International Journal of Cosmetic Science

International Journal of Cosmetic Science, 2012, **34**, 161–168 doi: 10.1111/j.1468-2494.2011.00696.x

Population genetic segmentation of MHC-correlated perfume preferences

A. Hämmerli*,†, C. Schweisgut† and M. Kaegi*,†

*Institute for Integrative Biology, ETH Zurich, Universitätstrasse 16, 8092 Zürich and †Basisnote Ltd., Sandrainstrasse 3, 3007 Bern, Switzerland

Received 8 December 2010, Accepted 1 November 2011

Keywords: individual preferences, major histocompatibility complex, mate choice behaviour, olfaction, perfume ingredients, perfumes, scent, sexual communication

Synopsis

It has become difficult to find a matching perfume. An overwhelming number of 300 new perfumes launch each year, and marketing campaigns target pre-defined groups based on gender, age or income rather than on individual preferences. Recent evidence for a genetic basis of perfume preferences, however, could be the starting point for a novel population genetic approach to better match perfumes with people's preferences. With a total of 116 participants genotyped for alleles of three loci of the major histocompatibility complex (MHC), the aim of this study was to test whether common MHC alleles could be used as genetic markers to segment a given population into preference types. Significant deviations from random expectations for a set of 10 common perfume ingredients indicate how such segmentation could be achieved. In addition, preference patterns of participants confronted with images that contained a sexual communication context significantly differed in their ratings for some of the scents compared with participants confronted with images of perfume bottles. This strongly supports the assumption that genetically correlated perfume preferences evolved in the context of sexual communication. The results are discussed in the light of perfume customization.

Introduction

'Perfumes must be tried on for fit just as much as clothing'. This quote from Chandler Burr [1], the perfume critics of the New York Times, pinpoints one of the crucial, almost paradoxical problems of today's perfume industry: Individuals greatly differ in their preference for scents [2]. The use of perfumes has been a documented part of human culture for over 5000 years. Many of the traditional perfume ingredients are still used in present-day perfumes. And before the rise of the modern perfume industry some 150 years ago, perfumes were to a large extent 'tried on for fit', with the help of a perfumer creating that individual perfume – a perfume for 'myself' [3]. Perfumes used to be an exclusive luxury for few individuals; today, the range of perfumes might be more representative for the range of perfume preference types in a given population because perfumes are less exclusive. New perfumes, however, do not target individuals, but larger groups of individuals or even whole segments of populations [4]. So what is the common denominator to match the preference of such a segment? This work is trying to offer a biological solution.

So far, the matching of scent preferences for defined groups has been a process of trial and error and the success for new perfumes largely unpredictable. For example, perfumes for men on the Western European market often contain woody notes because this is assumed to be a 'male' note [3]. At the same time, it is known that many women buy men perfume targeted at male customers [5]. Most of the new launches fail despite huge targeted marketing campaigns, while some other perfumes (e.g. Chanel No 5, l'Air du temps etc.) have persisted in the market for decades [2]. The reasons for this are largely unknown. Cult or the strength of a brand may explain part of this persistence; the preference for a scent, however, may explain the rest. And this could be the reason why the ever-increasing number of different perfume brands is turning the search for the preferred personal scent for many women and men into a random walk through retail stores [6]. The observation that young people often turn against their parents preferences might further weaken the importance of brand on preference. Marketing certainly is the most important factor to convince people to buy a perfume for the first time. To convince people to buy a perfume again, however, is still largely a question of the individual preference for a particular scent [3].

So what makes perfume preferences personal? The reason may be a biological one. An experiment with common perfume ingredients [7] showed that individual preference for scents for a personal perfume (a perfume for 'myself') was significantly correlated with the immune genes of the major histocompatibility complex (MHC). Throughout this work, the term 'self' is used with reference to the context of choosing a scent for one self and not for a partner. MHC genes have also been shown to influence the preference for body odour in human populations (see for example [8, 9]) and in populations of other vertebrates such as mice and fish (see review in [10]). Through olfactory cues, the MHC is facilitating sexual communication about the compatibility of the immune system between two potential mating partners to raise the immunocompetence of offspring. Sexual communication as used here means olfactory-based communication in the context of mate choice. And perfumes, as [7] conclude, might support sexual communication by enhancing in some way the immunogenetic signal, the olfactory cue for immunological compatibility. In other words, sexual communication sets the stage for evolutionary processes involved in perfume preferences, potentially shaping the art of perfume preparation, the choice or production of perfume ingredients and the individual use of perfumes. While there is a large body of literature investigating MHC-correlated odour preferences [10], the mechanistic back-

Correspondence: August Hämmerli, Institute for Integrative Biology, ETH Zurich, Universitätstrasse 16, 8092 Zürich, Switzerland. Tel.: +41 44 6323913; fax: +41 43 4119011; e-mail: August.haemmerli@env.ethz.ch

ground still remains largely unknown [11], and to the best of our knowledge, there is only one study looking at MHC-correlated perfume preferences thus far [7].

The main finding of [7] was a significant correlation between the MHC and the relative preference for a set of scents that have traditionally been used in perfumery. This correlation was only significant, if the ratings were related to perfume preference for 'myself'. Building on the results of [7], the aim of this study was to perform a genetic segmentation with common MHC alleles, forming genetically defined groups of preferences for common perfume ingredients. To achieve this, it was necessary to compare absolute scorings rather than only relative scorings as used in [7], because relative scorings do not allow the comparison of preference differences at the level of single perfume components. For customization of perfumes, however, it is necessary to make a quantitative statement about individual perfume components – a statement such as the following: to tailor a perfume to the preferences of carriers of the MHC allele A2, scent x has to be added and scent y has to be omitted. And to achieve this, the quality or valence of each scent (pleasantness or unpleasantness) has first to be separated from the variance of intensity perception [12].

Specifically, we addressed the following three questions: (i) Are absolute perfume preferences correlated to specific MHC alleles? This is expected, if intensity can be controlled for, as relative preferences have already been shown to be correlated [7]. (ii) Is population genetic segmentation into preference groups possible? Several common MHC alleles have been correlated with perfume preferences. The aim here is to look for preference differences between common alleles with a suitable statistical approach. Common MHC alleles cover a large portion of the population (>90%), and hence, preference groups will be relevant to large population segments. (iii) Does a visual context for sexual communication influence the rating of perfumes? Milinski and Wedekind (2001) found MHC-correlated perfume preferences when scents were rated for 'a perfume that you would use for yourself', as a possible amplifier of MHC-correlated body odour to 'enhance sexual communication'. Here, we test, as an extension to previous findings, whether the visual context for sexual communication can influence perfume preferences in a similar way, as a context for 'self'.

To this end, we extended part of the [7] study with a reduced set of the most suitable perfume ingredients, by adding a calibration to absolute scorings for preferences and presenting images with or without a context of sexual communication (images from a generic perfume context and from online dating marketing) to the participants during the study. In addition, we tested the reproducibility of results with a focus group after 1 month.

Materials and methods

The 10 scents that were chosen for the following experiments and the frequent alleles that were analysed are based on the findings of [7] on which the experimental plan to test for systematic effects is based. The scents were chosen according to the following criteria: variation in preferences, availability, handling, conservation and calibration of solutions. Scents from animal origin (e.g. civet, castoreum and ambra) were omitted because their availability is low, most of them have been replaced by synthetics in modern perfumes [13], and their concentrations in perfumes are much lower than the concentrations used in [7]. The large number of alleles in the MHC depicts a statistical challenge. And hence, only common alleles were selected according to their frequency in the study population. A list of both can be found in Table I. The study consists of a small-scale pre-experiment (i) to control for a potential effect of intensity on the ratings of quality for the main experiment (ii).

Intensity (pre-experiment)

For a genetic segmentation, it is necessary to have comparable absolute ratings. Therefore, the negative correlation between the ratings of intensity and quality (if a scent is too strong, most people will reject it independent of their preference) was assessed in a pilot study with 1-butanol as a reference substance. This common industry solvent is used to calibrate and compare the ratings of scents [12]. The sliding scales for scoring were adapted from [7].

Participants were recruited through basis note Ltd. from the larger Zurich area. All participants had been typed for their HLA A, B and DR loci (IMGM laboratories, Lochhamerstrasse, Martinsried, Germany). The pre-experiment took place in March 2010 including five women and five men. The experimental design was completely randomized and double blind. Vials were covered in tin foil and were coded. In each round, participants had to rate the scent of 10 vials. Each vial contained one scent in one of the three concentrations 1 : 10, 1 : 100 and 1 : 1000. Solvent was 1 mL EtOH and the rest deionized H_2O. The concentrations for all natural scents (only reference substance 1-butanol was synthetic) were derived from concentrations as delivered by Essencia AG, Horgen, Switzerland. The experiment took place in a well-aerated meeting room. All participants were instructed on how to perform the tests before the trials started. All participants were asked not to drink any beverages other than water and not to eat shortly before and during the experiment.

Table I Frequency of the most common alleles in this study compared with the frequencies of the same alleles in the Milinski & Wedekind (2001) study and as average over global data available on allelefrequency.net

	A2	A1	B7	B44	DR13	DR7
This study*	35.2% (20,5)	23.2% (14.2)	23.2% (15.1)	20.3% (14.0)	26.1% (16.2)	30.4%(18.3)
Milinski & Wedekind**	46.9, 50.4 (69, 52)	38.05, 33.62 (43, 38)	30.97, 23.89 (35, 27)		31.85, 25.66 (36, 29)	–
Web***	44.2% (65 populations)	29.5% (22 populations)	27.8% (16 populations)	27.1% (20 populations	26.8% (27 populations)	26.6% (11 populations)

Given in brackets:
*Percentage frequency for each allele (number of heterozygote carrier of allele, number of homozygous carrier).
**Percentage frequency for each allele for the first and second study (number of carrier for studies 1 and 2 respectively).
***Mean percentage for each allele (number of populations considered for mean).

Trials: A participant had to spray the scent from the first vial on a kim-wipe and rate the odour for intensity and quality each on a separate sliding scale between 0 (extremely weak/unattractive) and 10 (extremely strong/attractive) and then dispose of the kim-wipe in a plastic bag and continue on for the remaining nine vials. At the end of each round, participants disposed the plastic bag with all the kim-wipes outside the room and washed their hands. The room was aerated during 5 min between each round while the new set of vials was prepared for the next participant. To minimize adaptation of the olfactory system, each participant rated the scents in three different rounds (10 scents per round, three concentrations × 10 scents in total) with a break of about 1 h in between. Order of vials for each round was completely randomized.

In the analysis, eight values were missing because participants either crosses 'I do not smell anything' (which scored as zero for intensity and NA for quality, $n = 1$) or 'I don't know' (which scored NA for intensity and quality, $n = 7$). As expected, there was an overall significant negative correlation between the ratings of intensity and quality (two-way ANOVA. ($F_{2,274}$, $P << 0.01$), note, however, that observations for this analysis are not independent). Based on the later correlation, it seemed conservative to apply the following adjustments for concentrations: if the mean value of the intensity rating of a given scent was within ±1 standard error of the mean (SEM) of the intensity rating for 1-butanol for a given concentration (1 : 10, 1 : 100, 1 : 1000), the intensity was taken as valid for the main experiment. This was given for the intermediate concentration 1 : 100 for nine of the 10 scents. For cinnamon (Fig. 1), only the lowest concentration (1 : 10) ±1 SEM was within the range of 1-butanol, and hence, this concentration was taken for the main experiment.

Quality (main experiment)

The term 'Quality' in this work is used as valence of an odour *sensu* [12], which depicts a rating between 'pleasantness' and 'unpleasantness'. Based on the findings from the rating of intensity against quality for the studied perfume components, all scents were mixed into hydrogel (Proderma, Neblikerstrasse, Schötz, Switzerland) as is routinely done for perfume samples in promotions. The reasons for this procedure are better standardization for natural scents that are otherwise difficult to handle and better conservation for repeated measures (reproducibility of results). Based on the results from the pilot study, the concentrations for scents ranged between 1% and 3%, which was then mixed with 33% ETOH, 64% H_2O and 2% Dow Corning® (RM2051). A half-gram of this mix was packaged in foil pouches used for perfume samples. One pouch of each scent was attached on the right side of a questionnaire. The questionnaire had two separate sliding scales from extremely weak to extremely intensive (for intensity) and extremely unattractive to extremely attractive (for quality). The question asked (in German) was: 'How pleasant do you find this scent as an ingredient of a perfume for yourself?' Participants could also cross 'I do not smell anything', which scored zero for intensity and NA for quality, and/or 'don't know', which scored NA for both, intensity and quality. The rating of intensity was used to control the effects of the hydrogel that might have altered the findings from the pilot study on intensity. Each scent was coded with a 10-digit hexadecimal code such that they would not be recognizable even in the repetitive rounds. This code was also printed behind the pouch, to guarantee correct assignment to the right questionnaire in case it got detached from the sheet. Each questionnaire was accompanied with a colour image, a collage of 15 images either from a generic

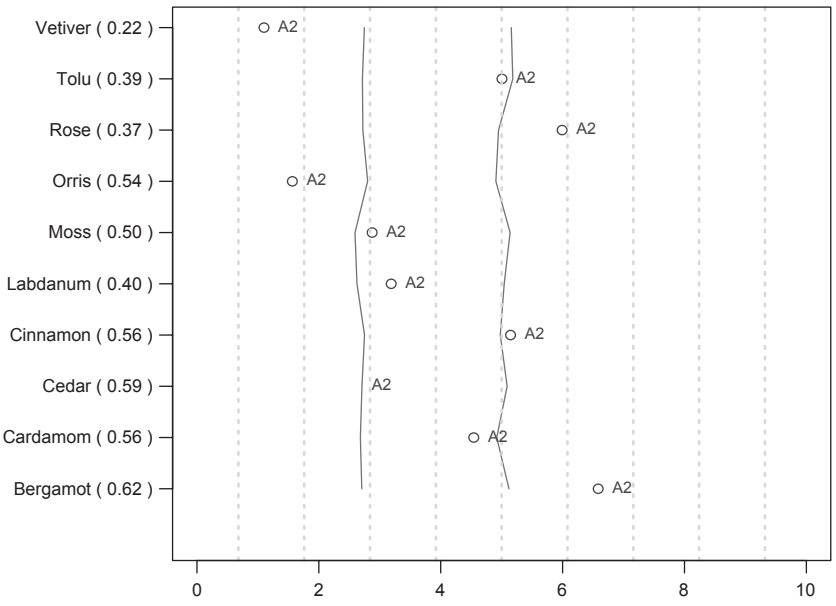

Figure 1 As an example illustrating the analysis underlying Table II, mean ratings for the quality of 10 perfume ingredients with 95% confidence intervals (solid line) estimated with bootstrap for random expectations are given for carriers of major histocompatibility complex (MHC) allele A2. The scale for the ratings ranges from 0 (extremely unattractive) to 10 (extremely attractive). Thus, the mean values left of the confidence intervals signify lower preference than random expectations and mean values right of the confidence intervals signify higher preference than random expectations. The same calculation was performed for the two most common alleles for all three considered MHC loci (A2, A1, B7, B44, DR13, DR7), see also Table I. The correlation coefficient between first and second round ratings (1 month later) is given as R^2 in brackets next to the perfume ingredients.

perfume context (imagery from perfume marketing) or with the context for sexual communication (imagery from online dating marketing) (see Appendix). To minimize the chance for background noise (colour, features, etc.) to become relevant to treatment effects, collage as a layout was preferred over showing only one image per participant. Trials were double blind. The people assembling and shipping the questionnaires did not know which scent was in which pouch, and the key to assign participants to a treatment group was only kept by Manuel Kaegi.

All participants gave their written consent to participate in the study after the content of the study had been explained to them (for more details on the recruitment of participants see (i)). All participants could optionally take part in a lottery to win a price, and this was indicated on each questionnaire. In total, 116 participants were sent a first round of perfume samples at the beginning of June 2010. From these 116 participants, 69 returned the questionnaire. With a subset of the participants from the first round, an additional round was performed 4 weeks after the first round to estimate the reproducibility of results (from 35 in total, 30 returned the questionnaire). In each round, the order of the scents was completely randomized. The images that were accompanying the questionnaire for each participant were the same for rounds 1 and 2.

Data analysis

All data analysis was performed with the scripting language R. The six most frequent alleles in our study population were A2 (n = 25), A1 (n = 16), B7 (n = 16), B44 (n = 14), DR13 (n = 18) and DR7 (n = 21) (see also [7]), which had been selected *a priori* for the analysis. On average, overall population frequencies from global data for these alleles were 44.2% (A2), 29.5% (A1), 27.8% (B7), 27.1% (B44), 26.8% (DR13) and 26.6% (DR7), which is in the same order of magnitude as the frequencies found in our study (see Table I). In addition, there were six cases with intensity = 0, quality = NA from participants choosing the sentence 'I do not smell anything'. In five cases, participants chose 'don't know'.

Because of the skewed frequency distribution across the different scent preferences and because the sample sizes where unequal among alleles, we used bootstrapping to approximate the theoretical distribution under the null hypothesis. Under the null hypothesis, the distribution was based on the assumption that preference ratings are independent of the genotype of participants and the degree of heterozygosity. The bootstrapping was performed with random sampling with replacement (n = 1000). In a next step, we calculated the simultaneous 95% confidence intervals (CI) of the bootstrapped distribution, which were multiplied by the number of intervals formed to correct for interdependence between intervals [14]. These CIs were then compared with the observed mean value for each scent and genotype. As Fig. 2 shows for genotype A2 for example, the observed mean values for six of 10 (60%) scents lie outside the 95% CI. This strongly suggests that genotype A2 has an effect on scent preferences. The same analysis was performed for the treatment group with the images containing context for sexual communication (dating) and the group with images containing only generic content (perfume).

Results

To make the results relevant to a given population, the frequency of common MHC alleles has to be considered. The most common MHC allele in the sample population is A2, its frequency is comparable with that of the study by [7] and with global average population frequencies (see Table I). Homozygosity, the same allele at a given gene locus, is <20% for all six common alleles chosen for the

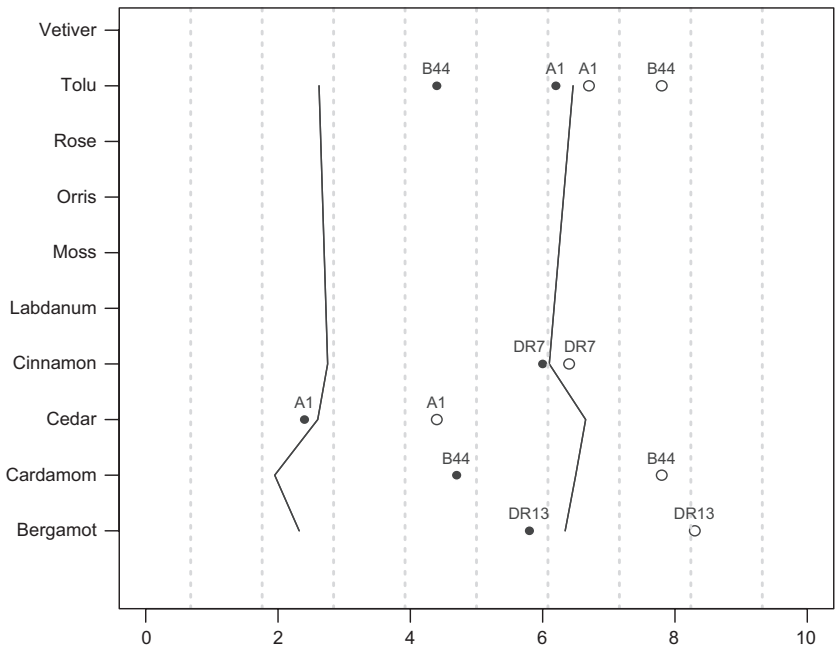

Figure 2 Mean ratings and 95% confidence intervals for the quality of perfume ingredients, for which the treatment groups for context between dating (open circles) and a perfume (filled circles) differed for common alleles.

analysis. The number and frequency of all participants in our study population carrying zero–six of these common alleles were the following: zero of six (n = 6, 8.7%), one of six (n = 23, 33.5%), two of six (n = 32, 46.4%), three of six (n = 6, 8.7%) and four of six (n = 2, 2.9%). No participant carried more than four of the six common alleles. It is interesting to note that for the loci B and DR, [7] found B8 and DR15 to be the most common alleles in the sample population, whereas this study and comparative global data on MHC allele frequencies find B7 and DR13 to be the most common alleles for the respective loci (Table I). The six most common alleles found in this study cover 91.3% of the population (100% – 0 of 6 = 8.7%). This shows that even though the MHC is one of the most diverse gene regions, the overrepresentation renders data on common alleles relevant to a large part of the population (>90%).

There was a significant difference in the ratings between men and women. Overall, women rated the quality of scents slightly but significantly lower than men (3.98 vs. 4.44, $F_{1,602} = 5.13$, $P = 0.023$). Oral contraceptives have been shown to influence MHC-correlated odour preferences (see review in [15]). Only six women in this study population indicated that they were taking oral contraceptives at the time of the experiment, and these women did not differ significantly in their ratings of scents. Therefore, the use of oral contraceptives was not considered further as a factor in this study.

We found mean ratings for the quality of some of the chosen scents to significantly deviate from random expectations for carriers of common alleles (see Fig. 2 for the example of the most common allele A2). Two scents, vetiver and bergamot, however, did not have discriminating power among carriers of common alleles, which means that these scents are less useful to segment a given population into preference groups. Vetiver was consistently rated lower than random expectations while bergamot was consistently rated higher than random expectations (Table II). Interestingly, the overall mean for the ratings of vetiver and bergamot was within the 95% confidence limits. This means that participants with less common alleles rated these two scents significantly different compared to participants with common alleles. The interindividual variation in preferences is reflected by the 95% confidence intervals.

Overall, orris, cedar and vetiver were rated lower than random expectations, and tolu, Rose and cinnamon were rated higher than random expectations for the chosen alleles. Table II read in column format (from top to bottom separate for each column) for each allele indicates how populations could be segmented into preference types. For example, a matching perfume for all carriers of allele A2 would have reduced relative concentrations for vetiver, orris and cedar (mean values for preference ratings were lower than random expectations) and an increase in concentration for rose, cinnamon and bergamot (mean values for preference ratings were higher than random expectations). Each of the preference types (or profiles) for the chosen common alleles are unique despite the fact that, for example, vetiver and bergamot do not have discriminating power.

Reproducibility of test results was estimated with a focal group of 30 participants typed for their MHC A, B and DR loci. Two weeks after the first round of ratings, this focus group had to rate the same 10 scents in completely randomized order again. The rating were surprisingly consistent with no overall significant difference in average ratings between rounds 1 and 2 ($t_{1,522} = 0.75$, $P = 0.45$, $\mu_1 = 4.13$, $\mu_2 = 3.95$). Corresponding R^2's for each scent are shown in Fig. 2.

The ratings for some of the common alleles for tolu, cinnamon, cedar, cardamom and bergamot differed between subject groups that were provided with an image with either the context for sexual communication or the context for perfumes (Appendix).

Preferences for common alleles compared to preferences for rare alleles ('all alleles minus the one's chosen for the analysis of common alleles') contrast preferences against the population mean. Significant differences between rare and common alleles were found for DR13 (tolu +, orris −, cinnamon +) and for B44 (rose +), again using the same bootstrapping procedure within 95% confidence limits, as described earlier.

Discussion

This work concentrates on the question whether frequent alleles from the MHC genes relevant to a large population segment could be used as markers, to distinguish groups with common preferences for perfumes using a biological approach. Specifically, the following three questions were asked: (i) Are absolute preferences correlated to specific MHC alleles? (ii) Is population genetic segmentation possible? (iii) Is the context for sexual communication influencing the rating of perfumes?

The immune genes of the MHC have been shown to correlate with individual body odour [8], with the preference for body odour

Table II Mean scoring of the chosen scents and scent notes (men = 33, women = 32 (women on pill = 6), valid scorings in total = 65). For each common allele (+) indicates that mean observed preferences were above random expectations and (−) indicates that mean observed preferences were below random expectations for a given scent and (r) indicates that mean observed preferences were not distinguishable form random expectations

Scent	Note	Mean scoring	SE mean	A2	A1	B7	B44	DR13	DR7
Vetiver	Woody	1.38	0.22	−	−	−	−	−	−
Tolu	Balsamic	5.33	0.34	+	+	r	r	r	+
Rose	Floral	5.1	0.38	+	r	r	r	r	r
Orris	Floral	2.25	0.26	−	−	−	−	r	−
Moss	Woody	3.98	0.36	r	r	r	r	r	r
Labdanum	Leathery	3.59	0.28	r	r	r	−	r	r
Cinnamon	Spicy	5.4	0.27	+	+	r	r	r	+
Cedar	Woody	3.54	0.33	−	r	r	r	−	r
Cardamom	Spicy	4.67	0.36	r	r	r	r	r	r
Bergamot	Citrus	6.7	0.33	+	+	+	+	+	+

Preference below (−) and above (+) random expectations for overall mean: −Orris, Cedar, Vetiver and +Tolu, Rose, Cinnamon

of potential mates [10, 16] and with the preference of perfume ingredients [7]. The significant deviation from random expectations for the preference of 10 chosen perfume ingredients with common MHC alleles is supporting the findings by [7] and is extending the context for 'self' with a visual cue, as context for sexual communication. According to the findings from [7] the context for 'self' is introduced in contrast to the context for 'partner'. Their data show a significant correlation of MHC with the rating of scents only, if people imagined to rate a given scent as component in a perfume for themselves and not, when rating a scent as perfume component for a partner. This is what is meant with 'self' also in this study. As long as the mechanisms behind these correlations are not fully understood, however, alternatives such as imprinting mechanisms during ontogenesis might also play a role.

Most scents remain constant in their quality over orders of magnitude of concentration [12]. Nevertheless, at high concentrations, quality tends to be negatively correlated with intensity, as was the case, for example, for the cinnamon oil used in this study. Hence, reliability of absolute scorings was achieved by calibrating the amount of perfume ingredients with initial ratings for intensity against a reference substance of known concentration. The final concentrations were in principal chosen in a way such that individual ratings showed variance among participants within the sliding scale between 0 and 10 (meaning that people could decide whether they liked a scent or not). This procedure seemed successful for most scents; however, the concentrations for bergamot (highest average ratings) and vetiver (lowest average rating) could probably been reduced even more, as both scents did not show any discriminating power at the level of common alleles (people agreed largely on the quality of these two scents) (see Table II). Interestingly, the pooled rare alleles showed discriminating power for some of the scents where common alleles did not. For example, the ratings for rare alleles were above random expectations for tolu (DR13) and rose (B44, DR13) and below random expectations for orris (DR13) (in brackets, corresponding common allele with no discrimination from random expectations).

Average percentage distance between consecutive scorings showed short-term repeatability of results but the corresponding R^2 values were relatively small. [7] showed high repeatability for the scorings of these same perfume ingredients within two consecutive years. Even though the mechanism by which preference differences are inherited is not known, this short-term (1 month) and longer-term (2 years) stability of MHC-correlated perfume preferences is in line with the finding that some scent preferences are indeed not acquired, but have a strong genetic background [12].

In a study by [7], they report a significant difference between the ratings of women and men independently of the MHC but the reason for this effect remained unclear. They suspected intensity of the perfumes to play a role. In our study population, women on average tended to rate the quality of scents lower than men for perfume ingredients that had been checked for intensity. The olfactory system of women has been shown to be more sensitive than the olfactory system of men, and this difference has been qualitatively estimated to be about 20% [3, 17]. Women in our sample population experienced seven of the 10 chosen perfume ingredients as more intense and consequently as less pleasant than men (data not shown). In sexual selection, often women are more selective in mate choice decisions in the face of biological asymmetry in resource allocation between women and men, visible for example in the size difference between egg cell and sperm and in the mothers tied resources during pregnancy and nursing [18]. A better sense of smell in women might reflect this circumstance because it would allow women to make a more qualified decision whenever scent is involved in sexual communication. It is interesting to note that a segmentation of perfumes in women and men perfumes is ambiguous and only reflected by a statistical difference in composition. Many cultures, for example, in the Middle East only use unisex perfumes [13], and some brands (e.g. Calvin Klein) also sell unisex perfumes in the Western markets. Tolu, however, the only scent that women rated higher than men in our study population is known in perfumery as a typical 'feminine scent'.

While female sexual responsivity, the chance for extra-pair copulations and other psychological traits have been shown to be MHC correlated in couples [11], visual cues have been shown to influence MHC-correlated preferences in a mate choice decision context through facial traits [19]. We used visual cues of sexual communication to extend the context of 'self'. The images were introduced as treatment in the present study to extend the discussion on 'context' from Milinski & Wedekind 2001. They claimed that the context of 'sexual communication' was apparent for example from marketing campaigns in perfumery a cue for end users that the given perfume product has something to do with love and attraction. This is why we chose generic images from perfumery (no 'sexual communication') and from online dating ('sexual communication' without perfumes) as background control to test for differences. The images were built from various Internet sources and 25 images for each context were concatenated. The reason for this latter step was, that any systematic effect detectable against the background variance is unlikely to come for example from just one background colour, one facial trait or one shape of a given perfume bottle. The newly composed context contained two levels. First, 'I choose a perfume for myself' and secondly, 'I am in the context of sexual communication'. The corresponding treatment groups differed in their ratings for tolu, cinnamon, cedar, cardamom and bergamot. Despite this limited differentiation among the chosen scents, this data provides a first indication, that visual cues building a context for sexual communication may also influence MHC-correlated perfume preferences. In a future study, it might be rewarding to measure the effect of visual cues simultaneously with respect to (i) the context for ratings: I might like the perfume of my partner but would never wear it myself; and (ii) the competence for ratings: a preference for 'self' has to match only one MHC type. The preference for a potential partner has to match many MHC types, and hence, a perfume-correlated signal will be lost statistically for that reason.

But how important is MHC-correlated odour preferences for mate choice in humans relative to other factors involved? As a factor directly influencing mate choice decisions, the effect of MHC linked odour cues is likely to be less important than, for example, visual cues or social background considering the low levels of significance in experiments controlling for confounding or enhancing factors (e.g. [8]). In mice, for example, the effect is strong also because mice have relatively bad eyesight and are generally active during night-time where olfaction becomes more important than vision. In humans, however, vision is predominant. Scent, however, is likely to become crucial, when partners engage in physical intimacy. This might be reflected by the circumstance that in couples counselling, partners reporting a disliking of the body odour of the other during physical contact are considered difficult or even impossible to treat [16], and hence, even a slight mismatch in scent preference might increase the likelihood to separate in long-term relationships.

Body odour is facilitating in some way a measurement of the differences in immune genes. While tremendous progress has been

made in understanding the olfactory system, the exact mechanism by which MHC signalling is achieved remains unclear. For example it is still debated, whether volatile compounds (carboxylic acids) contain an MHC signal or largely non-volatile compounds (peptide ligands) are responsible for this (see for example [20]). Böhm and Zufall 2006 state that there are two general ways by which individuals can generate unique chemical signals that reflect their genotype. In the first model, transmission of a common pool of signals is affected by the use of structurally diverse carrier molecules. Although each individual produces the same mixture of signals, its composition is quantitatively altered during transmission (see for example microbial decomposition of precursors to carboxylic acids in [21]). In an alternative model, the sender could have a metabolic pathway with polymorphic components, such that the original signals are unique among individuals of the same species (see, for example, MHC peptide ligands that selectively activate olfactory neurons in Leinders-Zufall et al. 2004). The microbial modification of perfume components itself is debated. While the base note in perfumery is defined as an unfolding of the scent in association with the skin with time, some authors have doubted this factor to be important (e.g. [22]).

It is interesting to note that thus far no odorant receptor gene for MHC-specific signals has been detected while for other genetically related odour preferences, they have. For example, the preference for androstenone, an odorous steroid derived from testosterone, which is perceived by different individuals as offensive, pleasant or even odourless can be traced back to the OR7D4 odorant receptor gene [12].

In addition, it is unclear whether in humans the distance in MHC between potential mates is maximized as has been suggested, for example, by [8] or rather optimized, as some recent evidence from animal model systems suggests [23–25]. A new study with the three spined stickleback even shows that an additional olfactory maleness signal might be necessary to produce a reliable MHC signal for women [26]. Therefore, the mechanism by which perfumes fit into the MHC signalling process must remain hypothetical at this stage. It is possible that perfumes, especially fine fragrances, act as a kind of amplifiers in surroundings where foreign scents might otherwise mask an MHC signal [7]. The perfumer Jellinek observed that some frequently used perfume components (such as the ones used in this study) contain so-called subscents or Beigerüche which mimic in their odour experience bodily odours. One example is indol, a chemical component present in rose or jasmine, with a buttery, sweaty smell. Hence, these subscents in natural perfume ingredients that act as reminiscents of human body odours may be selectively used by choosing a personal perfume [27].

A perfume is not the sum of its molecules, and one might question the use of individual perfume ingredients to actually measure perfume preferences. However, most natural ingredients are 'perfumes' themselves in the way that they consist of hundreds of different molecules [28]. And from these molecules, only few need to be responsible for MHC-correlated preferences to be effective [27]. Interestingly, only about a hundred plant species have been used to obtain perfume ingredients from ancient times to the present day representing a tiny fraction of the number of scented flowering plants in the world [29].

The approach of individual perfumery that was common in the nineteenth century still exists today, but it occupies only niches [27]. The fragrance market is dominated by a globally structured value chain from molecule producers to brand owners to the retail stores. Over the past years, the fragrance market has witnessed a drastic increase in new product launches and therefore increasing competition, while fragrance market growth has remained low with decreasing return on investment in new product development. The scent of a new perfume and the match between a perfume and its customers are largely decided in marketing campaigns and not at the level of the perfumer, except when the perfumer itself is part of the campaign. The image that the industry is still fiercely defending, however, is the traditional one, as this recent quote out of a perfume guide illustrates: 'Unfortunately for profits, perfume really is an art, not a science' [30]. But genetically correlated perfume preferences constitute a strong entry point for a scientific approach in perfume making. On the inside, the industry itself has already picked up the subject of MHC and scent preferences (see for example [21], and two patents on using peptides to customize scents have appeared in the past years, one by Milinski and Böhm (WO/2003/090705) and the other by Ziegler et al. (WO/2001/081374), the latter of which has been bought by Coty in 2009.

Every second perfume is bought as a gift (57%). Two-thirds of people who receive perfume as a gift are unsatisfied with its scent (75%). In repeat purchases, however, it is the satisfaction with the scent that counts ([6], www.fragrance.org). Therefore, a better-tailored match between the quality of a scent and the preference of its users could turn out to be crucial for the success of a new fragrance by supporting (by no means replacing) the making of a perfume. And a population genetic approach as suggested in this work could offer such support and might open a new, innovative approach for a young industry with ancient roots.

Acknowledgement

We thank Paul Schmid-Hempel for his ongoing support, Andreas Wilhelm for advice on the use of perfume ingredients, Mathias Wegner and Dominic Senn for comments on an earlier draft of this manuscript. This research was funded by CTI, the commission for technological innovation of Switzerland (CTI-Project Nr. 10754.1 PFLS-LS).

References

1. Burr, C. *The Perfect Scent*. Picador, New York (2009).
2. Toller, S.V. and Dodd, G.H. *Perfumery: The Psychology and Biology of Fragrance*. Chapman & Hall, London (1991)
3. Gilbert, A. *What the Nose Knows*. Crown Publishers, New York (2008).
4. Walker, C. *The Rise in Natural Functional Fragrances*. GCI Magazine, Carol Stream, IL (2008). Availabe at: www.gcimagazine.com.
5. PerfumerFlavorist.com, *Sensory and Fragrance Home Care and Personal Care Trends*. Datamonitor (2007).
6. Passikoff, R. and O'Connor, K. *Sense & Scent Building emotional bonds through engagement measurement*. Fragrance, Amsterdam pp. 165–173 (2007).
7. Milinski, M. and Wedekind, C. Evidence for MHC-correlated perfume preferences in humans. *Behav. Ecol.* **12**, 140–149 (2001).
8. Wedekind, C., Seebeck, T., Bettens, F. and Paepke, A.J. MHC-dependent mate preferences in humans. *Proc. R. Soc. Lond. B* **260**, 245–249 (1995).

9. Chaix, R., Cao, C. and Donnelly, P. Is mate choice in humans MHC-dependent? *PLoS Genet.* **4**, e1000184 (2008).
10. Havlicek, J. and Roberts, S.C. MHC-correlated mate choice in humans: a review. *Psychoneuroendocrinology* **34**, 497–512 (2009).
11. Garver-Apgar, C.E. *et al.* histocompatibility complex alleles, sexual responsivity, and unfaithfulness in romantic couples. *Psychol. Sci.* **17**, 830–836 (2006).
12. Keller, A. *et al.* Genetic variation in a human odorant receptor alters odour perception. *Nature* **449**, 468–472 (2007).
13. Morris, E.T. *Düfte: die Kulturgeschichte des Parfums*. Patmos Verlag, Düsseldorf (2006).
14. Hsu, J.C. *Multiple Comparison Theory and Methods*. Chapman & Hall, London (1996).
15. Alvergne, A. and Lummaa, V. Does the contraceptive pill alter mate choice in humans? *Trends Ecol. Evol.* **1178**, 171–179 (2010).
16. Ober, C. *et al.* HLA and mate choice in humans. *Am. J. Hum. Genet.* **61**, 497–504 (1997).
17. Hatt, H. and Dee, R. *Das Maiglöcken-Phänomen*. Piper, München (2008).
18. Smith, J.M. *Evolutionary Genetics*. Oxford University Press, New York (1989).
19. Roberts, S.C. *et al.* Manipulation of body odour alters men's self-confidence and judgements of their visual attractiveness by women. *Int. J. Cosmet. Sci.* **31**, 47–54 (2009).
20. Kwak, J. *et al.* In search of the chemical basis for MHC odortypes. *Proc. Biol. Sci.* **277**, 2417–2425 (2010).
21. Kuhn, F. and Natsch, A. Body odour of monozygotic human twins: a common pattern of odorant carboxylic acids released by a bacterial aminoacylase from axilla secretions contributing to an inherited body odour type. *J. R. Soc. Interface* **6**, 377–392 (2009).
22. Behan, J.M. *et al.* Insight into how skin changes perfume. *Int. J. Cosmet. Sci.* **18**, 237–246 (1996).
23. Wegner, K.M. *et al.* Parasite selection for immunogenetic optimality. *Science* **301**, 1343 (2003).
24. Reusch, T.B.H. *et al.* Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature* **414**, 300–302 (2001).
25. Milinski, M. The major histocompatibility complex, sexual selection, and mate choice. *Annu. Rev. Ecol. Evol. Syst.* **37**, 159–186 (2006).
26. Milinski, M. *et al.* Costly major histocompatibility complex signals produced only by reproductively active males, but not females, must be validated by a 'maleness signal' in three-spined sticklebacks. *Proc. Biol. Sci.* **277**, 291–398 (2010).
27. Jellinek, P. *Die psychologischen grundlagen der parfümerie*. Hüthig Verlag, Heidelberg (1994).
28. Parry, E.J. *Cyclopedia of Perfumery*. J. & A. Churchill, London (1925).
29. Stoddart, D.M. *The Scented Ape: The Biology and Culture of Human Odour*. Cambridge University Press, Cambridge (1986).
30. Turin, L. and Sanchez, T. *Perfumes: The Guide*. Penguin Books, London (2008).

Appendix

Collage of images used in the questionnaires for the context of perfumes and dating (see text for details on this layout).