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Original Article

The Intensity of Human Body Odors and the MHC: Should We Expect A Link?

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Abstract: It is now well established that genes within the major histocompatibility complex (MHC) somehow affect the production of body odors in several vertebrates, including humans. Here we discuss whether variation in the intensity of body odors may be influenced by the MHC. In order to examine this question, we have to control for MHC-linked odor perception on the smeller's side. Such a control is necessary because the perception of pleasantness and intensity seem to be confounded, and the causalities are still unsolved. It has previously been found that intense odors are scored as less pleasant if the signaler and the receiver are of MHC-dissimilar type, but not if they are of MHC similar type. We argue, and first data suggest, that an effect of the degree of MHC-heterozygosity and odor intensity is likely (MHC-homozygotes may normally smell more intense), while there is currently no strong argument for other possible links between the MHC and body odor intensity.

Keywords: Odor intensity, MHC homo- and heterozygosity, 'good genes' hypothesis, 'compatible genes' hypothesis, T-shirt experiment.

Introduction

The physiology and the biological significance of human body odors is multifaceted and not yet well understood yet (Beauchamp and Yamazaki, 2005; Grammer et al., 2005; McClintock et al., 2005; Yamazaki and Beauchamp, 2005; Ziegler et al., 2005). The reason for this lack of knowledge seems obvious: odors are often short-lived and difficult to store, hard to describe, and still very difficult to analyse chemically, at least in the quantities that can be relevant in social contexts.

Despite these analytical problems and the shortage of hard data, humans seem to vary in the intensity of their odors, even if we correct for behavioral and environmental factors. Among humans, there is variation in the size and the number of glands on the skin (Stoddart, 1990), variation in gland activity (Kreyden et al., 2002), and obvious variation in the number and density of those body hairs that amplify odors through their support of microorganisms and their role in odor diffusion. However, our personal perception can easily mislead us when it comes to the generality of odor quality and intensity.

Whether or not an odor is perceived as intense depends not only on the kind and the amount of volatile molecules that are emitted (the strength of the signal), but also on factors that influence the perception of the odor (the sensitivity of the receiver). With regard to the latter, there are many factors that may play a role. For example, it seems that women are usually more sensitive to body odors than men (Dalton et al., 2002), and that the sensitivity for odor components can be trained in women during their reproductive age (Dalton et al., 2002). Also, the perception of body odors varies within the menstrual cycle (Doty et al., 1981; Gangestad and Thornhill, 1998; Rikowski and Grammer, 1999), changes with pregnancy (Gilbert and Wysocki, 1991), generally decreases with age (Wedekind and Füri, 1997) and appears to be influenced by the contraceptive pill (Wedekind et al., 1995; Thorne et al., 2002).

The perception of body odors is also linked to genes of the MHC (Yamazaki et al., 1976; Penn and Potts, 1999; Ziegler et al., 2005). These genes can be quite important in explaining odor preferences. In T-shirt experiments, when the influence of many potentially confounding variables is reduced, up to 23 % of the variance in pleasantness could be explained by the degree of similarity at the loci of the MHC between T-shirt wearer and smeller (Wedekind & Füri 1997). In mice, the estimates are even higher and reach up to 50% (Ziegler et al., 2005). The intensity of one and the same body odor can be perceived very differently by different individuals. In Wedekind & Füri (1997), when 121 students rated the same six odors for intensity, pleasantness and sexiness, all the odors received nearly all possible scores from very weak to very intense. In one case (Wedekind et al. 1995), a women even reported nausea for several hours after smelling the experimental T-shirts. She specifically linked her nausea to one odor which she rated as very intense and very unpleasant, while other smellers scored this particular odor as less intense and quite pleasant. In this context, it may be interesting that this woman was using the contraceptive pill at the time of the experiment. Pregnancy, which is partly simulated by the pill, is often linked to nausea and vomiting as a response to odors (Heinrichs, 2002).

Wedekind et al. (1995) found that the perception of pleasantness and of intensity of an odor are correlated if smeller and odor source have dissimilar MHC types, but not if they are of similar MHC type. Since non-related individuals are normally dissimilar at the MHC , we would expect that, as a general tendency, weaker body odors are normally rated as more pleasant than more intense ones. This expectation was confirmed by Wedekind & Füri (1997). However, the causality

remains unclear. Is an odor perceived as unpleasant because it is intense, or as intense because it is unpleasant?

Not controlling for MHC-linked odor perception can introduce disturbing biases in an analysis of odor intensity. Vice versa, a lack of control for odor intensity can confound or weaken an analysis of MHC-dependent preferences (Wedekind and Seebeck, 1996; Wedekind, 2002; Roberts et al., 2005). It is therefore necessary to disentangle possible MHC effects on odor intensity from MHC-dependent odor perception. In a previous study we used an experimental method that allows to test for, and to control for, MHC-dependent variation in odor perception within a population (Wedekind et al., 1995). Here we use the data of Wedekind et al. (1995) to analyse, on the one hand, intensity ratings of receivers that are either dissimilar or similar to the signaller's MHC, i.e. we control for the effects of the MHC by analysing possible effects within the two experimental groups. On the other hand, we calculate MHC-neutral odor scores that can be used as estimates of the average intensity of body odors. We use these neutral scores to ask whether the intensity of a man's odor is in any way connected to *his* MHC genotype. We test three questions: 1) Is the degree of MHC heterozygosity linked to odor intensity? 2) Are the odors of carriers of most common MHC antigens generally perceived as differently intense than other body odors? 3) Are men with more intense odors also more similar to each other in their MHC genotypes than expected by chance? Effect size approximations are used to quantify the observed trends in the data and to estimate the significance of each possible link between MHC and odors intensity. We use our analyses to discuss several hypotheses on the biology of MHC-linked odor signalling in humans.

Methods

The 38 men whose odors are studied here were on average 24.5 years old (SD = 2.2) and students of the University of Bern at the time of the experiments. They all appeared to be of Caucasian origin, spoke the Swiss German dialect without any obvious accents, and had forenames and surnames that are common in the German speaking part of Switzerland. They were typed for their MHC (HLA-A, -B, and –DR) and asked to wear T-shirts during two nights. The odors of these worn T-shirts were subsequently evaluated by women who had an MHC type that was either similar (on average 2.7 dissimilar antigens) or dissimilar (on average 5.9 dissimilar antigens) to the T-shirt wearer's MHC. See Wedekind et al. (1995) for a detailed description of the methods and for an analysis of the general effects of the MHC on these odors and on female preferences for them.

We use three procedures to control for MHC-linked odor preferences. First, there is evidence for an interaction between MHC-linked preferences and the contraceptive pill (Wedekind et al., 1995). Since the exact nature of this interaction is not fully understood yet, we only used evaluations of women who did not use the contraceptive pill during the time of the experiments (we could therefore only use a subset of the data of Wedekind et al. (1995) in the present analyses). Second, each

odor was evaluated by on average 4.7 women (range 2 to 12) and at least once each by a woman with similar or dissimilar MHC type, respectively. We tested for possible links between odor intensity and the MHC within each to the two experimental groups ("similar" and "dissimilar"). Third, we averaged the ratings of all MHC-similar and MHC-dissimilar women and used the mean of these two average ratings to obtain scores that includes the ratings of many women but are neutral with respect to the degree of MHC similarity between the man and the smelling women.

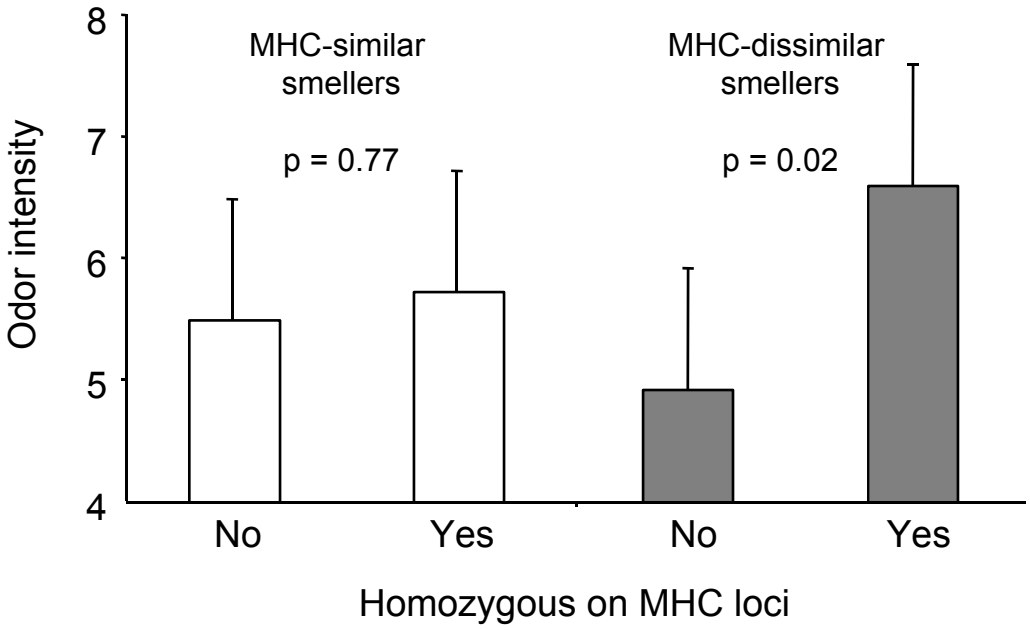
We used parametric test statistics when graphical inspections of the data suggested that the respective model assumptions were met. In order to test whether the men's MHC type is correlated to the intensity of their body odors, we used the following test procedures: (i) We tested whether the degree of MHC heterozygosity is linked to odor intensity in two-sample t-tests; (ii) we used again two-sample t-tests to test whether the presence or absence of the most common MHC antigens in the study population was linked to the average odor intensity; (iii) we grouped the men into two groups of N=19 each according to their odor intensity ("strong" versus "weak"). We then tested whether these two groups also clustered with respect to the average degree of similarity on the MHC. This was done by comparing the mean number of dissimilar antigens of all pairwise comparisons within each group to a null expectancy. This null expectancy was derived from 1000 random permutations of the above procedure (randomization test). Power analyses for two-sample t-tests were done online on www.stat.uiowa.edu/~rlenth/Power/, assuming equal variances and equal sample sizes.

Results

Of the 38 men tested, 17 (45%) were typed as homozygous for at least one MHC antigen. On average, their odors were not significantly more intense than those of MHC heterozygotes (Table 1). The estimated effect size (in average standard deviations) was $d = 0.552$. A power analysis revealed that if we missed an existing effect (type II error), one would have an 80% change of finding it at $p = 0.05$ if the sample size were about tripled (Table 1). However, three men were homozygous for two of the three loci, and their average body odor intensity was even slightly lower than the intensity of the other homozygotes.

Since we have two average scorings for each odor, one by MHC-similar and one by MHC-dissimilar smeller, we could also test for the effect of the signallers' MHC homozygosity within each group of smellers. We found that the intensity scores of MHC-similar smellers were not significantly influenced by homozygosities on the signaller's side, while MHC homozygotes smelled significantly more intense to MHC-dissimilar smellers than MHC heterozygotes (Figure 1). These intensity scores did not seem to be significantly influenced by the perception of pleasantness (within each experimental group, $|t|$ always < 0.82, p always > 0.42). However, when all mean scores per signaller were correlated, pleasant odors tended to be less intense ones (Spearman rank order correlation coefficient $r_s = -0.46$, $p = 0.003$).

Figure 1: The average odor intensity of T-shirts worn by men who are either heterozygous on all the MHC loci analysed, or who are homozygous on at least one of these loci. The figure gives the mean (+SE) scorings of MHC similar (open bars) and MHC-dissimilar smellers (closed bars) for the same 38 odors each. The p-values are two-tailed.



The most common MHC antigen in the population is by far HLA-A2, with a prevalence of 50% in the present sample, and around 50% in comparable samples (Grundschober et al. 1994, Wedekind & Füri 1997, Milinski and Wedekind 2001). We found no significant effect of this antigen (Table 1) or any of the eight other most common antigens that Milinski & Wedekind (2001) listed ($|t|$ always < 1.33, p always > 0.19). The group of homozygotes were not dominated by men who possessed HLA-A2 (only 37% of them are homozygous on any MHC locus, $\chi^2 = 0.47$, $p = 0.49$). Therefore, the second analysis in Table 1 is not confounded with the first. The estimated effect size for HLA-A2 as grouping factor was $d = 0.339$. We found analogous non-significant results if we tested for a possible effect of HLA-A2 within the scorings of the MHC-similar and MHC-dissimilar smellers, respectively ($|t|$ always < 0.8, p always > 0.42).

Men with more intense odors do not have significantly more similarities in their MHC genotypes than expected by chance (Table 1). The one-tailed randomization tests did not reveal any tendencies. It turned out that exactly half of all men with HLA-A2 were each in the group "intense" and the group "weak" ($\chi^2 = 0.0$, $p = 1.0$). Hence, the results of the third test in Table 1 can be seen as statistically independent of the second test.

Table 1: Tests for possible MHC effects on the intensity of male body odors. MHC-linked perception is controlled for by using weighted mean scorings of MHC-similar and MHC-dissimilar smellers.

Question	**Analysis***	**Test****	**N_{min}*****	**Conclusion if tendency indicated real effect**
(i) Effect of MHC-homozygosity? (17 of 38 men are homozygote)	$\mu_1 = 5.21$ (1.87) $\mu_2 = 6.17$ (1.59)	$t = 1.67$ $P_2 = 0.10$	106	MHC homozygotes would smell more intense than others.
(ii) Effects of most common MHC antigen? (18 of 38 men have HLA-A2)	$\mu_1 = 5.35$ (1.82) $\mu_2 = 5.96$ (1.76)	$t = 1.04$ $P_2 = 0.30$	276	Men with the most common MHC antigen would smell more intense than others.
(iii) Average similarities in MHC-types	Randomization test			
- among 19 men with relatively intense odors?	$\mu_{obs} = 4.62$, $\mu_{exp} = 4.61$	$P_1 = 0.51$		No tendency towards any clustering of MHC-type with odor intensity
- among 19 men with relatively weak odors?	$\mu_{obs} = 4.78$, $\mu_{exp} = 4.61$	$P_1 = 0.97$		

* Mean (SD)

** P_2 = two-tailed, P_1 = one-tailed

*** The number of male odors that would be needed to demonstrate an effect (if it exists) at $\alpha = 0.05$ with a probability of 80%.

Discussion

MHC-linked odor and mate preferences are typically seen as today's best example of sexual selection for compatible genes, i.e. for a choice of genetic complementarity (reviews in Mays and Hill, 2004; Neff and Pitcher, 2005). In such compatible-genes sexual selection models, the intensity of a signal is not necessarily expected to be linked to its information content (Trivers, 1972; Wedekind, 1994; Tregenza and Wedell, 2000). Alternatively, the so called "good-genes" models of sexual selection (Zahavi, 1975; Grafen, 1990; Møller and Alatalo, 1999) predict a correlation between signal intensity and health and vigor. The argument here is that health and vigor is expected to depend on overall genetic quality, and only individuals in good health and vigor can afford the costs of the signal.

We may often assume that the intensity of a signal is positively correlated

with health and vigor, because stronger signals are normally more expensive to produce or maintain. In the case of body odors, however, it is not immediately clear whether an odor that is perceived as intense (i) is an indicator of better overall health and vigor (Zahavi, 1975), or (ii) is perceived as intense because intensity rating is confounded with pleasantness rating, and unpleasant odors tend to be rated as intense regardless of the amount of volatiles that are involved. In the second case, high-quality odor components, i.e. components that are more expensive to the signaller and more attractive to the perceiver, may cause an odor to be rated as more pleasant and hence less intense. At the moment, we have to leave it open which scenario is more likely for human body odors. Nevertheless, the two types of sexual selection models mentioned above still make clear predictions for the case of body odors. If MHC-linked sexual selection is indeed about finding genetically compatible mates, we would not expect a link between odor intensity and the specificity of MHC antigens. If, however, some MHC-antigens or antigen-combinations are better adapted to current challenges than others, body odors pleasantness and/or intensities may be correlated to the MHC because they reveal an individual's health and vigor (as seems to be the case for spurs in pheasants (von Schantz et al., 1996)). As one possibility, we may then predict a link between odor intensity and the specificity of MHC antigens. Our effect-size estimations and the power analyses suggest that such a link does not exist or is very weak in the case of human body odors. This observation for itself contradicts the good-genes sexual selection hypothesis and is in agreement with the compatible-genes sexual selection hypothesis (Neff & Pitcher 2005).

Brown (1997) suggested that the overall degree of heterozygosity or the degree of heterozygosity on certain key loci may be positively linked with an individual's health and vigor, and that sexual signals may therefore reveal heterozygosity at these loci. In the case of the MHC, it seems that these loci are still under selection in many human populations, because MHC heterozygotes are normally more frequent than expected under Hardy-Weinberg equilibrium (Hedrick and Thomson, 1983; Black and Hedrick, 1997). However, it is not entirely clear whether this is due to natural or sexual selection, or both (Apanius et al., 1997; Prugnolle et al., 2005). If natural selection is responsible for these findings, MHC heterozygotes are generally in better health and vigor than people with homozygous MHC loci. Brown's (1997) hypothesis would then predict a link between MHC heterozygosity and characteristics that are used for mate selection. Indeed, Robert et al. (2005) recently found a link between MHC heterozygosity and facial attractiveness in humans. In their experiments, MHC heterozygosities were scored as more attractive, and when the scores were specifically asked for, faces of MHC heterozygous subjects were perceived as "healthier" than faces of people with homozygosities at the MHC locus.

In the case of body odors the direction of a possible correlation between MHC heterozygosity and signal intensity is not immediately obvious. Our first results and the effect size estimates suggest that there could be a difference in odor intensity between MHC heterozygotes and homozygotes, with homozygotes smelling more

intensely, especially for MHC-dissimilar smellers (i.e. for most individuals in an outbred and MHC-diverse population). However, we do not know whether MHC heterozygosity and health and vigor are correlated in our study population. Alternatively to Brown's (1997) hypothesis, it is possible that there is no link between MHC heterozygosity and the signaller's health and vigor, and that the differences between MHC homozygotes and heterozygotes are explained by some yet unknown constraints in the physiology of odor production.

In conclusion, if we control for MHC-linked perception we find few indications for a possible link between body odor intensity and MHC specificity. It appears that MHC homozygotes produce body odors that are on average perceived as more intense than those of heterozygotes. If such a link can be verified in future studies, it may indicate a statistical link between MHC homozygosity and general health and vigor or, alternatively, it may contribute to a better understanding of the physiology of MHC-linked odor production.

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