The artificially scented ape: Investigating the role of fragrances and body odours in human interactions

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Acknowledgements

I would like to start with a disclaimer. My mind is currently a sieve and all of the words that I own have been poured into the following pages, and so I truly hope that no one important (or even of no importance) has been missed from the following acknowledgements. If I have forgotten someone then I hope that these mitigating circumstances mean that I can be forgiven. When I began compiling a list of people to thank it really seemed to be never-ending, so much so that, being in ‘thesis mode’, I considered using a table to concisely and clearly allocate my thanks. After careful consideration I decided that some may find this slightly cold, and so I have opted for the more traditional format, which you can find below.

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Now sit back, relax, and (hopefully) enjoy the following thesis (not really sure who I am talking to at this point; who reads these things?).

Abstract

It was long believed that humans were unable to utilise the odours of conspecifics to co-ordinate social interactions in ways in which other species appear to be capable. However, a surge in interest in human social olfaction has recently challenged this view. The numerous studies conducted in this area have found that multiple state and trait related cues can be detected in body odour. Furthermore, many studies indicate that women are often more sensitive to these cues, and that sensitivity can be associated with fertility, findings that are consistent with sex differences in reproductive effort and benefits of choosiness in mate-searching.

Since previous studies in this area have usually addressed the potential for humans to use olfactory communication in a comparable manner to other mammals, they typically involve collection and assessment of 'natural' odour. That is, they explicitly exclude the possibility of 'contamination' of odour samples by artificial fragrances. However, humans have used artificial fragrances for millennia, across many different cultures. This raises the question of whether widespread fragrance use may affect or disrupt the detection of this information in modern humans.

The first aim of this thesis was to address this question by investigating how fragrance use may mediate the detection of olfactory information in humans. As well as providing further evidence for sex differences in the assessment of olfactory cues, and for the role of olfaction in real world partner choice, the findings herein suggest that fragrance may act differently on different information being assessed, potentially masking accurate assessment of certain traits (such as masculinity), while fragrance

choice and preferences may be important in complementing other olfactory information (such as the general distinguishability of an individuals' odour profile).

A second aim of the thesis was to develop a scale in order to more accurately describe the varying perceptual qualities of human body odour – in other words to map human body odours. This work was conducted alongside perfumers in order to benefit from their expertise in olfactory perception and semantic labelling of odours. The development of such a scale could enable improved understanding of the perceptual qualities of human odour, making it possible to link specific perceptual qualities to specific cues (e.g. symmetry, masculinity, sex) or to manipulate odours based on perceptual qualities in experimental settings, and has direct practical implications for fragrance designers and for improving the ability of individuals to choose fragrance products that suit their odour profile.

The second section of the thesis focuses on the effects of odours on the individual wearer as well as on perceivers in the environment. One study is presented which investigates the role of malodour reduction compared to the addition of fragrances in perceptions of confidence and attractiveness, finding that both the reduction of malodour and the addition of fragrance appear to be important for confidence as rated by others in the environment.

The final study presented in the thesis examines a hitherto un-investigated role of olfaction during human pregnancy. The rationale for the study is based on evidence suggesting that in certain non-human species, which also show bi-parental care of offspring, there may be a role for chemical, or odour based, communication which underpins behavioural and endocrinological changes related to infant care behaviours

in males. The study found little evidence to support the presence of analogous olfactory signalling during human pregnancy, though the findings are discussed in light of methodological changes which, if made in future studies, may result in different outcomes.

The thesis concludes with a discussion of the importance of continuing to investigate various forms of olfactory communication, as well as improving our understanding of odours through the mapping of their perceptual qualities, and finally further examining the ways in which various fragranced products, which are widely used in society, may affect all of this. Future directions for this area of research are discussed. This line of investigation will, I argue, enable us to finally establish the true role of olfaction in contemporary social environments.

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Chapter 1: General Introduction

Odour and non–verbal communication

It is well-established that many non-human species can detect information from conspecifics via olfaction. The information available from odour is used by both competitors and mates (Gosling & Roberts, 2001) and appears to be wide-ranging, including, but not limited to reproductive status (Clarke, Barrett, & Henzi, 2009; Miranda, Almeida, Hubbard, Barata, & Canário, 2005) competitive ability (Huck, Banks, & Wang, 1981; Rich & Hurst, 1998) and genetic compatibility (Ilmonen, Stundner, Thoss, & Penn, 2009; Ruther, Matschke, Garbe, & Steiner, 2009). Additionally, olfactory signals not only reveal characteristics of the individual, but have also been found to induce physiological and behavioural changes in the perceiver, such as accelerating or delaying the onset of puberty, inducing ovulation or abortion, increasing and decreasing sperm allocation, as well as affecting the performance of copulatory behaviours in many non-human animals (for a review see Petrulis, 2013).

Olfactory communication in humans

Though once largely disregarded, the role of olfaction in human communication has now developed into an area of great interest. The initial supposition that humans are chiefly visual creatures was reinforced by modern genetic discoveries showing that humans have a reduced number of olfactory receptor cells and functional olfactory receptor genes compared to other species, such as dogs and mice (Schaal & Porter, 1991; Young, 2002). However, while we may be inferior to other species regarding our ability to detect odours, we are in fact quite well endowed with sebaceous and apocrine glands (Kippenberger et al., 2012); this led Stoddart

(1990) to label humans as 'the scented ape'. Furthermore, these glands become active during puberty (Montagna & Parakkal, 1974), suggesting a role in sexual selection. Based on such information, it has been hypothesized that humans retain the ability to assess olfactory cues in mate choice scenarios, with body odour being posited as serving an analogous signalling function in humans to the urinary and glandular odour cues used in other animals (Comfort, 1971; Penn et al., 2007; Schleidt, Hold, & Attili, 1981; Stoddart, 1990).

In keeping with this, there is a wealth of evidence supporting the availability of various cues from human body odour, representing a wide range of traits. Humans, for example, have been found capable of detecting emotions that were present at the time of odour production (Chen & Haviland-Jones, 2000). Research has also suggested that humans are very good at discriminating between smells, being able to pick out a t-shirt worn by themselves amongst 100 worn by other individuals (Lord & Kasprzak, 1989), and humans also have the capacity to recognize kin via body odour (Weisfeld, Czilli, Phillips, Gall, & Lichtman, 2003), which is important in sexual selection in order to avoid inbreeding. Additionally, mothers are able to discriminate the smell of their own offspring from that of others (Ferdenzi, Schaal, & Roberts, 2010; Porter, Cernoch, & McLaughlin, 1983). Weisfeld and colleagues (2003) also found that non-related but known individuals could be identified via their odour as accurately as genetic kin could, suggesting an associative mechanism underlying odour recognition. Moreover, people appear capable of recognising and assessing unfamiliar human odours well, with Roberts and colleagues (2005) finding that people could match the odours of monozygotic but not dizygotic twins at an above chance level, even when the twins were living apart.

Traits related to mate choice and sexual selection

Individuals also appear to be capable of assessing mate choice relevant cues in body odour such as menstrual cycle stage (Havlíček, Dvorakova, Bartos, & Flegr, 2006; Singh & Bronstad, 2001) health status (Moshkin et al., 2012), sex (Schleidt et al., 1981), personality (Sorokowska, 2013), diet (Fialová, Roberts, & Havlíček, 2013) and genetic compatibility (Havlíček & Roberts, 2013). For example, individuals can discriminate olfactory cues of a woman's ovulatory stage, with studies finding that men perceive female odours collected during the follicular phase of the menstrual cycle to be more attractive than those from the luteal phase, the latter being associated with a low conception risk (Gildersleeve, Haselton, Larson, & Pillsworth, 2012; Kuukasjärvi et al., 2004; Singh & Bronstad, 2001).

This mate relevant information available in body odour is often also present in cues from other modalities. For example, studies have found that individuals who have low fluctuating asymmetry, which is believed to represent an individual's ability to cope with environmental challenges and may therefore act as a proxy of genetic quality, are found to be more attractive than their less symmetrical counterparts (Fink, Neave, Manning, & Grammer, 2006; Tovée, Tasker, & Benson, 2000). Symmetrical individuals are also rated as smelling more attractive (Rikowski & Grammer, 1999). These preferences additionally appear to alter in relation to fertility status, with women showing greater preferences for the scent of symmetrical men during the most fertile phase of the menstrual cycle, a time at which conception risk is highest (Gangestad & Thornhill, 1998; Garver-Apgar, Gangestad, & Thornhill, 2008; Thornhill & Gangestad, 1999).

Effects of olfactory signals on the perceiver

Furthermore, findings suggest that these olfactory cues may not only provide information, but, as found with non-human animals, could potentially alter the physiological state of the perceiver. For example, Bensafi and colleagues (2003) found that presentation of a human sex steroid derived compound led to increased physiological arousal in women and decreased arousal in men. As with other species (Perrot-Sinal, Ossenkopp, & Kavaliers, 1999), odours also appear to be capable of inducing endocrinological changes in humans, with one study finding that men who were exposed to the odour of ovulating women experienced increases in salivary testosterone concentrations (Miller & Maner, 2010).

Human relationships with body odour

In spite of the apparent value of olfactory cues in evaluating others, there are a number of cultures where the conscious detection of body odour is perceived negatively (e.g. Schleidt et al., 1981). This is echoed in the early development and use of fragrances and perfumes worldwide, which dates back to at least the ancient Egyptian and Greek civilisations (Stoddart, 1990). Indeed, the fragrance industry in western societies is worth billions of dollars, and personal fragrance use is widespread, with one study finding that 79% of women and 60% of men sampled in the UK used a deodorant every day (Roberts, Miner, & Shackelford, 2010), and sales in the flavour and fragrance industry have reportedly risen from $12.9 billion to $22 billion in an 11 year period (Lenochová et al., 2012). The use of such products raises the question of what effect they might have on the cues present in body odour, and in turn how this influences our social and sexual interactions with others. Indeed, it was reported that videos of men who used fragranced antiperspirants were judged as more attractive compared to videos of men who used a placebo; perhaps due to changes in self-confidence of the target men (Roberts et al., 2009). The widespread use of fragranced products makes it difficult to extend the previous findings, discussed above, to real world settings, as these studies often omit all extraneous fragrances. In order to effectively assess the role of body odour in contemporary society, then, we must incorporate fragranced products into experimental designs. This was one of the aims of the current thesis. The few studies which have already done this are detailed in the following chapters. Together, they provide evidence that, in fact, rather than masking odour and cues in body odour, fragrances may be complementing or enhancing the biological information for which we show evolved preferences. The first half of the thesis provides more evidence for this.

Thesis outline

The first section of this thesis (chapters 3, 4, 5 and 6) focuses on fragrance-body odour interactions. Chapter 3 investigates the effects of fragrances on the ability to discriminate between odours from different individuals, an ability that previous findings suggest humans are capable of when odours are unfragranced. The study utilises body odour samples alongside fragrance-body odour blends. It also compares discrimination rates when the blend involves the individual's own preferred fragrance or an experimenter-assigned fragrance. The results suggest that discrimination performance differs when body odour is presented in isolation or when blended with a chosen fragrance and with an assigned fragrance.

Chapter 4 investigates the effects of fragrance use on the detection of mate quality cues in body odour, specifically masculinity (in men) and femininity (in women). This study highlights a female sensitivity in the detection of these cues, and further suggests that male fragrance use may be masking cues of masculinity in male body odour, with those who are low in masculinity significantly improving their masculinity ratings when wearing a fragrance.

Chapter 5 presents a review of the literature concerning disassortative mating related to major histocompatibility (MHC) cues in human body odour. Against this background, a novel study is presented with the aim of investigating the presence of disassortative odour mating in real world romantic couples, as well as the effects of hormonal contraception use on this mate choice. Additionally, it aims to investigate whether fragrances alter these effects, perhaps by enhancing distinctive odour profiles of individuals, as suggested by findings from chapter 3. The work presented in chapter 6 presents an initial investigation into developing a verbal scale which could be used to reliably detect and describe various differences in the perceptual qualities of odours, with the aim of relating these differences to various odour cues (such as MHC and sex of donor). The initial pilot scale developed appears to be useful in relating sex to perceptual odour qualities.

The second section of the thesis (chapters 7 & 8) relates more generally to body odours and behaviours, addressing both how body odours can affect the individual and also how they may affect others in the immediate environment.

Chapter 7 reports a study which attempted to tease apart the positive effects of fragrance and the negative effect of perceived malodour in self-reported confidence and attractiveness of fragrance wearers. The findings from this study further suggest that fragrance is important in influencing non-verbal behaviour, and that, as predicted by our relationship with body odour, malodour is deleterious to self-confidence.

Finally, chapter 8 presents an ambitious and novel experimental investigation of olfactory communication during pregnancy. Studies with non-human mammals suggest that males of species showing bi-parental care may undergo endocrinological and behavioural changes prior to the birth of offspring, perhaps in order to prepare them for their paternal role. The current study found no evidence to suggest that exposure to pregnant women's odours has this effect on men's behaviour. However, the study represents only an initial foray into this intriguing possibility, and the chapter discusses potential confounds which, if addressed in future research, may result in different findings.

The thesis concludes with a general discussion (chapter 9) of the implications of the findings presented, as well as presenting suggestions for future research, and methodological improvements.

Chapter 2: Methodology

Axillary odour collection

Odour Donors

All participants recruited for the following studies reported being heterosexual, non-smokers who regularly wore deodorant, unless otherwise stated. They will be referred to as odour donors throughout the following chapters. Additionally, and unless otherwise stated, we restricted our recruitment of female odour donors to women who were using hormonal contraception, in order to control for cyclical hormonal changes which are known to influence women's body odour (Gildersleeve et al., 2012; Havlíček et al., 2006).

Odour collection restrictions

In line with previous research, we instructed our donors to avoid drinking alcohol, being in smoky places, exercising and eating certain strong-smelling foods (e.g. garlic, asparagus, curry) one day prior to, and during, odour collection periods (Roberts, Havlíček, & Petrie, 2013). They were additionally asked to refrain from sexual activity and to avoid sharing their bed with anyone during the odour collection phases (Kohoutová, Rubešová, & Havlíček, 2011; Lenochová et al., 2012; Roberts et al., 2011). Donors were also provided with fragrance free soap (Simple Pure™) and asked to use only this in place of any fragranced hygiene products for 24 hours prior to odour collection, and in between any odour collection periods.

Odour collection

Each donor was provided with an odour collection pack containing instructions, including a reminder to avoid the aforementioned behaviour/foods, as well as experimenter contact details. The pack also included 100% cotton oval shaped makeup pads (approximately 9.5cm x 6.5cm, 3mm thick, Cosmetic Oval Pads, The Boots Company PLC) and surgical tape (Finepore™, 2.5cm wide). Donors were instructed to apply the cotton pad onto their armpit, using the tape to hold this in place, and to remove it after 24 hours had passed. There is variation in sampling time across studies, though numerous studies to date have adopted 24 hour sampling periods for odour collection (Kohoutová et al., 2011; Martins et al., 2005; Santos, Schinemann, Gabardo, & Bicalho, 2005; Sorokowska, Butovskaya, & Veselovskaya, 2015). Furthermore Havlíček et al. (2011) found that 12 hour sampling yielded samples which were less intense, and less likely to be perceived, compared with a 24 hour sampling period. Donors were instructed to shower with the fragrance free soap prior to and following each 24 hour odour collection period.

For some studies, donors were required to wear deodorant during odour collection, and in this case they were instructed to spray their deodorant in their usual fashion onto their armpit before applying the cotton pad in the same way. After 24 hours, donors were told to remove the pads and seal them in small plastic zip lock bags provided, each individual pad in a separate bag. These bags were pre-labelled before being given to each donor, with spaces for them to provide information concerning the date/time odour collection began and ended, which armpit the sample was from, their sex, and their donor code which they had been given by the experimenter.

Odour Storage

The donors returned the samples, labelled and in sealed plastic bags, to the lab within 2 hours of removal, where they were stored in a freezer at -30˚C until use.

Samples were thawed at room temperature for 2 hours prior to test sessions and re-frozen between test sessions. Previous research suggests freezing and thawing of samples has minimal impact on the perceptual quality of the odour (Lenochová, Roberts, & Havlíček, 2009; Roberts, Gosling, Carter, & Petrie, 2008).

Odour presentation

Unless otherwise stated, all odour samples were presented in 500ml clear glass conical flasks, with aluminium foil caps. When handling samples, latex free gloves were worn in order to avoid contamination of odour, and in the majority of studies both the left and right axilla samples from one donor were placed in a flask together (e.g. the left and right fragranced samples together, and the left and right unfragranced samples together).

After being informed of the respective study and providing consent, participants would be instructed on odour exposure. They were instructed to remove the foil cap, smell the sample and then to replace the caps. Participants were usually given as much time to smell the samples as they wanted, and were free to return and re-smell any samples if they chose to. None of the studies presented involved deception regarding the samples – all participants were fully informed that the samples being presented were collected from human axillae.

Participants could only reasonably be expected to smell a certain number of samples at a time without becoming subject to sensory fatigue, and so many of the following studies involved multiple sessions of odour ratings. After sample use, each glass flask was cleaned using a fragrance free detergent (Neutracon, Decon

Laboratories Ltd) and allowed to dry prior to the next test session, in order to avoid any cross contamination of samples.

Olfactory ability

Additionally, in some of the following studies, participants were asked to complete the Sniffin' Sticks olfactory ability screening test. This is a 12-item cued identification test of nasal chemosensory functioning (Hummel, Kobal, Gudziol, & Mackay-Sim, 2007; Hummel, Bensafi, et al., 2007). It employs the use of odour dispensing devices, shaped like pens. Participants removed the lids from these, sniffed each one, and then had to select, from a score sheet provided, the correct label for the odour from a choice of four words. The resulting score is the sum of correct answers. This test was completed after odour rating/exposure tasks so as to avoid interfering with participants' olfactory perception.

Section One

Chapter 3

Effect of fragrance use on discrimination of individual body odour

This chapter is based on the following published manuscript;

Allen, C., Havlíček, J., & Roberts, S. C. (2015). Effect of fragrance use on discrimination of individual body odor. *Frontiers in Psychology,* 6, 1115.

Introduction

Individuals appear to be adept at body odour recognition. For example, as mentioned in the general introduction, Lord and Kasprzak (1989) found that individuals could select a t-shirt which had been worn by themselves out of 100 worn by other people. There are a multitude of benefits incurred by an individual who can discriminate between conspecifics using olfactory information. It has been suggested that in the mother-infant relationship, odour recognition and detection are important for both the forming of an attachment, and for inducing feeding (Raimbault, Saliba, & Porter, 2007). It has been found that mothers can discriminate the smell of their own offspring from others (Ferdenzi et al., 2010; Porter et al., 1983), with neonates also reportedly being capable of discriminating between their own mother's axillary odours and that of an unfamiliar lactating female (Cernoch & Porter, 1985).

Although these findings suggest that body odour discrimination is important, as highlighted in the general introduction, personal odour is often 'modified' with the use of artificial fragrances (Roberts & Havlíček, 2012), with the conscious evaluation of body odour having a long history of negative connotations within numerous cultures (Schleidt et al., 1981). Reduction of ones' ability to detect individual characteristics of body odour would, at first sight, appear to be problematic given the information that can be gained from an individuals' odour and its influence in various social interactions. However, recent research suggests that, rather than masking odour entirely, fragrances may in fact be chosen to complement and perhaps enhance the volatiles present in an individuals' body odour. For example, Milinski and Wedekind (2001) found that Major Histocompatibility Complex genotype correlated significantly with an individuals' 'liking' of a fragrance compound, which they argue suggests that humans choose fragrances to amplify genetic cues present in their odour. In keeping with this, Lenochová and colleagues (2012) found that mixtures of participants' body odour with their perfume of choice were perceived by female raters to be more pleasant than a mixture containing a randomly assigned perfume, even when controlling for the pleasantness of fragrances. This suggests that fragrances are chosen to work in tandem with individual body odour, potentially enhancing an individuals' personal olfactory fingerprint.

Study rationale

In light of this, the current study aimed to investigate the effect of fragrance use on the perceived individual quality of body odour, thus further investigating whether fragrances may mask or enhance idiosyncratic cues in body odour. To do this, odour samples were collected from individuals who were matched on deodorant brand use. In order to assess participants' ability to discriminate between these odours, triangle tests were conducted in which participants had to select the 'odd one out' from three odours in which two were from the same individual. This test was conducted with both unfragranced body odour samples and, from the same individuals, blended samples of body odour and fragrance where the fragrance was the donor's usual brand of choice. The former allowed us to assess underlying ability for discrimination of body odours, while the latter allowed us to assess the impact of fragrance on idiosyncratic information available in that body odour. Finally, the test was repeated using samples containing body odour and a fragrance that was assigned to the donor by the experimenters (following Lenochová et al., 2012). This enabled us to investigate whether fragrance is specifically chosen by an individual in order to enhance their idiosyncratic biological information.

Based on previous findings showing that humans are capable of discriminating between individual odours, we expected that, at least in the unfragranced body odour condition, participants would be able to identify the odd one out at an above chance level. Similarly, in view of the findings of Lenochová et al. (2012), we predicted that performance would be at above chance levels for assessments of body odour and donors' own deodorant blends. Indeed, if body odour and fragrance do combine to form a new emergent odour, task performance might even exceed that of the no fragrance condition. In contrast, we hypothesised that participants would perform worse in the condition employing samples containing an assigned deodorant, as this fragrance had not been chosen by the donor and so might clash with the idiosyncratic body odour.

Methods

The study received ethical approval from the University of Stirling Psychology Ethics Committee.

Odour Collection

All donors provided informed consent. Odour samples were collected from 6 men (mean age ± SD = 24.5 ± 5.24, range 19-32) and 6 women (mean age ± SD = 21.17 ± 2.93, range 18-26), all of whom reported being heterosexual, non-smokers who regularly wore deodorant. As cyclical hormonal changes related to the menstrual cycle can affect the perceptual quality of body odors (Havlíček et al., 2006; Kuukasjärvi et al., 2004) we recruited only female donors who reported using hormonal contraception. Donors were additionally selected based on their current deodorant use, with all males reporting using the same commercially available fragrance (Lynx

Africa – deodorant body spray). Female donors did not all use the same deodorant, but were selected so that there were two individuals each using the same deodorant (two using Sure Crystal Invisible, two using Nivea Pearl and Beauty and two using Dove Go Fresh Pomegranate and Lemon – all antiperspirant deodorants). This ensured that, for both men and women, triangle tests could be established utilizing donor pairs who used the same fragrance. All 6 female donors reported shaving their armpits during the study, whereas all male donors reported not shaving their armpits.

Each donor provided three axillary odor samples; one whilst wearing no deodorant (no fragrance), the second whilst wearing their own deodorant (own fragrance) and the third whilst wearing a deodorant provided by the experimenter (assigned fragrance). The assigned deodorant was chosen on the basis that it was not currently, or previously, used by any of the donors, with the six males receiving the same commercially available product which was designed for men (Adidas Ice Dive – a deodorant body spray), and the six female donors receiving the same commercially available deodorant which was designed for female use (Vaseline Active Fresh – an antiperspirant deodorant).

Odour collection took place on three consecutive days, with donors following collection instructions detailed in chapter 2.

Triangle test participants

All participants were visitors at the Centre for Life in Newcastle upon Tyne. The tests for male and female odour samples were completed by independent sets of participants. In total, 238 participants (65 men; mean age ± SD = 40.15 ± 16.15, range 16-76 and 173 women; mean age ± SD = 41.97 ± 13.36, range 17-79) completed the test with male odour samples. A set of 189 participants (66 men; mean age ± SD = 41.11 ± 14.75, range 16-76 and 123 women; mean age ± SD = 38.06 ± 14.83, range 16-78) completed the test with female odour samples.

Triangle test procedure

Participants provided informed consent and basic demographic information (age and sex). The nature of the task was explained in advance, and participants were told that they would be smelling samples of body odour and fragrance. Each participant was then presented with three 500ml clear glass conical flasks, with aluminium foil caps, containing odour samples. Two of these odour samples were from the same individual, and the third was from a different donor of the same sex. For the donor who only presented one sample, the right axillary sample was used. Participants were informed that one of these was different from the rest, and they were instructed to remove the tinfoil covering and smell each flask before identifying the odd one out.

Within each triangle test donor samples were paired so that each pair used the same deodorant (males paired with males and females paired with females). There were three odour conditions, with each triangle test having all three samples containing either no fragrance, own fragrance or assigned fragrance, and participants were blind to these. Each participant took part in one session during which they completed one triangle test in each of the three odour conditions, with each test involving a different donor pair. Each session used either all male or all female donor samples, and consequently each participant was exposed to either all of the female or all of the male samples (see Table 1). After sample use each glass flask was cleaned using a fragrance free detergent (Neutracon, Decon Laboratories Ltd) and allowed to dry prior to the next test session. Both male and female samples were used in three separate test sessions (Table 1) each of which was conducted over approximately a day and a half. This meant that samples were thawed and used for 5-6 hours before being refrozen and thawed the next day where they were used for a further 2-4 hours (depending on the number of visitors at the centre). Samples were treated in the same way (i.e. time of use) across the three conditions. Table 1 shows the number of participants who took part in each test session.

Table 1 Donor pairings used in each triangle test. Each participant took part in one session, and was therefore exposed to all three conditions, with three odours in each (two of the same, one of a different donor), all of which were of the same sex. Consequently each participant was exposed to all of the male donor samples OR all of the female donor samples. Mean participant age ± SD is shown for each test session.

			Donors used in each condition		
	Test session		No Fragrance	Own Fragrance	Assigned Fragrance
Male donor samples	A	n = 68 mean age± SD = 42.69 ± 13.45	1 & 2	3 & 4	5 & 6
Male donor samples	B	n = 74 mean age± SD = 41.62 ± 13.80	3 & 4	5 & 6	1 & 2
Male donor samples	C	n = 96 mean age± SD = 40.49 ± 14.97	5 & 6	1 & 2	3 & 4
Female donor samples	D	n = 59 mean age± SD = 42.76 ± 15.47	7 & 8	11 & 12	9 & 10
Female donor samples	E	n = 71 mean age± SD = 36.11 ± 14.43	9 & 10	7 & 8	11 & 12
Female donor samples	F	n = 59 mean age± SD = 39.10 ± 14.06	11 & 12	9 & 10	7 & 8

Additionally, each participant completed the Sniffin' Sticks Screening test. This is a 12-item cued odour identification test (Hummel, Bensafi, et al., 2007) which assess ability to verbally label common odours. It employs the use of odour dispensing devices, shaped like pens. Participants sniff each of these and then must select the correct label for the odour from a choice of four words. The resulting score is the sum of correct answers. This was completed after the triangle test.

Results

Binomial tests were first conducted to compare the observed frequency of correct scores against that expected by chance (in this case .33). For each condition, participants were able to discriminate between the odours at a level significantly above chance (all p's < .001, Figure 1). A Chi-squared test indicated that there was a significant difference between the number of correct responses achieved in the three odour conditions, $Chi^2(2) = 23.87$, $p < .001$.

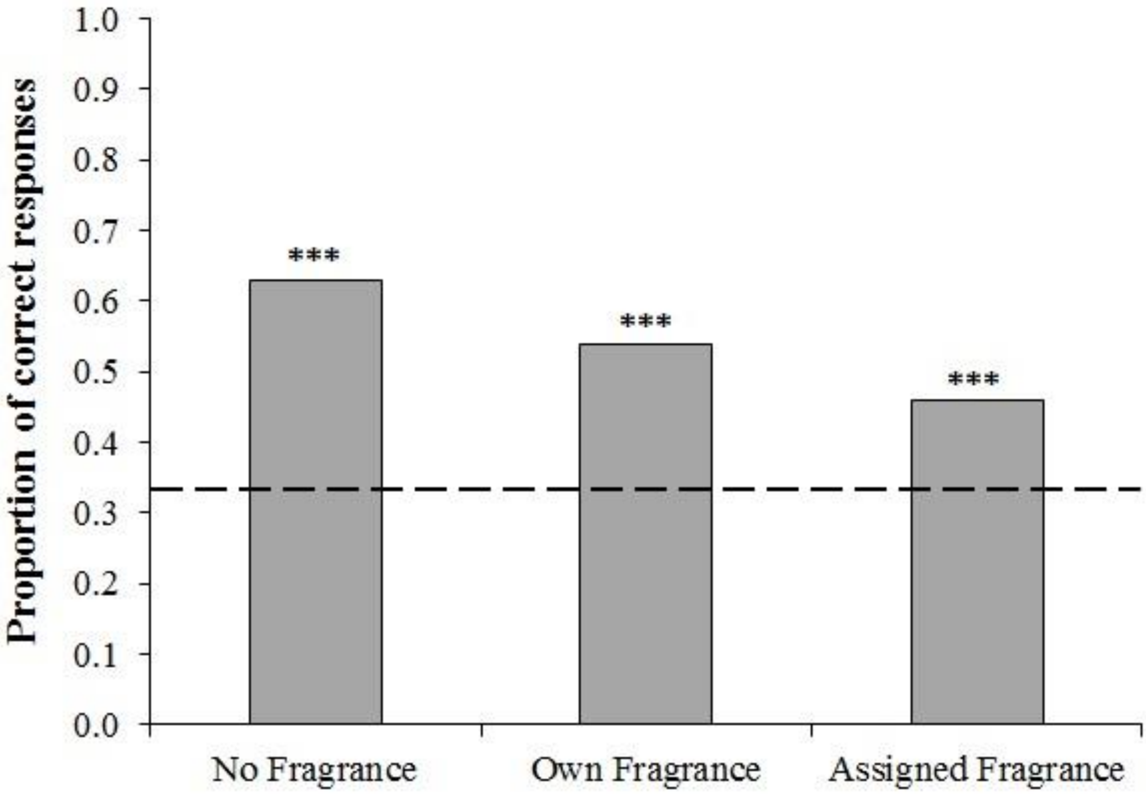


Figure 1 Proportion of participants who correctly chose the odd one out on the triangle test. Dashed line indicates the proportion of correct responses which would be expected by chance (0.33). Binomial tests indicate significance above chance level *** p < .001.

In order to investigate these differences further, a binary logistic regression was conducted. The dependent variable was the participants' response in each test (correct, incorrect) and five candidate predictor variables were included in the model; donor sex, participant sex, participants' scores on the Sniffin' sticks test, participants' age, and odour condition ('no fragrance', 'own fragrance', 'assigned fragrance').

Performance on the Sniffin' sticks test significantly and positively predicted participants' performance on the triangle tests, Exp (B) = 1.175, $p < .001$, as did participant sex, Exp (B) = .777, $p = .048$, (females having a higher proportion of correct responses, .57, compared to males, .48). The effect of donor sex was also significant, $p = .001$, Exp (B) = 1.503, such that there was a higher proportion of correct responses when assessing male samples (.59) compared with female samples (.49). Importantly, odour condition was found to be a significant predictor of test performance, $p < .001$. Orthogonal planned contrasts revealed that the proportion of correct responses was higher in the 'no fragrance' condition than that of the two fragranced conditions, Exp (B) = 1.749, $p < .001$, and higher in the 'own fragrance' condition than that of the 'assigned fragrance' condition, Exp (B) = 1.375, $p = .03$. The model also revealed a significant interaction between odour condition and donor sex, $p < .001$, with participants returning more correct responses when assessing female samples in the 'no fragrance' condition, Exp (B) = .175, $p < .001$, while the proportion of correct responses was higher in male samples in the 'own fragrance' and 'assigned fragrance' conditions, Exp (B) = 1.094, $p = .757$ (Figure 2). There was no significant interaction between participant sex and performance across the three conditions, $p = .603$. Interestingly, while it is well documented that olfactory ability declines with age (Hummel, Kobal, et al., 2007) though there was found to be no effect of participants' age on task performance, Exp (B) = .998, $p = .674$. It was, however, found that participants' age was significantly negatively correlated with performance on the olfactory ability test, $r = -.207$, $n = 420$, $p < .001$, with older individuals performing worse than younger individuals.

Finally, in order to further investigate the significant interaction between odour condition and donor sex, we repeated the analysis separately for responses to male and female samples by male and female participants (Figure 2). Binomial tests indicated that, for female odour samples, men correctly discriminated the odours at proportions above chance in the no fragrance condition, $p < .001$ (.68 correct), but not the own fragrance (.38 correct) or assigned fragrance condition (.30 correct), whereas women were correct at an above chance level in both the no fragrance, $p < .001$, (.75 correct), and the own fragrance conditions, $p = .03$, (.41 correct), but not the assigned fragrance condition (.36 correct, see Figure 2.a). However, performance was higher for male odour samples, with men performing at a significantly above chance level in both the no fragrance, $p =.001$, (.52 correct), and the own fragrance conditions, $p < .001$, (.58 correct), but not the assigned condition (.043 correct), and women performing above chance in all three conditions, no fragrance $p < .001$, (.57 correct), own fragrance $p < .001$, (.67 correct) and assigned fragrance $p < .001$, (.61 correct; Figure 2.b).

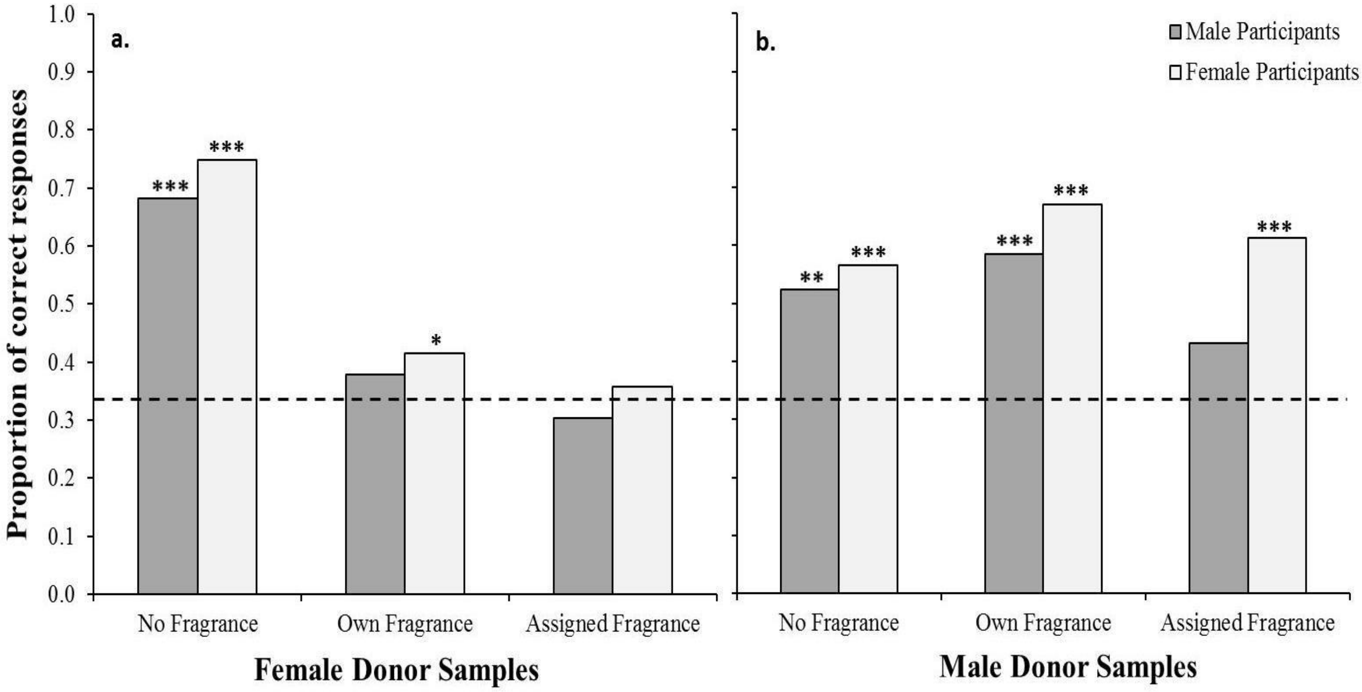


Figure 2 Proportion of male and female participants who correctly chose the odd one out on the triangle test when using female samples **(a.)** and male samples **(b.)**. Dashed line indicates the proportion of correct responses which would be expected by chance (0.33). Binomial tests indicated significance above chance * p < .05, ** p < .01, *** p < .001.

Discussion

The current study aimed to investigate the impact of artificial fragrances on the perception of individual body odours, and in turn, to investigate whether fragrances might either mask or enhance idiosyncratic information available in odours. This was achieved using a triangle test paradigm, with participants identifying the 'odd one out' from three odours, either with no fragrance, the donors' own fragrance, or an experimenter assigned fragrance. As expected, the discrimination rate was highest in the 'no fragrance' condition, followed by the 'own fragrance' and then the 'assigned fragrance' conditions. Furthermore, participants' performance on the triangle test was mediated by their olfactory ability, as assessed using the Sniffin' Sticks identification task. Individuals with higher identification scores performed better in the triangle tests. We found no relationship between participants' age and their performance on the task, which might at first sight be surprising given that olfactory ability tends to decline with age (Hummel, Kobal, et al., 2007). However, this is likely explained by the inclusion of scores from the Sniffin' Sticks task in the model. As would be predicted, these scores were negatively correlated with participants' age.

Our results also indicate that female participants performed better on the triangle tests than male participants did. This is perhaps unsurprising as it has repeatedly been reported that women tend to outperform men on various aspects of olfactory perception (Brand & Millot, 2001; Cardesín et al., 2006; Doty & Cameron, 2010). Additionally, previous work has also found women to outperform men in specific tasks of body odour identification (Schleidt, 1980) and self-recognition of body odours (Platek, Burch, & Gallup, 2001).

Irrespective of the participant sex differences reported, all participants were good at discriminating between odours, performing at a significantly above chance level in the no fragrance condition, supporting previous findings such as those of Lord and Kasprzak (1989). Furthermore, participants' performance was also at a significantly above chance level in both of the deodorant conditions, lending further support to the idea that fragrance does not mask information present in body odour. More importantly however, was the finding that performance was significantly better in the 'own fragrance' condition compared to the 'assigned fragrance' condition. This indicates that fragrance-body odour blends involving individually preferred fragrances are qualitatively different from blends involving randomly selected fragrances. Such findings further substantiate claims by Milinski and Wedekind (2001) and Lenochová et al. (2012) that fragrances may, perhaps unintentionally, be chosen to complement body odours. However it does appear that, while participants' performance when assessing blends with the fragrance of choice was better than with assigned fragrances, it was poorer than when assessing body odour alone. This suggests that the emergent quality of the blend does not appear to actively enhance individuality, even though it does not appear to mask it either.

It must be noted, however, that the current study raised some interesting questions regarding differences in discrimination between odours when using male and female samples. For female odours the findings were largely consistent with the overall analysis, such that unfragranced samples were the easiest to discriminate, followed by own fragranced samples and then assigned fragranced samples, and with discrimination of assigned fragrance samples being at about chance levels (though performance in the two fragranced conditions was not significantly different).

However, this pattern was not evident in male samples with participants performing in all conditions at a significantly above chance level, and with there being no significant difference between participants' performance across the three conditions.

It is possible that this finding was driven by the quality of the male odours. Male odours appear to be more intense and distinctive than female odours, and it may therefore be easier to discriminate between them even in the presence of a fragrance. In support of this, previous studies have suggested that discrimination between male and female odours is probabilistic, with sex classifications being related to the perceived intensity of the odours: stronger, more intense odours are more likely to be judged as male than weaker ones, regardless of the actual sex of the odour donor (Doty, Orndorff, Leyden, & Kligman, 1978; Doty, 1981). An alternative, or contributory explanation is that the male fragrances used here were all deodorants, containing only fragrance and compounds which reduce the presence of odour causing bacteria, whereas the female fragrances used were all antiperspirant deodorants, and thus additionally contained compounds which inhibit the production of sweat. This may have also contributed to different levels of intensity in the male and female samples, but intensity was not assessed by our raters and we therefore cannot confirm this. One further possible explanation is that the assigned fragrance for the male donors was in some way perceptually different than that given to the female donors, making discrimination of male odours easier. Either of these suggestions, in isolation or taken together, may provide an explanation for the improved performance with male samples, and future research should aim to investigate this further by including intensity ratings of the individual odours, with and without fragrances, as well as ratings of fragrance intensity in the absence of body odour, or perhaps by utilising a unisex fragrance for the assigned condition.

Furthermore, due to the setting in which the experiment took place we were somewhat restricted as participants did not have time to complete more than three tests (taking approximately 10-15 minutes per participant). Conducting the study in this environment presented a trade-off between the number of participants completing the test and the number of tests they each completed, which allowed us to obtain a very good sample size with a large and representative age range. Importantly the odour conditions were balanced, with each participant completing a test in each odour condition, which is the critical element of the experimental design. It should also be noted that while we recruited a large sample of participants, there were only six donors of each sex, and future research should employ a larger number of donors in order to present a more representative range of odours.

Despite this, the current study benefits from adopting a more ecologically valid methodology than has previously been used. Previous research investigating the effects of fragrances on body odour tend to use perfumes as opposed to deodorants (Havlíček & Roberts, 2013). There is a good reason for this; perfumes are solely fragrance, whereas deodorants combine fragrance and odour suppressants. However, deodorants are widely used, with one study reporting that between 82.7-93.3% of 17,000 individuals sampled in the UK indicated that they used a deodorant either daily or on most days (Rodriguez, Steer, Farrow, Golding, & Day, 2013). Thus, assessment of the effects of deodorants, as well as perfumes, are important to understand the cultural effects of modern patterns of fragranced products on odour perception. It is also noteworthy that individual discrimination was possible despite the odoursuppressing qualities of deodorants and their anti-microbial action, and that because of this the current findings may actually underestimate discrimination rates. Furthermore, it was in the odour samples provided by women, who used antiperspirant deodorants, that identification was improved with the use of a chosen versus an allocated fragrance, lending additional support to the importance of fragrance/body odour blends in identification, rather than a reduction of sweat or body odour.

The findings from this study help to reveal just how complex the perception and holistic affective response to fragrance users by other individuals around us is in real-life interactions. As mentioned above, the majority of people wear some form of fragrance on a daily basis (Rodriguez et al., 2013). It is also likely to be the case that when entering a mate choice arena, for example when going on a date or for a night out in a nightclub, that an even larger proportion of individuals will be wearing fragranced products. Given this, it is most likely that encounters with new individuals in many social settings, and perhaps especially in a mate-choice context, will involve the perception of fragrance and body odour blends, rather than either the fragrance or body odour alone. This, coupled with the findings from the current study and those of Lenochová and colleagues (2012), highlights the potential importance of the fragrance choice decision that individuals make. It has been shown, for example, that fragrance preferences are linked to idiosyncratic genetic traits such as MHC (Manfred Milinski & Wedekind, 2001), but future research should focus on elucidating the fragrance choice process that individuals undergo, assessing the relative role of genetics but also other factors such as commercial advertising, which are likely to be influential in this process.

Clearly more work is needed to further elucidate the effects of fragrance on individual discrimination, as well as understanding the process related to fragrance choice, but the current study has provided some ground work which will be useful for directing future research in this area. The main findings are in keeping with previous literature discussed, supporting the idea that individual fragrance choice does not mask information present in body odour, though further research is needed to clarify the difference between odour discrimination of male and female odours. Finally, while we have found evidence to suggest that personal fragrance choice does not prevent the overall discrimination of an individual, further investigation must be carried out to ascertain whether fragrance use masks other kinds of information that may be available in body odour, such as emotions, health status and fertility status.

Chapter 4

The impact of artificial fragrances on the assessment of mate quality cues in body odour

This chapter is based on the following manuscript which has been invited for resubmission;

Allen, C., Cobey, K. D., Havlíček, J. & Roberts, S. C. (invited resubmission). *Evolution and Human Behavior.*

Introduction

It has been posited that the contradiction regarding olfaction and fragrance use, discussed in the general introduction, may represent an interaction between culturally evolved practices and biologically evolved olfactory signals. Indeed it has been proposed that biologically evolved preferences might even shape cultural practices. Havlíček and Roberts (2013) discuss the use of cosmetics in this regard, an example of this being that individuals may wear foundation in order to improve the appearance of skin health – a biologically evolved preference being enhanced via a cultural practice. In support of this, one study found there to be greater contrast in the luminance of females' faces than males', and that gender assumptions of androgynous faces could be manipulated by increasing or decreasing the luminosity contrast of images (Russell, 2009). Furthermore, the authors found that the same face had higher levels of contrast when makeup was applied compared to having no makeup applied, lending support to the concept that facial cosmetics are used to enhance sexually dimorphic attributes, in this case femininity, which may play a role in human mate choice.

Recent research (see chapter 3) also suggests that rather than completely masking cues present in body odour, fragrances may instead be chosen (perhaps unintentionally) to enhance the unique qualities of an individual's body odour. Preference for common perfume ingredients relates to the Major Histocompatibility Complex (MHC), a set of genes involved in immune function (Hämmerli, Schweisgut, & Kaegi, 2012; Milinski & Wedekind, 2001). MHC is potentially an important cue of genetic compatibility in humans, as in other species, and MHC-disassortative odour and mating preferences have been recorded (Havlíček & Roberts, 2013). MHC-correlated perfume choice may thus enhance idiosyncratic immunogenetic cues available in body odour and be used in mate choice. In further support of this, Lenochová and colleagues (2012) found that mixtures of participants' body odour with their perfume of choice were perceived to be more pleasant than mixtures of body odour and an experimenter-assigned perfume, suggesting that choice of fragrance may complement underlying body odour. Additionally, Allen and colleagues (2015) found that odour discrimination was improved when using a fragrance of choice compared to an experimenter assigned fragrance. However, how fragrance use may interfere with odour-based discrimination of specific mate quality cues has not been explored.

In order to clarify this issue, we investigated the effects of fragrance use on the perception of masculinity and femininity in men and women. These traits have been previously linked to mate choice and sexual selection in humans, with masculinity potentially reflecting underlying genetic quality in males (Thornhill & Gangestad, 1999) and femininity being identified as a trait representing reproductive potential in human females (e.g. Fraccaro et al., 2010). Both traits are detectable across multiple modalities (Fraccaro et al., 2010; Little, Connely, Feinberg, Jones, & Roberts, 2011), with perceptions of facial masculinity having recently been found to correlate with morphological sexually dimorphic traits such as height and weight (Holzleitner et al., 2014). Additionally, both traits are central constructs used in the commercial development of fragrances, with most perfumes and deodorants being classified as either masculine or feminine (so-called unisex fragrances are in the minority; Lindqvist, 2012). This further cements the cultural relevance of these sexually dimorphic traits for males and females, making them prime candidates for cultural practices which may have emerged as a result of a biologically evolved preference. Fragrances, as with other cosmetics, may be designed and used to enhance the perception of these traits, thus making an individual more appealing to the opposite sex.

Study rationale

The current study aimed to investigate whether commercially available fragranced products lead to changes in ratings of masculinity/femininity, traits for which we show evolved, sexually dimorphic preferences. This cultural practice of applying fragrance might interfere with our perceptions of these traits for which we show evolved preferences. If this would be the case, fragranced products would decrease discrimination of masculinity/femininity. In order to assess these hypotheses, we aimed to first replicate previous findings that these mate-choice relevant, sexually dimorphic traits assessed using one modality are correlated with the assessments of the same trait in another modality. This was accomplished by examining the relationship between odour rated and facially rated masculinity/femininity. By comparison of these cross-modal relationships between faces and axillary odour, with and without the presence of a fragrance, we were able to investigate the impact that fragrance had on the assessment of individuals' odour, here taken as representing one aspect of their attractiveness to a potential mate. We hypothesized that fragranced odour samples would be rated as more masculine or feminine than unfragranced samples (as predicted if they are mimicking our biologically evolved preferences). Furthermore, we predicted that the ratings of masculinity and femininity given to male and female *unfragranced* axillary odours would be correlated with the ratings given to the same individuals' faces. Finally, we hypothesized that the presence of an artificial fragrance would lead to biased assessment of an individual's masculinity/femininity through body odour, thus resulting in no correlation being found between fragranced odour ratings and face ratings of masculinity/femininity, as fragrances are specifically designed to enhance these traits reducing the individual variation in these underlying body odour cues, and essentially their value as a cue (Lindqvist, 2012).

Method

The study received ethical approval from the University of Stirling's Psychology Ethics Committee.

Odour Donors

Odour samples were collected from 20 men (mean age ± SD = 23.25 ± 4.23; range: 19-33) and 20 women (21.2 ± 2.50; range: 18-27) recruited from the University of Stirling, all of whom were heterosexual non-smokers who regularly wore deodorant. We restricted our recruitment of female odour donors to women who were using hormonal contraception, in order to control for cyclical hormonal changes which are known to influence women's body odour (Gildersleeve et al., 2012; Havlíček et al., 2006).

We collected two axillary odour samples from each donor: one while donors were wearing no underarm fragrance (hereafter termed the 'unfragranced sample') and one while donors were wearing their usual underarm fragrance (hereafter termed 'fragranced sample'). The two odour collection periods were on consecutive days (unfragranced followed by fragranced), and donors were instructed to follow the sample collection procedure outlined in chapter 2.

Finally, digital color facial photographs were taken of each donor (head and shoulders) in standardized lighting conditions, at a standard 1.5m distance against a neutral grey background, using a Canon PowerShot G6 digital camera (7.1 megapixel, focal length range of 7.2 to 28.8mm). For the purpose of the photo, participants were instructed to adopt a neutral expression. All participants were requested to remove make-up beforehand, and to remove glasses, jewelry and facial piercings.

Odour Raters

Odour samples were rated by 275 same and opposite-sex raters. We excluded scores if raters did not complete all of the ratings (N = 23), indicated they were homosexual (N = 12) or answered 'prefer not to say' with regard to their sexual orientation (N = 1), leaving a total of 239 raters used in analyses.

Male odour samples were rated by a total of 75 women (mean age ± SD = 20.12 ± 2.39; range: 17-30), and by 45 men (21.26 ± 4.16; range: 18-40). Female odour samples were rated by an independent set of 75 women (21.67 ± 4.05; range: 18-49) and 44 men (21.25 ± 2.01; range: 19-26).

Face Raters

Participants were an independent set of 204 individuals recruited via online social networking sites, and were not familiar with the individuals they were rating. As with odour ratings, incomplete responses (N = 65) and those from raters who were homosexual (N = 6) or who chose 'prefer not to say' (N =3) when completing the sexual orientation question were excluded, leaving a total of 130 raters used in the analysis. For the male face rating task, the final sample of raters included 42 women (mean age ± SD = 28.26 ± 9.61; range: 21-62) and 16 men (30.81 ± 11.37; range: 23-

62). Female faces were rated by an independent set of 54 women (24.99 ± 8.28; range: 18-54) and 18 men (30.17 ± 10.39; range: 19-49).

Odour Rating Procedure

After providing informed consent, participants were asked for some basic demographic information. Each participant then rated odour samples presented in clear glass 500ml conical flasks with aluminum foil coverings. Participants were asked to rate the perceived masculinity or femininity of each odour on a 7-point scale (1 = below average, 4 = average, 7 = above average). Female samples were rated for femininity and male samples for masculinity. In order to avoid sensory overload, each rater judged samples from 5 donors (all male or all female), rating both the unfragranced and fragranced samples from these 5 donors (10 samples in total). In this way, the 20 male and 20 female donor samples were each divided into four groups of five. The four groups of male odour samples were judged by similar numbers of female raters (N = 19, 18, 18, 20 for groups 1-4, respectively) and male raters (N = 10, 11, 13, 11). This was also true of female raters (N = 20, 18, 20, 18) and male raters (N = 9, 13, 10, 12) assessing female odour samples. Mean values were computed for each donor separately from ratings given by same- or opposite-sex participants, for both face and odour ratings.

The order in which participants rated the unfragranced and fragranced samples was counterbalanced. As participants rated a subset of 5 of the 20 donors' samples, and as the focus of our study was on the comparison between fragranced and unfragranced samples, it was not deemed necessary to counterbalance the order of individual donor ratings within each odour condition.

Face rating procedure

Two online photograph rating tasks were created, one for male donors and one for female donors. Images appeared individually and participants rated faces for masculinity/femininity (depending on sex of the stimuli). The order in which each image appeared was randomized between participants. Participants who completed the face ratings also provided basic demographic information (age, sex, sexual orientation).

Results

Effects of fragrance on odour ratings

In order to investigate the effect of fragrance on sample ratings, we ran a repeated-measures ANOVA with two within-subjects factors, each with two levels (fragrance condition: fragranced, unfragranced; rater sex: same, opposite). As the male and female donor samples were assessed on an analogous but different scale (i.e., masculinity, femininity) we ran the analysis for each donor's sex separately.

For ratings given to male donors, there was a significant main effect of rater sex, with female raters giving higher ratings of masculinity to odour samples ($M = 3.51$, $SD = .62$) than male raters ($M = 3.31$, $SD = .68$), $F(1,19) = 5.657$, $p = .028$, $d = .31$. However, there was overall no significant difference between unfragranced and fragranced samples, $F(1,19) = .219$, $p = .645$. There was also a significant interaction between the sex of the rater, and the ratings given to the two fragrance conditions, $F(1,19) = 6.103$, $p = .023$ (Fig. 3). Post hoc paired sample t-tests revealed that there was no significant difference between the ratings given by females to fragranced and unfragranced samples, $t(19) = -.857$, $p = .402$, or between ratings given by males to fragranced and unfragranced samples, t(19) = 1.321, p = .202. However further analysis did reveal a significant difference between ratings given by males (M = 3.13, SD = .81) and females (M = 3.59, SD = .69) to fragranced samples, t(19) = 3.782, p = .001, d = .61, but not between the ratings of unfragranced samples by males and females, t(19) = -.337, p = .740 (Figure 3a).

The same analysis was then completed for the ratings obtained for female donors' odour samples. Here there was no significant main effect of rater sex, F(1,19) = 1.556, p = .227, but there was a significant main effect of fragrance, with the fragranced samples being rated as more feminine (M = 3.76, SD = .93) than the unfragranced samples (M = 3.06, SD = .64), F(1,19) = 17.450, p = .001, d = .88 (Figure3b). Unlike with the male donors, there was no significant interaction between rater sex and ratings given to the two fragrance conditions, F (1,19) = .029, p = .866. In exploratory post hoc analyses, we found that there were significant differences between ratings of fragranced and unfragranced samples given by both male and female raters, with fragranced samples being rated as more feminine than unfragranced samples, t (19) = -4.96, p < .001, d = .78 (figure 3b).

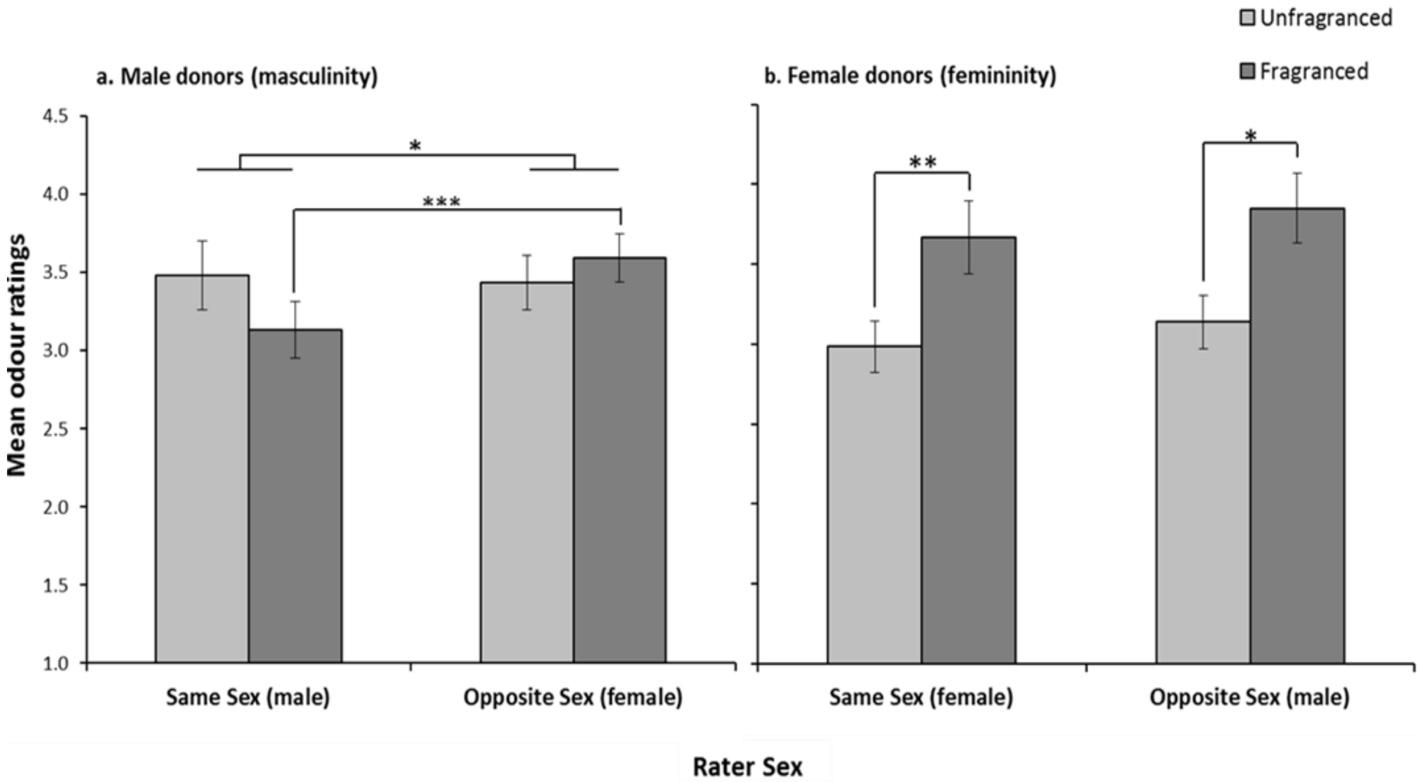


Figure 3 a. Mean odour ratings given by female raters to male odour samples (masculinity) and female odour samples (femininity). **b.** Mean odour ratings given by male raters to male odour samples (masculinity) and female odour samples (femininity). Bars represent mean ± 1 SEM. Lines represent post hoc tests, *p<.05, **p < .01, *** p < .001.

Relationship between face and odour ratings

Next, we investigated whether perception of femininity/masculinity was concordant across modalities by running correlational analyses using the mean ratings given to the odours and facial photographs of the donors.

For female raters, there was a significant and positive correlation between their ratings of unfragranced odours and face ratings of female donors, *r* (20) = .53, p = .02 (Figure 4a), as well as the fragranced odours and face ratings of female donors, *r* (20) = .50, p = .03 (Figure 4b). Furthermore, we found a significant and positive correlation between ratings given by females to unfragranced odours and male donors faces, *r* (20) = .45, p = .046 (Figure 4c), but the correlation between ratings of fragranced odour and male donors faces was not significant, *r* (20) = .005, p = .98 (Figure 4d).

For ratings given by male participants, there were found to be no significant correlations between unfragranced odour ratings and face ratings, *r* (20) = .34, p = .15 (Figure 5a), or fragranced odour ratings and face ratings given to female donors, *r* (20) = .17, p = .46 (Figure 5b.). Additionally there were no significant correlations found between unfragranced ratings of odour and face ratings, *r* (20) = .08, p = .74 (Figure 5c), or fragranced ratings and face ratings given to male donors samples, *r* (20) = .07, p = .77 (Figure 5d).

Figure 4 Mean ratings of faces and odours (fragranced and unfragranced) given by female raters to both male and female donors. Male donors were rated for masculinity; female donors for femininity. Panels **a** and **c** show ratings of unfragranced odour samples and faces; panels **b** and **d** show ratings of fragranced odour samples and faces.

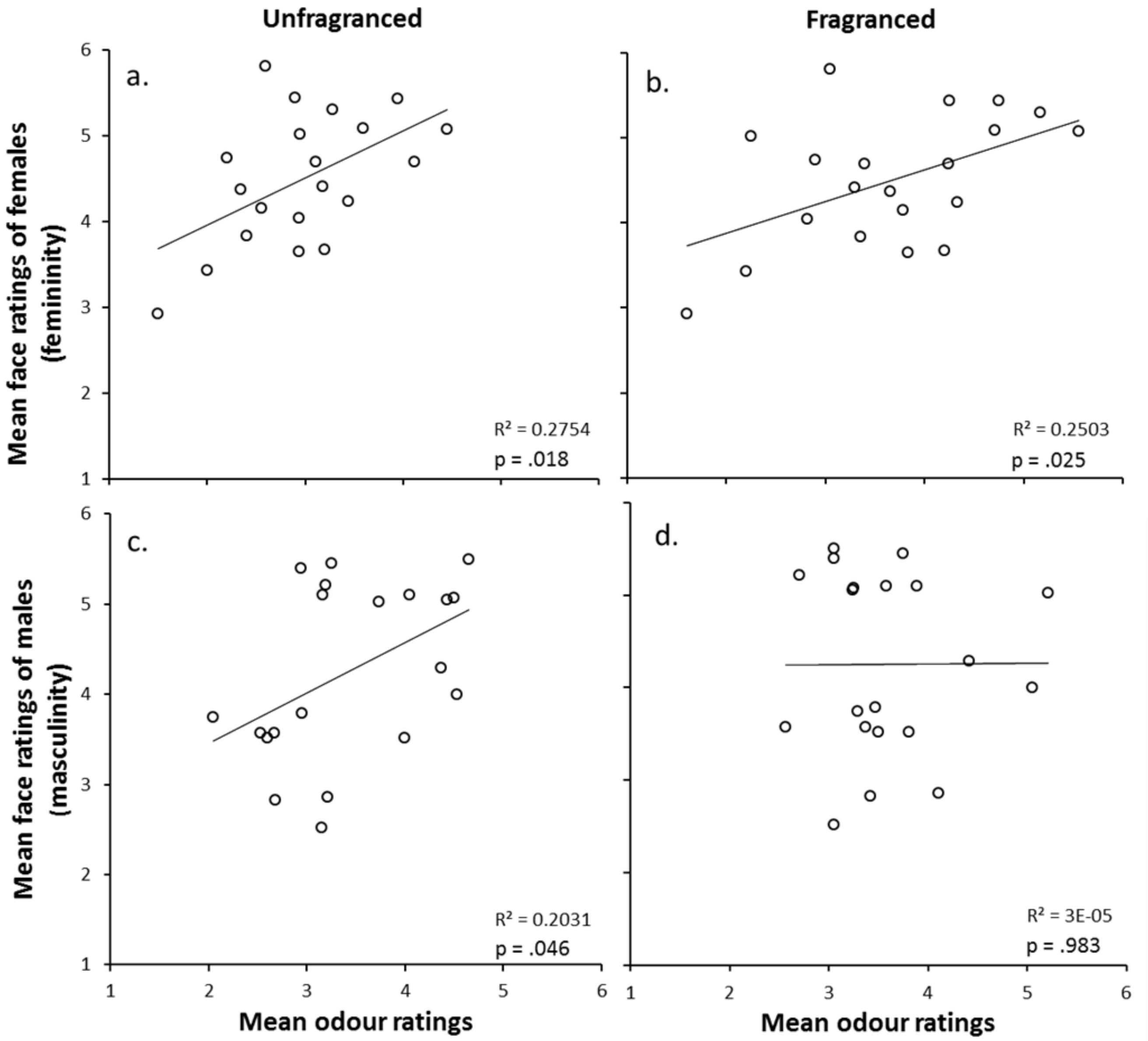
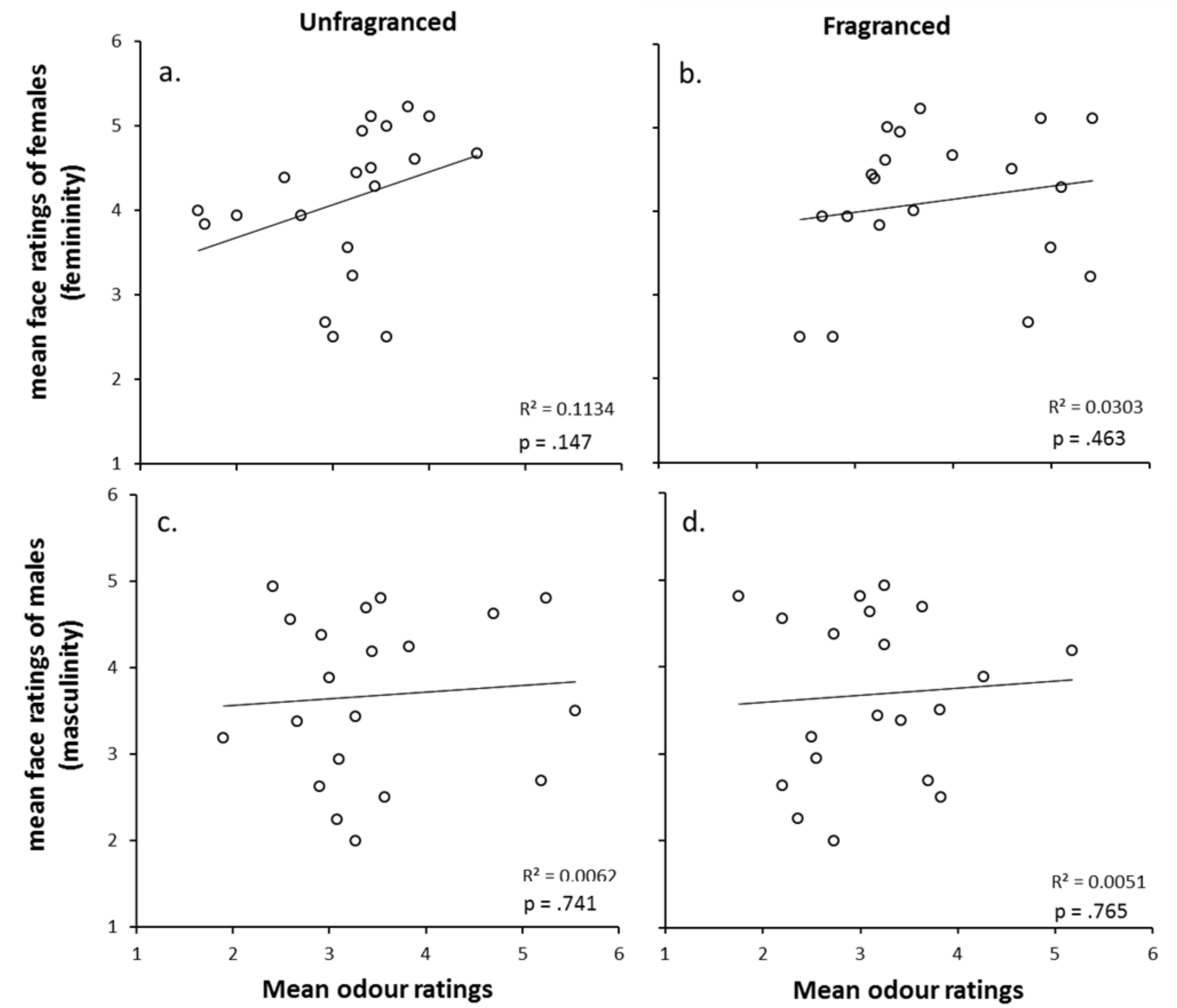


Figure 5 Mean ratings of faces and odours (fragranced and unfragranced) given by male raters to both male and female donors. Male donors were rated for masculinity; female donors for femininity. Panels **a** and **c** show ratings of unfragranced odour samples and faces; panels **b** and **d** show ratings of fragranced odour samples and faces.



In order to further understand the differential effect that fragrance appeared to be having on ratings of masculinity and femininity given by same- and opposite-sex raters, we used a median split to divide the male and female donors into two groups; those who had received relatively high face ratings of masculinity/femininity and those who had received relatively low ratings. We then ran a repeated measures ANOVA, including fragrance as a within-subjects factor (fragranced, unfragranced), and high/low masculinity/femininity face ratings (split by the median) as a between-subjects factor. This analysis was run separately for male and female donors' ratings, as well as for same and opposite sex raters.

There was no significant main effect of fragrance condition for women rating men, $F(1,18) = .88$, $p = .36$. However, there was a significant interaction between ratings given by women to the male fragranced and unfragranced samples and the high/low score for facial masculinity, $F(1,18) = 4.84$, $p = .04$ (Figure 6a). Post-hoc independent samples t-tests revealed that there was a significant difference between mean ratings given to the unfragranced samples of individuals in the high ($M = 3.83$, $SD = .65$) and low ($M = 3.03$, $SD = .74$) face masculinity groups, $t(18) = -2.55$, $p = .02$, $d = 1.13$, but not between the fragranced samples, $t(18) = -.17$, $p = .87$ (Fig. 6a). Paired samples t-tests further indicated that while there was a significant difference between the ratings for fragranced ($M = 3.56$, $SD = .66$) and unfragranced ($M = 3.04$, $SD = .74$) samples given to men grouped with 'low' facial masculinity, $t(9) = 3.36$, $p < .01$, $d = .74$, the same difference was not significant for the men grouped as having 'high' facial masculinity, $t(9) = -.71$, $p = .49$ (Figure 6a). This model was re-run using ratings given by males, and as before, there was no significant main effect of fragrance, $F(1,18) =$

1.66, p = .21, and there was no longer found to be a significant interaction between the ratings given to fragranced and unfragranced samples, and donors high/low face masculinity, F (1,18) = .08, p = .79 (Figure 6c).

The same analysis was conducted for female donors' ratings. For ratings of femininity from males we found that, unlike with male donors ratings by females, there was a significant main effect of fragrance, F (1,18) = 10.61, p = .004, d = .82 with fragranced samples receiving higher ratings of femininity than unfragranced. However there was no significant analogous interaction between face ratings and odour ratings, F (1,18) = .08, p = .79, as had been found with the male donors (Figure 6b). When analyzing responses from female raters there remained a main effect of fragrance, F (1,18) = 23.33, p < .001, with fragranced samples receiving on average higher ratings of femininity than unfragranced samples, and, as with male raters, there was no significant interaction between face and odour ratings, F (1,18) = .04, p = .84 (Figure 6d).

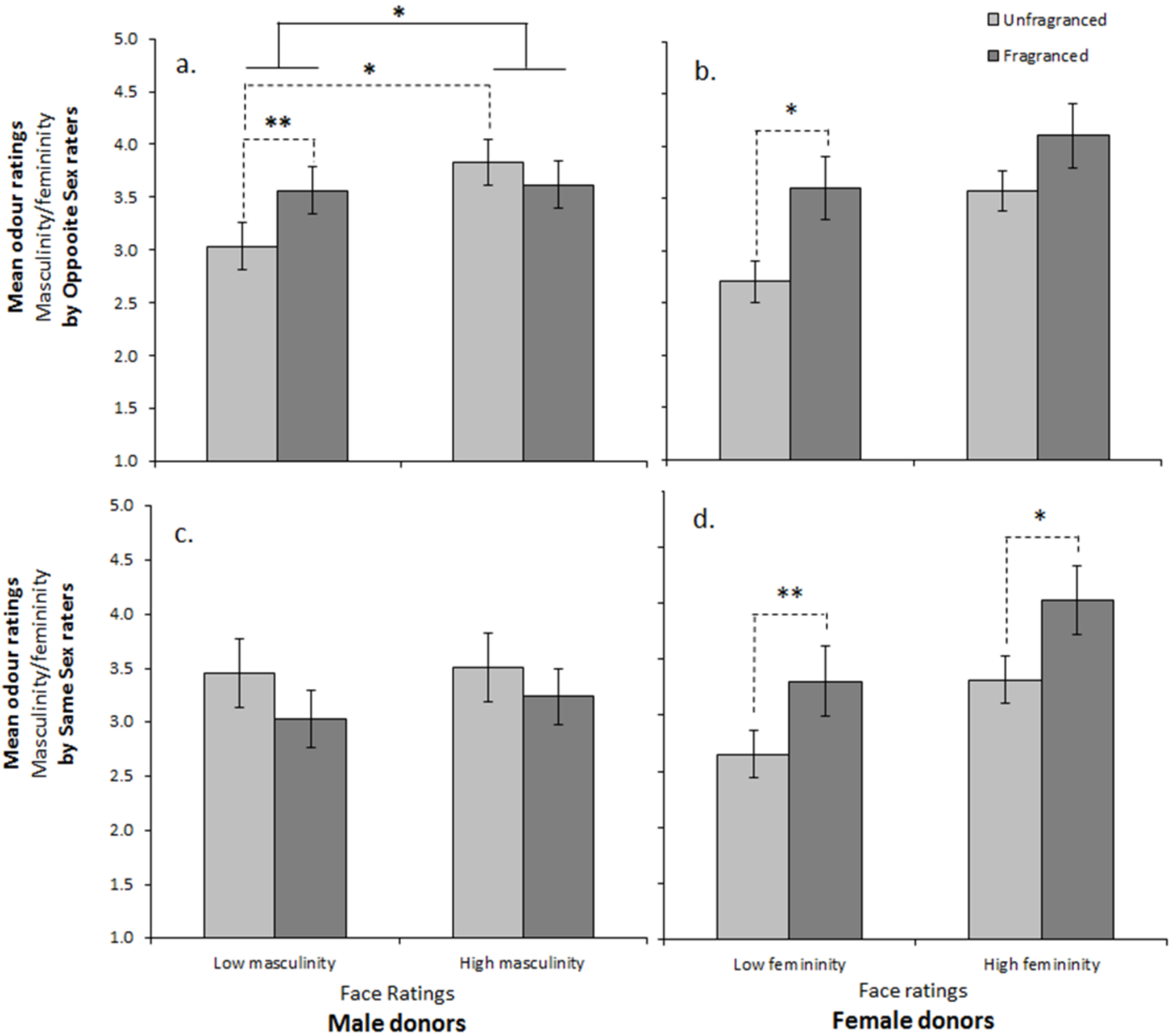


Figure 6 Mean ratings of masculinity/femininity given by same and opposite sex raters for male and female donor samples, split by high/low face masculinity/femininity ratings. Bars represent mean ± 1 SEM. Solid line represents significant interaction. Dashed lines represent post-hoc tests, * p < .05, ** p < .01. **b** and **d** depict main effects of fragrance which were not present in figures **a** and **c**.

Discussion

In this study we set out to investigate the effects of artificial fragrance use on the assessment of masculinity/femininity from body odour. In order to ascertain the impact of fragrance use, the relationships between face and odour ratings were investigated, both with and without fragrance.

Initially, we were interested in how fragrances influence the perception of body odour, and the current analysis suggests that this effect differs depending on the sex of the odour donor and of the rater. When looking at male odours, female raters tended to give higher ratings of masculinity than male raters, especially in the fragranced samples, suggesting that women are perhaps more attentive to perceptual changes in these traits. Despite this, fragranced samples were not rated as being significantly more masculine than unfragranced samples by either men or women, and ratings of femininity for female samples did not differ between male and female raters. However, female samples were found to be significantly more feminine with the addition of a fragrance, when rated by men and women, supporting the idea that fragrance may be used, as other cosmetics appear to be (e.g. Russell, 2009), to enhance potentially biologically evolved preferences.

This pattern of results potentially reflects some difference between fragrances designed for males and females – female fragrances may be designed to be more feminine than male fragrances are masculine. This explanation is still consistent with a evolutionary framework. For example, there are negative associations with being perceived as extremely masculine, with one study finding that masculine faces had decreased perceptions of warmth, emotionality, honesty, cooperativeness and parental quality (Perrett et al., 1998). Females have also been found to prefer a moderate level of masculinity over an extreme level (Rhodes, Hickford, & Jeffery, 2000). We know of no such studies that find analogous consequences of women being 'too feminine', with research suggesting that extreme feminization may not elicit these same negative responses (Rhodes et al., 2000), thus giving no reason to avoid over-feminizing a fragrance. Consequently, fragrance developers may avoid high levels of masculinity in male fragrances but not of femininity in female fragrances.

Our second prediction, that ratings of traits would be correlated across modalities, was partially supported, but this again appeared to be sex-dependent. There were significant correlations between ratings of masculinity and femininity given to unfragranced samples and faces which were rated by females (for both male and female samples), but this was not the case for ratings given by males (for both male and female samples). This finding builds on the one discussed above, further suggesting a sex-dependent attentiveness in perception of traits relating to masculinity/femininity. One potential explanation for this is that, due to sex differences in the physical/biological costs of reproduction (Trivers, 1972), it is more important for women to accurately assess these cues of potential mate quality, and so women show an increased sensitivity to the detection of this information. This is supported by previous work indicating that women are more sensitive in general than men are to odours (Brand & Millot, 2001), and that women place more importance on odour than males do in mate choice situations (Havlíček et al., 2008; Herz & Cahill, 1997). This sex difference may be exacerbated at certain times of a woman's menstrual cycle, as women's olfactory ability has been found to be heightened during the ovulatory phase of the cycle when estrogen levels and conception risk are high relative to other points in the cycle (Doty, 1981; Navarrete-Palacios, Hudson, Reyes-Guerrero, & Guevara-Guzmán, 2003; for a recent meta-analysis see Nováková, Havlíček, & Roberts, 2014). It could also be argued that women use more fragranced products than men do (Roberts et al., 2010) and that this additional experience may lead to an increased sensitivity/awareness. This is supported by recent findings that adults who reported higher engagement in odour-related activities during childhood show increased odour awareness and odour identification (Nováková, Valentova, & Havlíček, 2014). Though this argument could likewise be reversed; women are more sensitive to odours which leads them to use more fragranced products. While women may use more fragranced products, it is likely that the average man is exposed to a large number of fragranced products through daily interactions with women and their environment. In order to investigate this further future studies may benefit from measuring hygiene habits and individual differences in fragranced product use and exposure in raters.

The final hypothesis, that the addition of an artificial fragrance would prevent the accurate assessment of an individuals' masculinity/femininity through body odour, again partially supported by the current findings, also appeared to be dependent upon the sex of the rater. A significant correlation between facial masculinity ratings and odour masculinity ratings by women for unfragranced samples was no longer statistically significant when fragranced samples were assessed. Further analysis using a median split on men's facial masculinity also supported this: men with highly rated facial masculinity had significantly higher masculinity ratings of their unfragranced samples than those men with low face ratings. Importantly, this discrepancy between odour ratings in men with high and low facial masculinity disappeared with the addition of a fragrance. From an individual strategy perspective, and in support of the use of cultural practices to improve upon traits for which we show evolved preferences, this finding may suggest that those who already have desirable levels of masculinity achieve little benefit from wearing a fragrance. However, individuals low in these traits can potentially improve how others' perceive them through the application of a fragrance.

The story is less clear concerning the relationship between females' odours and face ratings. Unlike male raters, the significant correlation ratings of femininity of odours and faces by female raters, when assessing the unfragranced samples, also remained in the fragranced samples. Further analysis indicated that women rating female odours did not discriminate between donors who had received high or low scores for facial femininity. This pattern was also noted in male ratings of female odours, in keeping with the lack of concordance between face and odour ratings given by men as discussed above. This finding provides further evidence of a sex-specific attentiveness in assessing these olfactory cues, with heterosexual women appearing to have more acute perception of these traits than males. This increased olfactory attentiveness may be useful in a mate choice context, both for inter- and intrasexual selection, aiding the choice of a mate but also perhaps allowing accurate assessment of potential female competitors. However, it must be noted that fragrance use only appeared to *interfere* with accurate rating of male odours. Consequently, future research should investigate whether factors including current relationship status and relationship intent also play a role in an individuals' sensitivity/perception of these cues. Indeed, previous research has shown these factors are important contributors to mate preference. For instance, female preference for dominance in male body odour varies with relationship status (Havlíček, Roberts, & Flegr, 2005).

The current study provides evidence which further supports the cross-modality of mate quality cues in humans and their availability for use in a mate choice context, though it appears, at least with masculinity/femininity, to be specific to female perceivers. Additionally, the findings suggest that current widespread fragrance use might potentially interfere with evolved body odour-based preferences, with fragrances potentially being used in an analogous fashion to other cosmetic products such as makeup (Havlíček & Roberts, 2013). At least for men, fragrance use appears to be enhancing levels of body odour masculinity, and this in turn appears to make it harder for females to discriminate between individual males based on this trait.

One notable limitation in the generalisability of this study is that, while a large sample of participant raters were recruited (239 odour raters and 130 image raters), the sample did have quite a narrow age range of both donors and participants. Future research may benefit from establishing whether the findings are robust across a larger range of ages. Additionally, it is unclear how our findings can be extended to regularly cycling women, as all female donors were using hormonal contraceptives. This afforded us good control of the samples; however, it prevents us from generalizing our findings across all reproductive age women. There was also potentially some noise introduced into the data since our female raters included women both on and off hormonal contraception and analysis did not account for cycle stage. Furthermore, participants used fragranced deodorants rather than simple fragrances, so there may be a confounding factor of body odour suppression coupled with fragrance addition. Future research should address these issues and carefully control the commercial products used. Finally, it is difficult to predict from the current study whether use of fragrance would interfere with the assessment of other mate choice relevant traits (e.g., health, personality), which may be influenced differently by the addition of artificial fragrances. Recent research indicates that fragrance use might have significant impact on body odour-based personality judgments (Sorokowska, Sorokowski, & Havlíček, forthcoming) and individual discrimination of body odours (Allen et al., 2015, chapter 3). Future research will be important to determine the wider impact of fragrance use on these and other important social variables.

Chapter 5

An investigation of olfactory based disassortative mating in humans

Introduction

The previous chapters have demonstrated that the application of fragrances have varying effects on perceivers' ability to detect certain kinds of biologically relevant information in body odour. The effects vary with the sex of the wearer and perceiver, with women showing a greater ability to detect olfactory information. Additionally, fragrance appears to affect the perceptions of Individual traits which may be important in a mate-choice scenario, such as masculinity, as was found in chapter four. This chapter now investigates real life mate choice with regards to olfactory similarity, and the effect that hormonal contraception may have on this. It is predicted that individuals who meet and begin a relationship whilst the female is using hormonal contraception (HC) will smell more similar to one another than those who began a relationship whilst not using HC. The theoretical basis for this prediction is based on the literature pertaining to the Major Histocompatibility Complex (MHC), a group of genes related to immune function which have been implicated in mate choice, and appear to be detectable via human body odour. This is a large and active field of research, which has been reviewed on numerous occasions (Brown, 1997; Havlíček & Roberts, 2009; Kempenaers, 2007; Lie, Simmons, & Rhodes, 2010; Milinski, 2006; Neff & Pitcher, 2005; Penn & Potts, 1999; Setchell & Huchard, 2010; Tybur & Gangestad, 2011), and this chapter aims to review the main findings to date and additionally present a novel study aimed at further investigating the role of body odour in human mate choice, and how fragrance use may interact with this.

Immune function and sexual reproduction: The red queen hypothesis

One theory which has been proposed to explain the presence of sexual over asexual reproduction has been that of pathogen or parasite induced selection pressures. Initially posited by Van Valen (1973), the Red Queen hypothesis proposes that each generation will require new combinations of genes for resistance so as to deal effectively with parasites which are currently prevalent in the environment. From this it is proposed that a female should select the male whose own resistance genes, in combination with hers, provide any potential offspring with the optimum immune response for the current infectious environment.

Milinski (2006) points out that, in order to validate the Red Queen hypothesis, there are three requirements which must be met by the species in question. First, there must be large variation in these resistance genes in the environment. Second, the female must somehow be aware of her specific genes. Third, she must also be able to detect and assess the genes of any potential mates if she is to choose between them. There is in fact some substantial evidence supporting each of these three requirements, and therefore the Red Queen hypothesis, in humans.

The MHC (formally referred to as HLA, human leukocyte antigen, in humans) is a family of genes which has been linked to immune functioning in many vertebrates (Cooper & Alder, 2006; Kelley, Walter, & Trowsdale, 2005). Genes present in the MHC code for antigens that are responsible for recognising cells which contain proteins that are of foreign origin, the recognition of which is vital to initiating an immune response (Austyn & Wood, 1994). The MHC is a potential candidate for fulfilling the initial requirement outlined by Milinski (2006), as not only is it related to immune functioning, it is also extremely polymorphic in many vertebrates, to the point where it has been labelled a *supergene* by some (Hedrick, 1994).

There is also a growing body of evidence supporting Milinski's final two requirements for the Red Queen hypothesis, that individuals may in some way be 'aware' of their MHC, and furthermore, be able to select for a partner with a dissimilar MHC, which would increase the heterozygosity of potential offspring at this genetic locus. Research has found that this genetic trait may be detectable from visual cues related to facial appearance (Lie, Rhodes, & Simmons, 2008; Roberts et al., 2005; Thornhill et al., 2003), though findings are mixed with some studies showing preferences for assortative mating, going against the predictions of the Red Queen hypothesis (Roberts et al., 2005), and others finding no significant preferences for facial cues and MHC choice (Coetzee et al., 2007; Thornhill et al., 2003). This section will however focus on the literature concerning the role of olfactory signalling in MHC disassortative mating, as well as the evidence relating to real world mate choices.

Is MHC type detectable through olfaction?

Studies utilising twins have found that genetic information may be detectable via axillary odours in humans. Kalmus (1955) initially found that dogs could distinguish between the odours of identical twins, however, when conducting a retrieval task of axillary odours, dogs were found to accept the odour of either identical twin, suggesting that the odours were in some way perceptually similar. Building on this, Hepper (1988) found that dogs could discriminate between the odours of twins when they differed in their genetic relatedness or environmental factors (mostly diet), but that they could no longer discriminate between odours when twins where identical in both of these factors. Somerville and colleagues (1990) found that dogs matched the odours of twins at a greater rate than they did the odours of unrelated individuals, further suggesting that genetic information is available in human body odour. Roberts and colleagues (2005) also found that humans were capable of matching odours of monozygotic twins, but not dizygotic twins, at above chance levels, even when the twins had been living apart. Furthermore, matching of monozygotic twin odours was not significantly different from participants' ability to match duplicate odours from a single individual, suggesting that humans can perceive genetic cues in body odour. Gilbert and colleagues (1986) also discovered that humans were capable of distinguishing between the odours of mice who differed genetically only at the MHC, providing evidence that humans are capable of detecting olfactory differences in odours that relate to this genetic information. Research employing a chemical sensor device, or 'electronic nose', has also been used to detect MHC dependent odour types (Montag, Frank, Ulmer, Wernet, & Go, 2001).

Do humans show preferences for certain MHC types?

A number of experimental studies have also been conducted utilising human odours and investigating preferences for MHC type, the first of which was conducted by Wedekind and colleagues (1995). They typed 44 male and 49 female students on antigens coded by three HLA genes. They then provided the male participants with t-shirts to wear for two nights, with the now standard instructions to avoid fragranced products, strong-smelling foods, alcohol, smoking and bed sharing. After odour collection, the female participants were presented with a personalised set of six of these t-shirts, three of which had been identified as being dissimilar to the individual female participants' MHC (with an average of 5.9 dissimilar HLA antigens), and three of which had been classified as similar in MHC (an average of 2.7 dissimilar MHC antigens). The women were then asked to smell and rate these 6 t-shirts for pleasantness. It was found that the male odours were rated as being more pleasant when they were being rated by a female with a more dissimilar MHC profile, and vice versa. In further support of this preference for dissimilar odour, the women taking part also more often reported being reminded of a current, or ex-partner, when smelling the t-shirts that belonged to the MHC dissimilar men, than when smelling those belonging to the MHC similar men, suggesting that the experimental preferences for MHC dissimilar odours may reflect real world partner choices. One caveat to this was that, if the female participants were currently using hormonal contraceptives, they were not found to show this preference for the odour of MHC dissimilar men. This is in keeping with findings discussed in the previous chapters which suggest that female sensitivity and improved olfactory ability may be related to fertility and hormonal fluctuations which are tied to the menstrual cycle. It would be most adaptive for women to be able to access and assess MHC information in a potential mate when they were at an increased risk of pregnancy.

Wedekind and Füri (1997) found further support for MHC-correlated odour preferences. They also had participants rate t-shirts for pleasantness, and found a negative correlation between MHC similarity of t-shirt wearer and rater and the pleasantness ratings given to the t-shirts. They also replicated the finding that this correlation did not appear to be present when using ratings given by females who were using hormonal contraceptives, a finding which has also been demonstrated more recently by Roberts and colleagues (Roberts et al., 2008). Wedekind and Füri additionally found that individuals were more likely to be reminded of a current/ex-partner when sniffing a t-shirt which had significantly fewer MHC alleles in common with themselves than would be expected by chance. This study was also interesting as they found that the correlation was also present when men rated t-shirts for pleasantness, and additionally that the gender of the t-shirt wearer did not have an effect on this. However, while the study had a large sample size they only utilised 6 individuals to provide odour, and therefore these findings must be extrapolated with caution to the general population. Finally, a study by Jacob and colleagues (2002) found that preferences for odours were higher when there was a greater level of allelic matching between the sample odour and the participant.

While these findings seem quite clear cut, it must be noted that some studies have produced contradictory results. For example, Thornhill and colleagues (2003) found that male participants did show a preference for the scent of a t-shirt that was dissimilar in MHC, but there was no association between MHC similarity and odour pleasantness in female raters. They also found that the fertility status of the female rater was in no way correlated with the scent preferences, as had been found in previous studies. Though it should be noted that this study adopted a correlational design, unlike the studies of Wedekind and colleagues which used categorical odour preferences and this may potentially underlie the differences in findings. Furthermore, Santos and colleagues (2005) did not find a correlation between pleasantness of donors' odours and level of MHC dissimilarity with raters, but they did find that as the MHC similarity between odour and rater increased, so too did the raters' difficulty in deciding whether the odour was pleasant or unpleasant.

A more recent study, by Sorokowska and colleagues (2015) has however lent further support to the original findings of Wedekind (1995) and Wedekind and Füri

(1997), regarding preferred odours and odours of ex- and current partners. They asked 76 women to sniff axillary odour samples collected on cotton pads and then rate these for similarity to family members, and also to romantic partners. Participants were also asked to rate these odours on a number of traits such as sexiness, attractiveness, as well as whether the individual would make a good father. They found that, when an odour was deemed to be similar to that of a partner, it was rated as being more pleasant, as well as being associated with sexiness, tenderness, physical attraction, reliability, and the potential to be a good father. These traits were not associated with the odours deemed to be similar to that of a family member, which the authors take as further support for the hypothesis that women would not consider men as potential mates if they are high in genetic similarity. However, it must be noted that in this study each female participant only smelled one odour sample and furthermore, that only an assumption of genetic similarity was made regarding the donors and the raters.

So, overall the findings from studies of human odour preferences in laboratory settings are somewhat mixed, but do mostly appear to support the idea that individuals show preferences for odours related to MHC dissimilarity (Havlicek & Roberts, 2009). An important validation of these findings would be finding negative consequences of MHC assortative mating in humans and investigating real world mate choices and MHC dissimilarity; findings relating to both of these points will now be reviewed.

Are there negative outcomes related with high MHC homozygosity?

The red queen hypothesis states that each generation will require new combinations of resistance genes in order to deal with evolving parasite risks, and by this logic, one would expect that those individuals with a more heterozygous MHC genotype should deal more efficiently with pathogen and parasite threats. This has been found to be the case in numerous vertebrates. For example, Penn and colleagues (2002) introduced multiple strains of avirulent pathogens to mice and found that MHC heterozygous mice were more likely to survive and clear an infection and had a higher weight than homozygous mice. Additionally, in non-human primates, homozygosity of certain MHC genotypes has been linked to a more rapid progression of the Simian Immunodefficiency virus (SIV), with Sauermann and colleagues (2000) finding that only one Rhesus monkey died rapidly after infection with SIV, as opposed to five of the homozygous animals (e.g. Sauermann, Krawczak, Hunsmann, & Stahl-Hennig, 1997). Findings supporting this have also been noted in humans with HIV. Tang and colleagues (1999) studied 342 individuals who had HIV/Aids and typed them for various HLA genotypes. They found that homozygosity at the HLA –A and –B loci were more common among those individuals who had a more rapid progression to late stage HIV related conditions.

MHC compatibility also appears to play a role in pregnancy outcomes in humans. Ober and colleagues (1998) conducted a ten year prospective study in 111 Hutterite couples, following 251 pregnancies. They observed significantly increased foetal loss rates in couples who matched for all 16 HLA loci typed, and additionally that couples who matched for HLA –A and HLA –B also suffered increased foetal loss rates. This finding that rates of spontaneous abortions are often higher when couples share a larger proportion of MHC alleles has been replicated numerous times (e.g. Beer, Semprini, Zhu, & Quebbeman, 1985) as well across multiple cultures (e.g. Ho, Gill, Nsieh, Hsieh, & Lee, 1990; Koyama et al., 1991). Furthermore, evidence from fertility clinics and failed fertility treatments supports the relationship between MHC homozygosity and difficulties with conception rates. For example Weckstein et al. (1991) found that couples who had two or more failed in vitro fertilisation (IVF) treatment attempts also shared a significantly greater number of HLA antigens with one another than did control couples who reached a viable pregnancy during their first round of fertility treatment. Balasch and colleagues (1999) also found that 15 couples who failed three attempts at IVF shared a statistically greater proportion of HLA genes than those couples who conceived on the first treatment attempt and than a control group of 100 fertile couples. This appears to be a robust phenomenon, again being present cross culturally, with other studies also reporting associations between failed IVF treatment and increases in partners levels of shared HLA haplotypes (Creus et al., 1998; Ho et al., 1994).

In sum, findings from human and non-human animal studies do suggest that there are negative consequences of increased MHC homozygosity. This includes both an individuals' ability to resist infection and effects of partner MHC similarity on conception and pregnancy outcomes.

Real world mate choice in humans and MHC

Given the negative outcomes of MHC homozygosity, and the research suggesting that individuals appear to, mostly, prefer the smell of those with a dissimilar MHC, it would then be predicted that we would find real world examples of disassortative mating in humans based on their MHC profiles. However, the findings appear to be mixed. Some of the earliest studies found there to be little evidence of non-random mate choice in humans. For example, Pollack and colleagues (1982) typed 61 couples for their HLA antigens and found no evidence that HLA phenotypes were involved in human mate preferences. Nordlander and colleagues (1983) similarly found no evidence of HLA disassortative mating in 826 Swedish couples. Rosenberg and colleagues (1983), in their large sample of 1017 couples, found evidence to support assortative mating at the MHC, with couples sharing more MHC alleles than would be expected by chance, but concede that their large sample was very ethnically diverse and suggest that their findings may in fact simply reflect mating preferences for same ethnicity. However, studies using ethnically homozygous populations have also failed to find evidence for MHC disassortative mating preferences. Using a solely Caucasian data set of 500 couples, Jin and colleagues (1995) did not find any evidence of disassortative MHC mate choices, nor did Sans et al. (2008) in their sample of 183 Uruguayan couples. In keeping with this, Ihara and colleagues (2000) found in their two sample populations (150 couples from northeast Japan, and 300 couples from other various regions of Japan) that the proportion of shared HLA alleles in romantic partners was not significantly different from what would be expected by random choice.

These studies do not bode well for the MHC disassortative mating hypothesis, but in their review, Havlíček and Roberts (2009) point out that these studies have all been conducted in parts of the world where they believe modern life styles could have obscured findings, for example with increased population mobility, which they suggest would potentially lead to increased heterogeneity of various MHC alleles. Additionally, these studies fail to take into account use of hormonal contraception, which we know may have an effect on the detection of olfactory cues of MHC. So then the best evidence would come from communities which avoid these problems. As Havlíček and Roberts note, there are very few studies investigating this. One study conducted by Hedrick and Black (1997) focussed on 194 couples from 11 Amerindian tribes, finding that the proportion of HLA sharing between couples was very close to that which would be expected by chance. Ober and colleagues (1997) did, however, find support for MHC disassortative mating. They typed 411 Hutterite couples, an isolated North American society, and found that there were fewer HLA matches between spouses than would be expected by chance.

Some more recent work has, however, been found to lend support to the MHC disassortative mating hypothesis. Using genome wide data from the HapMap II data set Chaix, Cao and Donnelly (2008) investigated the frequency and similarity between spouses of 3,214,339 single nucleotide polymorphisms, of which 9,010 were located at the MHC loci, in a group of 30 European American Mormon couples, and 30 African couples of the Yoruba population in Nigeria. They found that the European couples were significantly more dissimilar at MHC loci than randomly paired individuals, and furthermore, they found that this level of dissimilarity was ‘extreme’ when compared to similarity in the rest of the genome. However, they found no such evidence for MHC disassortative preferences in the African couples, finding no significant pattern for similarity or dissimilarity at the MHC region, but instead finding a genome wide pattern of assortative mating. They posit that the differences seen between these two groups may reflect socio-demographic processes, with the Yoruba population being a patrilineal exogamous society. The authors suggest that marriages here occur more frequently between genealogically related lineages than unrelated ones, which could explain this global pattern of genetic similarity, though they provide no data to support this. It must also be noted that this study has been criticised, namely by Derti and colleagues (2010) who believed that the initial effect reported was weak, and when they applied their own similar analysis of the data set found no significant effect in the European American sample. Laurent and Chaix (2012) responded to this criticism, correcting the significance threshold to account for multiple hypothesis testing, as had been suggested by Derti, and they claimed to still find that dissimilarity at the MHC loci in European American spouses was extreme compared to the entire genome. It therefore appears that, although the use of the HapMap data may be useful in shedding light on the presence or absence of disassortative mating in humans, there is still work to be done, which is to be expected when tackling such a large and complicated data set as the entire human genome. Nonetheless, it could be cautiously concluded that the findings do suggest that, at least in some societies, disassortative MHC partner preferences may be present.

Garver-Apgar and colleagues (2006) conducted a particularly interesting study, which did not investigate the level of MHC disassortative mating in the population, but instead investigated the effects of MHC similarity in real world couples. In their sample of 48 romantic couples it was found that women's sexual satisfaction and responsiveness to their partners was negatively correlated with the number of MHC alleles shared between the two. Intriguingly, the authors also found that the number of extra-pair affairs reported by females was correlated positively with the number of shared MHC alleles between the couple. This correlation appeared to be specific to MHC similarity, as the effect remained when measures of sexual attitudes and promiscuity were taken into account, and as the number of shared MHC alleles in the current relationship was not found to be correlated with the number of extra-pair affairs that were reported from past relationships. Finally, it was additionally found that rejection of the male partners' sexual advances and the tendency to be unfaithful were found to be strongest during the phase of the menstrual cycle when probability of conception is highest (late follicular phase). These findings suggest then that MHC similarity within relationships may have a significant impact on a woman's behaviour, in a way that is unexpected given the individuals' general behaviour or their behaviour in previous relationships.

To summarise, the research on the role of MHC in real world partner choice remains mixed, though the findings from Garver-Apgar and colleagues (2006) suggest that increased MHC similarity in romantic couples may have negative effects on the relationship. Perhaps in certain societies, as Havlíček and Roberts (2009) suggest, MHC disassortative preferences are prevented due to certain societal factors. We have already discussed the use of hormonal contraception and how this may affect MHC detection or preferences, and next we will focus on fragrance use and how this may impact MHC detection.

Interaction between fragrance use and MHC olfactory cues

Further evidence for the role of MHC in odour preferences, and perhaps also human mate choice, is provided by studies which show interesting associations between fragrance use and MHC types. Milinski and Wedekind (2001) recruited 63 women and 74 men who had been typed for three HLA haplotypes, and asked them to initially rate 36 perfume ingredients for whether they would like to smell like that, and a following set of 18 for whether they would like to smell like that and additionally whether they would like a partner to smell like that. They found a significant association between two HLA types and preferences for self in both rating tasks, but not for preferences for partner. They suggest that this supports the hypothesis that fragrances are chosen to enhance personal biology and enhance the availability of MHC related immunogenetic cues in mate choice (as discussed already in chapters three and four). In further support of this hypothesis, Hämmerli, Schweisgut and Kaegi (2012) found that some of the more common MHC alleles could be used to subdivide a population into groups holding differential preferences for various perfume ingredients.

Furthermore, Lenochová and colleagues (2012) found that mixtures of an individuals' body odour with their fragrance of choice were rated as more pleasant than when mixed with an assigned fragrance, even when there was no difference in pleasantness of the fragrances in isolation of body odour. These studies together suggest, as Milinski and Wedekind (2001) first hypothesised, that artificial fragrances are perhaps being used to amplify the important olfactory cue of MHC type in humans. The current study aims to investigate these partner and fragrance preferences further, with the aim of providing more evidence for this hypothesis.

Study rationale

Although the evidence is at times contradictory, the fact that there are findings from multiple different sources (odour preferences in laboratory settings, effects of homozygosity on health and pregnancy outcomes, real world relationships and genome analysis) that support the importance of MHC in human mating suggest that further research is needed to illuminate the situation. Unlike, for example, masculinity, MHC dissimilarity preferences potentially present a much more complex adaptation to be investigated, especially given the scale of polymorphism within this genetic locus. To complicate matters further, as highlighted by previous researchers (Havlíček & Roberts, 2009), there are potential cultural influences which make this phenomenon harder to research, and the current study aims to investigate two of these further. One of these is the role of hormonal contraception and how this may be affecting mate choice in certain societies, a factor which was only considered in some of the more recent studies. The other is the role of fragrances, which, as stated in the general introduction, are prevalent in many societies, used on a frequent basis, and present in numerous personal care products, and the use of which has been specifically linked to MHC types. The current study aims to investigate both the effects of hormonal contraception and fragrance use on the similarity of odours from real world couples.

The literature to date suggests that expression of disassortative preferences for MHC in mates would be potentially beneficial. It also suggests that MHC information is available in body odour, and from this it can be inferred that dissimilar MHC complexes smell different to one another. Given this, we can predict that individuals in romantic relationships will on average smell dissimilar to one another. Furthermore, it has been found in some studies that women who are using hormonal contraception do not show this preference for MHC dissimilarity, so we can further predict that individuals in heterosexual relationships who meet and begin a romantic relationship when the female is using hormonal contraception, should on average smell more similar to one another compared with individuals who meet and begin a romantic relationship when the female is not using hormonal contraception. Finally, if preferred fragrances reflect an individuals' personal immunogenetics, then we might expect the same pattern of similarity and differences when smelling odour samples mixed with fragrances of couples who met whilst using or not using hormonal contraceptives. The study aimed to investigate this by comparing perceived similarity of odours (with or without fragrance) from real world couples, who either met whilst using or not using hormonal contraception (HC). As a control, we also used odour pairs from 'fake' (experimenter created) couples.

The hypotheses for this study were that ratings given to 'fake' couples (created from pairing odours from individuals who are not in relationships) will be rated less dissimilar than actual couples, and that couples who met whilst using HC would be rated on average as smelling more similar than those who met and began their relationship when not using HC. Additionally, it was predicted that these findings would hold true when using fragranced samples. Finally, given the findings from previous chapters, it was expected that there would be a difference in ratings given by male and female raters, perhaps with female raters being more able to differentiate between the three groups with their similarity ratings (HC, no HC and Fake) than males.

Methods

Odour Donors

Thirty heterosexual couples who had been in a romantic relationship for at least 6 months, and in which the female partner had not yet reached menopause, were recruited to provide odour samples. Fifteen of these had begun their relationship whilst the woman was using hormonal contraception, and fifteen had met whilst the woman was not using any form of hormonal contraception (mean age of women = 28, SD = 8.59, range 20-51 years; mean age of male partners = 29.47, SD = 9.21, range 20-51 years).

Each individual underwent two 24 hour odour collection periods on consecutive days as detailed in chapter two, the first of which was without any fragranced products and the second was collected whilst wearing the individuals' usual deodorant or perfume.

Donors also completed an online questionnaire to collect basic demographic information, as well as information on length of relationship, cohabitation status and current and past contraceptive use. Of those who met whilst using hormonal contraception, 10 were cohabiting and 5 were not. Of those who were not using hormonal contraception when they met, 12 were cohabiting and 3 were not. Couples also completed the Relationship Assessment Scale (Hendrick, 1988), consisting of 7 phrases, which participants rate using a 1-5 scale, where 1 = low and 5 = high (see appendix A).

Raters

Ratings of odours took place at the Centre for Life in Newcastle upon Tyne, where 437 visitors participated (280 women, 157 men). After excluding those individuals who did not complete the ratings for all odour samples, there were 261 female (mean age = 40.89, SD = 10.35, range: 17-76) and 152 male raters (M = 42.67, SD = 12.26, range = 17-78; participants were included if they had completed all samples ratings, even if they had not completed the Sniffin Sticks 12 item olfactory ability identification test, see chapter two, n = 30).

Procedure

In all, 15 test sessions were carried out over 5 days (3 sessions each day). Each session lasted between 1.5 and 3 hours depending on recruitment rate, and contained samples from different couples.

Each participant took part in one test session only. After providing informed consent, they were presented with 6 pairs of conical flasks containing body odours (12 flasks in total). Participants were only presented with this many pairs of odours in order to reduce any potential effects of sensory overload or olfactory fatigue. Pair 1 contained the unfragranced odour samples from one donor couple who began their relationship whilst using HC (male and female odours in separate flasks) and pair 2 were the same couples' fragranced samples. Pairs 3 and 4 were the odour samples of a couple who began their relationship whilst not using HC, with pair 3 containing the unfragranced and pair 4 the fragranced sample. Pairs 5 and 6 were from a 'fake' couple. A male and a female from separate relationships were assigned as a pair by the experimenter, with pair 5 again using the unfragranced sample of one man and one woman, and pair 6 using the fragranced samples from the same man and woman. The individuals chosen for the 'fake' couples were those whose samples were already being used in one of the other two test sessions from that day. In this way we were able to reduce the amount of time that samples were unfrozen. Each sample was thawed and used for one day (6-8 hours, before being re-frozen. Research to date suggests that perceptual qualities of odour samples remain robust to freeze-thaw cycles (Lenochová et al., 2009). Samples were stored in a cool box with ice packs when not in use during the day.

Participants did not know the sex of the samples or that the samples came from individuals in romantic relationships. Participants were instructed to remove the tin foil caps from each pair of flasks (e.g. flask 1 and 2), sniff both samples, and then to rate from 1 (completely) to 9 (not at all) how similar the two smelled to one another.

Finally each participant also completed the Sniffin Sticks olfactory ability test.

Results

Donor as the unit of analysis

Independent samples t-tests indicated that there was no significant differences between relationship length, cohabitation length, age difference, RAS scores, or RAS difference scores (calculated by subtracting females score from corresponding males score) between the couples who met whilst using HC or not (Table 2).

Table 2 Demographic and relationship data from couples who met whilst using HC and those who met when not using HC. Data are means ± SEM; differences were tested using independent-samples t tests.

Variable	Mean HC	Mean No HC	t	df	p
Relationship Length (months)	66.40 ± 20.26	85.07 ± 19.52	.63	28	.513
Cohabitation length (months)	36.40 ± 18.11	59.87 ± 20.59	1.20	28	.240
Male partner age	26.47 ± 1.64	32.47 ± 2.78	1.86	28	.074
Female partner age	26.13 ± 1.80	29.87 ± 2.3	.86	28	.399
RAS Female	4.46 ± .23	4.69 ± .09	.93	27	.359
RAS Male	4.58 ± .14	4.64 ± .35	.35	26	.732
RAS difference score	.44 ± .13	.28 ± .07	-1.15	26	.262

Mean similarity scores were calculated for each couple. In order to assess if there were any differences between the ratings of similarity given to couples in the three conditions, a repeated measures ANOVA was conducted with a within-subjects factor of Fragrance (fragranced, unfragranced) and of Rater sex (male, female) and a between-subjects factor of Condition (met on HC, met off HC, 'fake' couples). There was no main effect of Fragrance, F (1, 42) = .91, p = .346. There was a significant main effect of Rater sex, F (1, 42) = 7.79, p = .008, with men giving on average higher scores of similarity to odour pairs (mean = 4.65) than women did (mean = 4.27). However, contrary to expectation, there was no significant Fragrance x Condition interaction, F (2, 42) = .85, p = .435. Helmert planned contrasts also revealed no significant difference between the ratings of similarity given to fake and real couples, p = .346, or between the couples who had met whilst using HC and those who had not been using HC when they met, p = .483 (Figure 7).

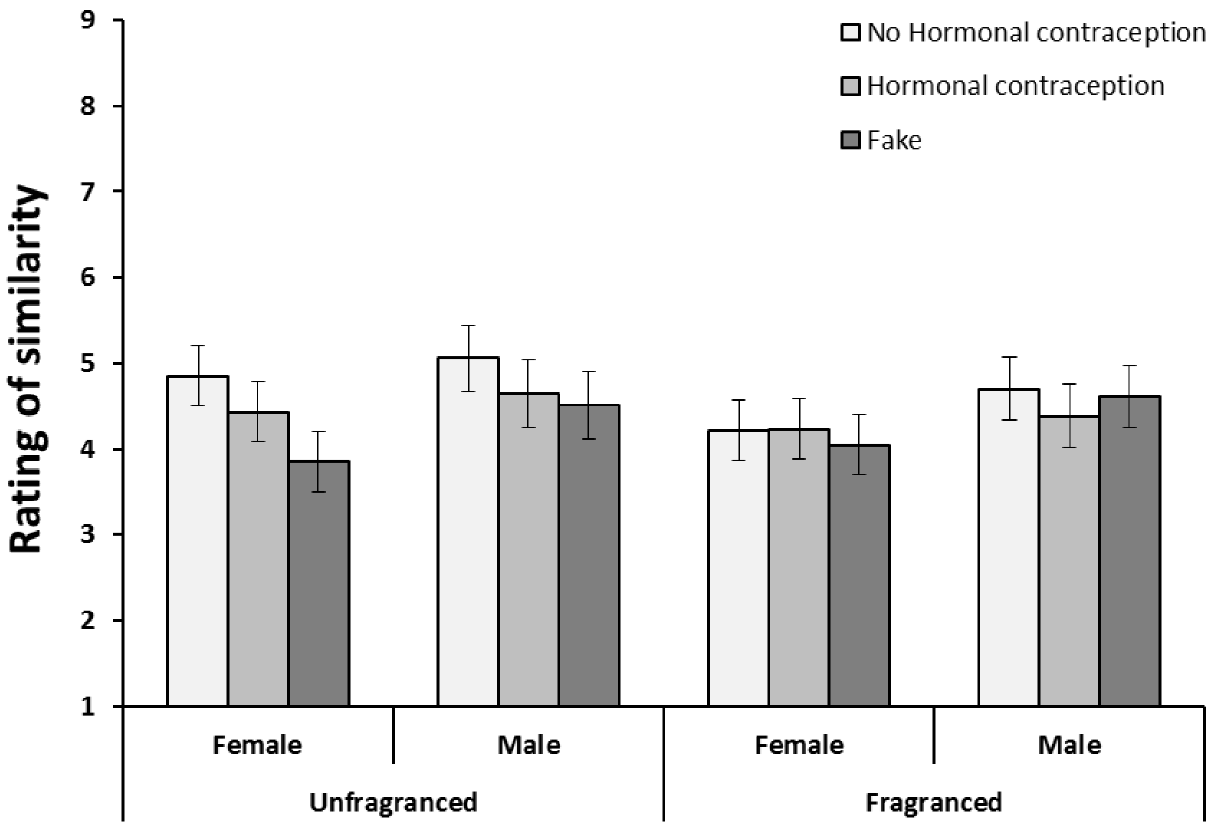


Figure 7 Mean (± SEM) ratings of similarity for odours donated by couples. Mean scores are shown for both male and female raters to fragranced and unfragranced categories of the three couples conditions. Ratings were given on a 9-point scale (1 = not at all similar, 9 = completely similar).

Raters as the unit of analysis

The data were further examined using the individual rater as the unit of analysis. The benefit of this approach is that it enabled inclusion, as a covariate, rater scores on the Sniffin Sticks test, thus controlling for potential variation in raters' olfactory ability. We conducted a repeated measures ANOVA with Fragrance (fragranced, unfragranced) and Condition (No HC, HC, Fake) as within-subject factors and with rater sex as a between-subjects factor, and a covariate of Sniffin Sticks score. Again, there was no main effect of Fragrance, $F(1,379) = .31$, $p = .576$, and there were no significant interactions between ratings given to the fragranced and unfragranced samples and the raters' ages, sex, or score on the Sniffin Sticks task. There was also no main effect of condition, $F(2, 758) = 1.42$, $p = .241$. However, in this analysis, there was a significant interaction between Condition and Fragrance, $F(2, 758) = 3.89$, $p = .021$. Post hoc paired samples t tests revealed that there was no significant difference between similarity ratings given to the HC couples' fragranced and unfragranced samples, $t(412) = 1.27$, $p = .206$, or between similarity ratings of the fragranced and unfragranced fake couples, $t(412) = -1.02$, $p = .309$, but that in the no HC couples unfragranced samples were rated as being significantly more similar than the fragranced samples, $t(412) = 2.91$, $p = .004$, $d = 0.18$. Furthermore, while for the fragranced samples there were no significant differences between the three couple conditions, mean ratings given to unfragranced No HC and HC samples were significantly different to one another, $t(412) = 2.02$, $p = .044$, as were the mean ratings given to No HC and Fake couples, $t(412) = 3.76$, $p < .001$, but there was no significant difference between mean ratings received by the HC and Fake couples, $t(412) = 1.77$, $p = .078$ (Figure 8).

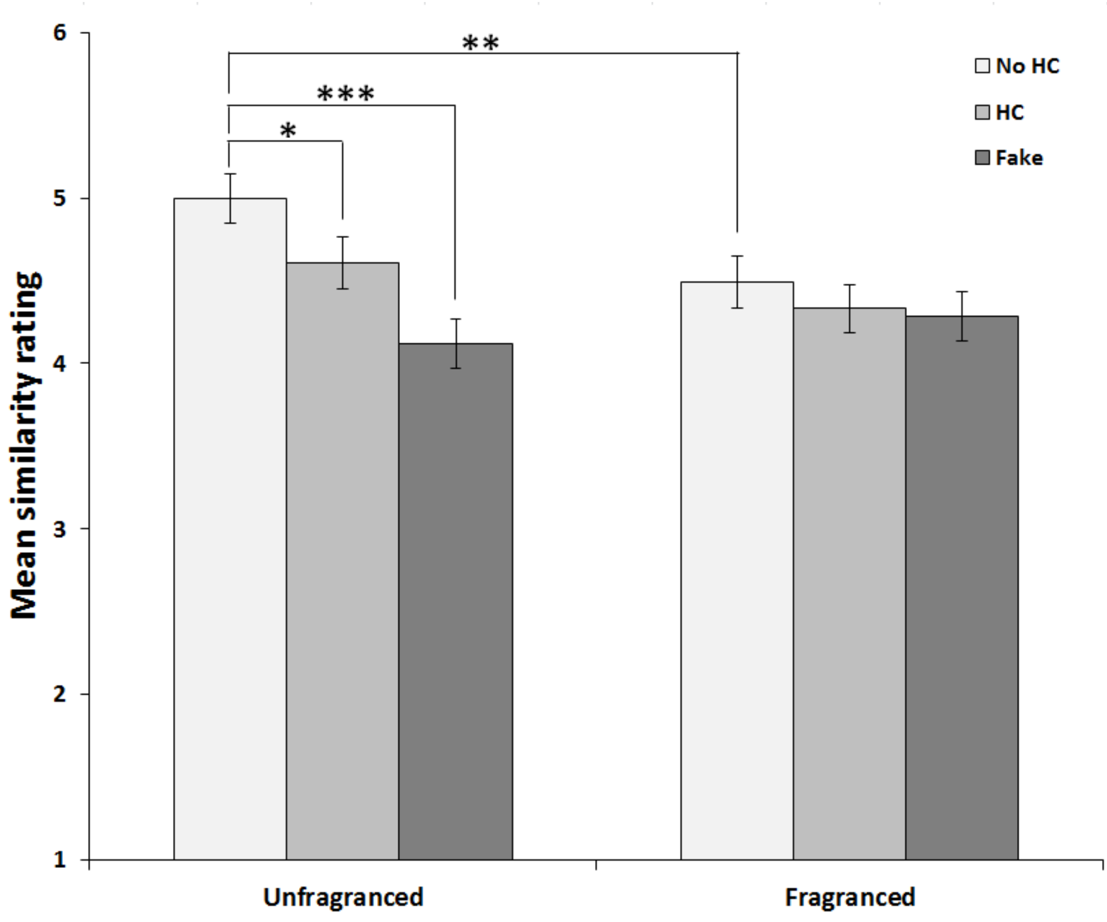


Figure 8 Mean ratings (±SEM) given to Unfragranced and Fragranced samples from the three couple conditions. There was a significant interaction between couple condition and fragrance, p = .021. Lines indicate post hoc paired samples t tests, *p < .05, * p <.01, *** p < .001.

There were also significant interactions between Couple Condition and participants' scores on the Sniffin Sticks task, F (2, 758) = 3.46, p = .032, and between Couple Condition, Fragrance and Sniffin sticks score, F (2,758) = 4.32, p = .014. In order to further investigate the interaction between couple condition, fragrance and Sniffin Sticks scores, a median split analysis was performed on the participants Sniffin Sticks scores (30 participants had not completed the SS task and so were not included in the following analyses). As there were now 383 participants the median split did not produce even groups. The split was conducted twice, once with the median value being classed in the low SS score category, and once with the median value being classed in the high SS category. As can be seen from Table 3, there were no significant differences between the means for any of the similarity ratings when the median was in different categories, and so it was arbitrarily decided to classify the median in the high SS score category for the following analyses (low SS n = 191, high SS n = 192).

Table 3 Results from independent samples t tests assessing the comparability of mean scores (± 1 SEM) for high and low SS categories when the median value was included in either the low SS or high SS category. No significant differences were found.

	Median split group	Mean when median in low SS score	Mean when median in high SS score	t	df	p
Unfragranced	Low SS	4.73 ± .221	4.72 ± .221	.04	381	.484
HC	High SS	4.47 ± .202	4.48 ± .201	-.05	381	.480
Fragranced	Low SS	4.00 ± .201	4.01 ± .201	.05	381	.476
HC	High SS	4.68 ± .198	4.66 ± .198	.07	381	.472
Unfragranced	Low SS	5.05 ± .207	5.06 ± .207	-.05	381	.480
No HC	High SS	4.90 ± .202	4.89 ± .201	.05	381	.480
Fragranced	Low SS	4.55 ± .215	4.53 ± .215	.06	381	.476
No HC	High SS	4.34 ± .210	4.35 ± .210	-.06	381	.476
Unfragranced	Low SS	4.11 ± .210	4.12 ± .211	-.02	381	.492
Fake	High SS	3.96 ± .201	3.95 ± .200	.02	381	.492
Fragranced	Low SS	4.57 ± .206	4.58 ± .207	-.03	381	.488
Fake	High SS	3.90 ± .203	3.89 ± .202	.02	381	.492

A repeated measures ANOVA was conducted, as before, with a within subjects factors of fragrance (unfragranced, fragranced) and of couple condition (no HC, HC, fake), with a between subjects factor of rater sex, and rater Sniffin' Sticks score (high vs low using median split), and a covariate of rater age. This revealed no main effects of condition, $F(2,756) = 2.08$, $p = .126$, or of fragrance, $F(1,378) = .00$, $p = .996$, and no significant interactions. Exploratory post-hoc repeated measures ANOVAs were then conducted separately for the raters who had high SS scores, and those who had low SS scores, again with two within subjects factors of fragrance and couple condition, a between subjects factor of rater sex and a covariate of rater age. For the raters who received low SS scores there was found to be no main effects of condition, F (2,376) = .06, p = .942, or of fragrance, F (1,188) = 1.04, p = .309, and there were no significant interactions. However, for the raters who received high SS scores there was a significant main effect of couple condition, F (2,378) = 3.70, p = .026, with Helmert planned contrasts revealing that ratings of similarity in the No HC condition (mean = 4.69) were significantly different from those in the other two couple conditions, p = .028, but that there was no significant difference between the HC (mean = 4.57) and fake couple (mean = 4.04) ratings of similarity, p = .105. There was however no significant main effect of fragrance, F (1,189) = 1.26, p =.263, and there were no significant interactions.

In order to attempt to further interpret the three way interaction noted previously (couple condition x Fragrance x Sniffin' Sticks found prior to the median split) post hoc paired samples t tests were conducted (see figure 9). It was found that there were significant differences between the similarity ratings of No HC and fake unfragranced samples given by both high SS scoring and low SS scoring raters, but not between No HC and HC, or between HC and Fake (see table 4, and figure 9). Additionally, when looking at the fragranced samples, there were found to be significant differences in similarity ratings given to HC and fake couples by both high and low SS scoring raters (though this difference was in the opposite direction), but not between HC and No HC, or between No HC and Fake (see Table 4, and figure 9).

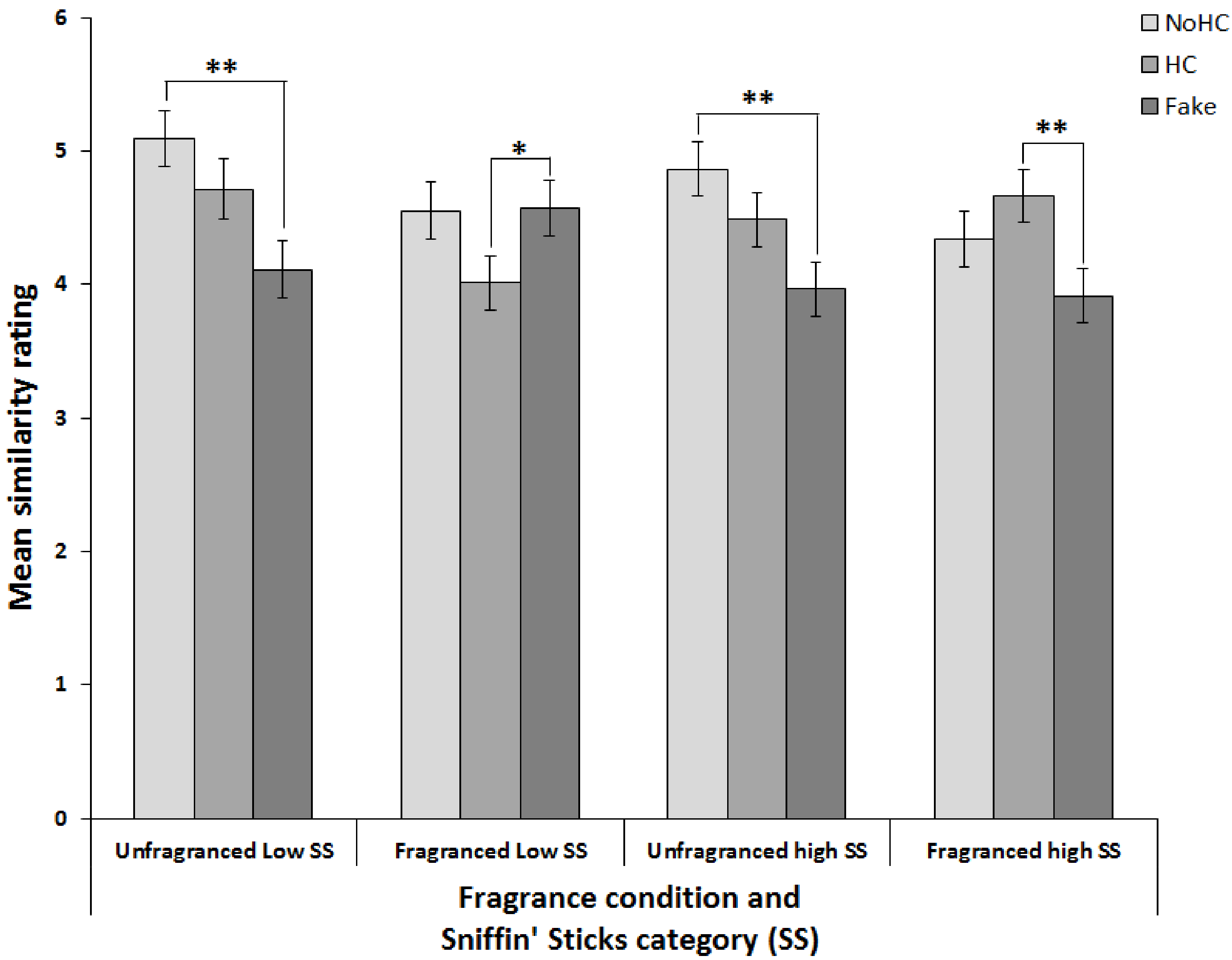


Figure 9 Mean similarity ratings (1, not at all similar, 9 very similar) for couples who met whilst using HC and those who met whilst not using HC, for both fragranced and unfragranced samples, given by raters who had high and low scores on the Sniffin Sticks test (established using a median split). The significant three way interaction between couple condition, fragrance and Sniffin Sticks scores is shown. Lines indicate post hoc independent samples t tests, *p < .05, ** p < .01.

Table 4 Post hoc paired samples t tests investigating the differences between the mean similarity scores (± 1 SEM) three couple conditions (HC, No HC, Fake) across the two fragrance conditions (fragranced and unfragranced) by raters who were classed as having high or low Sniffin' sticks (SS) scores (using a median split).

SS median split	Fragrance Condition	Variable pair	Mean	t	df	p
Low SS score	**Unfragranced**	HC	4.72 ± .221	-1.18	190	.240
		No HC	5.07 ± .207			
		HC	4.72 ± .221	1.94	190	.054
		Fake	4.12 ± .211			
		No HC	5.07 ± .207	3.32	190	.001
		Fake	4.12 ± .211			
High SS score	**Unfragranced**	HC	4.48 ± .201	-1.49	191	.139
		No HC	4.89 ± .201			
		HC	4.48 ± .201	1.789	191	.079
		Fake	3.95 ± .200			
		No HC	4.89 ± .201	3.09	191	.002
		Fake	3.95 ± .200			
Low SS score	**Fragranced**	HC	4.02 ± .201	-1.77	190	.079
		No HC	4.53 ± .215			
		HC	4.02 ± .201	-2.04	190	.043
		Fake	4.58 ± .207			
		No HC	4.53 ± .215	-.15	190	.880
		Fake	4.58 ± .207			
High SS score	**Unfragranced**	HC	4.67 ± .198	1.09	191	.278
		No HC	4.36 ± .210			
		HC	4.67 ± .198	2.71	191	.007
		Fake	3.90 ± .202			
		No HC	4.36 ± .210	1.56	191	.121
		Fake	3.90 ± .202			

Discussion

The aim of this study was to investigate whether there would be differences in the similarity of odours between romantic partners who met whilst using or not using hormonal contraception. Additionally, the odours from these couples were compared to those of 'fake' couples, which were created from pairing male and female odours of individuals who were not in a relationship, in order to see if these differed in similarity from the actual couples' odours.

In the initial analysis each couple was used as the unit of analysis, and it was found that there were no significant differences between ratings of similarity given to the three couple groups (HC, no HC, fake). There was however a significant main effect of rater sex, finding that men tended to give ratings of increased similarity compared to women. We had predicted that women may be more able to discriminate between the couple groups using similarity ratings, based on the findings of previous chapters, and previous literature (Brand & Millot, 2001; Doty & Cameron, 2010) showing that women tend to outperform men on tests of olfactory ability. This finding does not fully support that prediction, but it does suggest that men and women potentially perceived the odours differently.

In order to investigate the question further, while taking into account individual variability in raters' ability, an additional analysis was conducted with rater as the unit of analysis. This analysis revealed a significant interaction between ratings of similarity given to the three couple conditions (no HC, HC, fake) and the fragranced and unfragranced samples. Post hoc tests found that there were significant differences in ratings of similarity between the three couple conditions in the unfragranced samples, with couples who were not using HC when they began their relationship smelling more similar than those who were using HC, who again smelled more similar than the fake couples. In contrast, these differences were not evident in fragranced samples.

The results in this second analysis support the prediction that HC use would affect the similarity of couples' odours. The effect is, however, in the opposite direction of the original prediction, which, based on previous literature, stated that couples who met whilst not using HC should smell more dissimilar than those who were using HC when they began their relationship. This is based on the findings that women tended to show preferences for odours of men with dissimilar MHC types (Roberts et al., 2008; Wedekind & Furi, 1997; Wedekind et al., 1995) but that this preference wasn't noted in women who were using hormonal contraceptives (Roberts et al., 2008; Wedekind & Furi, 1997).

One final point to note is that the majority of HC and no HC couples were cohabiting (Hc 10, no HC 12). It may be that, upon initially meeting a partner, the perceptual qualities of each individuals' odours are distinct, but upon living together in a shared environment, likely with similar diets and activities which may affect odour, odours may become more similar. This would be very simple to test in future studies either by recruiting couples who have just recently begun their romantic relationship, or are not yet cohabiting.

Potentially these findings provide evidence for assortative mating based on odour in humans. However, it must be noted that one potential confound of the study is the measurement of similarity, which was a simple 1-9 scale. It could be the case that this is too simplistic a tool to detect subtle differences related to the extremely polymorphic MHC complex, with odours themselves being extremely multi-faceted and containing a wealth of information, as has been described in previous chapters, concerning fertility status, health status, personality traits and potentially genetic quality and compatibility. It is possible that participants were basing their similarity ratings on different perceptual qualities of the odours, for example some may have rated similarity based on their pleasantness while others may have made similarity judgements based on the intensity of the odours, which again could have confounded the results. In support of this, Wedekind and colleagues (2006) did report finding differing ratings of odour intensity for individuals classified as being MHC homozygotes and heterozygotes. Moreover other researchers have noted difficulties in accurately describing MHC related odour differences in laymen, with Wedekind and colleagues (2007) finding that a trained perfumer could describe odours in a way which revealed specific MHC alleles, but that people without this odour expertise could not. Perhaps a better way to tackle this area would be to develop and validate an accurate and more sensitive scale for rating and describing odours. In this way, the similarity between individuals' odours could be more effectively compared.

Interestingly, there was no difference in similarity ratings in the fragranced samples, with ratings of the three couple conditions not differing significantly from one another. It had been predicted that the same pattern of ratings should be seen, even with the presence of a fragrance, as fragrance choice has been linked to MHC type (Lenochová et al., 2012; Manfred Milinski & Wedekind, 2001), but the current findings suggest that fragrance is disrupting the ability of raters to detect similarity of odours. Previous literature which has linked fragrance preferences to MHC types have mostly used perfumes or perfume ingredients, whereas the current study employed deodorants. This might explain the discrepancy between the findings because the deodorants contained fragrance as well as compounds which target bacteria responsible for odour production, and potentially compounds which reduce the overall production of sweat (if antiperspirants were used). It is possible that the additional properties of deodorants and antiperspirants do in fact mask MHC relevant information in odour cues, as it has not been established which volatile compounds in odour are specifically linked to the MHC profile of an individual. If this is the case, then it may provide an explanation as to why certain studies investigating modern, western societies, have often failed to find MHC disassortative mating patterns. In other words, due to the prevalent use of deodorants and antiperspirants in these societies, MHC related olfactory cues might be obscured at the initiation of a romantic relationship. This explanation should be taken with caution as, to the author's knowledge; this is the first study investigating the effects of deodorants and antiperspirants on olfactory cues which may relate to MHC in romantic pairings in humans. Additionally, it is important to note that the current study did not actually measure MHC in odour donors and so more work would be needed to substantiate such claims.

In conclusion, the hypotheses were not supported by the current findings, although these findings reflect the difficulty with replication that has been seen across the literature relating to disassortative MHC mating preferences in humans. The findings here suggest that there are assortative patterns of mate choice related to body odour and that hormonal contraceptive use may interfere with this. Additionally the current findings contradict research with perfume and perfume ingredients, suggesting that perhaps the more commonly used deodorants and antiperspirants are interfering with the perception of olfactory cues, at least with regard to similarity of odours. The next step in further understanding these results is to investigate a more sensitive scale for the measurement of perceptual odour qualities, with which to re-examine the questions explored in this chapter.

Chapter 6

Perfumers' perceptions of body odours: a new scale for odour description

Introduction

The previous chapter highlighted a potential issue with lay ratings of similarity between odours. Human odours are multi-faceted, as reflected by the range of information which appears to be detectable, from stable traits such as MHC (Wedekind et al., 1995) and symmetry (Rikowski & Grammer, 1999), through to those which fluctuate such as emotions (Chen & Haviland-Jones, 2000), health (Moshkin et al., 2012), and fertility status (Cobey, Buunk, Pollet, Klipping, & Roberts, 2013). The complexity of odours is one potential explanation for the null findings of the previous chapter, showing no difference in similarity ratings between couples who began their relationship whilst using hormonal contraception (HC) and those who were not.

The similarity scale adopted in the previous chapter to rate odours was utilised as a proxy for measuring variation in MHC between romantic partners. As discussed in the previous chapter, there are relatively simple tests to identify the MHC type of an individual, but knowing an individuals' MHC type brings us no closer to identifying the specific volatile compounds which are being perceived by conspecifics. This is true not only for MHC but for all of the other 'information' which research has demonstrated can be detected from odours. There are of course other, more sensitive methods for measuring compounds related to MHC types, such as gas chromatography mass spectrometry (GC/MS). This analytical method allows the identification of different compounds present in samples. A great deal of research has been conducted using this method in order to attempt to identify the volatile chemical compounds which may be responsible for individual differences in odour, however, it has been noted that there are issues concerning this area of research. In a recent review, Kwak and colleagues (2010) point out that a number of studies investigating the urinary volatile profiles of congenic mice, while appearing to be regulated by MHC genes, are also influenced by non-MHC genes and environmental factors. They go on to argue that while these findings are interesting they must be taken with caution, as behavioural data relating to MHC types remain consistent across genetic backgrounds, suggesting that these volatile profiles have not been found to actually underlie the perceptual odour differences between mice. Given these problems, perhaps then it would be beneficial to initially investigate perceptual variation in odour, and then relate this to MHC types, and potentially to other traits such as health and symmetry.

How then can we improve upon the ratings of the perceptual qualities of odours and increase the sensitivity of a measure? One solution would be to develop a scale for describing odours which would allow more facets of odours to be considered. Additionally, it may be beneficial to develop and utilise such a scale with those who are trained sniffers – namely perfumers and perfume evaluators. Perhaps they can provide us with more detailed descriptions of odours than the general public, allowing us to further investigate the potentially fine-grained differences between our odour fingerprints. Research following this line of investigation, while uncommon, does show some promise. One study found that while there was no difference in hedonic ratings of odours given by laymen and trained perfumers, that perfumers gave richer verbal descriptions of odours (Sezille, Fournel, Rouby, Rinck, & Bensafi, 2014). Additionally, as detailed in the previous chapter, a study by Wedekind and colleagues (2007) found that trained perfumers were capable of describing human body odours in such a way that MHC allelic specificity could be revealed, though untrained laymen could not. These findings suggest then that the olfactory training and experience with odours which perfumers gain may lead to better, more accurate descriptions of odours than can be achieved through ratings collected from laymen.

Study rationale

The aim of the current study was to work alongside perfumers in order to establish a descriptive scale which could be used to describe body odours. In addition to providing more detailed ratings of odours which may relate to immunogenetic information, we were interested in what other information may be assessed from these descriptions, namely sex of the odour donor, which can be identified based on the volatile chemical compounds present in axillary odours (Penn et al., 2007).

Method

Two perfumers (1 male and 1 female) and two perfume evaluators (both female) volunteered to take part in the study. The individuals were aged 29-45 (mean = 38.25, SD = 7.27) and had been working in the industry for between 6-18 years (mean = 11.75, SD = 5.05). Both perfumers and perfume evaluators work together to meet client briefs for fragrances. Evaluators are heavily involved in smelling the fragrances, in order to ascertain if these meet the brief, but it is the perfumer who is responsible for designing the composition of compounds, and as such perfumers have more knowledge of raw ingredients, and consequently have more years of training before becoming a perfumer.

They were provided with 62 axillary odour samples (including the 30 couples used in the previous chapter, as well as those from one additional couple). They initially, as a group in one session, assessed four samples (a man and woman who met whilst using HC, and a man and woman who met whilst not using HC), and together came up with a list of 15 descriptors to use to rate these and the rest of the samples. These were Musty, Mouldy, Earthy, Onion, Spicy, Fatty, Oily, Greasy, ChipFat, Animalic, Vegetable, Heavy, Milky, Sweet, and Metallic. Having established this common semantic inventory, they then smelled each of the 62 samples and rated each of them according to each descriptor using a 10-point scale (0 = no presence of this descriptor, 10 = extreme presence of descriptor). The category 'other' was also included in case any important descriptors may have been missed from the original list. Additionally, for each sample, they indicated whether they thought it was from a man or a woman.

The four assessors smelled all samples over the space of a week. These were rated in groups of 5, with assessors rating no more than 10 samples in a day. The samples were removed from the freezer and allowed to defrost for 30 minutes before use. As this was done across the assessors work day, sometimes samples were smelled in isolation and at other times more than one assessor may have been present. They typically spent a few minutes smelling each sample. The samples were left out of the freezer for an hour to allow all four assessors to complete this during their working day. Assessors were blind to the sex of the samples as well as to the relationship pairings.

Results

The category 'other' was only used 11 times across all samples and all assessors, suggesting that the original 15 descriptors were sufficient. Additionally, no one clear descriptor came out of the other category. 'Other' descriptors used were: Green (1), Chocolate (3), Salty (1), Cumin (1), Grass (1), Maltol (1), Cheese (1), Cotton (1) and Sharp (1).

Identifying sex from odour

Binomial tests were used to compare the observed frequency of correct guesses (assessors' guesses of sample sex) against that expected by chance (.5). It was found that only assessor 1 (evaluator) was capable of correctly inferring the sex of the samples at a significantly above chance level, p = .003 (.69 correct), with assessor 3 (perfumer) showing only a marginal significance above chance, p = .056 (.63 correct) and assesors 2 (perfumer) and 4 (evaluator) performing at a close to chance level, assessor 2 p = .374 (.56 correct), assessor 4 p = .899 (.52 correct, see figure 10).

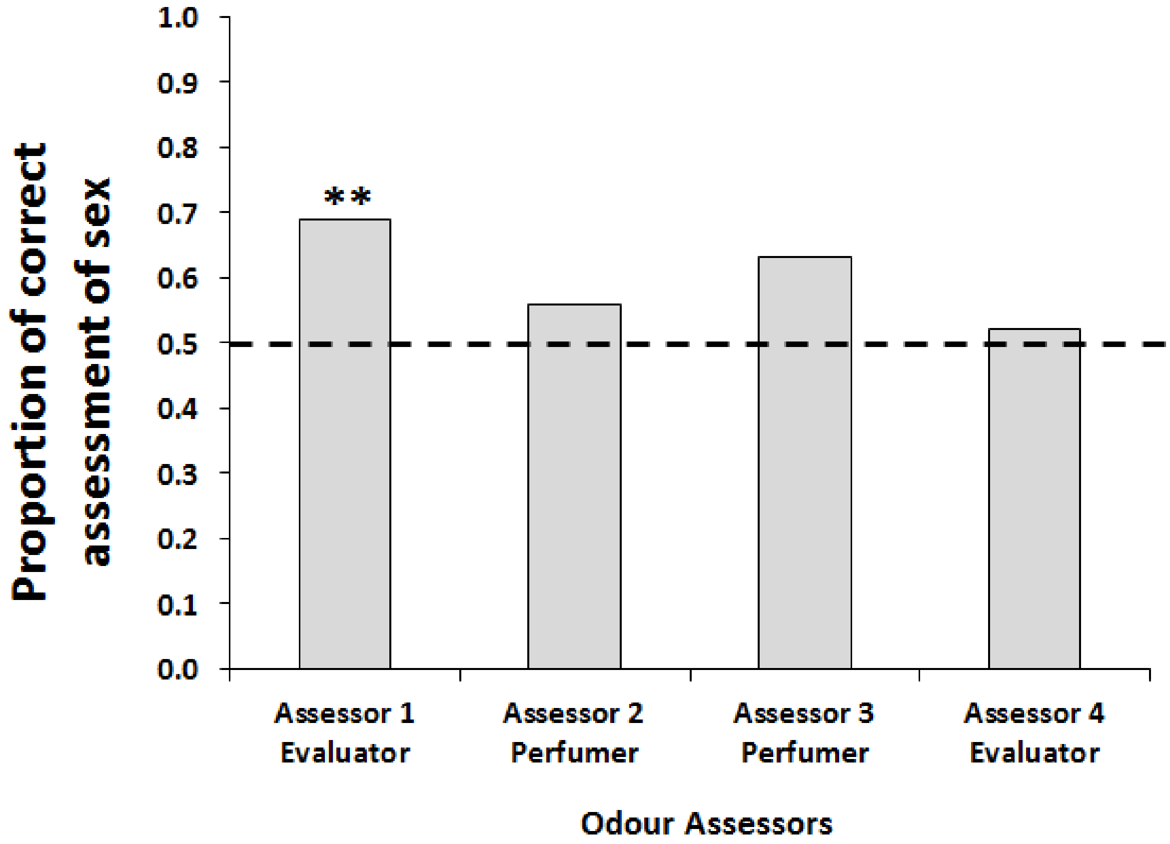


Figure 10 Proportion of correct guesses of donor sex by each assessor. Assessors 1 and 4 are evaluators, assessors 2 and 3 are perfumers. Dashed line indicates chance level. ** p < .01.

To control for differences in use of the assessment scales across assessors, their individual scores were standardised by computing z scores for each assessors' scores for each descriptor. Intraclass correlation coefficients were conducted in order to establish the inter assessor reliability across the scale. As can be seen from table 5, only 6 of the 15 descriptors had ICI's above .4 (.40-.59 = fair, .60-.74 = good, > .74 = excellent, Cicchetti & Sparrow, 1981; Fleiss, 1981).

Table 5 Intraclass correlation coefficients for the 4 assessors Z score ratings across the 15 descriptors (not including 'other'). 95% confidence intervals are shown. ICI values above .4 are deemed acceptable and are indicated in bold.

Descriptor	ICI Z scores	95% CI lower bound	95% CI upper bound
Musty	.155	-.249	.453
Mouldy	-.043	-.590	.338
Earthy	.080	-.361	.404
Onion	**.552**	**.338**	**.710**
Spicy	**.589**	**.393**	**.734**
Fatty	-.135	-.679	.265
Oily	.160	-.242	.456
Greasy	.301	-.034	.547
Chipfat	.324	.001	.562
Animalic	**.531**	**.284**	**.702**
Vegetable	-.281	-.894	.171
Heavy	**.598**	**.405**	**.740**
Milky	**.475**	**.224**	**.660**
Sweet	**.633**	**.457**	**.762**
Metallic	-.155	-.917	.304

To further investigate sex differences in donors' odours, the differences between male and females' descriptor ratings were analysed. The mean *z* score from all assessors for each donor, for each descriptor, was then calculated.

A repeated measures ANOVA was conducted, with descriptor as the within-subjects factor (15 levels, excluding 'other' category) and donor sex as the between-subjects factor. There was no main effect of descriptor, F (14,840) = .000, p = 1.00, but there was a significant interaction between descriptor ratings and donor sex, F (14, 840) = 2.110, p = .010. Post hoc independent samples t-tests revealed that there were significant differences between male and female donors in mean standardised rating scores of Spicy, Animalic and Metallic, with men receiving higher ratings for all three of these descriptors, though it must be noted that only 2 of these received acceptably high intraclass correlation coefficients (see table 6).

Table 6 Mean standardised scores for each descriptor for male and female samples. P values are taken from post hoc independent samples t tests. Significant values shown in bold. * indicates mean was calculated from only 3 of the 4 assessor, ** indicate mean was calculated from only 2 of the 4 assessors, as some assessors simply did not use a certain descriptor across any of the samples.

Descriptor	Male mean rating	Female mean rating	p
Musty	.0094	-.0094	.891
Mouldy*	.0821	-.0821	.260
Earthy	-.0175	.0175	.792
Onion	.0670	-.0670	.424
Spicy	**.1782**	**-.1782**	**.035**
Fatty	.0150	-.0150	.806
Oily	-.0879	.0879	.197
Greasy	-.0936	.0936	.197
ChipFat	-.0502	.0502	.497
Animalic*	**.2559**	**-.2559**	**.004**
Vegetable	-.0940	.0940	.104
Heavy	.1471	-.1471	.085
Milky	.0039	-.0039	.961
Sweet	.0058	-.0058	.948
Metallic**	**.1738**	**-.1738**	**.044**

***mean calculated from 3 assessors, **mean calculated from 2 assessors**

Couples' similarity

Using the mean *z* scores across descriptors, odour difference scores were then calculated for each romantic couple by subtracting the females score from the males for each of the descriptors. In order to investigate the similarity of couples odours a repeated measures ANOVA was conducted using these scores, with a within-subjects factor of descriptor (15 levels, excluding 'other') and a between subjects factor of pill use (using HC when they met vs. not using HC when they met). There was found to be no significant main effect of descriptor, $F (14,406) = .56$, $p = .894$, and there was no significant interaction between descriptor difference scores and pill use, $F (14,406) = 1.27$, $p = .266$. The unsigned differences for each couple were summed across all descriptors (excluding 'other'), and an independent samples t test was conducted finding there to be no significant difference between the summed difference scores for couples who met whilst using HC (mean difference score = 7.78, SD = 2.85) and those who weren't (mean = 6.36, SD = 2.41), $t (29) = -1.49$, $p = .147$. Finally the unsigned differences were summed across only the 6 descriptors which had shown the highest intraclass correlation coefficients and the t test was conducted again, still finding there to be no significant difference between HC (mean = 3.41, SD = 2.11) and No HC couples (mean = 3.10, SD = 1.87), $t (29) = -.49$, $p = .622$.

Finally males and females scores for each of the descriptors (not including 'other') were correlated. This was done separately for couples in the No HC (table 7) and HC (table 8) condition, in order to investigate levels of disassortative/assortative mating. There were was only found to be one significant correlation between the same descriptor in both males and females, and that was for milky ratings, in HC couples (.764), however this correlation was positive, and we would expect to see negative correlations to provide support for disassortative mating based on perceptual odour qualities. There were found to be three significant negative correlations between different descriptors (NoHC – Vegetable and Spicy, -.539; HC – Fatty and Milky, -.687, Chipfat and Milky, -.578) which potentially provide tentative evidence of disassortative mating; however these correlations include descriptors which had low intraclass correlation coefficient. Additionally, due to the large number of variables correlated here, there is an increased likelihood of type I errors occurring, and so these findings should be interpreted with caution.

Table 7 Correlations between males' and females' ratings from couples who met whilst not using HC across the 15 odour descriptors excluding 'other (1 = Musty, 2 = Mouldy, 3 = Earthy, 4 = Onion, 5 = Spicy, 6 = Fatty, 7 = Oily, 8 = Greasy, 9 = ChipFat, 10 = Animalic, 11 = Vegetable, 12 = Heavy, 13 = Milky, 14 = Sweet, 15 = Metallic). * $p < .05$, † $p = .051$ (also highlighted in bold).

No HC	**1M**	**2M**	**3M**	**4M**	**5M**	**6M**	**7M**	**8M**	**9M**	**10M**	**11M**	**12M**	**13M**	**14M**	**15M**
1F	.286	.295	.052	-.271	-.227	-.086	.312	.295	.219	-.260	**.621***	-.304	-.082	.158	-.452
2F	-.232	-.191	-.130	-.199	.004	-.307	-.473	.011	-.320	-.194	.054	-.271	-.081	.304	.073
3F	-.021	-.067	-.314	.181	.008	-.057	.376	.418	-.152	.028	.180	.170	-.063	.058	.145
4F	-.211	-.384	.096	.065	.182	-.283	-.155	.167	-.359	.227	.327	-.076	-.359	-.095	-.138
5F	-.104	-.327	-.111	.106	.202	-.036	-.280	.043	-.245	**.496†**	-.059	-.022	-.111	-.112	.119
6F	.202	.145	-.025	-.242	-.138	.220	.002	.122	-.097	-.157	.388	.030	.076	.217	-.331
7F	-.186	.327	-.255	-.043	-.038	.314	.364	.330	.265	-.343	.303	.228	-.197	.018	.237
8F	.179	.281	-.165	-.295	-.222	.357	.441	.121	.224	-.273	.372	.356	.336	.239	-.213
9F	-.270	-.184	-.007	.149	.462	-.264	-.162	.244	-.109	-.001	-.155	.116	-.279	.010	-.065
10F	.257	-.176	-.009	-.175	.077	-.009	-.184	-.001	-.360	.314	.276	.104	.193	.059	-.370
11F	.131	.034	-.020	-.413	**-.539***	.068	.121	.121	.073	-.469	.460	-.298	.227	.334	-.410
12F	.306	.188	**.500***	.015	.119	-.118	.044	.173	-.222	.145	-.105	.295	-.238	-.310	.041
13F	-.238	-.249	-.255	.053	.003	-.117	-.193	-.227	.157	-.205	-.311	-.018	.074	.349	-.090
14F	-.267	-.164	-.173	-.127	-.247	-.243	-.088	-.344	-.046	-.238	-.225	-.114	.451	.401	.119
15F	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000

Table 8 Correlations between males' and females' ratings from couples who met whilst using HC across the 15 odour descriptors excluding 'other (1 = Musty, 2 = Mouldy, 3 = Earthy, 4 = Onion, 5 = Spicy, 6 = Fatty, 7 = Oily, 8 = Greasy, 9 = ChipFat, 10 = Animalic, 11 = Vegetable, 12 = Heavy, 13 = Milky, 14 = Sweet, 15 = Metallic). * p < .05, ** p < .01, † p = .051 (also highlighted in bold).

HC	1M	2M	3M	4M	5M	6M	7M	8M	9M	10M	11M	12M	13M	14M	15M
1F	.352	**.585***	.133	.302	.434	.021	-.294	-.123	-.226	**.600***	-.026	.518*	-.089	-.105	-.181
2F	-.139	-.086	.146	-.061	.259	.139	-.083	.189	.268	.024	.379	.072	-.275	-.174	-.160
3F	-.108	-.167	-.125	.015	.121	.104	.109	.464	.387	.209	.316	.235	-.167	-.031	-.125
4F	-.368	.007	-.204	.397	.387	-.116	.209	.123	-.059	-.033	.079	.245	-.173	-.361	.088
5F	-.192	.244	-.169	.124	.375	-.179	.130	.072	-.065	-.248	.157	-.007	-.099	-.196	-.128
6F	.046	-.269	-.049	-.077	-.044	.244	-.259	.278	.073	.393	-.376	.183	**-.687****	-.262	.218
7F	-.056	-.404	.215	-.011	-.014	-.211	-.146	-.099	-.215	.248	-.159	.258	-.324	-.025	.355
8F	.180	-.081	-.022	.032	.229	-.188	-.029	-.014	-.281	.169	-.260	.193	-.381	-.242	.080
9F	.074	-.433	-.003	-.273	-.301	.378	-.163	.451	.278	.027	-.220	-.175	**-.578***	-.238	.077
10F	-.137	.139	.499	.132	.399	-.129	-.466	-.072	-.367	.133	-.074	.227	-.455	-.172	.305
11F	-.281	-.189	.304	-.124	-.081	-.053	.017	.491	.113	-.055	-.124	-.044	-.087	.168	.278
12F	-.097	**.512†**	-.113	.255	**.550***	-.194	.008	.049	-.196	-.034	.079	.169	-.055	-.258	-.253
13F	.022	.234	-.109	-.054	-.236	.146	.088	-.144	.121	-.194	.429	-.219	**.764****	.102	-.181
14F	.040	.229	-.146	-.059	-.284	-.089	-.057	-.308	-.072	-.176	.313	-.146	**.844****	.297	-.199
15F	-.021	.406	.138	.085	.232	-.093	.251	-.229	.016	.032	.436	.074	.263	-.023	-.061

Exploratory factor analysis of descriptors

As only 6 of the descriptors showed good inter-rater reliability as measured via intraclass correlation coefficients (see table 5) only these 6 were included in the following analyses. In order to try and improve the accuracy of the descriptors a factor analysis was conducted. Suitability of the 6 items for factor analysis was initially examined, using several well recognised criteria.

First, all 6 items were found to be somewhat correlated ($r > .3$) with at least one other item (see table 9). Second, the Kaiser-Meyer-Olkin measure of sampling adequacy (.806) was above the recommended value of .6, and Bartlett's test of sphericity was significant, $x^2(15) = 148.46$, $p < .001$. Furthermore, the diagonals of the anti-image correlation matrix were all found to be over .5, and finally all variables had communalities above .3, suggesting common variance with other items. These analyses suggest the data are suited to further analysis using factor analysis.

Table 9 Correlations between the 6 descriptors which were included in the factor analysis.

	Onion	Spicy	Animal	Heavy	Milky
Spicy	.703				
Animalic	.549	.568			
Heavy	.635	.700	.546		
Milky	-.268	-.285	-.171	-.105	
Sweet	-.461	-.386	-.313	-.255	.522

Using the mean z-scores for each of the 6 descriptors and for each donor an exploratory factor analysis (principal axis factoring) was conducted using varimax rotation. Initial eigenvalues found that the first two factors explained 54.05% and 19.97% of the variance respectively. When utilising the varimax rotations the total percentage variance explained by factors one and two became 40.42% and 20.19%, with the two factor solution explaining 60.62% of the variance in total. Upon further inspection it was found that all 6 items had primary factor loadings of above .4, and only one factor was found to cross load onto another factor at above .3 (Onion, cross loaded with -.328), but this was deemed acceptable as the primary factor loading was high (.753), so all 6 variables were retained and two factors were extracted from the model (see table 10).

Table 10 Loadings and communalities for the 6 descriptor items based on mean Z scores from the 4 assessors.

Descriptor	Factor 1	Factor 2	Communalities
Onion	.753	-.328	.675
Spicy	.815	-.265	.735
Animalic	.645	-.180	.448
Heavy	.836	-.042	.701
Milky	-.095	.665	.451
Sweet	-.263	.747	.627

Composite scores were created for each donor for each of these two factors which were included as within-subjects factors in a repeated measures ANOVA with donor sex as the between-subjects factor. This analysis revealed that there was no main effect of factors, $F\ (1,60) = .000$, $p = 1.00$, nor a significant interaction between factors and donor sex, $F\ (1,60) = 1.69$, $p = .198$. Despite this, exploratory post hoc independent analyses were conducted to compare males and females scores on the two extracted factors.

Independent samples t tests were conducted utilising these two extracted factors and it was found that there was no significant difference between males and females ratings of factor two, $t\ (60) = -.36$, $p = .724$, but that there was for factor 1, t

(60) = -2.23, p = .029, with men scoring higher in this factor than women (see figure 11).

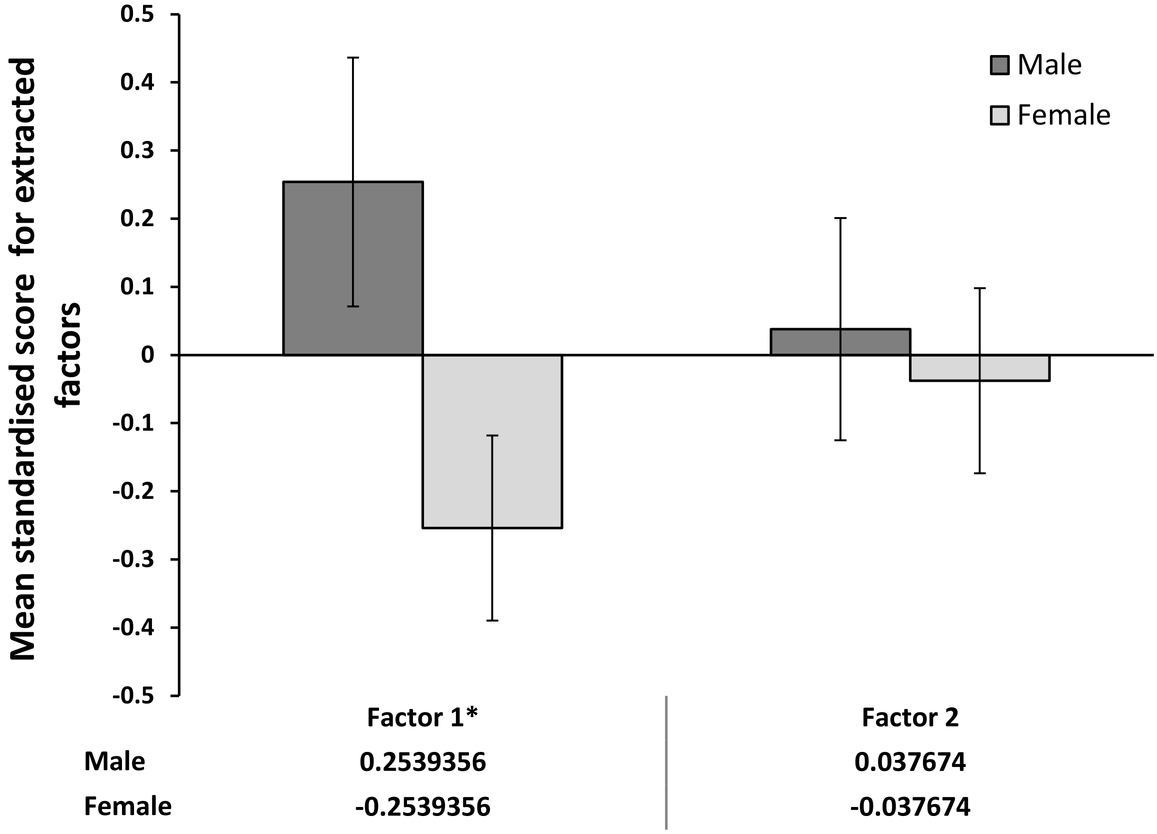


Figure 11 Mean ratings for males and females for the two factors generated from the factor analysis. Error bars represent ± 1SEM. * p < .05.

Next, using the scores computed for the two extracted factors, we again calculated difference scores for each couple by subtracting the female's score from the male's score, for each couple. We then re-ran the repeated measures ANOVA with a within subjects factor of 'factor' with two levels, and a between subjects factor of HC use at the initiation of the relationship (HC use vs. no HC use). As before we found no main effect of factor, F (1,29) = .1.59, p = .216, and no significant interaction between this and HC use at relationship start, F (1,29) = .000, p = .999. Post hoc independent samples t tests also indicated that there was no significant difference between the summed difference scores of the two factors between those couples who met whilst using, or not using HC, $t(29) = .69$, $p = .498$.

To further investigate the presence of assortative/disassortative mating the two factors were correlated between males and females who had met when using HC and between males and females who were not using HC. It was found that there were no significant correlations between any of the factors or within either of the couple groups (HC and No HC, see Table 11). Presence of significant negative correlations between males and females would have provided evidence of disassortative mating based on odour cues.

Table 11 Bivariate correlations between male and females ratings of the two factors from the above PAF analysis, presented separately for couples who met whilst using HC and those who met when not using HC. No significant correlations were found.

Couple Condition	**Factor**	**Factor 1 Female**	**Factor 2 Female**
No HC	Factor 1 Male	.186	-.099
	Factor 2 Male	-.202	.400
HC	Factor 1 Male	.234	.078
	Factor 2 Male	-.231	.341

Discussion

Although findings suggest that there are chemical differences between male and female odours (Penn et al., 2007) our assessors were not all successful at discriminating sex of the odour donors at an above chance level, though we did only have a small sample of 4 assessors. This is in keeping with the one previous study which investigated this, finding that only a small proportion (22% female and 44% male) of observers could predict donor sex at above chance levels from body odour samples (Doty et al., 1978). Despite this, we did see some significant sex differences in descriptor ratings, with male odours receiving higher ratings of Spicy, Animalic and Metallic than female odours. In order to further investigate this, an exploratory factor analysis was conducted with the aim of improving the accuracy of the descriptors used. Two factors were extracted from this the first of which contained the descriptors Onion, Spicy, Animalic and Heavy, the second contained Milky and Sweet.

However, we no longer found a significant interaction between donor's sex and the descriptor ratings when using the scores which had been computed for these three factors. As this study represents the very early stages of an investigation into validating a scale for verbally describing odours, exploratory post hoc analyses were conducted despite the lack of a significant interaction. This did find that there was a significant difference between men and womens' ratings on factor 1 (Onion/Spicy/Animalic/Heavy), which was in keeping with the differences found before the factor analysis had been conducted, with men scoring more highly on the Spicy and Animalic traits than women.

We also investigated if these more detailed descriptors could be used to differentiate between couples who met whilst using HC and those who met whilst not using HC by calculating differences scores, thus aiming to improve upon the measure of similarity used in the previous chapter. We originally did this using the mean z scores averaged across assessors for the original 15 descriptors and found there to be no significant difference between the two groups of couples. This remained the case when using difference scores calculated from the two extracted factors.

While the scale used found no evidence of odour differences between romantic partners in relation to HC use at the start of the relationship as would be predicted by different mate choice in these two groups, it did reveal sex differences in axillary odours, in line with the findings of Penn and colleagues (2007). It may be that within our sample disassortative mating based on odour cues was not present, or that the scale, which is in the very early stages of development, needs more refinement to detect the relevant perceptual odour traits. Clearly further work is needed to investigate the role of disassortative mate choice based on odour profiles in humans, especially given the lack of agreement in the findings to date. Further testing and validation of the current scale will be important for this line of investigation, as well as for the investigation of other olfactory cues present in body odour.

Section two

Chapter 7

The influence of deodorant on self-confidence, attractiveness and body language

Introduction

A growing body of research has investigated the effects of various odours on behaviours and mood (Herz, 2002), but as Roberts and colleagues (2009) note, far fewer studies have described the effects of fragrances designed to reduce personal malodour, either on the wearers or on perceivers in the social environment.

Effects of fragrance on perceivers

Gueguen (2001) found that female confederates were more likely to be offered help by passers-by when they dropped a glove/handkerchief if they were wearing perfume than if they were not. Higuchi et al. (2005) also found that women being interviewed while wearing a perfume that they liked were subsequently rated as having higher self-confidence compared to those wearing no perfume at all, perhaps directly resulting from a positive self-reported mood change in these women while wearing perfume. It has also been found that fragrance type can influence impressions of personality (Fiore, 1992). Fiore found that fragrances which were more closely related, based on fragrance genealogies (for example Chypre and Oriental are more closely related than either are to floral fragrances), also shared more descriptors of personality than their dissimilar counterparts, when rated by a group of 90 female undergraduates. Furthermore, a more recent study conducted by Retiveau and colleagues (2004) found that the presence of three fine fragrances decreased negative affect and increased vigor in wearers, and that additionally each of the fragrances elicited specific 'mood patterns'. The woody/citrus/coniferous scent increased hostility and tension, whereas the floral/woody scent lowered depression, tension and confusion, and finally the floral/chypre/citrus scent led to decreased anger and confusion.

Other studies report that effects of fragrance can be mediated by various factors such as the attire of the wearer. Baron and colleagues (1981), for example, had 94 male participants take part in a study which they were told was investigating the formation of first impressions. These participants had to respond to a series of questions asked by the experimenter along with a study partner who was in fact a female confederate. Female confederates were dressed either neatly (blouse and skirt) or informally (jeans and sweatshirt), and were either wearing perfume or were not. After the study, male participants were asked to rate their partner (the female confederate) for a number of traits related to personal appearance, as well as how much they liked the individual, and whether they thought she was attractive. The researchers found that the presence of perfume increased male participants' attraction towards the confederates as well as leading to positive shifts in social judgements, but only when the confederate was dressed informally. In fact, when the confederate was dressed neatly, opposite effects were reported. In a further study, Baron (1983) additionally found that the effects of fragrance may be mediated by the sex of the perceiver. In this study, Baron had participants interview male and female applicants for a job – these applicants were in fact confederates who were either wearing, or not wearing, perfume or cologne. After the interviews, participants had to rate the applicants on a number of job-related and personal characteristics. It was found that men gave lower ratings for both job and personal characteristics to candidates who wore a fragrance (both male and female) than to those who didn't, but the opposite was true for female interviewers. In a further study, Baron also found that, when participants were provoked by a confederate, they were more likely to be aggressive towards this person when a fragrance was being worn than when there was no fragrance, or when there was simply an ambient scent (from an air freshener) in the room (Baron, 1980).

Together, these studies demonstrate the impact which a fragrance can have on others in the environment, with fragrances appearing capable of manipulating behaviour as well as informing judgements. Interestingly, these studies also demonstrate that there are sex differences in the effects which can be observed from fragrance use, which is in keeping with the findings of previous chapters, which show differential sensitivity to odours by male and female perceivers (chapters 3 and 4).

Effects of fragrance on wearers

Perhaps surprisingly, there is comparatively little research on the effects of fragrance on wearers. Abriat and colleagues (2007) reported that using a fragranced skin care product for a week lead to significantly improved measures of self-reported mood, as well as producing a relaxing effect as measured by facial electromyography. As described above, Higuchi et al. (2005) found that perfume also increased self-reported self-confidence. In a similar vein, Freyberg and Ahren (2011) found that fragrances were related to social enjoyment. They recruited 27 adolescent girls and asked them to wear their favourite perfume or an assigned perfume on alternate days. They found that the assigned fragrance was rated as less pleasant than the preferred one, and that this was associated with reduced social enjoyment as measured by questionnaires. Furthermore, it was associated with a reduction in the use of words related to intimacy which the girls used in their narratives, as measured in writing samples.

Study Rationale

It is apparent from the research discussed above that fragrances worn on the body appear to have important effects on the emotions of the wearer, as well as altering the perception of several wearer characteristics by perceivers, with most of this research focussing on the latter. As well as presenting only a limited investigation of the effects of fragrance use on the wearer, most of this research has been conducted using perfumes. While this is a valid and important line of questioning, it fails to account for a large range of deodorant and antiperspirant products which are used on a frequent basis by a large proportion of individuals in contemporary society. To date, only one study (Roberts et al. 2009) has investigated the psychological effects of deodorant use on both the wearer and the perceiver.

Roberts et al. report broadly similar effects as those discussed above. Men were given a deodorant to wear for 2 days, half receiving a commercially available product (full deodorant, presented in a plain white spray can), while the other half were given an identically packaged spray which lacked both the fragrance and antimicrobial agent (placebo deodorant). Participants were therefore blind to the experimental condition. Participants in the full deodorant condition showed a significant increase in self-reported self-confidence compared to baseline after just 15 minutes of deodorant exposure, and this was not seen in the placebo condition. After two days of use, participants receiving the full deodorant again showed increased self-confidence, and also showed increased self-reported attractiveness compared to the placebo condition, though this latter pattern was not statistically significant. Finally, although there was no overall difference in visual attractiveness, as rated by an independent female rater group who were shown photographs of the men taken under standard conditions, men in the full deodorant condition were judged as more attractive in video footage (played without sound) than those in the placebo condition. This suggested that deodorant use, perhaps through increased self-confidence and self-perceived attractiveness, also had an effect on their non-verbal behaviour.

These findings are extremely interesting, however they raise further questions. The study unfortunately does not pinpoint exactly what aspect of the deodorant formulation led to the observed changes in self-perception. In other words, it could be due to either masking of underlying malodour by the antimicrobial ingredient in the full deodorant, or to the addition of fragrance. The current experiment aimed to investigate which of these may underpin these changes in self-confidence, using a similar methodology to that of Roberts et al. (2009), but with the addition of conditions in which fragrance only, or anti-microbial agent only, were presented to participants.

Based on the earlier study, it was predicted that participants in the full deodorant condition would show increases in self-confidence and potentially self-rated attractiveness, and would be judged more attractive in photos, compared to those in the placebo (ethanol only) condition, after 5 days of deodorant use. It was further predicted that self-report scores and ratings given by those in the fragrance-only or anti-microbial agent-only conditions would fall somewhere in between these.

Method

Participants

Wearers

Forty-four heterosexual, non-smoking male volunteers (aged 19-32, mean age 23.09, SD 3.21) were recruited from the University of Liverpool and the University of Stirling. Ethical approval was obtained from both Universities and all participants gave informed consent before taking part. All participants were blind to the experimental design. Participants were asked if they had any allergies/sensitivities to fragrance products and were instructed not to take part if this was the case.

Raters

A further 89 participants were recruited to rate the photos of the wearers. Of these, 75 reported themselves as heterosexual, 4 bisexual, and 9 homosexual. There were 19 men (age 19-42, mean = 22.95, SD = 4.79) and 65 women (age 18-65, mean = 25.48, SD = 9.4). Raters were recruited via Facebook and email to take part in an online rating task. Participants were asked only to take part if they were not from the Stirling or Liverpool area, to ensure the images they saw were of unknown individuals.

Procedure

Wearers attended two laboratory sessions, one on Monday and one on Friday of the same week. During this time they completed questionnaires, had photos taken, and in the Monday session were given a deodorant. Participants were also required to complete an online questionnaire in their own time on the Wednesday, via a hyperlink sent within an email. Participants were required to wear the deodorant they were given for the duration of the week, starting on the Monday, after questionnaire completion, and to refrain from using any other scented product during this time. They were provided with fragrance-free soap to use in place of their normal products when bathing. All men confirmed that they used the sprays each day. Finally, wearers rated the pleasantness of the allocated spray.

Materials

Questionnaires for wearers were created using Qualtrics online software (www.qualtrics.com). Items included basic demographic information (age, sexuality, relationship status, frequency of deodorant use). They also included measures of physical attractiveness, dominance, assertiveness, self-efficacy, competence, and extraversion items (Goldberg, 1999), and a self-esteem scale (Rosenberg, 1965), all of which were taken from the International Personality Items Pool (http://ipip.ori.org/ipip) and were the same as those which were used by Roberts and colleagues (2009) to assess self-confidence (see appendix B). Responses to these were recorded on a 5-point Likert scale (1= strongly disagree, 5 = strongly agree). In addition, three questions assessed self-reported attractiveness on a 7-point Likert scale (see Figure 12), again as completed by participants in Roberts et al.'s (2009) study. During the first (Monday) session, participants completed the questionnaire before any deodorant was applied, in order to establish a baseline measure. Total self-confidence scores (TSC) were calculated by summing the responses for each participant on the questions taken from the IPIP, following Roberts et al. (2009). In addition, the three questions assessing attractiveness were also summed for each participant to create a composite measure of self-rated attractiveness (SRA).

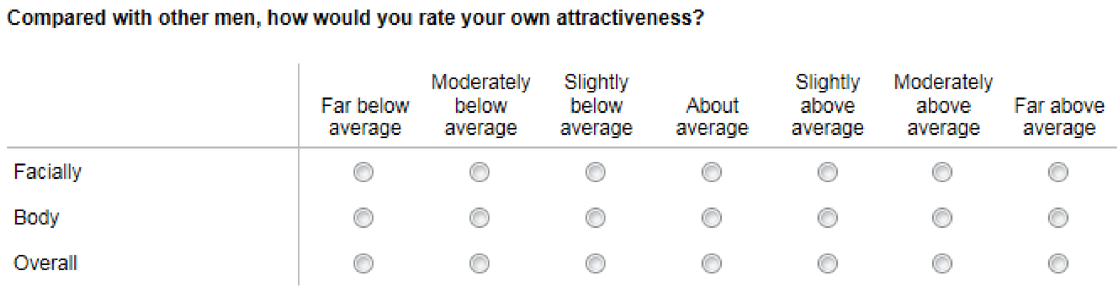


Figure 12 Screenshot taken of the online questionnaire undertaken by lab participants. Image depicts the question relating to self-reported attractiveness.

The deodorants used were provided by Unilever plc. The full deodorant was a commercially available product used only in the United States, and so was unlikely to be used by any of the wearers. However it was presented in plainly packaged aerosol cans, containing only the instructions for use. There were four conditions, namely: Full deodorant, Fragrance only, Antimicrobial agent only, and Placebo (ethanol only). There were 11 participants in each condition, with participants being assigned to condition in alternate order of recruitment.

After providing informed consent and completion of the online questionnaire, photographs were taken of participants. Photographs were taken at a set distance of 2 metres, in the same room and lighting conditions for each participant, using a Canon Powershot G6 digital camera with 7.1mp. Participants were given a white t-shirt to wear and were seated in front of a white wall. Images were cropped so that they showed the whole body and face, from the knees up. This was done in an attempt to capture body posture, which was absent in the head-and-shoulder-only images in the Roberts et al. (2009) study.

In the second part of the study, raters completed an online forced choice task using the photographs taken of the participants before and after deodorant use. All photos were resized to 448 X 336 pixels before use. Images from the same participant were presented side by side, and raters were required to choose the image in which they judged the man to appear most confident (pre-use vs. post-use). The image pairs were presented in randomised order, and the side (left, right) on which pre- and post-use images appeared was also randomised between and within raters. At the end of the survey, raters were asked if they knew any of the men in the images – no raters reported recognising any of the participants. Subsequently, the proportion of post-use selections was calculated for each online rater for each photograph condition (full deodorant, fragrance only, antimicrobial only, and ethanol only/placebo) for analysis.

Results

Following Roberts and colleagues (2009), participants' scores on the 7 IPIP items were averaged to create a total self-confidence score (TSC). As a validity check, this TSC score was then correlated with each of the 7 individual measures, showing that all correlations with TSC were significant (Table 12). Summed TSC scores were therefore used in the following analyses in order to reduce issues relating to multiple testing.

Table 12 Matrix of correlations between the 7 primary constructs relating to self-confidence and the average total self-confidence score (TSC). Data are Pearson correlation coefficients (above) and exact two-tailed p values (below). Significant correlations are highlighted in bold. Ass, assertiveness; Comp, competitiveness; Dom, dominance; Ext, extraversion; Phys.Att, physical attractiveness; SEf, self-efficacy; SEs, self esteem.

	Comp	Dom	Ext	Phys.Att	SEf	SEs	TSC
Ass	.051	**.503**	**.434**	.224	**.351**	.078	**.583**
	.744	**.001**	**.003**	.145	**.019**	.614	**<.001**
Comp		-.090	.047	.269	**.442**	**.743**	**.594**
		.560	.760	.077	**.003**	**<.001**	**<.001**
Dom			.064	.105	.233	-.178	**.329**
			.682	.496	.129	.247	**.029**
Ext				**.370**	.192	**.348**	**.646**
				.013	.212	**.021**	**<.001**
Phys.Att					.234	**.343**	**.650**
					.126	**.023**	**<.001**
SEf						**.456**	**.639**
						.002	**<.001**
SEs							**.704**
							<.001

Effects on self-confidence

A repeated measures ANOVA was conducted using the TSC scores, with Session as a within-subjects factor (3 levels: Monday, Wednesday, Friday) and Condition as the between-subjects factor (4 levels: full deodorant, fragrance only, microbial only, and ethanol only). The expressed pleasantness of the deodorant by wearers was also included as a covariate. This analysis revealed no significant main effect of Session, $F (2,68) = 1.619$, $p = .206$, and no significant interaction between Session and Condition, $F (6,68) = .529$, $p = .785$ (Figure 13), or between session and pleasantness of the deodorant, $F (2,68) = 2.223$, $p = .116$.

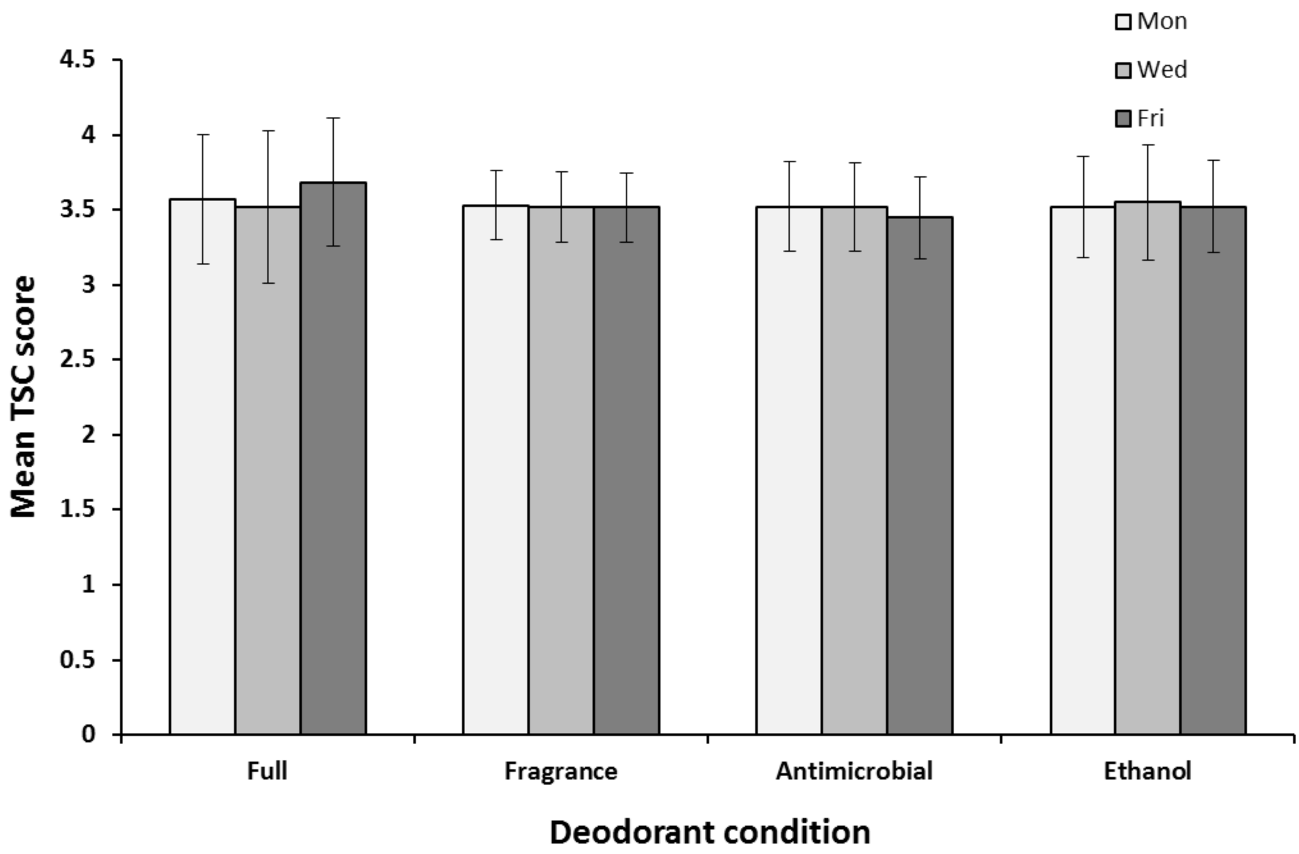


Figure 13 Mean self-confidence (TSC) scores (±SEM) for participants in the four deodorant conditions, across the three time points (Monday, Wednesday, Friday).

A similar analysis was carried out with self-rated attractiveness (SRA) scores as the dependent variable. This analysis revealed no significant main effect of Session, $F(2,72) = .402$, $p = .670$, and no significant interaction between Session and Condition, $F(6,72) = .172$, $p = .984$ (Figure 14), or between session and pleasantness of deodorant, $F(2,72) = .271$, $p = .764$.

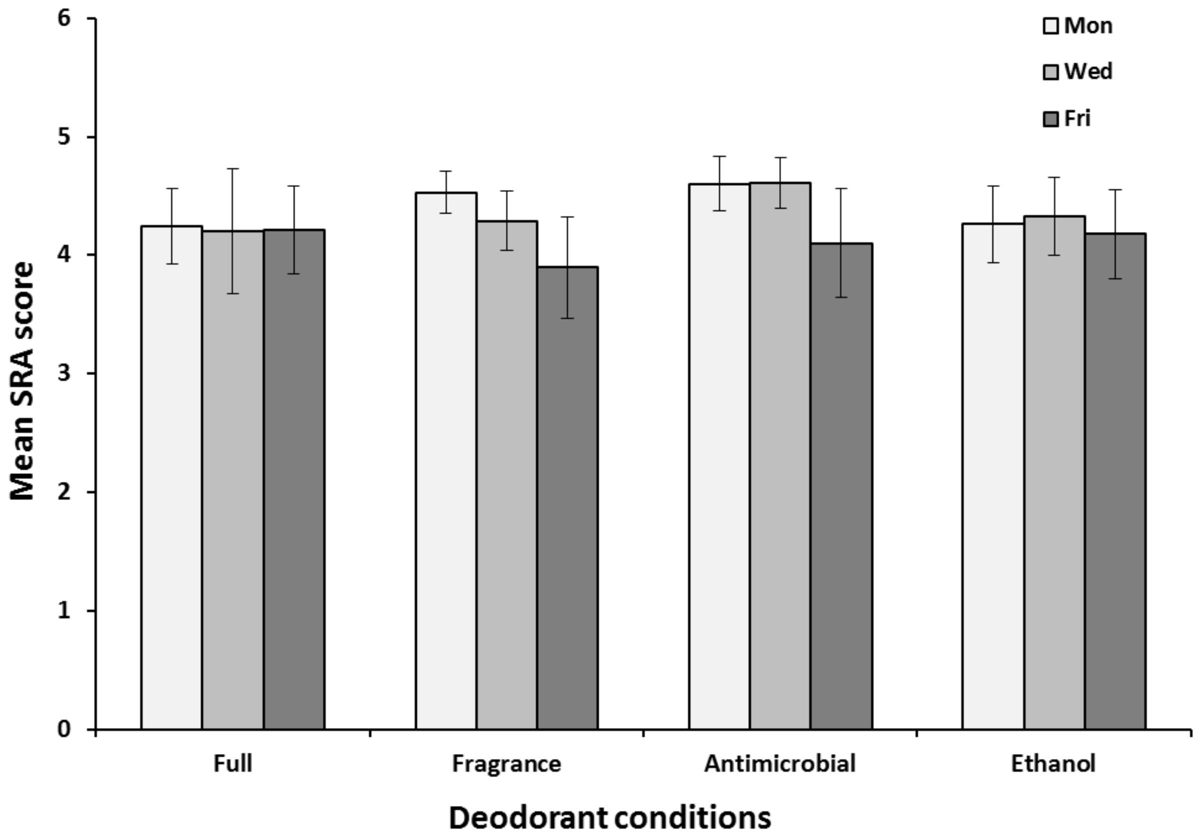


Figure 14 Mean self-rated attractiveness scores (SRA) ±SEM for participants in the four deodorant conditions across the three time points (Monday, Wednesday, Friday).

Photo ratings

Binomial tests were conducted in order to compare the observed frequency with which Monday and Friday photographs were selected as most confident against that expected by chance (.5). Tests indicated that Monday photographs were chosen at a significantly above chance level in the Ethanol condition (proportion .58, $p < .001$), and in the Fragrance only condition Friday photographs were chosen at a significantly above chance level (proportion .60, $p < .001$). Neither Monday nor Friday photographs were chosen at a significantly above chance level in the Full or Antimicrobial conditions (See figure 15).

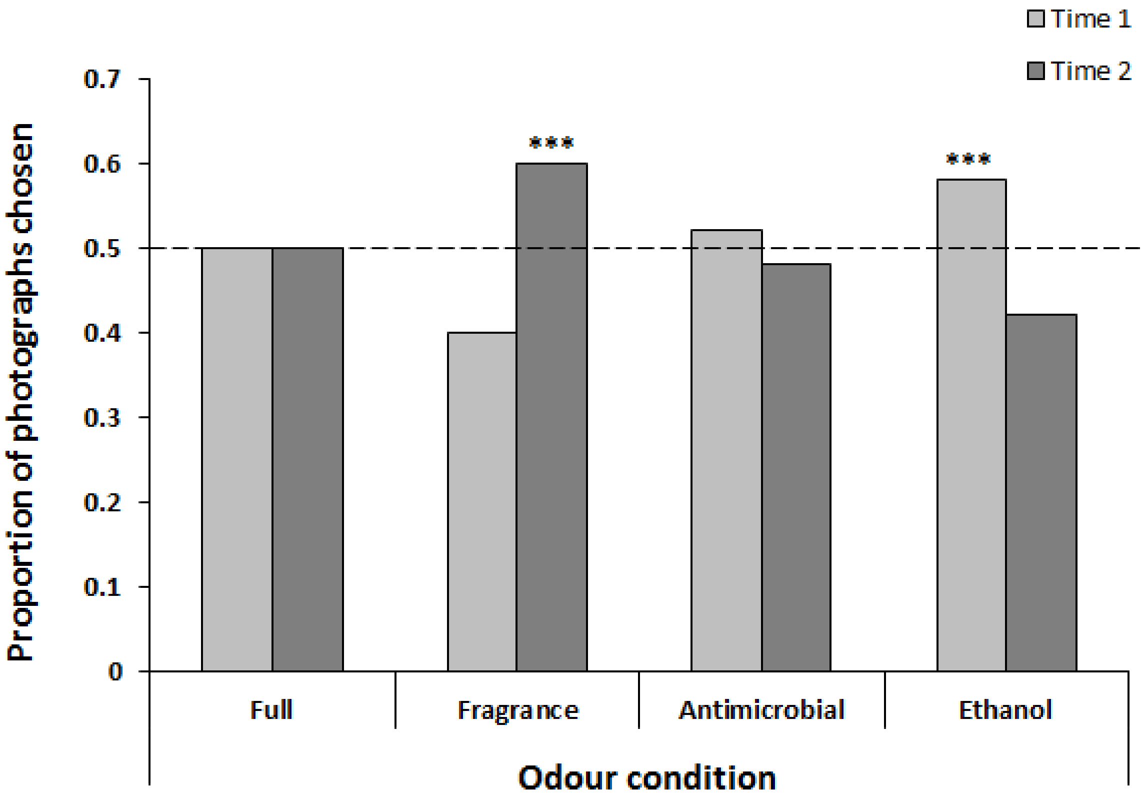


Figure 15 Proportion of Monday (time 1) and Friday (time 2) photographs chosen as being most confident by online participants. Dashed line indicates chance level (.50). Binomial tests indicated significance above chance level, *** $P < .001$.

Discussion

We found no main effect or any significant interactions relating to total self-confidence measures or self-rated attractiveness measures, in contrast with the findings of Roberts et al. (2009). Differences in the methodologies of the two studies may underly the different results obtained. In the study conducted by Roberts et al., participants completed an initial baseline measure of questionnaires, followed by deodorant application and then after a 15 minute interval they repeated the questionnaire battery. However participants in the current study only completed a baseline measure on the first day, with the second questionnaire being completed two days later. Furthermore, the current study included a smaller sample size of deodorant wearers per condition (n = 11) , while Roberts et al. had 35 participants split between two conditions; sample size is something which should be addressed in future research. It may also be useful for any future replications to require that participants undergo a washout period wearing no deodorant for 1 or 2 days prior to testing, in order to maximise the effects of deodorant use on self-confidence and attractiveness.

Despite this, there were some interesting findings related to others' perceptions of self-confidence of the images in the online study. In the full and anti-microbial conditions, there was no difference in the perceived confidence of the images between the Monday and the Friday sessions. However, participants chose the Friday photographs as more confident at a significantly above chance level in the fragrance only condition, suggesting that the fragrance used had a positive effect on non-verbal behaviour, at least as judged in the photographs taken. Furthermore, participants chose the Monday images as more confident at a significantly above chance level in the ethanol (placebo) condition, suggesting that those men who had gone for 5 days without additional fragrance or any antimicrobial agent to help reduce malodour, were perceived as less confident than they were at baseline.

While this study aimed to tease apart the effects of fragrance and reduction of malodour on self-confidence and attractiveness, more work is needed. The findings lend support to the idea that lack of both fragrance and the presence of malodour (in the ethanol/placebo condition) are deleterious to an individual's confidence, at least as perceived by others, but the findings do not allow us to further understand the relative roles of fragrance and malodour reduction in this effect. Future studies should aim to continue this important line of research, in order to understand which aspects of perception are affecting confidence. Does fragrance matter, or is it simply necessary to reduce malodour? Findings from the previous chapters (3 and 4) do suggest that fragrance may play an important role alongside the reduction of malodour, but further work is needed to elucidate this. Additionally, future research should continue this line of investigation utilising both male and female participants, as previous studies (Baron, 1983) and previous chapters (3 and 4) have highlighted potential sex differences in the perceptions of fragrances and of fragrance wearers.

It is also important to note that the previous chapters have highlighted that deodorants can have an effect on the accurate judgement of various mate quality cues in body odour (chapter 4). As well as investigating the effects of deodorant on other cues than those which have currently been addressed, further research should be conducted, as with this study, in order to differentiate between the effects of fragrance and antimicrobial agents on the perception of these important biological cues.

Chapter 8

Preparation for fatherhood: The role of olfactory communication during pregnancy

Introduction

In species where bi-parental, cooperative care of offspring occurs it may be adaptive to be able to signal the presence of a pregnancy to the paired male. Communication of pregnancy status may allow for physiological and behavioural changes, on the part of the male, which would be adaptive in aiding offspring care and paternal motivation.

Evidence for olfactory communication during pregnancy in non-human species

There is evidence suggesting that cotton-top tamarins (*Saguinus oedipus*) – a monogamous species showing bi-parental care of offspring - utilise olfactory communication between male and female partners during pregnancy which may be responsible for physiological changes in the male. Around mid-pregnancy, female cotton-top tamarins show a rise in urinary concentrations of glucocorticoids which coincides with the development of foetal adrenal glands. Ziegler et al. (2004) found that, 1-2 weeks after this, males with paternal experience showed a peak in cortisol and corticosterone themselves, suggesting a potential communication mechanism in pregnant female urine. Previous studies with cotton-top tamarins have also found that there is a peak of prolactin at mid-pregnancy in male partners (Ziegler & Snowdon, 2000). This led Ziegler and colleagues (2004) to hypothesise that glucocorticoid changes may act as signals in male cotton-top tamarins, initiating the onset of hormonal changes which may encourage adaptive parental behaviour and motivation.

However, males with no previous parental experience only showed a peak in prolactin levels within the last month of pregnancy (Ziegler & Snowdon, 2000), indicating that there may be some role of experience or learning involved in the operation of any potential chemical signals. In a follow-up study investigating the effects of paternal experience in male cotton-top tamarins, Almond et al. (2008) paired experienced males with primiparous pregnant females. They found that males still showed a mid-pregnancy rise in glucocorticoids but only showed a peak in prolactin in the final month of pregnancy. The fact that there is still a prolactin peak, albeit at a later point, nevertheless suggests some kind of chemical communication between the pregnant female and her mate. This may perhaps be mediated by the presence of previous offspring which would be in the environment of an experienced but not an inexperienced father, potentially having an additive effect on any chemical signals present. It is important to bear in mind however that many of the studies mentioned above have very small sample sizes.

Primates are not the only animals in which evidence for chemical communication during pregnancy has been found. Brown et al. (1995) found that male gerbils (*Meriones unguiculatus,* who are monogamous and show paternal care of offspring), when housed with their pregnant mates, had elevated plasma prolactin levels compared to unmated males. Smorkatcheva and colleagues (2009) found that in monogamous biparental voles (*Microtus mandarinus*), male faecal testosterone levels were reduced after the birth of a litter. Furthermore, Simoncelli and colleagues (2010) found that they could manipulate paternal behaviour in biparental monogamous male prairie voles (*Microtus ochrogaster*) by altering the level of contact that was maintained with the pregnant female partner. After having mated with a female, male voles were either allowed to remain in full contact with the female, or given only distal cues of the female (housed in the same room but a separate cage), or given no cues of, and prevented from contact with, the female. One group of males were also left unmated and allowed distal cues of females. At mid-gestation, males were exposed to infants and their responses were videotaped. It was found that most of the males behaved paternally towards the infants, but those males who had been mated and received tactile or distal cues of pregnant females approached the infants faster, and additionally were more likely to care for them, than those males who were unmated but had received distal female cues. Moreover, the males with experience of tactile cues showed the highest level of infant contact, and they had the lowest levels of observed non-social behaviour, suggesting that close physical contact with a pregnant female in some way altered males' paternal behaviours.

However, not all studies have found contact to be necessary for the display of paternal care behaviours. Jones and Wynne-Edwards (2001) found no effect of female contact during pregnancy on expression of male paternal and midwifery behaviours. They utilised Djungarian hamsters (*Phodopus campbelli*), which show biparental care and are known to undergo hormonal changes several days prior to the birth of pups, which has been linked to the expression of infant care and midwifery behaviours. They initially allowed the hamsters to mate, before removing the male to be housed in separate cages in the same room. Male hamsters were then returned to their partners' cage either after 5 minutes, or the evening prior to their pups' birth, or not at all. Additionally one group of males were returned just prior to the birth of the pups, but after having been housed in a separate room with independent airflow. They found that there was no subsequent difference between these groups in the display of paternal behaviours and in their involvement with the birth.

Furthermore, evidence for chemical communication specifically during pregnancy has not been found in all species which are monogamous and show biparental care of offspring. For example, in a study by Gubernick and Nelson (1989), male California mice (*Peromyscus californicus*) housed with their pregnant mates showed a rise in prolactin after the birth of their pups, but not prior to this. It has also been found that Mongolian gerbils (*Meriones unguiculatus*), who show biparental care, exhibit no decreases in testosterone with the arrival of offspring, and that the lack of this decrease appears to have no impact on the display of infant care behaviours (Juana et al., 2010). Interestingly they also found that decreases in aggression were not associated with increase in paternal behaviours, suggesting that, at least in this species, there is no trade-off between aggression and care behaviours. Moreover, studies have found that Siamang gibbons (*Symphalangus syndactylus*) display direct paternal care to offspring (unlike all other gibbons), but hormonal changes which may underpin these behaviours, such as decreases in androgens, appear to be specific to the post-partum period and father-infant proximity, rather than during pregnancy (Rafacz, Margulis, & Santymire, 2012).

Evidence for olfactory communication during pregnancy in humans

Like marmosets, tamarins and gerbils, humans are generally monogamous and tend to show cooperative care of offspring, making them potential candidates for the use of chemical signalling between mates during pregnancy.

In support of this, many studies have found associations between various hormone levels in men and their parental status. The main hormone which has been investigated in this regard is testosterone (Wynne-Edwards, 2001), which is central in the 'challenge hypothesis', first proposed by Wingfield and colleagues (1990), which states that testosterone facilitates reproductive effort at the expense of parenting effort. Consequently, in monogamous species showing biparental care of offspring, it is predicted that testosterone levels must be down-regulated in order to initiate effective infant care behaviours in males. Gray and colleagues (2006) found, in their sample of 126 Chinese men, that those men who were fathers had significantly lower testosterone levels than married and unmarried non-fathers. While it could be argued that this effect could arise because men who have lower testosterone are more likely to become fathers, Gettler and colleagues (2011) have found evidence to suggest that this is not the case. In a longitudinal study of 624 Philippine men, they found that those who were not fathers at baseline and had higher levels of testosterone upon waking were more likely to have become partnered fathers at follow-up, four and a half years later, compared with those who had lower levels of testosterone at baseline. Additionally, these men were found to show larger declines in testosterone levels over this time frame than their single, non-father counterparts.

It is also possible that these changes in testosterone may relate more directly to the formation of a committed romantic relationship, rather than as a response to a pregnancy or the presence of a child. For example, one study found that, of 122 male students, those who reported being in committed romantic relationships had significantly lower testosterone levels than those who were not (Burnham et al., 2003). However, Perini and colleagues (2012) controlled for this potential confound of relationship status. They found that fathers showed lower testosterone levels than non-fathers who were in committed relationships, both one month prior to and one month following the birth of their first child. While this study does not show exactly when hormonal changes occur, it does suggest that these may appear during pregnancy.

In further support of hormonal changes occurring during pregnancy, Storey et al. (2000) found that both males and females who were co-habiting and expecting a child together showed higher plasma prolactin and estradiol levels in late gestation compared to early gestation, and that these levels were strongly correlated within relationships. Additionally, Edelstein and colleagues (2015) reported longitudinal declines in men's testosterone levels during their partners pregnancy. There are however contradictions in the research. Berg and Wynne-Edwards (2002) found no correlation between estradiol saliva concentrations during pregnancy in partners, although only nine couples participated in this research and only estradiol (not prolactin) was measured. These contradictory findings may also result from the different chemical sampling methods (i.e. saliva vs. plasma).

The research to date appears to suggest that it is at least plausible that human males may undergo hormonal changes prior to parturition. Furthermore, a potential mechanism for this communication has been identified. Vaglio et al. (2009) found that pregnant women developed distinctive patterns of five volatile chemical compounds in sweat samples taken from the para-axillary and areolar regions. These chemicals were not found in non-pregnant, non-lactating women and there was a change in the patterns of their concentrations in early compared to late gestation, suggesting that they could provide information on pregnancy status.

Study rationale

While the literature to date suggests that testosterone levels are reduced in individuals showing biparental care of offspring prior to parturition, and that there appears to be a potential olfactory mechanism, this has not yet been experimentally tested in humans. The current study aimed to address this by exposing male participants to pregnant female odours using a repeated measures design, whereby men completed a questionnaire and computer task (time 1) followed by an odour exposure period before completing the questionnaire and computer task again (time 2). It was predicted that exposure to pregnant female odours would reduce interest in mating effort (from time 1 measurement to time 2), as measured by the sociosexual orientation inventory (Penke & Asendorpf, 2008), in comparison to those exposed to non-pregnant female odours, and those exposed to a control condition with no odours presented (from this we would predict a statistical interaction between time 1/time 2 measures and odour exposure condition). It was also predicted that exposure to pregnant female odours would increase paternal motivation (from time 1 to time 2), which we measured using a 'pay-per-view' key-press task, in which participants were exposed to infant and other stimuli (again predicting a statistical interaction between time 1/time 2 performance on this task with odour exposure condition).

Methods

Odour donors

Five pregnant woman aged 27-33 years (mean = 29.8, SD = 2.59) were recruited to provide axillary odour samples. Each woman provided samples from three time points: early gestation (20-23 weeks, mean = 21.4, SD = 1.14), late gestation (31-39 weeks, mean = 33.83, SD = 3.49) and post-pregnancy (25-43 weeks post-partum, mean = 30.6, SD = 7.67). At each time point, each donor provided two axillary samples over a 24hr period, twice on consecutive days (except one donor who only provided one sample from each axilla per time frame). Odour collection followed the protocol laid out in chapter 2, with the small change that cotton pads were sewn into the armpits of cotton t-shirts (washed with a fragrance-free detergent) instead of being taped to the underarms, in order to make the pregnant donors as comfortable as possible during odour collection.

Additionally, 5 non-pregnant woman, aged 24-29 (mean = 26.4, SD = 1.95), all of whom were using hormonal contraception, provided two 24 hour axillary odour samples (again one donor only provided one 24 hour sample). All 10 of the female donors were non-smokers.

Composite odours were then created from pads worn in each of the conditions: early pregnancy, late pregnancy, post-pregnancy, and control (non-pregnant) women. A further control condition was included, using blank (unworn) pads. For each condition, two identical composites were created. This was done by cutting in half each cotton pad and placing the two halves in separate glass jars with screw top lids. In this way there were two jars for each odour condition containing one half of every sample (both left and right axilla for all donors in that condition) that had been provided for that condition, ensuring that each jar contained the same number of identical samples. These were stored in the freezer until testing.

Participants

Ninety-one men aged 18-44 (mean = 22.63, SEM = .544) were recruited to participate in a lab-based study. Eighty of these men reported being heterosexual, with 6 being homosexual and 5 bisexual; 47 were in a romantic relationship at the time of the study.

Materials

Participants completed a questionnaire, comprising three scales and basic demographic questions using Qualtrics software. Participants completed the relationships assessment scale (RAS, Hendrick, 1988), a 7 item questionnaire with one reverse scored item, which is used to measure general relationship satisfaction, for example 'How well does your partner meet your needs?' (see appendix A). This is usually completed using a 1-5 rating scale, with one equalling low agreement with the statement and 5 equalling complete agreement, but for the purposes of this study the scale was changed to 0-100 in order to allow for greater variance in responses. Participants only completed this scale if they indicated that they were currently in a romantic relationship. Additionally, participants completed the revised sociosexual orientation index, a nine item measure, consisting of sections relating to behaviour, attitudes and desire (SOI-R, Penke & Asendorpf, 2008, see appendix C). The three behavioural questions utilise a nine point scale indicating varying numbers of sexual partners which can then be coded and aggregated to form the behavioural score. The attitude facet adopts a 1-9 scale with participants selecting whether they strongly disagree (1) or strongly agree (9) with a statement, and the final facet asks how often participants have specific desires, answering on a 1 (never) to 9 (at least once a day) scale. The attitudes and desires scale were changed from 1-9 to 0-100, as with the RAS scale, to again allow for greater variance in responses. Finally, the participants completed an 11 item (1 reverse coded) dominance scale taken from the International Personality Item Pool (Goldberg et al., 2006, see appendix B). Participants respond with their level of agreement to each statement which is presented, and again a 0-100 point scale was utilised.

Participants also completed a ‘pay-per-view’ key-press task measuring incentive salience (Hahn, Xiao, Sprengelmeyer, & Perrett, 2013). At a computer, participants are presented with a face, with a default viewing time of 4 seconds, and they are able to increase this viewing time by pressing ‘N’ and ‘M’ keys on the keyboard, or to decrease the viewing time by pressing ‘Z’ and ‘X’ keys. A timer bar is presented on the screen next to the image indicating the time which remains before the image is changed. Each alternate key-press pair is coded as one keypress unit. This paradigm was developed by Hahn and colleagues (2013) and quantifies the incentive salience of an image via the amount of effort (key-presses) that is exerted to keep or remove the image. Twenty adult male faces, twenty adult female faces and twenty baby faces were presented across two blocks in a counterbalanced order. Participants were informed that the task length was predetermined; however, this was in fact determined by their keypress behaviour. This was done in order to dissuade participants from pressing only the decrease viewing time keys in order to finish the task more quickly. After completing this task, participants were also asked to rate the faces which had been previously presented for attractiveness (1 = not at all attractive, 7 = very attractive, male and female faces) and for cuteness (1 = not at all cute, 7 = very cute, baby faces). An average rating score was subsequently calculated for each participant for each of the 3 face types (baby, female, male), both before and after odour exposure.

Procedure

Participants attended a lab session which lasted approximately 45 minutes to an hour. They provided informed consent, knowing that they would be exposed to human odours (but not knowing that these were specifically from pregnant women). They initially completed the online questionnaire providing basic demographic information (age, sexual orientation, relationship and cohabitation status and length), completed the RAS, the SOI-R and a brief dominance questionnaire. They then completed the computer keypress and face rating tasks (session A).

Next, they were presented with the composite odour in a jar. Participants were allocated to a condition based on the time that they signed up for the study. On screen instructions were given to guide them through the exposure procedure. They were instructed to remove the lid and smell the sample for 20 seconds (with a 40 second break afterwards). They did this ten times (lasting ten minutes in total), with on screen instructions and a timer to notify them of when to start and stop smelling. After this, the onscreen instructions asked them to sit quietly for 5 minutes (this was timed for them) before retrieving the experimenter. A brief odour exposure was chosen over a longer one for this initial investigation as research has found that even short odour exposure sessions can lead to endocrinological changes (Miller & Maner, 2010; Perrot-Sinal et al., 1999).

After the odour exposure they completed the online questionnaire again (this time excluding the demographic questions and the first three SOI-R questions related to behaviour, as it was not expected that this information would change with odour exposure – session B). Finally, they completed the computer based keypress and rating tasks a second time before finishing the experiment and being debriefed.

It was noted that not all participants had completed all ratings of faces. Four participants had rated 59 out of 60 faces, two had rated 58 out of 60, and one participant was missing all face ratings as well as having missed 13 faces for the keypress task in session A. In session B three participants completed 59 out of 60 face ratings, with the remainder of the participants completing all ratings and the keypress task for all face stimuli. It was decided that all of these participants would be retained for analysis except for the one participant who missed all of the face ratings and a substantial number of keypress task stimuli. All 91 participants completed each section of the questionnaire and so were included in the following analyses.

Results

Participants

There was an approximately even split between single and partnered males in each of the odour conditions (Table 13).

Table 13 Number and relationship status of participants in each odour condition.

Condition	Number of participants	Frequency of Participants in a relationship	Frequency of participants not in a relationship
Blank pads	18	8	10
Control female	18	9	9
Early pregnancy	18	11	7
Late pregnancy	18	7	11
Post-pregnancy	19	12	7

Computer task responses

Face Ratings

A repeated measures ANOVA was conducted with face type (baby, female, male) and time (before and after exposure) as within-subject factors, and condition (blank pad, control female, early pregnancy, late pregnancy and post pregnancy) and relationship status as between-subject factors. This analysis revealed a significant main effect of face type $F(2,160) = 36.19$ $p < .001$. Planned contrasts revealed that Baby faces were rated as significantly more cute ($M = 3.88$) than male faces were attractive ($M = 3.29$), $p < .001$, but that female faces were rated as significantly more attractive ($M = 4.19$) than baby faces were rated as cute, $p = .006$. The ANOVA revealed no main effect of time, $F(1,80) = 1.38$, $p = .244$, and no significant interactions with odour condition, as would be predicted by the hypotheses (see figure 16), or participant relationship status. There was, however, a significant interaction between face type and time, $F(2,160) = 7.77$, $p = .001$. Post hoc paired samples t tests revealed that baby faces were rated significantly lower at time 2 than time 1, $t(89) = 2.77$, $p = .007$, female faces were rated as significantly more attractive at time 2, $t(89) = -2.46$, $p = .016$, and that male faces were rated marginally lower at time 2 than time 1, $t(89) = 1.96$, $p = .053$ (figure 17). Additionally, post hoc paired samples t-tests revealed that female faces received significantly higher ratings than baby faces, $t(89) = -3.01$, $p = .003$, and than male faces, $t(89) = 8.68$, $p < .001$, and that baby faces received significantly higher ratings than male faces did, $t(89) = 5.46$, $p < .001$ (figure 17).

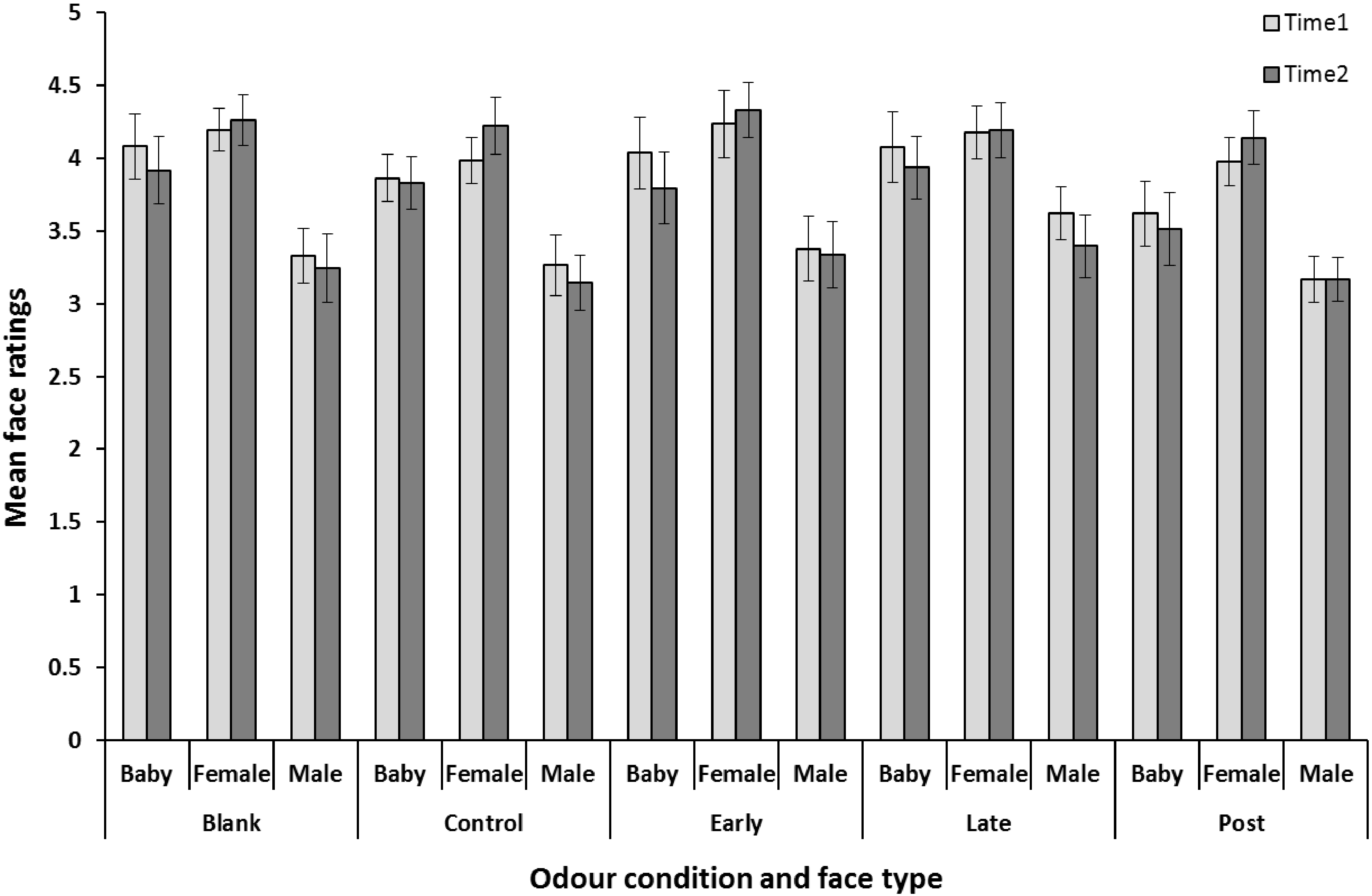


Figure 16 Mean face ratings given for each face type (baby, female, male) in each odour condition (blank pads with no odour, control female odour, early pregnancy odour, late pregnancy odour and post-pregnancy odour) at time 1 (before odour exposure) and time 2 (after odour exposure). Baby faces were rated for cuteness and male/female faces for attractiveness. Error bars represent ±1 SEM.

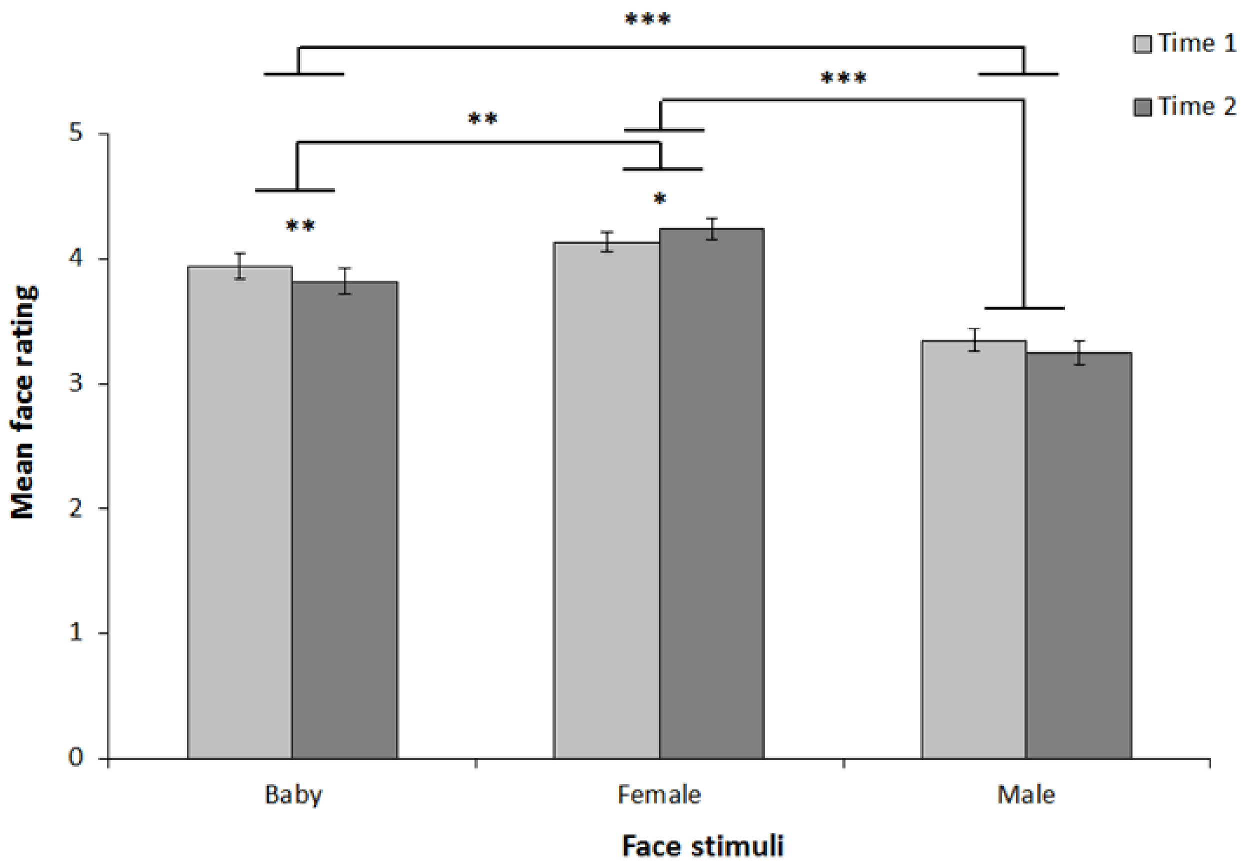


Figure 17 Estimated marginal mean ratings given to the three face types both before (time 1) and after (time 2) experimental exposure, when controlling for participant relationship status, averaged across all experimental odour conditions. Error bars represent ± 1 SEM. Post hoc paired samples t tests, * < .05, ** < .01, *** < .001.

The ANOVA was repeated, this time including only the blank, control female, and late pregnancy odour conditions, as these three presented the main contrasts of interest between the conditions. This revealed the same main findings as before: a significant main effect of face type, $F(2,94) = 22.64$, $p < .001$, and an interaction between face type and time, $F(2,94) = 4.59$, $p = .013$. As before there was no main effect of time, $F(1,47) = .97$, $p = .331$, and no significant interactions with relationship status of participants.

Finally the ANOVA was repeated again, this time separately for males who reported being single and those who reported being partnered. For single men, there was a significant main effect of face type, $F(2,54) = 15.58$, $p < .001$, with planned contrasts revealing that baby faces were rated significantly higher than male faces, $p < .001$, and that female faces were rated significantly higher than male faces, $p < .001$. There was no significant main effect of time, $F(1,27) = 2.40$, $p = .133$, and there were no significant interactions. When looking at partnered men there was again found to be a main effect of face type, $F(2,40) = 8.58$, $p = .001$, with baby faces being rated significantly higher than male faces, $p = .017$, and with female faces being rated as significantly higher than male faces < .001. There was no main effect of time, $F(1,20) = .02$, $p = .886$, but there was a significant interaction between face type and time, $F(2,40) = 4.38$, $p = .019$ (the same pattern as in Figure 2), and finally there was a significant interaction between face type, time, and odour condition, $F(4,40) = 3.27$, $p = .032$. In order to interpret this interaction, individual ANOVA's including within-subjects factors of face type and time were conducted for each of the three odour conditions (Blank, Control female, and Late pregnancy) for partnered men. The blank odour condition revealed a marginally significant main effect of face type, $F(2,14) = 3.56$, $p = .056$, with post hoc paired samples t tests revealing that females were rated as significantly more attractive than males, $t(7) = 4.58$, $p = .003$, but that there was no significant difference between the ratings given to baby and female faces, $t(7) = -.83$, $p = .435$, or between the ratings given to baby and male faces, $t(7) = 1.51$, $p = .174$. There was also no significant main effect of time, $F(1,7) = .36$, $p = .568$, and there were no significant interactions. The late pregnancy odour condition revealed no significant main effect of face, $F(2,12) = 2.91$, $p = .094$, or of time, $F(1,6) = .48$, $p =$

.516, and no significant interactions. Finally, the control female condition revealed no significant effect of face type, F (2,14) = 3.01, p = .082, or time, F (1,7) = .19, p = .671, however there was found to be a significant interaction between face and time, F (2,14) = 6.42, p = .010. Post hoc paired samples t tests revealed that there was a marginally significant difference between male and female face ratings, with females receiving higher ratings, t (7) = 2.32, p = .053, but that there was no difference between male and baby ratings, t (7) = 1.07, p = .320, or between baby and female ratings, t (7) = -1.44, p = .194. There were no significant difference between time 1 and time 2 ratings for any of the faces (figure 18).

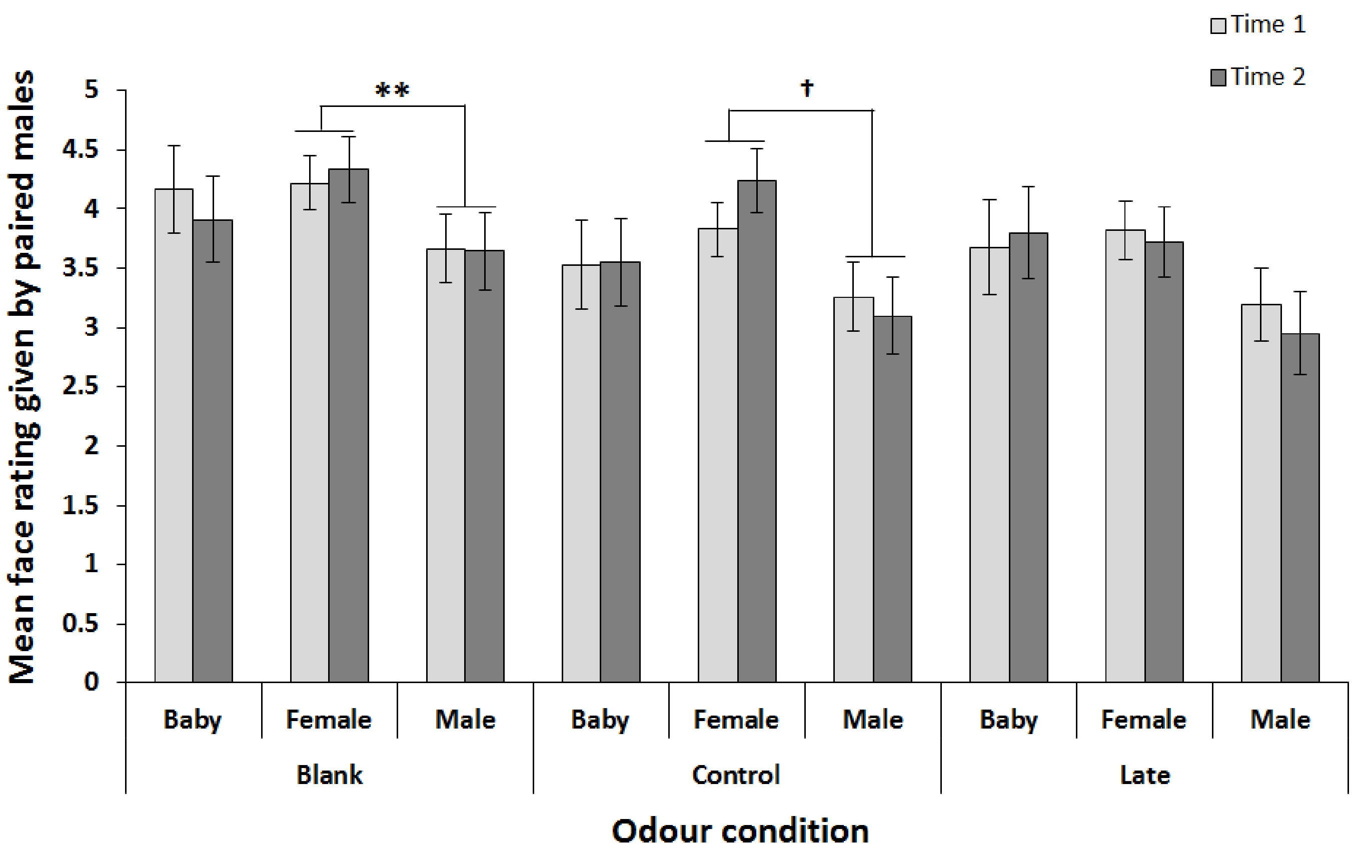
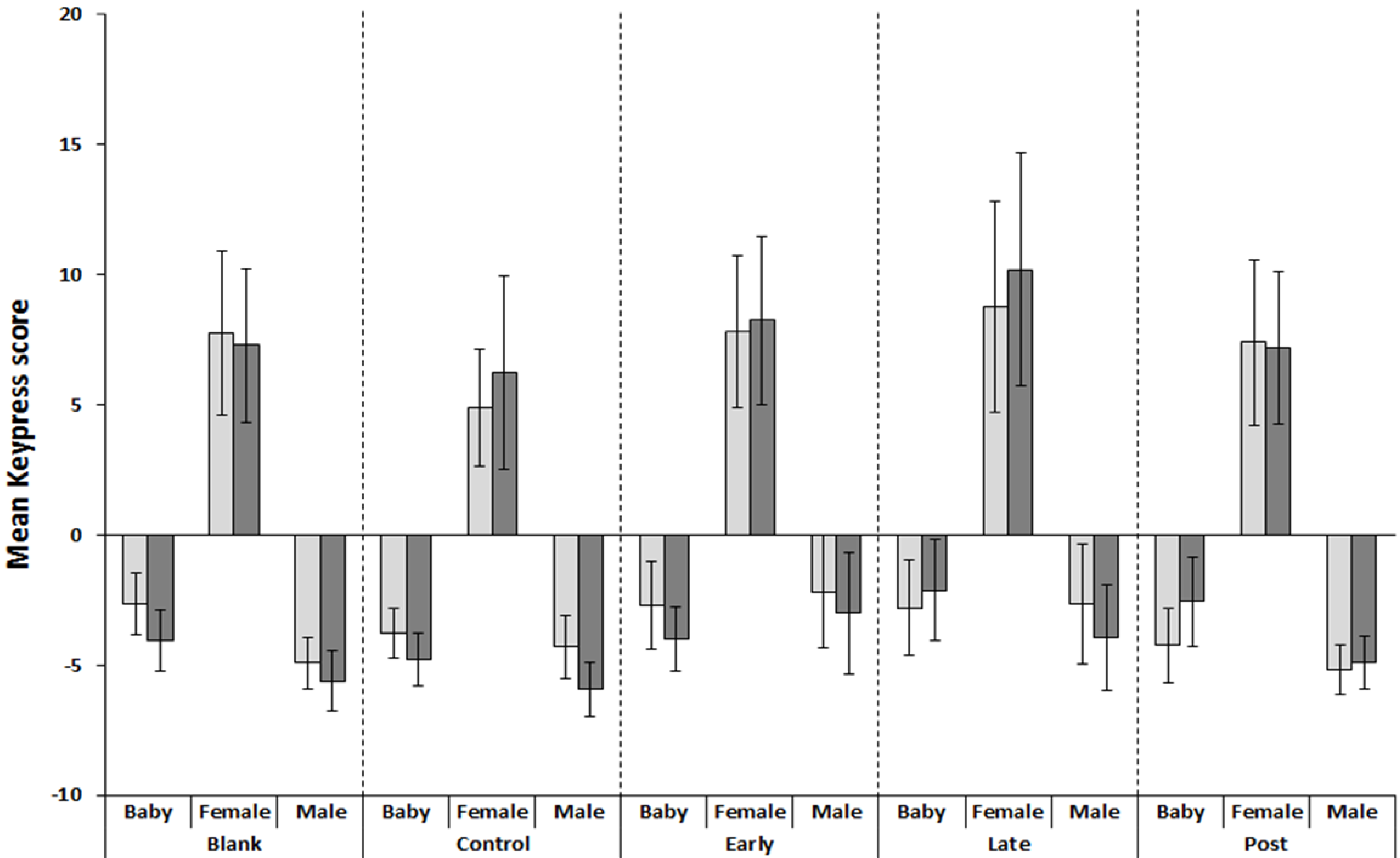


Figure 18 Mean face ratings given by paired men, showing the significant interaction between face type, time and odour condition. Post hoc paired samples t tests, ** < .01, † = .053

Keypress task

For each face that each participant viewed, the number of negative keypresses was subtracted from the number of positive keypresses. These values were then averaged across face types in order to create a keypress score for each participant for each of the three face types. These scores were analysed in a repeated measures ANOVA, with a within subjects factor of time (time 1 or time 2) and face type (baby, female, male), and a between subjects factor of odour condition (blank, control female, early pregnancy, late pregnancy, post-pregnancy) and of participant relationship status (single or partnered). This analysis revealed a significant main effect of face type, $F (2,160) = 41.59$, $p < .001$, with post hoc paired samples t-tests revealing that there was no significant difference between the keypress scores of baby faces and male faces, $t (89) = 1.26$, $p = .213$, but that baby faces received significantly lower keypress scores on average ($M = -3.36$) compared to female faces ($M = 7.59$), $t (89) = -6.99$, $p < .001$, and that female faces also received significantly higher keypress scores on average than male faces did ($M = -4.26$), $t (89) = 7.05$, $p < .001$ (see figure 19).

As with the rating data, this analysis was repeated this time using only data from the blank, control female and late pregnancy odour conditions. This revealed the same main effect of face as found before, $F (2,94) = 21.63$, $p< .001$. Additionally there were significant interactions between time and relationship status, $F (1,47) = 4.93$, $p = .031$, face type and time, $F (2,94) = 4.09$, $p = .020$, and between face type, time and relationship status, $F (2,94) = 3.22$, $p = .044$. Post hoc paired samples t tests indicated that there were no significant differences between time 1 and time 2 keypress scores for any face type across the three conditions in partnered men. However in single men it was found that in the blank condition that baby faces received significantly lower keypress scores at time 2 compared to time 1, t (9) = 3.11, p = .013. In the control female condition it was found that Baby faces also received significantly lower keypress scores at time 2 than time 1, t (8) = 2.79, p = .024, and additionally that male faces received significantly lower key press scores at time 2 compared to time 1, t (8) = 2.55, p = .034. Finally in the late pregnancy condition it was also found that male faces received significantly lower keypress scores at time 1 compared to time 2, t (10) = 2.26,p=.047(Figure20).



	Blank Baby	Blank Female	Blank Male	Control Baby	Control Female	Control Male	Early Baby	Early Female	Early Male	Late Baby	Late Female	Late Male	Post Baby	Post Female	Post Male
Time1	-2.6444	7.7472	-4.9222	-3.7882	4.8794	-4.3088	-2.7139	7.8167	-2.175	-2.7944	8.7639	-2.6333	-4.2447	7.3868	-5.1944
Time2	-4.05	7.2806	-5.6194	-4.8059	6.2176	-5.9324	-3.9861	8.2306	-3.0111	-2.125	10.1944	-3.9417	-2.5605	7.1895	-4.9

Odour condition and face type

Figure 19 Mean keypress scores given for face types (baby, female, male) across the odour exposure conditions (blank pads with no odour, control female odour, early pregnancy odour, late pregnancy odour, post-pregnancy odour). Error bars represent ±1 SEM.

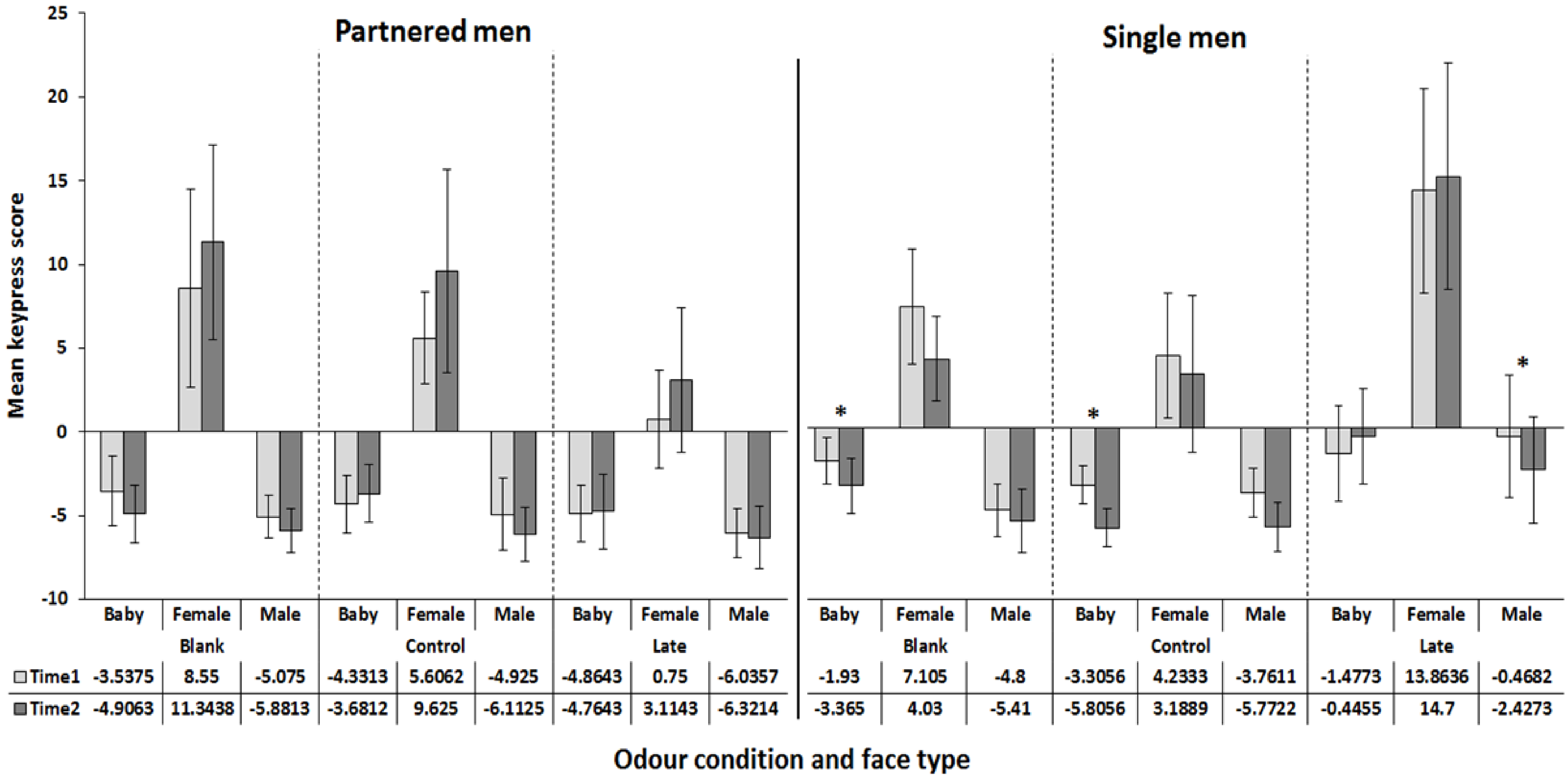


Figure 20 Mean keypress score for each face type (baby, female, male) in each condition (blank, non-pregnant female odour, late pregnancy female odour) for single and partnered men. Error bars represent ± 1 SEM. Post hoc paired samples t tests, * < .05.

Questionnaire data

A repeated measures ANOVA was conducted for the dominance scores with a within subjects factor of time (before and after odour exposure) and a between subjects factor of odour condition and relationship status. There was no main effect of time, F (1,81) = .55, p = .460, and there were no significant interactions. This finding remained when only considering the blank, control female, and late pregnancy conditions, and also when looking at single and partnered men separately (see figure 21). The same analysis was repeated, this time using the SOI-R attitudes scores, and again there was found to be no main effect of time, F (1,81) = .74, p = .391, and no significant interactions. This finding remained the same when only including blank, control female and late pregnancy conditions. However when looking at single and partnered men separately there was found to be a significant main effect of time, F (1,21) = 4.47, p = .047, with partnered men showing an increase in SOI-R attitudes scores after the odour exposure (M = 73.12) compared to before (M = 69.92), which was not found in single men, F (1,27) = .11, p = .744 (figure 21). The repeated measures ANOVA was again repeated using SOI-R desires scores, with the between subjects factor of relationship status and condition, and a within subjects factor of time, finding no significant main effect of time, F (1,81) = 1.67, p = .200, and no significant interactions. This finding remained the same when looking at the three odour conditions as opposed to all five (blank, control female, late pregnancy), and also when looking at single and partnered men separately (figure 21).

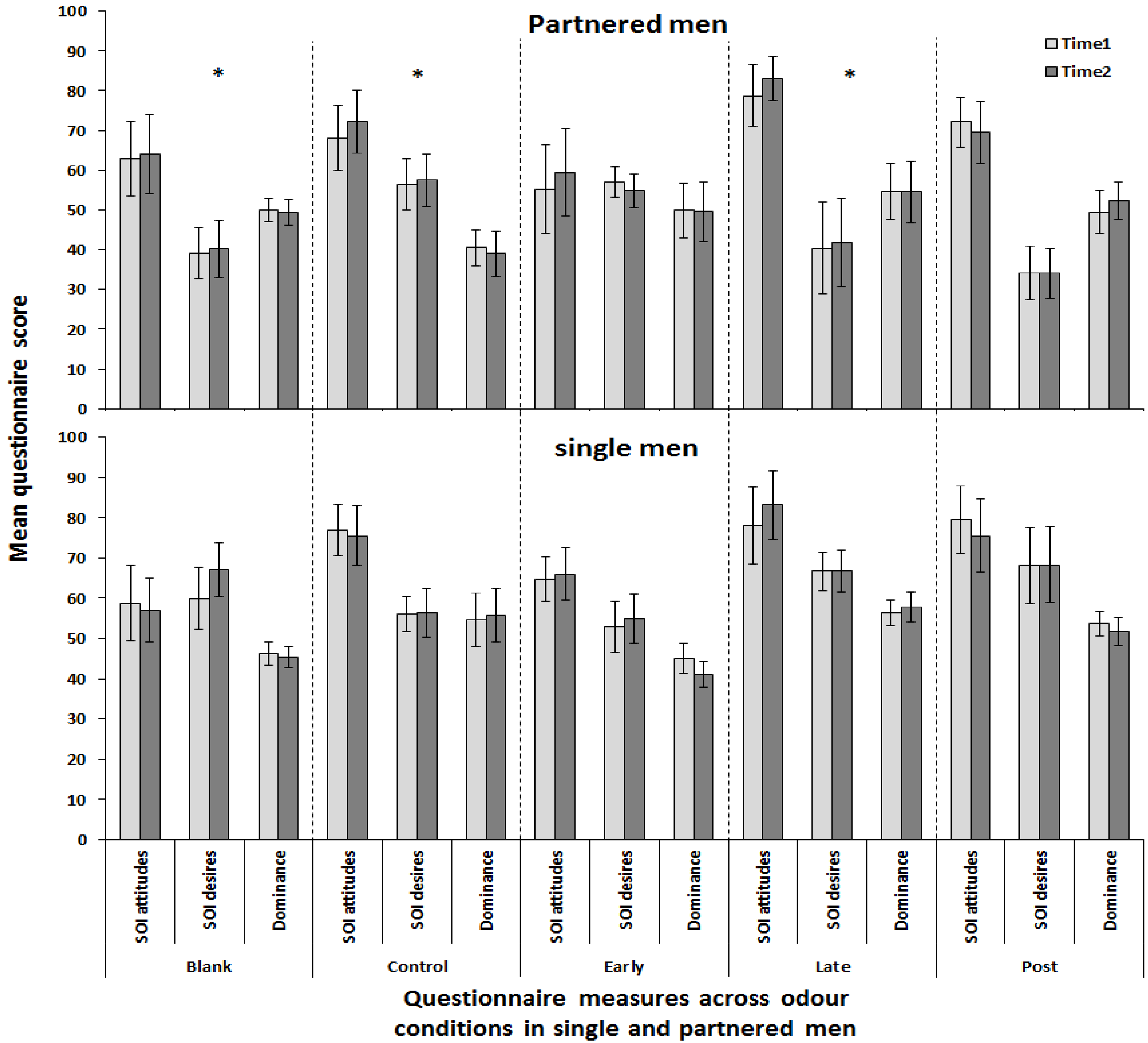


Figure 21 Mean scores for SOI-R attitudes, SOI-R desires and dominance scales, for single and partnered men in the 5 odour conditions (blank, control female odour, early pregnancy, late pregnancy and post pregnancy odours), before (time 1) and after (time 2) odour exposure. * indicate conditions included in RM ANOVA which found significant main effect of time for SOI-R attitudes. Error bars represent ± 1 SEM.

Finally a repeated measures ANOVA was conducted using the RAS scores from before and after odour exposure with time as a within subjects factor, and a between subjects factor of odour condition. This revealed a significant main effect of time, F (1,42) = 4.64, p = .037, with RAS scores being higher (M = 78.9) after odour exposure than before (M = 77.58). Post hoc paired samples t tests revealed that RAS scores significantly increased after exposure to late pregnancy odours, t (6) = -3.06, p = .022, but not in any of the other odour conditions (figure 22).

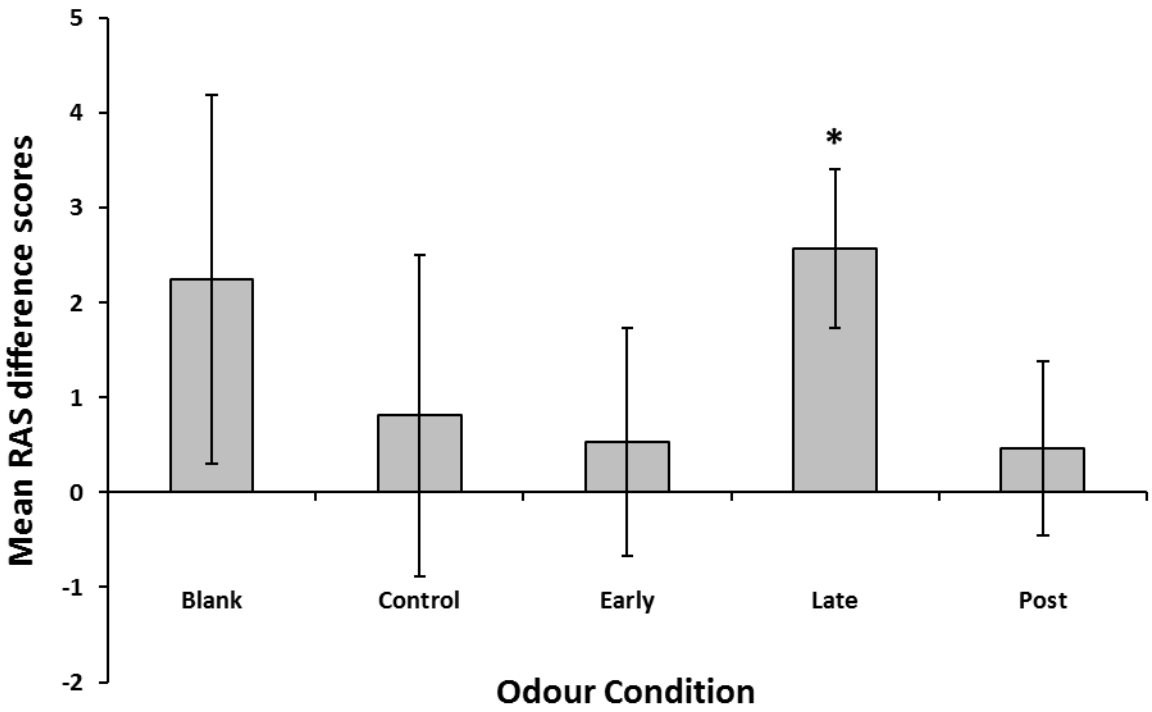


Figure 22 Mean RAS difference scores calculated by subtracting scores given by partnered men before (time 1) away from those given after (time 2) experimental odour exposure (blank pads, control female odour, early pregnancy, late pregnancy and post-pregnancy odour). Post hoc paired samples t tests were conducted on time 1 and time 2 responses from men in each of these conditions, * p < .05. Error bars represent ±1 SEM.

Discussion

Based on previous findings, the current study predicted that exposure to pregnant female odour would affect male participants' physiology and psychology in such a way that might prepare them for providing parental investment. This prediction was based on the discovery of specific volatile compounds in body odour of pregnant women but not non-pregnant women (Vaglio, Minicozzi, Bonometti, Mello, & Chiarelli, 2009), which may present a mechanism for inducing physiological hormonal changes, which may in turn result in psychological and behavioural changes. One of the main hormonal measures which have been implicated in potentially underlying these behavioural changes related to infant care-giving is testosterone (Wingfield et al., 1990; Wynne-Edwards, 2001).

Three psychological measures were employed in the current design. It was predicted that dominance would decrease after exposure to pregnant female odours but not after exposure to female odours or blank control pads as dominance has been related to mating effort, and also to testosterone levels (Mazur & Booth, 1998; Mehta & Josephs, 2010; Qvarnström & Forsgren, 1998; Swaddle & Reierson, 2002). However the current study found no effect of odour condition on dominance levels. Additionally the study employed two sections of the SOI-R, which are related to interest in mating (Penke & Asendorpf, 2008), which were again predicted to decrease after exposure to pregnant odours. The current study in fact found that there were no significant changes in measures of SOI-R attitudes or desires across the odour conditions. Post hoc analyses did reveal that partnered men showed increases in SOI-R attitude scores after the experimental manipulation; however this was across all odour conditions, including the blank, no odour, condition, and so cannot be attributed to the presence of female, or pregnant females' odours.

Participants who reported being in a romantic relationship at the time of the study also completed the RAS, a measure of relationship quality, and it was found that RAS scores increased from time 1 to time 2. Additional post hoc analysis indicated that these scores only significantly increased after exposure to late pregnancy odours. This could be interpreted as supporting the current hypothesis that pregnant odours may influence the physiology and psychology of men, increasing their feelings of contentment within their current relationship. However, it must be noted that there were only 7 partnered men in the late pregnancy odour condition and so this finding should be interpreted with caution, and future work will need to verify this with a larger sample of partnered men.

It was also predicted that exposure to pregnant female odours would increase the incentive salience of infant stimuli, as measured using a 'pay-per-view' key-press task (Hahn et al., 2013).The current study found little evidence of changes in viewing habits of baby or adult faces in relation to specific odour exposure. There appeared to be a decrease in the effort expended to view male and baby faces in the second repetition of the task in the no odour condition which may simply reflect boredom with the task, as there was no odour presented. Additionally, it was found that male and baby keypress scores also decreased after exposure to control female odours, and so did male face keypress scores after exposure to late pregnancy odours. This again should be interpreted with caution as it may simply represent boredom with the task, though it warrants further investigation. It was however found that female faces had significantly higher keypress scores, indicating an increased amount of effort in maintaining the image on the screen, compared to baby or adult male faces, though this finding was irrespective of odour condition. This indicates that the majority of participants preferred to look at female faces than baby or male faces, which is not surprising as the majority of the sample reported being heterosexual.

The final measure utilised in the study was that of face ratings, with the prediction that ratings of cuteness of baby faces would increase after exposure to pregnant female odours, but not after exposure to blank, or control female odours. Additionally participants rated male and female faces for attractiveness. It was found that generally female faces received higher ratings than babies and males faces, and additionally that babies received higher ratings than males. Additionally, baby and male ratings decreased at time 2 and female faces increased, though this was again found to be irrespective of odour condition. This pattern was maintained in single men, however partnered men in blank condition and the late pregnancy condition showed no significant difference between female and baby face ratings babies were not significantly different from females, or babies from males. This then does not support the current hypothesis, finding no clear relation between odour type and face ratings. The finding does however further suggest that relationship status may be important, though again this would need to be further investigated as the current study only presents ratings from relatively small groups of single and partnered men across the odour conditions. One additional point to note with the keypress data is that there was much individual variation present in these measures, as can be seen from the error bars in Figures 19 and 20. This is not necessarily surprising, but it does mean that we must be careful in drawing any firm conclusions from the differences across odour conditions.

With the possible exception of the effect of late pregnant odour on RAS scores, these findings may seem to dispute the role of olfactory communication during pregnancy in humans, but it must be noted that this is the first study to experimentally investigate the role of these odours and so future designs may more adeptly address this line of questioning. For example, the current study only used a short-term odour exposure. This decision was made based on findings that odours can affect hormone levels, specifically testosterone levels which we had hypothesised to be important in underlying changes related to infant interest and reduced mating effort (Wingfield et al., 1990; Wynne-Edwards, 2001), within a short amount of time (Miller & Maner, 2010; Perrot-Sinal et al., 1999). Perhaps, then, if we had measured actual testosterone levels in our participants we may have found that this varied after exposure to pregnant odours compared to non-pregnant and blank conditions, even though our survey and keypress/rating measures did not. It may simply be that long-term rather than short-term changes in hormone levels are required to initiate changes in infant interest. Furthermore, longer odour exposure would present a more ecologically valid experimental design as pregnancy lasts for approximately 40 weeks, which, if expectant parents are living together, is a very long odour exposure time compared to that of the current study. However, attempting a design which incorporates long-term odour exposure presents additional difficulties as more odour samples and therefore donors would be required and participants would likely have to visit the lab on numerous occasions.

Although the current study did not provide strong evidence supporting olfactory communication during pregnancy it did highlight that future studies should incorporate relationship status into any further investigation. While there was no clear distinction between single and partnered males with regards to odour exposure, there were some differences between the two groups of men which will require further investigation with a more adequate sample size to interpret more fully. Given this, and findings which show that previous paternal experience may mediate potential chemical communication (Ziegler & Snowdon, 2000), future studies should also focus on including sub groups of single and partnered men, as well as men with and without children. This would likely also lead to recruitment of a sample with a more representative range of ages. The current study had a relatively large age range, however the majority of the sample were aged between 18-22, and this could be improved upon in future work.

Finally, although our predictions were not supported, the findings from the current study can be seen as providing evidence that brief exposure to pregnant females' body odour is not sufficient to induce psychological changes related to infant care. Additionally, the study design benefited from using composite odours over single samples, and from collecting odour samples from the same women at various pregnancy time points. Future work should aim to maintain these advantageous design features whilst investigating odour exposure over a longer time frame, and obtaining hormonal measures, before we can rule out an effect of odour on the behaviour of fathers-to-be.

Chapter 9: General Discussion

Introduction

As presented in chapter 1, there are numerous findings supporting the role of body odours in human olfactory communication. However, there is currently a lack of research incorporating fragrance use, making it difficult to generalise these findings to the population at large where the use of fragranced products is widespread. The current thesis sought to address this gap in the literature by experimentally assessing the impact of fragranced products on the detection and assessment of olfactory traits. The thesis also presented two studies investigating further effects which body odour may have on behaviours, both of the wearer and of perceivers in the environment.

Section One: summary

The first part of the thesis (chapters 3, 4, 5 and 6) aimed to specifically investigate the effects which the presence of artificial fragrances may have on the assessment of body odours.

Chapter 3 was based on the recently published manuscript by Allen, Havlíček and Roberts (2015) which investigated the way in which discrimination of body odours may be impacted by the presence of deodorants. The findings to date (presented in chapters 1 and 3) suggest that humans are adept at distinguishing between, and recognising, conspecifics on the basis of body odour (Ferdenzi et al., 2010; Lord & Kasprzak, 1989; Porter et al., 1983; Weisfeld et al., 2003), and that this ability appears to be present from an early age, with neonates having been found to be able to discriminate between the smell of their mother and that of an unfamiliar, lactating female (Cernoch & Porter, 1985). Intuitively, it seems as though the presence of a fragrance would mask the individuals' odour profile, in such a way that one would expect discrimination based on odours to become more difficult. However, the few studies that have investigated this seem to suggest that fragrances may be, in some unconscious way, chosen to enhance an individuals' body odour. For example, fragrance preferences have been linked to genetic information (Hämmerli et al., 2012; Milinski & Wedekind, 2001), and preferred fragrances have been found to result in increased ratings of pleasantness when mixed with an individual's body odour, compared to that of assigned fragrances (Lenochová et al., 2012).

The study presented in chapter 3 aimed to experimentally test whether discriminatory ability is affected by the presence of a fragrance, and furthermore whether fragrance choice also mediates this. Using a triangle task paradigm, participants were presented with three axillary odours (two from one individual and the third from another) and had to select the odour which was different. This was done three times with body odour in isolation, and then with body odour and a fragrance of choice blend, and with body odour and an assigned fragrance blend. In keeping with previous findings that women appear to outperform men on tests of olfactory ability (Brand & Millot, 2001; Cardesín et al., 2006; Doty & Cameron, 2010; Platek et al., 2001; Schleidt, 1980) it was found that women performed better in the task than men did. Irrespective of sex, participants were found to perform at significantly above chance levels in all three of the odour conditions, providing further evidence that fragrances may not be masking body odour, as well as providing more general support for our olfactory discriminatory ability. Importantly, it was found that performance in the own fragrance condition was significantly better than in the assigned fragrance condition, supporting previous claims that chosen fragrances are in some way complementing an individuals' odour profile (Lenochová et al., 2012;

Milinski & Wedekind, 2001). However, it must be noted that when looking at males' and females' performance separately, this pattern was not as clear, and so future work will be needed to clarify this.

Chapter 4 continued this line of investigation by assessing the effect of fragrance use on the ability to detect more specific information from body odour. The study presented examined the cross-sensory consistency (across faces and odours) in the perception of masculinity and femininity in men and women, and whether this is influenced by the use of artificial fragrance. Both masculinity and femininity (in men and women, respectively) are traits which have been linked to underlying genetic quality (Thornhill & Gangestad, 1999) and reproductive potential (Fraccaro et al., 2010) in humans. These traits also presented a unique opportunity for investigation as they both appear to be traits which are detectable across multiple modalities (Fraccaro et al., 2010; Holzleitner et al., 2014; Little et al., 2011), allowing correlations across modalities to be examined both with and without the presence of an artificial fragrance. Interestingly, as noted in chapter 4, they also represent central constructs in the classification of fragrances (Lindqvist, 2012). This provides further evidence of the cultural relevance of these sexually dimorphic biological traits, lending support to the claim, initially posited by Havlíček and Roberts (2013), that this cultural practice may have emerged as a result of a biologically evolved preference.

The study presented in chapter 4 provided evidence that artificial fragrances increased the femininity of women's odours, as would be predicted if fragrances are being used to enhance potentially biologically evolved preferences (as other cosmetics appear to be, see Russell, 2009). However, this did not seem to be the case with male odours and masculinity. In chapter 4 it is suggested that this may reflect the differing value of masculinity and femininity to opposite-sex partners. I suggest that sex-specific preferences for these traits may potentially be translated into fragrance design, with female fragrances perhaps being designed to be more feminine than male fragrances are masculine. In support of this hypothesis, there are findings suggesting that women prefer moderate levels of masculinity over extreme levels (Rhodes et al., 2000) and more masculine male faces tend to be perceived more negatively (Perrett et al., 1998); in contrast, there are no such analogous findings relating to levels of femininity in women's faces. Additionally, as in chapter 3, the results suggested that women may be more sensitive (or attentive) to body odour cues, as it showed that women's ratings of unfragranced odour samples and faces (of both men and women) were significantly correlated with one another (as predicted from previous findings) but men's ratings were not. It appeared that, at least with women rating men, the presence of a fragrance prevented the accurate assessment of masculinity from body odour, as assessed by the lack of significant correlation between fragranced odour and face ratings, but not between unfragranced odour and face ratings. Further investigation revealed that, for those men whose odour was highly masculine without fragrance, the addition of a fragrance did not significantly alter their perceived masculinity. However, for the men whose unfragranced odour was rated low on masculinity, the addition of a fragrance significantly improved their ratings of this trait. This was not seen with women's odours, with the significant correlation between body odour and ratings of facial femininity also being found with the addition of a fragrance (at least when rated by other women). Taken together, these findings further support the hypothesis that the differential optimum level of masculinity and femininity may have impacted the ultimate levels of masculinity and femininity within fragrances possibly leading to the ceiling effect seen in the men's results.

Chapter 5 presented a novel study investigating the presence of disassortative mating based on olfactory cues in humans. This study was based on findings which provide evidence for the presence of olfactory cues in body odour related to information regarding an individuals' major histocompatibility complex, or MHC (Gilbert et al., 1986; Hepper, 1988; Kalmus, 1955; Montag et al., 2001; Roberts, Gosling, et al., 2005; Sommerville et al., 1990) which is involved in immune functioning. Though previous findings are somewhat mixed (see chapter 5; also Havlíček & Roberts, 2009), studies have found that individuals prefer the smell of someone who has a dissimilar MHC to themselves (Jacob et al., 2002; Roberts et al., 2008; Thornhill et al., 2003; Wedekind & Furi, 1997; Wedekind et al., 1995). As discussed in chapter 5, this can be seen as representing an adaptive preference, as any potential offspring from such a union would likely possess increased heterozygosity at the MHC. The one caveat to this finding is that hormonal contraception use has been found to interfere with this odour preference (Roberts et al., 2008; Wedekind & Furi, 1997; Wedekind et al., 1995), and so couples who begin relationships when the female partner is using hormonal contraceptives may not show this pattern of disassortative mating.

The study presented in chapter 5 investigated the level of similarity between the body odours of real world romantic couples, by having participants rate pairs of odours from couples for their level of similarity. If individuals are choosing romantic partners based on disassortative odour preferences and this adaptive preference is disrupted by hormonal contraception, then we would have expected to see increased levels of odour similarity between couples who met and began their romantic relationships whilst using hormonal contraception (HC couples) compared to those couples who were not using hormonal contraception at the formation of their relationship (No HC couples). The opposite was in fact found, with both categories of couples being rated as smelling more similar to one another than fake (experimenter created) couples, but with No HC couples smelling more similar to one another than HC couples. An additional aim of this study was to test whether within-couple levels of odour similarity (or dissimilarity) were influenced by fragrance choice. If fragrance is being used to complement an individual's odour profile, as suggested by chapters 3 and 4 as well as previous research, then we would also expect to see the same pattern of results in both unfragranced and fragranced body odour samples (in this case, fragrances were the individual's preferred brand and were not assigned by the experimenter). However, this prediction was also not supported, as there was no difference between similarity ratings given to fragranced odour samples across the three couple categories (HC, No HC, fake couples). Though, it must be noted that in this particular study odour similarity was simply acting as a proxy for genetic similarity at the MHC (based on previous findings) and in fact no genetic measures were taken.

The unexpected findings presented in chapter 5 were instrumental in the development of the work presented in chapter 6. The study presented in chapter 5 had adopted a very simple scale for rating the similarity of the odours, and one criticism of this may be that information in body odour was potentially missed in the ratings. This seems likely given the wide range of information available in body odour (see chapter 1) coupled with the large number of volatile compounds which are present (Penn et al., 2007). In order to attempt to rectify this for future studies, work was conducted alongside perfumers, with the aim of creating a more detailed scale, or map, for describing the perceptual qualities of body odours. This work is presented in chapter 6. The odours collected from the donors in chapter 5 were smelled by the perfumers, who developed a scale containing 15 descriptors. Each sample was then rated on a 0-10 scale for the presence of each descriptor. Factor analysis was then conducted, which led to the extraction of two factors, one consisting of the descriptors onion/spicy/animalic/heavy, and the other of milky/sweet. It did appear that the extracted factors may be useful in indicating the sex of an odour donor, with men scoring significantly higher than women on ratings of onion/spicy/animalic/heavy. Further investigation, however, did not find any significant differences between the HC and No HC couples' scores as measured using either the extracted factors or the original 15 descriptors. Again, this would be predicted by effects of HC on odour-based disassortative mating, though as mentioned earlier, there were no genetic measures taken to assess actual disassortative mating at the MHC, odour similarity was simply being used as a proxy for this, and future research should aim to incorporate these measures where funding will allow.

Section One: conclusions, limitations and future directions

The work presented in chapters 3 and 4 further supports the need to include fragrances in the investigation of olfactory communication in humans, as argued in chapter 1. Findings in chapter 3 suggest that fragrance may complement an individuals' entire odour profile, allowing easier discrimination of an individual (at least compared to an assigned fragrance). On the other hand, the findings from chapter 4 suggest that when looking at more specific traits, such as masculinity, fragrance may actually enhance these traits to the point that it is more difficult to distinguish between individuals. The differential effect of fragrances in these two contexts (and between masculinity and femininity) suggest that, in order to fully understand the role of olfactory communication in the current environment, future work needs to focus on investigating how fragrance may be mediating other well-established instances of olfactory communication, such as that of personality, health, and cyclical odour changes related to the menstrual cycle.

A strength of the current thesis is that the work presented in section one has adopted the use of deodorants and antiperspirants as opposed to perfumes. In the literature, perfumes are the most commonly used fragranced product when incorporating fragrances into studies investigating olfactory communication. While it is an entirely valid design to use perfumes in studies, it must be noted that effects of perfumes may differ from that of antiperspirants and deodorants which are additionally designed to reduce malodour and sweat (as discussed in chapter 3). Again, in order to fully elucidate the role of fragrance in olfactory communication, future work should be conducted utilising the range of fragranced products which are available to the population.

There were however, some limitations in the current studies which should be addressed in future research. First, while in chapters 3 and 4 menstrual cycle stage was controlled for in odour donors (through recruitment of HC users only), this was not the case in chapter 5, and this may have had an impact on the similarity ratings which the couples received. Additionally, while it is important to control for menstrual cycle stage during odour collection, findings cannot necessarily be extended to regularly cycling women when only HC users are recruited.

Furthermore, menstrual cycle stage and HC use were also not controlled for in the raters/participants in chapters 3, 4 and 5. These chapters all showed differences in the ratings/performance from men and women, which led to the conclusion (discussed above) that women are more sensitive to odours and olfactory information. One possible explanation for this is that due to the differential physiological and biological costs of reproduction for males and females (Trivers, 1972), females are the choosier sex, in line with Bateman's principle (Bateman, 1948), and so it is more important for women to accurately assess any cues of potential mate quality (such as masculinity). It could also be argued that women use more fragranced products than men (Roberts et al., 2010), and this additional experience with fragrances could lead to an increased sensitivity, or attentiveness to odours in the environment. However, an alternative possibility is that this olfactory sensitivity is underpinned by hormonal changes related to the menstrual cycle, rather than being a more general female ability and so future research should control for this in both donors and raters/participants.

Additionally, while the olfactory ability of participants/raters was measured across the work presented in chapters 3, 4, and 5, it was never measured in the odour donors. This is potentially very important, specifically when investigating the effects of an own versus an assigned fragrance. If, for example, a donor has an extremely poor sense of smell, they may not be choosing a fragrance which best complements their body odour, as findings suggest, and this could then bias results.

Furthermore, some points concerning odour collection and presentation must be considered. With regards to the application of deodorant in the current studies, the decision was made to instruct participants to apply these in their usual way. This is potentially open to criticism as it allows variation in the amount of deodorant applied by each participant. This was not an easy decision to make given the lack of research incorporating fragrances, but, in keeping with the attempts made in the presented work to increase the ecological validity of findings related to odour perceptions it was decided that this would be the most valid instruction for participants to be given.

This is not to say that there is no room for improvement with regards to odour collection and presentation. For example, Chapter 8 (discussed in the next section) benefitted from creating identical samples by cutting odour pads in half, and, with hindsight, this method would have perhaps been beneficial in the work presented in chapter 3. In this chapter the samples presented in the three conical flasks were either the left or right axillary sample. By cutting the left and right samples in half, and adding half of each to each flask, we would have avoided any variation in the perceptual qualities of left and right samples, resulting in truly identical samples for comparison. Though it should be noted that this is only has a bearing on one chapter, as odour presentation for the remaining chapters involved presentation of both left and right axillary samples in the same flask.

A further potential criticism of the current work involves the order of sample collection. In the studies where multiple samples were collected from one participant (chapters 3, 4 & 5) the order of collection (fragranced and unfragranced) was not counterbalanced across armpits and days. In chapter 3 it would have been difficult to employ this odour collection strategy as 3 samples were required (No deodorant, own deodorant & assigned deodorant) and would need balancing across the three days and both armpits (left and right). Additionally, while this may have provided good control over the samples, the studies all also adopted clear and detailed instructions for participants in order to minimise the effects of extraneous variables such as food intake, sexual contact and potential exercise effects. Given this, and the fact that all samples were collected across consecutive days, it was deemed acceptable to not counterbalance sample collection across armpits in all of the studies.

Finally, it must be noted that the work presented in chapter 6 is in its very early stages. The scale needs revised as well as validated both with other perfumers as well as with laymen. This is an extremely important line of work which, if continued, may have profound impacts on both the research field and the perfume industry. As mentioned earlier, and throughout the thesis, human body odours are extremely complex, containing information relating to multiple stable traits as well as more transitory states (such as health and menstrual cycle stage). The fact that we do not have a validated, objective scale for measuring the perceptual qualities of body odours is, arguably, holding the research back. For example, when looking at research investigating faces, studies have found that there are numerous morphological and aesthetic cues (such as facial width to height ratio, skin texture and colour, etc.) which are related to various states and traits such as health, personality, diet, stage of menstrual cycle – in short, faces provide the same level of information that may be assessed from one's odour. However, the difference between the face and odour research is that these differences between faces can be objectively measured, which in turn allows for the manipulation of such traits in experimental settings. Unfortunately, we do not currently have this ability in the field of human olfactory communication, and I believe the first step towards this is to map and understand the perceptual differences in body odours, before attempting to relate these to specific volatile compounds, which would then enable the manipulation (or even creation) of odours for use in experiments. Moreover, mapping of body odours will allow further work to be conducted which investigates which specific fragrances work best to complement or enhance specific odour profiles. This would allow for the replication of previous work (for example the work conducted in chapter 3, Allen, Havlíček, & Roberts, 2015, and that of Lenochová et al., 2012) in a more predictive fashion, by being able to select a 'best match' and 'worst match' fragrance. Finally, if successful, this would also enable fragrances to be specifically designed for certain body odour types.

Section Two: summary, limitations and future directions

The second section of the thesis presented two studies (chapters 7 and 8) which focussed on investigating more generally body odours and behaviours, addressing both the ways in which body odours may affect the individual, and also how they may affect others in the immediate environment.

Chapter 7 presented a study which aimed to illuminate the various effects which the addition of fragrance and reduction of malodour may have on self-confidence and attractiveness, so as to better understand how deodorants and perfumes may differ from one another in their effects on the wearer and on perceivers in the environment. Participants were given either a full deodorant, only a fragrance, only the antimicrobial compound (used for malodour reduction) or an ethanol (control) solution, which they wore for a week. In contrast to previous research (detailed in chapter 7) there was found to be no variation in self-rated confidence or self-rated attractiveness across the deodorant conditions. Additionally wearers had their photo taken on the first day of participation, and on the last day (after a week of wearing the product), and these were used in an online forced choice task where participants had to choose the most confident image of the two. There was found to be no significant difference in the choice of photos selected as most confident for the full deodorant and antimicrobial only condition, which would be expected as both of these conditions reduced the malodour of the individual. In the ethanol only condition, as predicted, the first image (before spending a week using a placebo deodorant) was rated as more confident more often than the image taken at the end of the week, suggesting that malodour can have an impact on self-confidence, at least as rated by others. Finally, and most interestingly, in the fragrance only condition, the second image, taken after the week of wearing a fragrance only solution was rated as more confident than the image at the start of the week. This suggests that the addition of a fragrance may be as important as malodour reduction in positively impacting perceptions of perceivers and possibly wearers. These findings must be taken with caution however, as to date this is the only study to investigate separately the impact of these deodorant components. Further work is needed to replicate and validate these findings.

Chapter 8 presents the final study of the thesis; a novel investigation into olfactory communication during human pregnancy. This study is different from the others presented herein, as it involved no investigation of the effects of fragrance. While many odour cues have been identified and experimentally tested, the role of odours during pregnancy has not yet been well investigated, and this must occur before the addition of fragranced products into the experimental design. The rationale for the study was based on findings in non-human species who present similar levels of bi-parental care as humans do (see chapter 8). In these species it has been hypothesised that preparation, in the form of endocrinological and behavioural changes in fathers prior to parturition would be adaptive for infant survival, and research indeed suggests that there may be changes in various urinary and glandular secretions during pregnancy that might allow for the communication of this state to the male partner. In humans, it is known that certain volatile compounds present in axillary samples are found in different concentration during pregnancy compared to non-pregnant samples (Vaglio et al., 2009), suggesting a potential mechanism for olfactory communication. The study presented in chapter 8 represents the first attempt to experimentally induce behavioural changes in men via exposure to pregnant and non-pregnant females' odours, utilising a repeated measures design.

The majority of the measures adopted in chapter 8 showed no change in relation to odour exposure across the conditions (pregnant odours, non-pregnant odours). There was one potentially interesting finding, whereby partnered (but not unpartnered) men showed a significant increase in relationship satisfaction, as measured using the relationship assessment scale (Hendrick, 1988) after exposure to odours collected during late pregnancy. This could be taken as suggesting that these individuals became more invested in their relationships, which could be seen as useful prior to the birth of a child. However, this finding should be interpreted with caution as there were only 7 male participants in this category; future work will be needed to replicate and validate this.

The study presented in chapter 8 has also highlighted specific aspects of the experimental design which, if altered, may benefit future research. For example, the current findings, while not being conclusive, suggest that relationship status may be important and so future studies should incorporate a larger sample size split between single and partnered men. In a similar vein, it would potentially be worthwhile including parental status, as in some species parental experience has been implicated in mediating potential chemical communication during pregnancy (Ziegler & Snowdon, 2000). This would likely also increase the age range of the participants, which in the current study was rather narrow, 18-44, and would in turn make findings more generalisable to the population. Finally, and most importantly, future studies should investigate longer term odour exposure, as well as incorporating hormonal measures alongside behavioural measures. It was decided to investigate short term exposure in this initial experiment, as research suggests that certain endocrinological changes in response to olfactory signals can occur over short periods of time (Miller & Maner, 2010; Perrot-Sinal et al., 1999). However, it may be the case that longer term exposure is required to result in behavioural changes.

If enough evidence is accumulated to support the presence of olfactory communication during pregnancy in humans, then, as with the work presented in the first section of the thesis, research will need to be conducted investigating how fragrances are impacting this and what consequences this may have for paternal care.

Section Two: conclusions

Although the findings from the final two chapters do not permit firm conclusions, as yet, it must be noted that both of these experiments represent initial investigation of novel research questions. As such, a degree of trial and error with regards to experimental design is to be expected, and I believe future research can address various issues in order to continue these lines of investigation, for example addressing sample size, number of odour donors, and length of odour exposure. Both studies present important questions and merit continuing investigation.

Overall conclusion

While the research presented provides interesting findings regarding the role of odours in human social interactions, it is important to note that there are some limitations and improvements which could be addressed in future research, specifically regarding study design and statistical analyses. The investigation of olfactory perception is plagued with difficulties, as discussed in chapter 2. Samples are difficult to collect, store and present. It has been suggested that researchers should collect a minimum of 20 observations per cell (Simmons, Nelson, & Simonsohn, 2011) in order to provide enough power for the detection of a significant effect, and while the current work aimed for this minimum benchmark it was not always achieved (male raters in chapter 4, chapter 7, and the male participants of chapter 8). Simmons and colleagues do however point out that a lower number of observations may be justified if there is a compelling cost of data collection, which I believe is the case when conducting research focussing on odour collection and presentation. Simmons and colleagues also suggest that care is taken when designing and analysing research in order to reduce the likelihood of type 1 errors occurring. For example, they stress that multiple testing and exploratory testing concerning the inclusion/exclusion of various covariates should be avoided where possible, or at the very least made transparent to the reader/reviewer. Given the exploratory nature of the work presented herein, and the educational nature of a postgraduate research degree, there are instances when I may have fallen foul of these recommendations (for example running multiple correlations in chapter 6, and multiple testing in chapter 8). With this in mind it is important to stress that the findings presented do not represent hard and fast conclusions, but rather allow us to develop hypotheses for future testing, and, additionally, that future work should aim to avoid these methodological and analytical pitfalls by following the guidance of those such as Simmons and colleagues.

Though there has recently been a surge in research investigating the role of olfaction in humans, I hope that this thesis demonstrates that there is still much to be investigated. We may have evidence of numerous states and traits which are detectable via body odour, but as the literature cited in chapter 8 suggests, there are still stones left unturned. Additionally, as evidenced in chapters 3 and 4, more work is needed to fully understand the impact that these findings have in a world full of artificial fragrances. Furthermore, work is needed to disentangle the various effects which different types of fragranced products may be having on olfactory communication, as evidenced in chapter 7. I believe that furthering our understanding of the perceptual qualities of body odours, as attempted in chapter 6, will open the door for much of this research. This will hopefully lead, eventually, to a comprehensive knowledge on the relationship between volatile compounds and perceptual odour qualities, as well as improving our understanding of the interaction between our own odours and the fragrances we apply to ourselves. Stoddart (1990) labelled us *The Scented Ape,* and we are not short of researchers investigating the various roles of our natural odours. However, many of us are in fact also *artificially* scented apes, and this should not be ignored in our quest to understand the role of odours across social contexts.

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Appendix A

Relationship assessment scale (RAS)

Items 4 and 7 are reverse-scored.
Scoring is kept continuous. The higher the score, the more satisfied the respondent is with his/her relationship. Items taken from:

Hendrick, S. S. (1988). A generic measure of relationship satisfaction. *Journal of Marriage and the Family, 50,* 93–98.

How well does your partner meet your needs?

1	2	3	4	5
Poorly		Average		Extremely well

In general, how satisfied are you with your relationship?

1	2	3	4	5
Unsatisfied		Average		Extremely satisfied

How good is your relationship compared to most?

1	2	3	4	5
Poor		Average		Excellent

How often do you wish you hadn't gotten into this relationship?

1	2	3	4	5
Never		Average		Very often

To what extent has your relationship met your original expectations:

1	2	3	4	5
Hardly at all		Average		Completely

How much do you love your partner?

1	2	3	4	5
Not much		Average		Very much

How many problems are there in your relationship?

1	2	3	4	5
Very few		Average		Very many

Appendix B

Personality Questionnaires

Constructs, items and reliability estimates are taken from the International Personality Items Pool. See Goldberg, L. R. (1999). A broad-bandwidth, public domain, personality inventory measuring the lower-level facets of several five-factor models. In I. Mervielde, I. Deary, F. De Fruyt, & F. Ostendorf (Eds.), *Personality Psychology in Europe*, Vol. 7 (pp. 7-28). Tilburg, The Netherlands: Tilburg University Press or www.ipip.ori.org

Assertiveness	+ keyed	Take charge. Want to be in charge. Say what I think. Am not afraid of providing criticism. Take control of things. Can take strong measures.
	– keyed	Wait for others to lead the way. Never challenge things. Let others make the decisions. Let myself be pushed around.
	Notes	Construct similar to Factor E (Dominance) in Cattell's Personality Factors Questionnaire (α=0.81)
Competence	+ keyed	Come up with good solutions. Complete tasks successfully. Carry out my plans. Accomplish a lot of work. Get things done quickly.
	– keyed	Feel that my life lacks direction. Am not sure where my life is going. Hang around doing nothing. Do just enough work to get by. Mess things up.
	Notes	Construct similar to Hogan Personality Inventory (α=0.81)

Dominance	+ keyed	Try to surpass others' accomplishments. Try to outdo others. Am quick to correct others. Impose my will on others. Demand explanations from others. Want to control the conversation. Am not afraid of providing criticism. Challenge others' points of view. Lay down the law to others. Put people under pressure.
	– keyed	Hate to seem pushy.
	Notes	Construct similar to Narcissism, California Psychological Inventory (α=0.82)
Extraversion	+ keyed	Am the life of the party. Feel comfortable around people. Start conversations. Talk to a lot of different people at parties. Don't mind being the center of attention.
	– keyed	Don't talk a lot. Keep in the background. Have little to say. Don't like to draw attention to myself. Am quiet around strangers.
	Notes	Big Five Factor Marker, Factor 1 (Surgency or Extraversion) (α=0.87)
Physical Attractiveness	+ keyed	Am considered attractive by others. Attract attention from the opposite sex. Have a pleasing physique. Like to look at my body. Like to look at myself in the mirror. Like to show off my body.
	– keyed	Don't consider myself attractive. Dislike looking at myself in the mirror. Dislike looking at my body.
	Notes	Construct similar to Personality

		Attributes Survey (α=0.87)
Self-efficacy	+ keyed	Can handle complex problems. Think quickly. Formulate ideas clearly. Have excellent ideas. Am quick to understand things.
	– keyed	Never challenge things. Undertake few things on my own. Let others determine my choices. Let myself be directed by others. Do not have a good imagination.
	Notes	Construct similar to Independence, California Psychological Inventory (α=0.81)
Self-esteem	+ keyed	Feel comfortable with myself. Just know that I will be a success. Seldom feel blue. Like to take responsibility for making decisions. Know my strengths.
	– keyed	Dislike myself. Am less capable than most people. Feel that my life lacks direction. Question my ability to do my work properly. Feel that I'm unable to deal with things.
	Notes	Construct similar to that measured in the Personality Attributes Survey

Appendix C

The revised Sociosexual Orientation Inventory (SOI-R)

Items 1-3 are aggregated to form the behaviour facet of the scale (α = .85). Items 4-6 (reverse code item 6) can be aggregated to form the attitude facet of the scale (α = .87) and finally, items 7-9 can be aggregated to form the Desire facet (α = .86). Constructs, items, and reliability estimates are taken from:

Penke, L., & Asendorpf, J. B. (2008). Beyond global sociosexual orientations: A more differentiated look at sociosexuality and its effects on courtship and romantic relationships. Journal of Personality and Social Psychology, 95, 1113-1135.

Please respond honestly to the following questions:

1. With how many different partners have you had sex within the past 12 months?

□	□	□	□	□	□	□	□	□
0	1	2	3	4	5-6	7-9	10-19	20 or more

2. With how many different partners have you had sexual intercourse on *one and only one* occasion?

□	□	□	□	□	□	□	□	□
0	1	2	3	4	5-6	7-9	10-19	20 or more

3. With how many different partners have you had sexual intercourse without having an interest in a long-term committed relationship with this person?

□	□	□	□	□	□	□	□	□
0	1	2	3	4	5-6	7-9	10-19	20 or more

4. Sex without love is OK.

1 □	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □
Strongly disagree								Strongly agree

5. I can imagine myself being comfortable and enjoying "casual" sex with different partners.

1 □	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □
Strongly disagree								Strongly agree

6. I do *not* want to have sex with a person until I am sure that we will have a long-term, serious relationship.

1 □	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □
Strongly disagree								Strongly agree

7. How often do you have fantasies about having sex with someone you are *not* in a committed romantic relationship with?

□ 1 – never
□ 2 – very seldom
□ 3 – about once every two or three months
□ 4 – about once a month
□ 5 – about once every two weeks
□ 6 – about once a week
□ 7 – several times per week
□ 8 – nearly every day
□ 9 – at least once a day

8. How often do you experience sexual arousal when you are in contact with someone you are *not* in a committed romantic relationship with?

□ 1 – never
□ 2 – very seldom
□ 3 – about once every two or three months
□ 4 – about once a month
□ 5 – about once every two weeks
□ 6 – about once a week
□ 7 – several times per week
□ 8 – nearly every day
□ 9 – at least once a day

9. In everyday life, how often do you have spontaneous fantasies about having sex with someone you have just met?

□ 1 – never

□ 2 – very seldom

□ 3 – about once every two or three months

□ 4 – about once a month

□ 5 – about once every two weeks

□ 6 – about once a week

□ 7 – several times per week

□ 8 – nearly every day

□ 9 – at least once a day