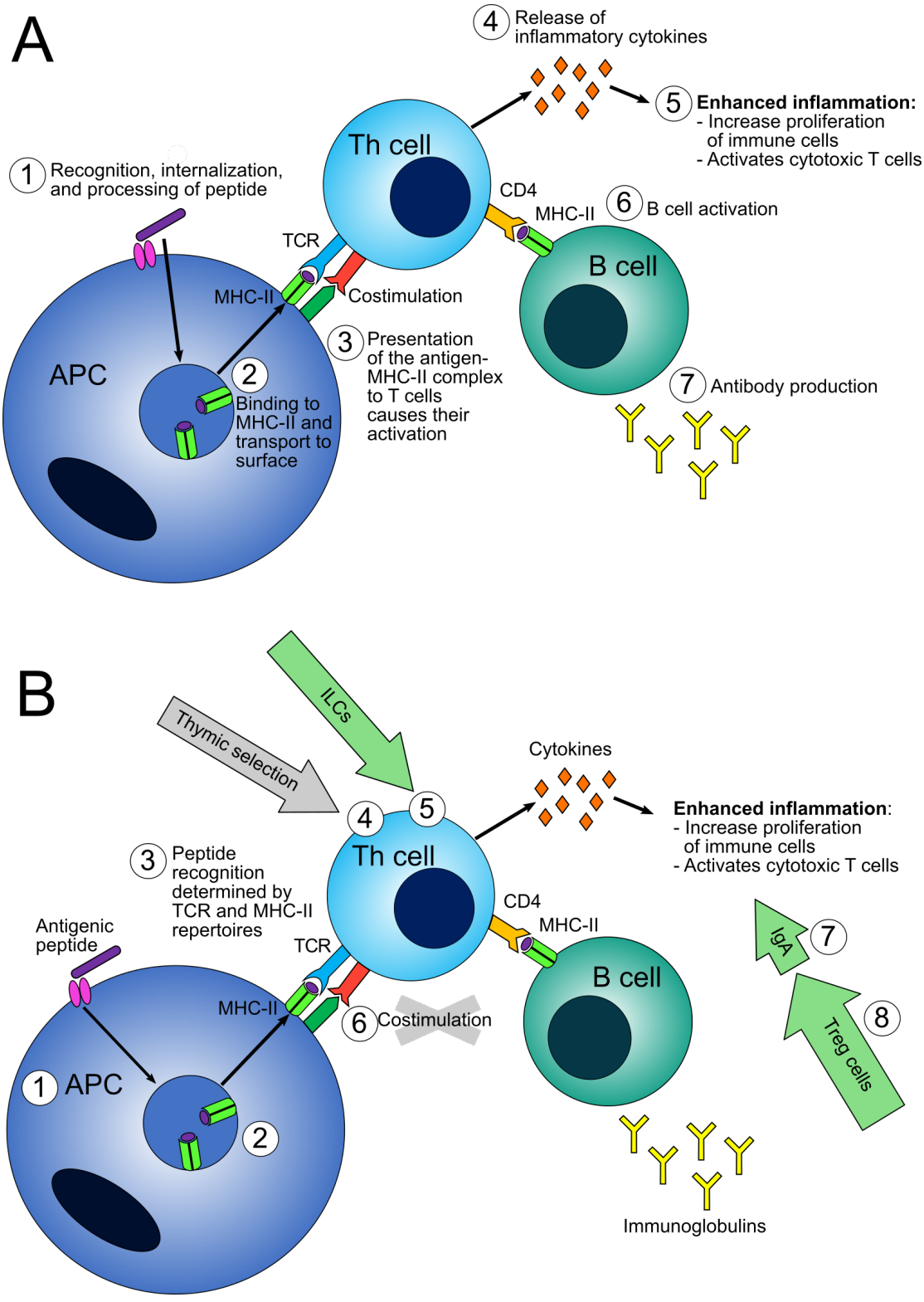
Apart from mounting immune responses aimed at eliminating pathogens, the immune system must be capable of tempering inflammation to protect tissues from oxidative damage, to promote tolerance to benign foreign entities, and to enable symbiotic relationships with commensals. Hence the immune system includes anti-inflammatory components such as regulatory T (Treg) cells (Fontenot et al. 2005) and innate lymphoid cells (ILCs), which are involved in maintaining homeostasis towards commensal microbiota (Hepworth et al. 2013; Hepworth et al. 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 towards 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 (for example 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 (Fig. 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 towards 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 towards a broader community of microbes has yet to be answered.



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532 Figure 3. Immune response. Steps of immune response involving MHC-II leading to (A)

533 elimination and (B) tolerance of the pathogen. (A) (1) After recognition by an APC, the peptide

534 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.

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 (Tab. S1-S3, Fig. S1-4, supplementary methods).

Overall, we screened 577 publications (from both search engines combined, no duplicates) and retained 64 publications relevant for our review (listed in Table S1-S3). These were subdivided into those on the relationship between the microbiota

and odor (n = 6 studies; Table S1), the MHC and odor (n = 51 studies; Table S2), and the MHC and the microbiota (n = 7 studies; 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 3 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, Table 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

587 Bengal cat (*Felis catus* × *Prionailurus bengalensis*)) (Table S1). Support for a
588 relationship between microbes and volatile chemicals that compose odor profiles
589 comes from studies on spotted hyenas (*Crocuta crocuta*) (Theis et al. 2013),
590 European badgers (Buesching et al. 2016), meerkats (*Suricata suricatta*) (Leclaire et
591 al. 2017) and South American tree frogs (*Boana prasina*) (Brunetti et al. 2019), in
592 which odor and microbiota profiles, obtained from secretions from the subcaudal
593 scent pouch or gland, anal glands and skin respectively, showed significant
594 covariation. However, this was not the case in great tits (*Parus major*) (Jacob et al.
595 2018) and Carolina dark-eyed juncos (*Junco hyemalis carolinensis*) (Whittaker et al.
596 2016). Despite missing covariation between odor and microbiota profiles in Carolina
597 dark-eyed-juncos, which might be caused by either only a subset of the microbiota
598 contributing to odor or redundancy in the odor-producing members of the microbial
599 community, the ability of members of the microbiota community to produce volatiles
600 found in secretions has been demonstrated in northern dark-eyed juncos (*Junco*
601 *hyemalis hyemalis*) (Whittaker et al. 2019). Likewise studies on meerkats (Leclaire et
602 al. 2017) and a Bengal cat (Yamaguchi et al. 2019) found microbes associated with
603 volatile production, suggesting that microbes contribute to odor in these species.
604 Evidence for the involvement of bacteria in odor generation also comes from African
605 elephants (*Loxodonta africana*), where Goodwin et al. (2016) showed that removal of
606 bacteria from exogenously aging urine of African Elephants hindered the formation of
607 odorous compounds.

608 Evidence for a causal mechanism linking the microbiota community and odor was
609 found in a study conducted by Whittaker et al. (2019) in which antibiotics were used
610 to artificially perturb the microbiota in northern dark-eyed juncos. This treatment
611 affected the volatile odor profile, which had been linked to the presence of particular
612 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 (Table S2). A criticism of odor

638 discrimination trials is that the ability to discriminate odors could arise due to training,
639 resulting in laboratory animals discriminating cues that their untrained counterparts
640 cannot distinguish in a natural situation (Penn and Potts 1998b). Our literature search
641 found 19 preference trials testing untrained animals (both wild or wild-caught (n = 14)
642 and laboratory (n = 5)) in flow chambers or y-mazes, and these studies
643 predominantly support an important role for MHC-based cues in mate choice or kin
644 recognition (for example Grieves et al. 2019). Importantly, preference trials have
645 since been complimented by habituation/dishabituation trials under naturalistic
646 settings, fortifying evidence for the discriminability of MHC-based odor differences
647 (Brown et al. 1989; Penn and Potts 1998b) with a certain minimum distance at the
648 peptide-binding site (Carroll et al. 2002) and odor formation based on soluble MHC
649 molecules (Pearse-Pratt et al. 1998; Janssen et al. 2001).

650 Although underrepresented, studies on MHC-odor interaction have also been
651 conducted on animals living in the wild or on wild-type animals held in captivity (n=
652 18 of 51 studies), and generally show support for a link between MHC and odor. For
653 example, in song sparrows (*Melospiza melodia*), black-legged kittiwakes (*Rissa*
654 *tridactyla*), and mandrills (*Mandrillus sphinx*) (Setchell et al. 2011; Leclaire et al.
655 2014; Slade et al. 2016; Grieves et al. 2019), there are positive correlations between
656 MHC genetic distance and chemical distance of the odor profile, the latter being
657 established using GC-MS. Of the two studies on captive ring-tailed lemurs (*Lemur*
658 *catta*), one found a statistically non-significant relationship between the absence of
659 certain MHC sequences and the concentration of volatile compounds in samples
660 obtained from the brachial gland and the tail (Knapp et al. 2006) while the other
661 found that MHC diversity and similarity is signaled via genital secretions in a sex- and
662 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; Schellinck et al. 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 (Fig. 2). In European plaice (*Pleuronectes platessa*), a weak but significant correlation between MHC-IIB matrices and pathogen abundance matrices of gill

688 microbiota was observed with certain alleles being positively linked to the presence
689 of certain bacterial genera (Wegner et al. 2012). In male Leach's storm petrels
690 (*Oceanodroma leucorhoa*) MHC-II DAB homozygosity explained 72% of variation in
691 the microbiota community structure of the uropygial gland (Pearce et al. 2017).
692 Similarly, Holstein dairy cows expressing two different MHC variants exhibit a
693 different composition of microbiota in their mammary glands on the day of calving but
694 not on following days (Derakhshani et al. 2018). These studies provide evidence for a
695 link between the MHC and the microbiota community, but they do not offer insights
696 into the mechanisms acting in MHC-based microbiota structuring.

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

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

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

726

727 **Composition of retrieved studies regarding study type and species**

728 Overall, results of our systematic review show that most studies focus on
729 correlational rather than causal investigation of interactions between MHC and
730 microbiota (n = 6 correlational vs n = 3 experimental studies). However, this pattern
731 is reversed for studies linking MHC and odor (n = 6 correlational vs n = 46
732 experimental studies; plus one observational/methodological publication), caused by
733 the great number of experimental studies on laboratory animals. For publications
734 investigating the relationship between microbiota and odor the proportion is almost
735 equal (n = 5 correlational vs n = 6 experimental studies). Altogether, publications
736 using laboratory-reared animals, mostly mice and rats, make up a similar portion
737 (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, Fig. 4) with the remaining portion of study species stemming from 8 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.

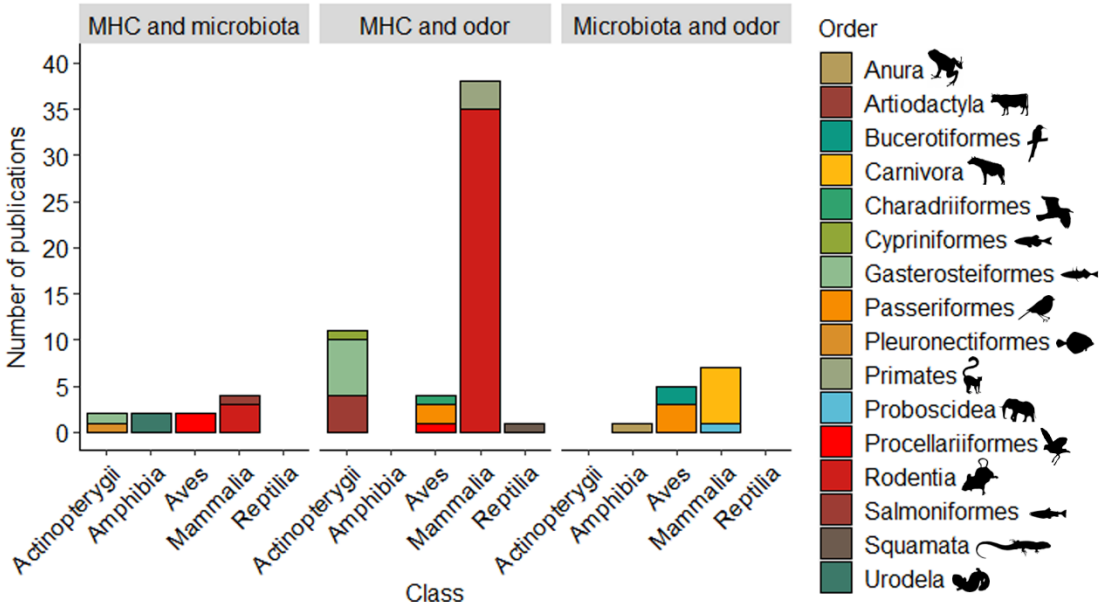


Figure 4. Study species used in studies investigating the links between MHC and microbiota, between MHC and odor, and between microbiota and odor. Number of publications that investigated either the link between MHC and microbiota, the MHC and odor, and the

754 microbiota and odor is represented for the different classes. Within classes, publication
755 numbers are further broken down into taxonomic orders.

756

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

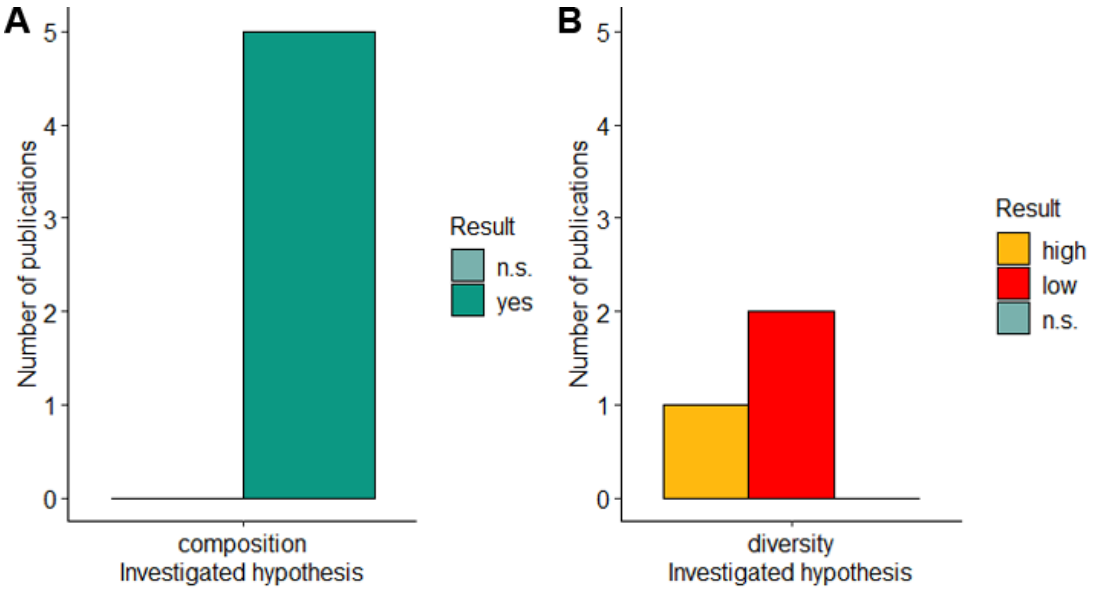


Figure 5. Empirical evidence for the relationship between MHC composition or diversity and the microbiota community. Number of publications investigating the link between MHC diversity or composition and the composition of the microbiota community (A) and MHC diversity or composition and microbiota diversity (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 non-significant 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 non-significant relationship between MHC and microbiota diversity ("n.s.").

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 results do not yield clear patterns

792 explaining the relations. Thus, we list several suggestions and recommendations for
793 future studies to develop credible evidence for the proposed mechanisms (Fig. 1 &
794 2).

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

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

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

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

836 (iv) Researchers should be aware that both microbiota and odor are affected by
837 genetic loci other than the MHC as well as exogenous factors. Studies have reported
838 that other proteins, such as MUPs, play an important role in odor discrimination in
839 mice (Cheetham et al. 2007) and that the mouse laboratory strain appears to have an
840 even stronger impact on odor than the MHC (Zomer et al. 2009). However, MUPs are
841 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.

(v) 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 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 (for example 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.

(vi) 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.

(vii) 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 have 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.

915 **References**

916 Aeschlimann PB, Häberli MA, Reusch TBH, Boehm T, Milinski M. 2003. Female
917 sticklebacks Gasterosteus aculeatus use self-reference to optimize MHC allele
918 number during mate selection. Behav Ecol Sociobiol. doi:10.1007/s00265-003-0611-
919 6.

920 Albone ES, Eglinton G, Walker JM, Ware GC. 1974. The anal sac secretion of the
921 red fox (Vulpes vulpes); its chemistry and microbiology. A comparison with the anal
922 sac secretion of the lion (Panthera leo). Life Sci. doi:10.1016/0024-3205(74)90069-1.

923 Alfonso C, Karlsson L. 2000. Nonclassical MHC Class II Molecules. Annu Rev
924 Immunol. doi:10.1146/annurev.immunol.18.1.113.

925 Ammann AJ, Hong R. 1971. Selective IgA deficiency: Presentation of 30 cases and a
926 review of the literature. Med (United States). doi:10.1097/00005792-197105000-
927 00004.

928 Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, Young VB. 2009.
929 Reproducible community dynamics of the gastrointestinal microbiota following
930 antibiotic perturbation. Infect Immun. doi:10.1128/IAI.01520-08.

931 Arakawa H, Arakawa K, Deak T. 2010. Sickness-related odor communication signals
932 as determinants of social behavior in rat: A role for inflammatory processes. Horm
933 Behav. doi:10.1016/j.yhbeh.2010.01.002.

934 Archie EA, Theis KR. 2011. Animal behaviour meets microbial ecology. Anim Behav.
935 doi:10.1016/j.anbehav.2011.05.029.

936 Artis D. 2008. Epithelial-cell recognition of commensal bacteria and maintenance of
937 immune homeostasis in the gut. Nat Rev Immunol. doi:10.1038/nri2316.

938 Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, Yagita H,
939 Ishii N, Evans R, Honda K, et al. 2008. ATP drives lamina propria TH17 cell
940 differentiation. Nature. doi:10.1038/nature07240.

941 Bard J, Yamazaki K, Curran M, Boyse EA, Beauchamp GK. 2000. Effect of B2m
942 gene disruption on MHC-determined odortypes. Immunogenetics.
943 doi:10.1007/s002510000165.

944 Beauchamp GK, Yamazaki K, Curran M, Bard J, Boyse EA. 1994. Fetal H-2
945 odortypes are evident in the urine of pregnant female mice. Immunogenetics.
946 doi:10.1007/BF00188613.

947 Benchareau J, Steinman R. 1998. Dendritic cells and the control of immunity. Nature.

948 Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. 1987.
949 Structure of the human class I histocompatibility antigen, HLA-A2. Nature.
950 doi:10.1038/329506a0.

951 Boehm T, Zufall F. 2006. MHC peptides and the sensory evaluation of genotype.
952 Trends Neurosci. doi:10.1016/j.tins.2005.11.006.

953 Bolnick DI, Snowberg LK, Caporaso JG, Lauber C, Knight R, Stutz WE. 2014. Major
954 Histocompatibility Complex class IIb polymorphism influences gut microbiota
955 composition and diversity. Mol Ecol. doi:10.1111/mec.12846.

956 Bour-Jordan H, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M,
957 Bluestone JA. 2011. Intrinsic and extrinsic control of peripheral T-cell tolerance by
958 costimulatory molecules of the CD28/B7 family. Immunol Rev. doi:10.1111/j.1600-
959 065X.2011.01011.x.

960 Braud VM, Allan DS, McMichael AJ. 1999. Functions of nonclassical MHC and non-
961 MHC-encoded class I molecules. Curr Opin Immunol. doi:10.1016/S0952-
962

963 7915(99)80018-1.

964 Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, Wiley DC.
965 1993. Three-dimensional structure of the human class II histocompatibility antigen
966 HLA-DR1. Nature. doi:10.1038/364033a0.

967 Brown RE, Roser B, Singh PB. 1989. Class I and class II regions of the major
968 histocompatibility complex both contribute to individual odors in congenic inbred
969 strains of rats. Behav Genet. doi:10.1007/BF01066029.

970 Brown RE, Schellinck HMI, West AM. 1996. The influence of dietary and genetic
971 cues on the ability of rats to discriminate between the urinary odors of MHC-congenic
972 mice. Physiol Behav. doi:10.1016/0031-9384(96)00030-3.

973 Brown RE, Singh PB, Roser B. 1987. The Major Histocompatibility Complex and the
974 chemosensory recognition of individuality in rats. Physiol Behav. doi:10.1016/0031-
975 9384(87)90186-7.

976 Brunetti AE, Lyra ML, Melo WGP, Andrade LE, Palacios-Rodríguez P, Prado BM,
977 Haddad CFB, Pupo MT, Lopes NP. 2019. Symbiotic skin bacteria as a source for
978 sex-specific scents in frogs. Proc Natl Acad Sci U S A.
979 doi:10.1073/pnas.1806834116.

980 Buesching CD, Tinnesand HV, Sin Y, Rosell F, Burke T, Macdonald DW. 2016.
981 Coding of Group Odor in the Subcaudal Gland Secretion of the European Badger
982 Meles meles: Chemical Composition and Pouch Microbiota. In: Chemical Signals in
983 Vertebrates 13.

984 Carroll LS, Penn DJ, Potts WK. 2002. Discrimination of MHC-derived odors by
985 untrained mice is consistent with divergence in peptide-binding region residues. Proc
986 Natl Acad Sci U S A. doi:10.1073/pnas.042244899.

987 Charpentier MJE, Boulet M, Drea CM. 2008. Smelling right: The scent of male lemurs
988 advertises genetic quality and relatedness. Mol Ecol. 17(14):3225–3233.
989 doi:10.1111/j.1365-294X.2008.03831.x.

990 Cheetham SA, Thom MD, Jury F, Ollier WER, Beynon RJ, Hurst JL. 2007. The
991 Genetic Basis of Individual-Recognition Signals in the Mouse. Curr Biol.
992 doi:10.1016/j.cub.2007.10.007.

993 Chen L, Flies DB. 2013. Molecular mechanisms of T cell co-stimulation and co-
994 inhibition. Nat Rev Immunol. doi:10.1038/nri3405.

995 Chung H, Kasper DL. 2010. Microbiota-stimulated immune mechanisms to maintain
996 gut homeostasis. Curr Opin Immunol. doi:10.1016/j.coi.2010.06.008.

997 Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO. 2009. A dominant, coordinated
998 T regulatory cell-IgA response to the intestinal microbiota. Proc Natl Acad Sci U S A.
999 doi:10.1073/pnas.0812681106.

1000 Cullender TC, Chassaing B, Janzon A, Kumar K, Muller CE, Werner JJ, Angenent
1001 LT, Bell ME, Hay AG, Peterson DA, et al. 2013. Innate and adaptive immunity
1002 interact to quench microbiome flagellar motility in the gut. Cell Host Microbe.
1003 doi:10.1016/j.chom.2013.10.009.

1004 Davenport ER, Mizrahi-Man O, Michelini K, Barreiro LB, Ober C, Gilad Y. 2014.
1005 Seasonal variation in human gut microbiome composition. PLoS One.
1006 doi:10.1371/journal.pone.0090731.

1007 Derakhshani H, Plaizier JC, De Buck J, Barkema HW, Khafipour E. 2018.
1008 Association of bovine major histocompatibility complex (BoLA) gene polymorphism
1009 with colostrum and milk microbiota of dairy cows during the first week of lactation.
1010 Microbiome. doi:10.1186/s40168-018-0586-1.

1011 Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. 2005.
1012 Regulatory T cell lineage specification by the forkhead transcription factor Foxp3.
1013 Immunity. doi:10.1016/j.immuni.2005.01.016.

1014 Fransen F, Zagato E, Mazzini E, Fosso B, Manzari C, El Aidy S, Chiavelli A, D'Erchia
1015 AM, Sethi MK, Pabst O, et al. 2015. BALB/c and C57BL/6 Mice Differ in Polyreactive
1016 IgA Abundance, which Impacts the Generation of Antigen-Specific IgA and
1017 Microbiota Diversity. Immunity. doi:10.1016/j.immuni.2015.08.011.

1018 Gaigher A, Burri R, San-Jose LM, Roulin A, Fumagalli L. 2019. Lack of statistical
1019 power as a major limitation in understanding MHC-mediated immunocompetence in
1020 wild vertebrate populations. Mol Ecol. doi:10.1111/mec.15276.

1021 Gautier P, Miaud C. 2003. Faecal pellets used as an economic territorial marker in
1022 two terrestrial alpine salamanders. Ecoscience.
1023 doi:10.1080/11956860.2003.11682759.

1024 Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M,
1025 Van Treuren W, Knight R, Bell JT, et al. 2014. Human genetics shape the gut
1026 microbiome. Cell. doi:10.1016/j.cell.2014.09.053.

1027 Goodwin TE, Harelimana IH, MacDonald LJ, Mark DB, Juru AU, Yin Q, Engman JA,
1028 Kopper RA, Lichti CF, Mackintosh SG, et al. 2016. The Role of Bacteria in Chemical
1029 Signals of Elephant Musth: Proximate Causes and Biochemical Pathways. In:
1030 Chemical Signals in Vertebrates 13.

1031 Gorman ML, Nedwell DB, Smith RM. 1974. An analysis of the contents of the anal
1032 scent pockets of Herpestes auropunctatus (Carnivora: Viverridae). J Zool.
1033 doi:10.1111/j.1469-7998.1974.tb04115.x.

1034 Greene LK, Drea CM. 2014. Love is in the air: Sociality and pair bondedness

influence sifaka reproductive signalling. Anim Behav. 88:147–156. doi:10.1016/j.anbehav.2013.11.019. http://dx.doi.org/10.1016/j.anbehav.2013.11.019.

Grieves LA, Gloor GB, Bernards MA, MacDougall-Shackleton EA. 2019. Songbirds show odour-based discrimination of similarity and diversity at the major histocompatibility complex. Anim Behav. doi:10.1016/j.anbehav.2019.10.005.

Grieves LA, Kelly TR, Bernards MA, MacDougall-Shackleton EA. 2018. Malarial infection alters wax ester composition of preen oil in songbirds: Results of an experimental study. Auk. doi:10.1642/auk-17-242.1.

Grogan KE, Harris RL, Boulet M, Drea CM. 2019. Genetic variation at MHC class II loci influences both olfactory signals and scent discrimination in ring-tailed lemurs. BMC Evol Biol. doi:10.1186/s12862-019-1486-0.

Hamilton WD. 1964. The genetical evolution of social behavior, parts I and II. J Theor Biol.

Harris RL, Boulet M, Grogan KE, Drea CM. 2018. Costs of injury for scent signalling in a strepsirrhine primate. Sci Rep. doi:10.1038/s41598-018-27322-3.

Harris RL, Holland BR, Cameron EZ, Davies NW, Nicol SC. 2014. Chemical signals in the echidna: Differences between seasons, sexes, individuals and gland types. J Zool. doi:10.1111/jzo.12133.

Hauber ME, Sherman PW. 2001. Self-referent phenotype matching: Theoretical considerations and empirical evidence. Trends Neurosci. doi:10.1016/S0166-2236(00)01916-0.

Hepworth MR, Fung TC, Masur SH, Kelsen JR, McConnell FM, Dubrot J, Withers DR, Hugues S, Farrar MA, Reith W, et al. 2015. Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific $CD4^+$ T cells. Science

(80-). doi:10.1126/science.aaa4812.

Hepworth MR, Monticelli LA, Fung TC, Ziegler CGK, Grunberg S, Sinha R, Mantegazza AR, Ma HL, Crawford A, Angelosanto JM, et al. 2013. Innate lymphoid cells regulate CD4 + T-cell responses to intestinal commensal bacteria. Nature. doi:10.1038/nature12240.

Hernández-Gómez O, Briggler JT, Williams RN. 2018. Influence of immunogenetics, sex and body condition on the cutaneous microbial communities of two giant salamanders. Mol Ecol. doi:10.1111/mec.14500.

Hiinig T, Schimpl A. 1979. Studies on the generation and expression of H-2-controlled T helper function in chimeric mice: Evidence for two levels of H-2 restriction. Eur J Immunol. doi:10.1002/eji.1830090912.

Hinz C, Namekawa R, Behrmann-Godel J, Oppelt C, Jaeschke A, Müller A, Friedrich RW, Gerlach G. 2013. Olfactory imprinting is triggered by MHC peptide ligands. Sci Rep. doi:10.1038/srep02800.

Honda K, Littman DR. 2016. The microbiota in adaptive immune homeostasis and disease. Nature. doi:10.1038/nature18848.

Howard JC. 1977. H-2 and mating preferences. Nature. 266(5601):406–408. doi:10.1038/266406a0.

Huseby ES, White J, Crawford F, Vass T, Becker D, Pinilla C, Marrack P, Kappler JW. 2005. How the T cell repertoire becomes peptide and MHC specific. Cell. doi:10.1016/j.cell.2005.05.013.

Jacob S, Sallé L, Zinger L, Chaine AS, Ducamp C, Boutault L, Russell AF, Heeb P. 2018. Chemical regulation of body feather microbiota in a wild bird. Mol Ecol. doi:10.1111/mec.14551.

Jami E, Mizrahi I. 2012. Composition and similarity of bovine rumen microbiota across individual animals. PLoS One. doi:10.1371/journal.pone.0033306.

Janssen E, Göhlen B, Behrens D, Richter K, Zavazava N. 2001. Allogeneic recombinant soluble MHC class I molecules modify urinary odor cues in rats. Physiol Behav. doi:10.1016/S0031-9384(00)00389-9.

Johnson RP. 1973. Scent marking in mammals. Anim Behav. doi:10.1016/S0003-3472(73)80012-0.

Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, Umetsu DT, Rudensky AY. 2012. Extrathymically generated regulatory T cells control mucosal T H 2 inflammation. Nature. doi:10.1038/nature10772.

Jurewicz MM, Stern LJ. 2019. Class II MHC antigen processing in immune tolerance and inflammation. Immunogenetics. doi:10.1007/s00251-018-1095-x.

Kaufman J. 2018. Unfinished Business: Evolution of the MHC and the Adaptive Immune System of Jawed Vertebrates. Annu Rev Immunol. doi:10.1146/annurev-immunol-051116-052450.

Kawamoto S, Maruya M, Kato LM, Suda W, Atarashi K, Doi Y, Tsutsui Y, Qin H, Honda K, Okada T, et al. 2014. Foxp3 + T Cells Regulate Immunoglobulin A Selection and Facilitate Diversification of Bacterial Species Responsible for Immune Homeostasis. Immunity. doi:10.1016/j.immuni.2014.05.016.

Khan AA, Yurkovetskiy L, O'Grady K, Pickard JM, de Pooter R, Antonopoulos DA, Golovkina T, Chervonsky A. 2019. Polymorphic Immune Mechanisms Regulate Commensal Repertoire. Cell Rep. doi:10.1016/j.celrep.2019.09.010.

Kimball BA, Yamazaki K, Kohler D, Bowen RA, Muth JP, Opiekun M, Beauchamp GK. 2013. Avian Influenza Infection Alters Fecal Odor in Mallards. PLoS One.

doi:10.1371/journal.pone.0075411.

Klein J. 1986. Natural history of the major histocompatibility complex. New York: John Wiley & Sons.

Klein L, Kyewski B, Allen PM, Hogquist KA. 2014. Positive and negative selection of the T cell repertoire: What thymocytes see (and don't see). Nat Rev Immunol. doi:10.1038/nri3667.

Knapp LA. 2005. The ABCs of MHC. Evol Anthropol. doi:10.1002/evan.20038.

Knapp LA, Robson J, Waterhouse JS. 2006. Olfactory signals and the MHC: A review and a case study in Lemur catta. In: American Journal of Primatology.

Kobayashi T, Voisin B, Kim DY, Kennedy EA, Jo JH, Shih HY, Truong A, Doebel T, Sakamoto K, Cui CY, et al. 2019. Homeostatic Control of Sebaceous Glands by Innate Lymphoid Cells Regulates Commensal Bacteria Equilibrium. Cell. doi:10.1016/j.cell.2018.12.031.

Kohl KD. 2012. Diversity and function of the avian gut microbiota. J Comp Physiol B Biochem Syst Environ Physiol. doi:10.1007/s00360-012-0645-z.

Kubinak JL, Stephens WZ, Soto R, Petersen C, Chiaro T, Gogokhia L, Bell R, Ajami NJ, Petrosino JF, Morrison L, et al. 2015. MHC variation sculpts individualized microbial communities that control susceptibility to enteric infection. Nat Commun. doi:10.1038/ncomms9642.

Kwak J, Opiekun MC, Matsumura K, Preti G, Yamazaki K, Beauchamp GK. 2009. Major histocompatibility complex-regulated odortypes: Peptide-free urinary volatile signals. Physiol Behav. doi:10.1016/j.physbeh.2008.10.003.

Kwak J, Willse A, Matsumura K, Opiekun MC, Yi W, Preti G, Yamazaki K,

1131 Beauchamp GK. 2008. Genetically-based olfactory signatures persist despite dietary
1132 variation. PLoS One. doi:10.1371/journal.pone.0003591.

1133 Kwak J, Willse A, Preti G, Yamazaki K, Beauchamp GK. 2010. In search of the
1134 chemical basis for MHC odourtypes. In: Proceedings of the Royal Society B:
1135 Biological Sciences.

1136 Lanyon C V., Rushton SP, O'Donnell AG, Goodfellow M, Ward AC, Petrie M, Jensen
1137 SP, Morris Gosling L, Penn DJ. 2007. Murine scent mark microbial communities are
1138 genetically determined. FEMS Microbiol Ecol. doi:10.1111/j.1574-
1139 6941.2006.00252.x.

1140 Leclaire S, Van Dongen WFD, Voccia S, Merkling T, Ducamp C, Hatch SA,
1141 Blanchard P, Danchin É, Wagner RH. 2014. Preen secretions encode information on
1142 MHC similarity in certain sex-dyads in a monogamous seabird. Sci Rep.
1143 doi:10.1038/srep06920.

1144 Leclaire S, Jacob S, Greene LK, Dubay GR, Drea CM. 2017. Social odours covary
1145 with bacterial community in the anal secretions of wild meerkats. Sci Rep.
1146 doi:10.1038/s41598-017-03356-x.

1147 Leclaire S, Strandh M, Dell'Ariccia G, Gabirot M, Westerdahl H, Bonadonna F. 2019.
1148 Plumage microbiota covaries with the major histocompatibility complex in blue
1149 petrels. Mol Ecol. doi:10.1111/mec.14993.

1150 Leinders-Zufall T, Brennan P, Widmayer P, Chandramani S. P, Maul-Pavicic A, Jäger
1151 M, Li XH, Breer H, Zufall F, Boehm T. 2004. MHC class I peptides as chemosensory
1152 signals in the vomeronasal organ. Science (80-). doi:10.1126/science.1102818.

1153 Lenz TL, Eizaguirre C, Scharsack JP, Kalbe M, Milinski M. 2009. Disentangling the
1154 role of MHC-dependent "good genes" and "compatible genes" in mate-choice
1155

1156 decisions of three-spined sticklebacks Gasterosteus aculeatus under semi-natural
1157 conditions. J Fish Biol. doi:10.1111/j.1095-8649.2009.02410.x.

1158 Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. 2008. Worlds within worlds:
1159 Evolution of the vertebrate gut microbiota. Nat Rev Microbiol.
1160 doi:10.1038/nrmicro1978.

1161 Lively CM, de Roode JC, Duffy MA, Graham AL, Koskella B. 2014. Interesting open
1162 questions in disease ecology and evolution. Am Nat. doi:10.1086/677032.

1163 Luckey TD. 1972. Introduction to intestinal microecology. Am J Clin Nutr.

1164 Maraci Ö, Engel K, Caspers BA. 2018. Olfactory communication via microbiota: what
1165 is known in birds? Genes (Basel). doi:10.3390/genes9080387.

1166 Marietta E, Rishi A, Taneja V. 2015. Immunogenetic control of the intestinal
1167 microbiota. Immunology. doi:10.1111/imm.12474.

1168 Marneweck C, Jürgens A, Shrader AM. 2017. Dung odours signal sex, age, territorial
1169 and oestrous state in white rhinos. Proc R Soc B Biol Sci.
1170 doi:10.1098/rspb.2016.2376.

1171 Martín-Vivaldi M, Peña A, Peralta-Sánchez JM, Sánchez L, Ananou S, Ruiz-
1172 Rodríguez M, Soler JJ. 2010. Antimicrobial chemicals in hoopoe preen secretions are
1173 produced by symbiotic bacteria. Proc R Soc B Biol Sci. doi:10.1098/rspb.2009.1377.

1174 Mason RT, Parker MR. 2010. Social behavior and pheromonal communication in
1175 reptiles. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol.
1176 doi:10.1007/s00359-010-0551-3.

1177 Migalska M, Sebastian A, Radwan J. 2019. Major histocompatibility complex class i
1178 diversity limits the repertoire of T cell receptors. Proc Natl Acad Sci U S A.
1179

1180 doi:10.1073/pnas.1807864116.

1181 Milinski M, Croy I, Hummel T, Boehm T. 2013. Major histocompatibility complex
1182 peptide ligands as olfactory cues in human body odour assessment. Proc R Soc B
1183 Biol Sci. doi:10.1098/rspb.2012.2889.

1184 Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, Boehm T.
1185 2005. Mate choice decisions of stickleback females predictably modified by MHC
1186 peptide ligands. Proc Natl Acad Sci U S A. doi:10.1073/pnas.0408264102.

1187 Milinski M, Griffiths SW, Reusch TBH, Boehm T. 2010. Costly major
1188 histocompatibility complex signals produced only by reproductively active males, but
1189 not females, must be validated by a “maleness signal” in three-spined sticklebacks.
1190 Proc R Soc B Biol Sci. doi:10.1098/rspb.2009.1501.

1191 Mitchell J, Cant MA, Vitikainen EIK, Nichols HJ. 2017. Smelling fit: Scent marking
1192 exposes parasitic infection status in the banded mongoose. Curr Zool.
1193 doi:10.1093/cz/zox003.

1194 Møller AP, Alatalo R V. 1999. Good-genes effects in sexual selection. Proc R Soc B
1195 Biol Sci. doi:10.1098/rspb.1999.0607.

1196 Nakagawa S, Lagisz M. 2019. How good does our map of knowledge have to be?: A
1197 comment on Berger-Tal et al. Behav Ecol. doi:10.1093/beheco/ary137.

1198 Nakagawa S, Noble DWA, Senior AM, Lagisz M. 2017. Meta-evaluation of meta-
1199 analysis: Ten appraisal questions for biologists. BMC Biol. doi:10.1186/s12915-017-
1200 0357-7.

1201 Neefjes J, Jongsma MLM, Paul P, Bakke O. 2011. Towards a systems understanding
1202 of MHC class I and MHC class II antigen presentation. Nat Rev Immunol.
1203 11(12):823–836. doi:10.1038/nri3084.

1204 Nowak MA, Tarczy-Hornoch K, Austyn JM. 1992. The optimal number of major
1205 histocompatibility complex molecules in an individual. Proc Natl Acad Sci U S A.
1206 doi:10.1073/pnas.89.22.10896.

1207 Oh J, Conlan S, Polley EC, Segre JA, Kong HH. 2012. Shifts in human skin and
1208 nares microbiota of healthy children and adults. Genome Med. doi:10.1186/gm378.

1209 Olsén KH, Grahn M, Lohm J, Langefors Å. 1998. MHC and kin discrimination in
1210 juvenile Arctic chart, Salvelinus alpinus (L.). Anim Behav.
1211 doi:10.1006/anbe.1998.0837.

1212 Overath P, Sturm T, Rammensee HG. 2014. Of volatiles and peptides: In search for
1213 MHC-dependent olfactory signals in social communication. Cell Mol Life Sci.
1214 doi:10.1007/s00018-014-1559-6.

1215 Pabst O. 2012. New concepts in the generation and functions of IgA. Nat Rev
1216 Immunol. doi:10.1038/nri3322.

1217 Pasparakis M, Haase I, Nestle FO. 2014. Mechanisms regulating skin immunity and
1218 inflammation. Nat Rev Immunol. doi:10.1038/nri3646.

1219 Pearce DS, Hoover BA, Jennings S, Nevitt GA, Docherty KM. 2017. Morphological
1220 and genetic factors shape the microbiome of a seabird species (Oceanodroma
1221 leucorhoa) more than environmental and social factors. Microbiome.
1222 doi:10.1186/s40168-017-0365-4.

1223 Pearse-Pratt R, Schellinck H, Brown R, Singh PB, Roser B. 1998. Soluble MHC
1224 antigens and olfactory recognition of genetic individuality: The mechanism. Genetica.
1225 doi:10.1023/A:1026489524199.

1226 Penn D, Potts WK. 1998a. How do major histocompatibility complex genes influence
1227 odor and mating preferences? Adv Immunol. doi:10.1016/s0065-2776(08)60612-4.

Penn D, Potts WK. 1998b. Untrained mice discriminate MHC-determined odors. Physiol Behav. doi:10.1016/S0031-9384(98)00052-3.

Perez-Muñoz ME, Arrieta MC, Ramer-Tait AE, Walter J. 2017. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: Implications for research on the pioneer infant microbiome. Microbiome. doi:10.1186/s40168-017-0268-4.

Peterson DA, McNulty NP, Guruge JL, Gordon JI. 2007. IgA Response to Symbiotic Bacteria as a Mediator of Gut Homeostasis. Cell Host Microbe. doi:10.1016/j.chom.2007.09.013.

Pierini F, Lenz TL. 2018. Divergent allele advantage at human MHC genes: Signatures of past and ongoing selection. Mol Biol Evol. doi:10.1093/molbev/msy116.

Pusey A, Wolf M. 1996. Inbreeding avoidance in animals. Trends Ecol Evol. doi:10.1016/0169-5347(96)10028-8.

Rajakaruna RS, Brown JA, Kaukinen KH, Miller KM. 2006. Major histocompatibility complex and kin discrimination in Atlantic salmon and brook trout. Mol Ecol. doi:10.1111/j.1365-294X.2006.03113.x.

Rammensee HG, Bachmann J, Emmerich NPN, Bachor OA, Stevanović S. 1999. SYFPEITHI: Database for MHC ligands and peptide motifs. Immunogenetics. doi:10.1007/s002510050595.

Rammensee HG, Bachmann J, Stevanović S. 2013. MHC ligands and peptide motifs. Springer Science & Business Media.

Rao X, Hoof I, Fontaine Costa AICA, Van Baarle D, Keşmir C. 2011. HLA class i allele promiscuity revisited. Immunogenetics. doi:10.1007/s00251-011-0552-6.

Reusch TBH, Häberli MA, Aeschlimann PB, Milinski M. 2001. Female sticklebacks

count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature. doi:10.1038/35104547.

Rosshart SP, Vassallo BG, Angeletti D, Hutchinson DS, Morgan AP, Takeda K, Hickman HD, McCulloch JA, Badger JH, Ajami NJ, et al. 2017. Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell. doi:10.1016/j.cell.2017.09.016.

Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, et al. 2018. Environment dominates over host genetics in shaping human gut microbiota. Nature. doi:10.1038/nature25973.

Ruff JS, Nelson AC, Kubinak JL, Potts WK. 2012. MHC signaling during social communication. Adv Exp Med Biol. doi:10.1007/978-1-4614-1680-7_17.

Schellinck HM, Monahan E, Brown RE, Maxson SC. 1993. A comparison of the contribution of the major histocompatibility complex (MHC) and Y chromosomes to the discriminability of individual urine odors of mice by Long-Evans rats. Behav Genet. doi:10.1007/BF01082464.

Schellinck HM, Slotnick BM, Brown RE. 1997. Odors of individuality originating from the major histocompatibility complex are masked by diet cues in the urine of rats. Anim Learn Behav. doi:10.3758/BF03199058.

Scordato ES, Dubay G, Drea CM. 2007. Chemical composition of scent marks in the ringtailed lemur (Lemur catta): Glandular differences, seasonal variation, and individual signatures. Chem Senses. 32(5):493–504. doi:10.1093/chemse/bjm018.

Setchell JM, Vaglio S, Abbott KM, Moggi-Cecchi J, Boscaro F, Pieraccini G, Knapp LA. 2011. Odour signals major histocompatibility complex genotype in an Old World monkey. In: Proceedings of the Royal Society B: Biological Sciences.

Simons RR, Felgenhauer BE, Jaeger RG. 1994. Salamander scent marks: Site of production and their role in territorial defence. Anim Behav. doi:10.1006/anbe.1994.1215.

Singer AG, Beauchamp GK, Yamazaki K. 1997. Volatile signals of the major histocompatibility complex in male mouse urine. Proc Natl Acad Sci U S A. doi:10.1073/pnas.94.6.2210.

Singer AG, Tsuchiya H, Wellington JL, Beauchamp GK, Yamazaki K. 1993. Chemistry of odortypes in mice: Fractionation and bioassay. J Chem Ecol. doi:10.1007/BF00994326.

Singh PB, Herbert J, Roser B, Arnott L, Tucker DK, Brown RE. 1990. Rearing rats in a germ-free environment eliminates their odors of individuality. J Chem Ecol. doi:10.1007/BF01014099.

Slade JWG, Watson MJ, Kelly TR, Gloor GB, Bernards MA, Macdougall-Shackleton EA. 2016. Chemical composition of preen wax reflects major histocompatibility complex similarity in songbirds. Proc R Soc B Biol Sci. doi:10.1098/rspb.2016.1966.

Smith T. 2006. Individual Olfactory Signatures in Common Marmosets (Callithrix jacchus). Am J Primatol. doi:10.1002/ajp.

Spehr M, Kelliher KR, Li XH, Boehm T, Leinders-Zufall T, Zufall F. 2006. Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. J Neurosci. doi:10.1523/JNEUROSCI.4939-05.2006.

Spence-Aizenberg A, Kimball BA, Williams LE, Fernandez-Duque E. 2018. Chemical composition of glandular secretions from a pair-living monogamous primate: Sex, age, and gland differences in captive and wild owl monkeys (Aotus spp.). Am J Primatol. doi:10.1002/ajp.22730.

1299 Spor A, Koren O, Ley R. 2011. Unravelling the effects of the environment and host
1300 genotype on the gut microbiome. Nat Rev Microbiol. doi:10.1038/nrmicro2540.

1301 Sprent J. 1984. T/B collaboration in vivo vs. in vitro. Ann l'Institut Pasteur - Immunol.
1302 doi:10.1016/S0769-2625(84)80085-9.

1303 Stewart JA, Chadwick VS, Murray A. 2005. Investigations into the influence of host
1304 genetics on the predominant eubacteria in the faecal microflora of children. J Med
1305 Microbiol. doi:10.1099/jmm.0.46189-0.

1306 Stoffel MA, Caspers BA, Forcada J, Giannakara A, Baier M, Eberhart-Phillips L,
1307 Müller C, Hoffman JI. 2015. Chemical fingerprints encode mother-offspring similarity,
1308 colony membership, relatedness, and genetic quality in fur seals. Proc Natl Acad Sci
1309 U S A. doi:10.1073/pnas.1506076112.

1310 Strauch UG, Obermeier F, Grunwald N, Gürster S, Dunger N, Schultz M, Griese DP,
1311 Mähler M, Schölmerich J, Rath HC. 2005. Influence of intestinal bacteria on induction
1312 of regulatory T cells: Lessons from a transfer model of colitis. Gut.
1313 doi:10.1136/gut.2004.059451.

1314 Sylvain FÉ, Derome N. 2017. Vertically and horizontally transmitted microbial
1315 symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish
1316 progeny. Sci Rep. doi:10.1038/s41598-017-05662-w.

1317 Teahon K, Webster AD, Price AB, Weston J, Bjarnason I. 1994. Studies on the
1318 enteropathy associated with primary hypogammaglobulinaemia. Gut.
1319 doi:10.1136/gut.35.9.1244.

1320 Theis KR, Schmidt TM, Holekamp KE. 2012. Evidence for a bacterial mechanism for
1321 group-specific social odors among hyenas. Sci Rep. doi:10.1038/srep00615.

1322 Theis KR, Venkataraman A, Dycus JA, Koonter KD, Schmitt-Matzen EN, Wagner AP,

Holekamp KE, Schmidt TM. 2013. Symbiotic bacteria appear to mediate hyena social odors. Proc Natl Acad Sci U S A. doi:10.1073/pnas.1306477110.

Toivanen P, Vaahtovuo J, Eerola E. 2001. Influence of major histocompatibility complex on bacterial composition of fecal flora. Infect Immun. doi:10.1128/IAI.69.4.2372-2377.2001.

Tremaroli V, Bäckhed F. 2012. Functional interactions between the gut microbiota and host metabolism. Nature. doi:10.1038/nature11552.

Trevelline BK, MacLeod KJ, Knutie SA, Langkilde T, Kohl KD. 2018. In ovo microbial communities: A potential mechanism for the initial acquisition of gut microbiota among oviparous birds and lizards. Biol Lett. doi:10.1098/rsbl.2018.0225.

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. 2009. A core gut microbiome in obese and lean twins. Nature. doi:10.1038/nature07540.

Vaglio S, Minicozzi P, Romoli R, Boscaro F, Pieraccini G, Moneti G, Moggi-Cecchi J. 2016. Sternal gland scent-marking signals sex, age, rank, and group identity in captive mandrills. Chem Senses. doi:10.1093/chemse/bjv077.

Wadud Khan MA, Zac Stephens W, Mohammed AD, Round JL, Kubinak JL. 2019. Does MHC heterozygosity influence microbiota form and function? PLoS One. doi:10.1371/journal.pone.0215946.

Wegner KM, Kalbe M, Kurtz J, Reusch TBH, Milinski M. 2003. Parasite selection for immunogenetic optimality. Science (80-). doi:10.1126/science.1088293.

Wegner KM, Reusch TBH, Kalbe M. 2003. Multiple parasites are driving major histocompatibility complex polymorphism in the wild. J Evol Biol. doi:10.1046/j.1420-9101.2003.00519.x.

1347 Wegner KM, Shama LNS, Kellnreitner F, Pockberger M. 2012. Diversity of immune
1348 genes and associated gill microbes of European plaice Pleuronectes platessa. Estuar
1349 Coast Shelf Sci. doi:10.1016/j.ecss.2011.09.001.

1350 Whittaker DJ, Gerlach NM, Slowinski SP, Corcoran KP, Winters AD, Soini HA,
1351 Novotny M V., Ketterson ED, Theis KR. 2016. Social environment has a primary
1352 influence on the microbial and odor profiles of a chemically signaling songbird. Front
1353 Ecol Evol. doi:10.3389/fevo.2016.00090.

1354 Whittaker DJ, Reichard DG, Drouilly M, Battle K, Ziegenfus C. 2014. Avian olfactory
1355 displays: a hypothesis for the function of bill-wiping in a social context. Behav Ecol
1356 Sociobiol. doi:10.1007/s00265-014-1829-1.

1357 Whittaker DJ, Slowinski SP, Greenberg JM, Alian O, Winters AD, Ahmad MM, Burrell
1358 MJE, Soini HA, Novotny M V., Ketterson ED, et al. 2019. Experimental evidence that
1359 symbiotic bacteria produce chemical cues in a songbird. J Exp Biol.
1360 doi:10.1242/jeb.202978.

1361 Willse A, Kwak J, Yamazaki K, Preti G, Wahl JH, Beauchamp GK. 2006. Individual
1362 odortypes: Interaction of MHC and background genes. Immunogenetics.
1363 doi:10.1007/s00251-006-0162-x.

1364 Winternitz J, Abbate J. 2015. Examining the evidence for major histocompatibility
1365 complex-dependent mate selection in humans and nonhuman primates. Res Rep
1366 Biol. doi:10.2147/rrb.s58514.

1367 Woelfing B, Traulsen A, Milinski M, Boehm T. 2009. Does intra-individual major
1368 histocompatibility complex diversity keep a golden mean? Philos Trans R Soc B Biol
1369 Sci. doi:10.1098/rstb.2008.0174.

1370 Woodhams DC, Bletz MC, Becker CG, Bender HA, Buitrago-Rosas D, Diebboll H,

Huynh R, Kearns PJ, Kueneman J, Kurosawa E, et al. 2020. Publisher Correction: Host-associated microbiomes are predicted by immune system complexity and climate (Genome Biology (2020) 21 (23) DOI: 10.1186/s13059-019-1908-8). Genome Biol. doi:10.1186/s13059-020-01955-y.

Yamaguchi MS, Ganz HH, Cho AW, Zaw TH, Jospin G, McCartney MM, Davis CE, Eisen JA, Coil DA. 2019. Bacteria isolated from Bengal cat (Felis catus × Prionailurus bengalensis) anal sac secretions produce volatile compounds potentially associated with animal signaling. PLoS One. doi:10.1371/journal.pone.0216846.

Yamazaki K, Beauchamp GK, Imai Y, Bard J, Boyse EA. 1992. Expression of urinary H-2 odortypes by infant mice. Proc Natl Acad Sci U S A. doi:10.1073/pnas.89.7.2756.

Yamazaki K, Beauchamp GK, Kupniewski D, Bard J, Thomas L, Boyse EA. 1988. Familial imprinting determines H-2 selective mating preferences. Sci Sci. doi:10.1126/science.3375818.

Zala SM, Potts WK, Penn DJ. 2004. Scent-marking displays provide honest signals of health and infection. Behav Ecol. doi:10.1093/beheco/arh022.

Zoetendal EG, Akkermans ADL, Akkermans-van Vliet WM, De Visser JAGM, De Vos WM. 2001. The host genotype affects the bacterial community in the human gastrointestinal tract. Microb Ecol Health Dis. doi:10.1080/089106001750462669.

Zomer S, Dixon SJ, Xu Y, Jensen SP, Wang H, Lanyon C V., O'Donnell AG, Clare AS, Gosling LM, Penn DJ, et al. 2009. Consensus multivariate methods in gas chromatography mass spectrometry and denaturing gradient gel electrophoresis: MHC-congenic and other strains of mice can be classified according to the profiles of volatiles and microflora in their scent-marks. Analyst. doi:10.1039/b807061j.

Figure captions

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 non-volatile 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.

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.

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.

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.

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 non-significant 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 non-significant relationship between MHC and microbiota diversity (“n.s.”).

Figures

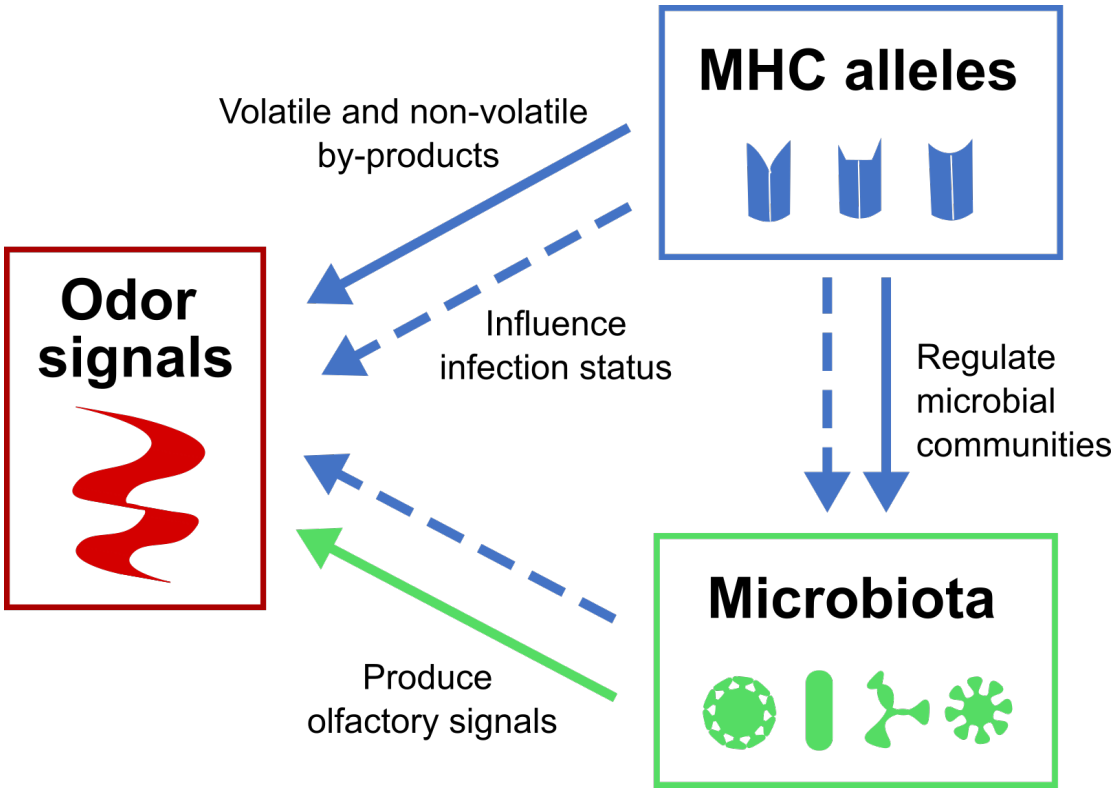


Figure 1

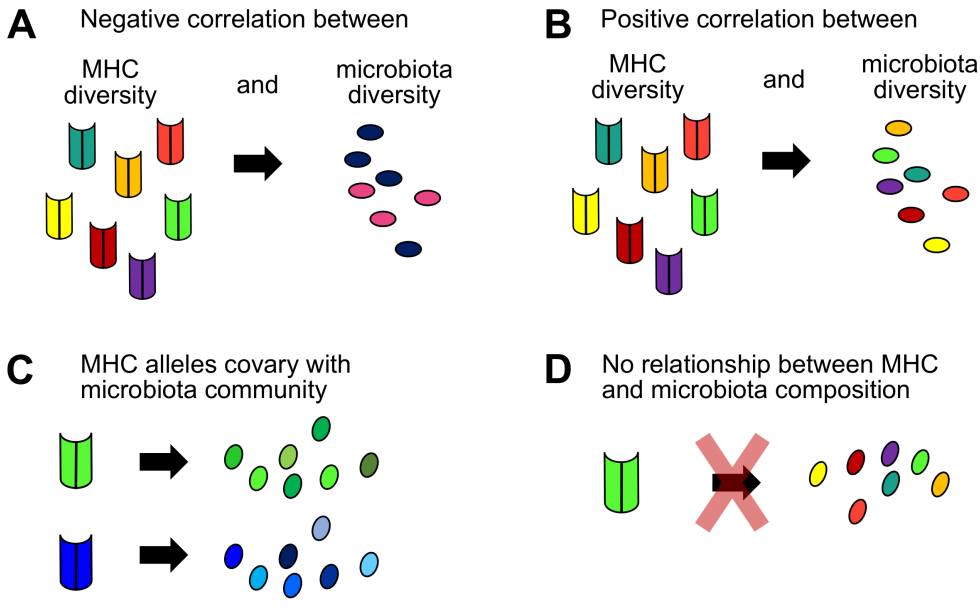
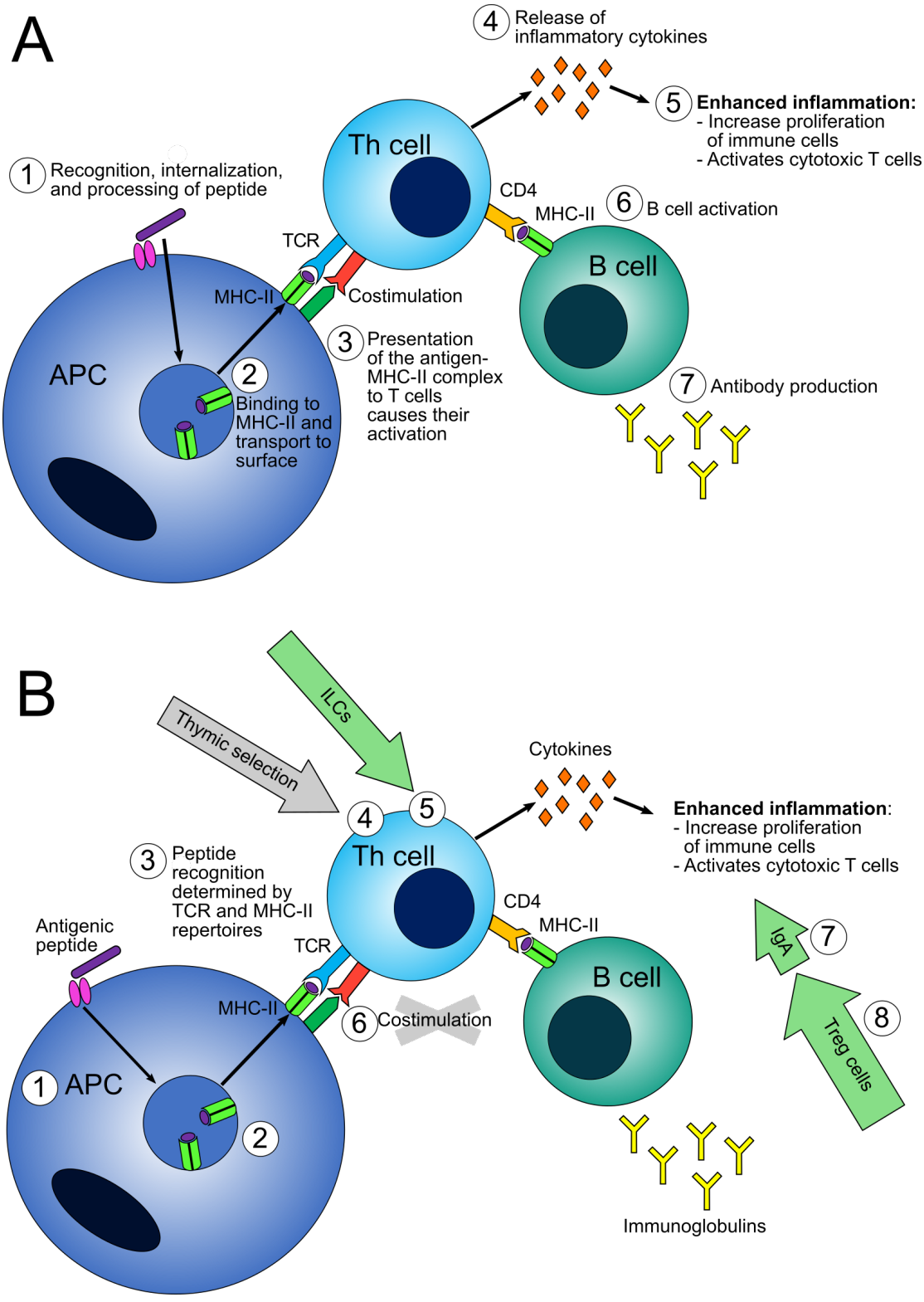


Figure 2



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1472 Figure 3

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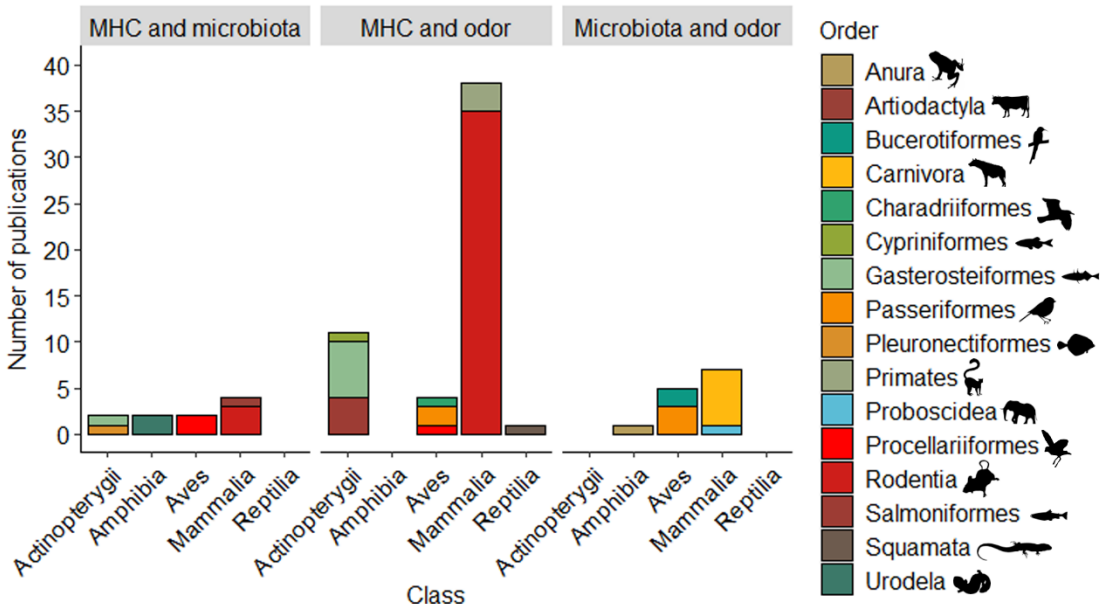

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1475 Figure 4

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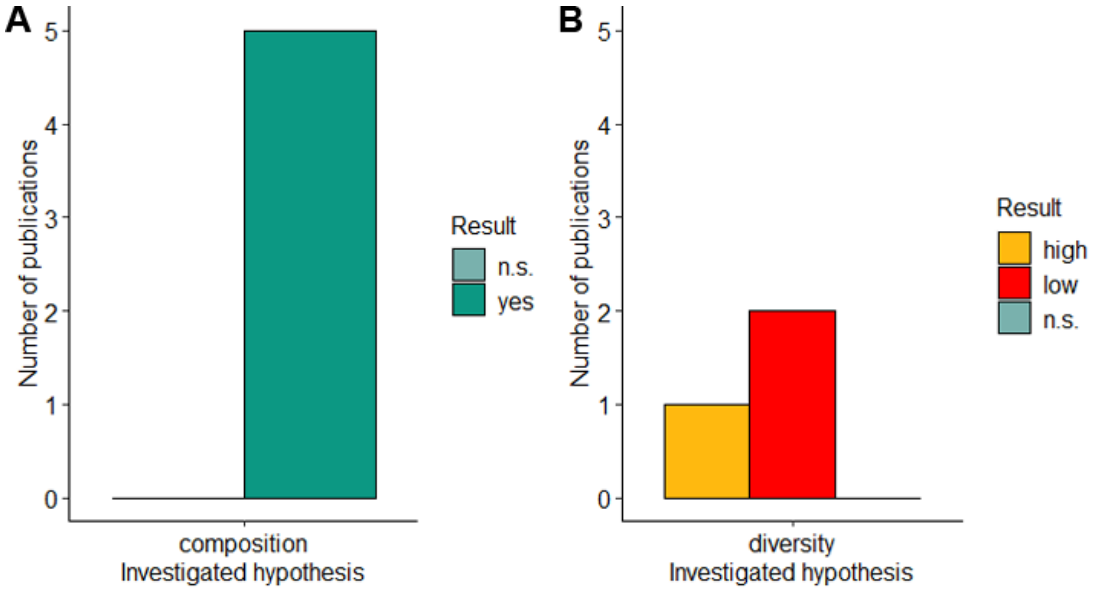

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1478 Figure 5