

## **Let's keep alternative hypotheses on the table: a response to comments on Schubert et al.**

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Odor-based communication between animals is widespread and both the major histocompatibility complex (MHC) and microbiota have been implicated in governing chemical cues relevant for social communication. A proposed sequence of links, defined succinctly in the commentary by Milinski (2021), proceeds as MHC–microbiota–odor signal. Our review (Schubert et al. 2021) aims to summarize the existing evidence for the involvement of microbiota in generating odors that could be used for social communication and the potential involvement of the MHC in governing microbiota. Reiterated in commentaries on our review (Leclaire 2021; Milinski 2021), we emphasize that the existence as well as the underlying mechanism of the proposed pathway have yet to be settled by research, therefore a key component of our review is suggesting ways to thoroughly test the MHC–microbiota–odor signal sequence. We appreciate the unique perspectives of the commentaries of our review, respond to points of contention and consensus, and highlight important points for consideration for future studies.

First, the line attributed to the last paragraph in our review (Schubert et al. 2021) is misquoted in the commentary by Milinski (2021). The actual phrasing is “... we hope that this review stimulates advances in the investigation and understanding of this potential key pathway for social communication.” We emphasize that we consider the MHC–microbiota–odor signal only a potential key pathway for social communication, and we believe that rigorous investigation with a clear outline of hypotheses will allow research to settle this question. It is true that none of the 577 publications retrieved by our systematic search investigated the interaction of all three components (MHC, odor, and microbiota) and so causal links showing odor generated via MHC-mediated microbiota to be the social signal are absent (Milinski 2021). Indeed, our review highlights the need to investigate the links between all three components in focal species, which is necessary to demonstrate whether odor produced by microbiota signals MHC genotype and is socially relevant. It would be profitable to focus research efforts on systems that allow controlling for confounding maternal (to an extent), environmental, and background genomic effects (Maraci and Caspers 2021; Whittaker 2021) for example, using congenic birds with well-characterized MHC (Leclaire 2021; Maraci and Caspers 2021). Care must also be taken so that lab

environments do not create artificial selective pressure on microbiota that overrides the effect of host genotype (Whittaker 2021).

A second criticism by Milinski (2021) assumes that odor signals are used during MHC-mediated mate choice for dissimilar/complimentary mates to produce optimally diverse offspring with highest immunocompetence. While this has been elegantly shown in studies on three-spined sticklebacks (Milinski et al. 2005), meta-analyses have indicated that mate choice for MHC-dissimilarity is not universal for all species (Kamiya et al. 2014; Winternitz et al. 2017), nor even for all populations within a species (Winternitz et al. 2017). Instead, preferences for MHC-diverse individuals appear more common (Kamiya et al. 2014; Winternitz et al. 2017). Therefore, since many females do not base their mate preferences on dissimilarity, not all females would need to know the odors of all microorganisms present and absent during mate choice. Preferences for diversity, for instance, could be realized by perceiving more complex odor profiles present in MHC heterozygous individuals.

A final criticism asks why we should consider other MHC signaling mechanisms when an established mechanism that provides allele-specificity has already been identified: peptide ligand-based odor signals (Milinski 2021). We argue that this mechanism has not yet been shown to apply broadly and its generality is unclear, considering many studies documenting alleles with largely overlapping peptide sets (e.g., Rao et al. 2011). If alleles cannot be reliably distinguished by the peptides they release, then in these species there is no adaptive benefit for peptide-based odor signaling specific alleles and so it is not expected to evolve. Indeed, depending on the social system affecting MHC-based social communication, allele-specificity in odor signals may not be required. For example, in familiarity-based systems, recognizing the smell of littermates could serve as a sufficient “rule of thumb” for individuals to detect and discriminate kin for cooperative and mating interactions.

Why would multiple signaling mechanisms that transmit the same information evolve (Milinski 2021)? Other researchers have speculated that complementary chemosignals (volatiles and nonvolatile peptides) are useful in unpredictable environments where together they provide more robust signals and can transmit information at different distances or in different behavioral contexts (Restrepo et al. 2006). More generally, many examples of multimodal mating signals exist and are thought to have evolved because they serve as “back-ups” or convey “multiple messages” or “unique messages” when deployed simultaneously. Microbiota may amplify signals from peptides through

metabolization/volatilization, they may muddy signals as a side effect of their existence, or they may simply not affect the signals of the MHC at all. We do not take sides in our review nor do we claim to know whether MHC-mediated microbiota contribute to social odor or not, in consensus with other commentaries that skepticism is important (Leclaire 2021; Milinski 2021).

If we want to rigorously test the MHC–microbe–social odor sequence, we agree that studies should aim for fine-scale characterization of the chemical odor profiles using state of the art methods (Leclaire 2021). In this vein, computer learning methods (e.g., Artificial Intelligence) might be valuable for chemosignal characterization and prediction methods to identify microbes and MHC alleles underlying specific chemical signatures. If clear links can be established, then they still must be confirmed. This could be demonstrated, for example, by using knock-out studies of MHC alleles or modifying their expression (Leclaire 2021), identifying consequent changes in odor profile via changes in microbial community, and then validating that changes in odor profile mediate changes in physiology or behavior. With recent developments in chemical analytical technology and data science, substantial progress can be made to identify the mechanistic basis of social odors.

## FUNDING

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

Conflict of Interest: We declare we have no competing interests.

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