The Discordant Development of Sexual Orientation in Identical Twins

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Declaration

I declare that this thesis, *The Discordant Development of Sexual Orientation in Identical Twins*, represents my own work, except where otherwise stated. None of the work referred to in this thesis has been accepted in any previous application for a higher degree at this or any other University or institution. All quotations have been distinguished by quotation marks and the sources of information specifically acknowledged.

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General Abstract

The present thesis investigated how genetically identical twins with discordant sexual orientations differed in correlates of their sexual orientation in order to understand to what degree non-genetic factors affect the formation of sexual orientation. Because identical twins share 100% of their genes, factors other than genetics may contribute to any differences within these pairs. Chapter 1 gives an overview of the literature. Chapter 2 focuses on childhood gender nonconformity (femininity in males, masculinity in females), which predicts a non-straight (gay, lesbian, or bisexual) sexual orientation in adulthood. In order to avoid the limitations of self-report measures, gender nonconformity of these twins was assessed via observer ratings of their photographs from childhood and adulthood. In addition, although genetically identical twins can differ in their self-reported sexual orientations, it is unclear to what degree these self-assessments reflect observable differences in sexual arousal such as genital response or pupil dilation patterns while viewing sexual stimuli. Chapter 3 focuses on these responses in identical twin pairs with discordant sexual orientations. Finally, differing intrauterine environments during the early development could result in a discordant development of sexual orientation in identical twins. This includes varied prenatal hormonal exposures between twins. Chapter 4 highlights a putative biomarker of early hormonal exposure: the ratio of index to ring finger length (2D:4D), within these twin pairs In combination, findings suggested that these twins differed in many (but not in all) correlates of their sexual orientation, suggesting non-genetic influences. However, there were also subtle similarities within pairs that pointed to potential familial (e.g., genetic) influences.

Author Note

Chapters 2 through to 4 of this thesis were written as independent pieces of research, with the aim of being submitted as peer-reviewed manuscripts for publications. As such, there is some overlap between chapters. For example, the General Introduction gives an overview of the literature that motivated each study, and therefore overlaps strongly with parts of the introductions provided in each chapter (manuscript). Likewise, there are sections in each chapter's methods that necessarily have to be similar across chapters. Chapter 2 is currently under review in *Developmental Psychology*; Chapter 3 is being prepared for submission to *Psychological Science*; and Chapter 4 is being prepared for submission to *Archives of Sexual Behavior*. Each of these journals conforms to APA formatting guidelines. In addition, given that these are North American journals, some chapters use US English rather than UK English as the default language.

Chapter 1 General Introduction

1.1 Motivation for Thesis

The present thesis investigates how genetically identical twins with discordant sexual orientations differed in correlates of their sexual orientation in order to understand to what degree non-genetic factors affect the formation of sexual orientation. Because identical twins share 100% of their genes, factors other than genetics may contribute to any differences within these pairs. This chapter gives an overview of the literature and the several correlates of sexual orientation that are investigated across the different studies.

Gender nonconformity describes the way in which behaviour diverges from what is considered gender stereotypical. That is, men who are more feminine, and less masculine, in their behaviours and interests than most other men can be defined as gender nonconforming; similarly, gender nonconforming women are more masculine and less feminine than most other women (Lippa, 2005). Gender nonconformity is characteristic of a homosexual orientation in both childhood (Bailey, Dunne, & Martin, 2000; Bailey & Zucker, 1995; Rieger, Linsenmeier, Gygax, & Bailey, 2008) and adulthood (Lippa, 2005, 2008; Rieger et al., 2008; Rieger, Linsenmeier, Gygax, Garcia, & Bailey, 2010). This co-development of sexual orientation and gender nonconformity is not fully determined by an individual's genes (Bailey et al., 2016). Therefore, factors other than the genetic makeup must be important. Evidence for this comes from pairs of identical twins who share all of their genes but differ with respect to their sexual orientation and degree of gender nonconformity. In previous work, identical twins with discordant sexual orientations have differed in their reported childhood gender nonconformity (Bailey & Pillard, 1991). However, self-report may be prone to cognitive biases and distort actual differences in gender nonconformity (Gottschalk, 2003). To avoid such limitations of self-report, the present research measures gender nonconformity via observer ratings of photos from childhood and adulthood.

Similar to the above potential limitations about self-reported gender nonconformity, self-report measures of sexual attraction and arousal may be prone to several biases in the context of sexuality research (Friedman et al., 2004; Ragins, Singh, & Cornwell, 2007). The following work therefore employs measures of attraction and arousal that did not rely on self-report. This includes the measure of genital arousal and pupil dilation to sexual stimuli; measures that are highly automatic and are not consciously controlled by the participants. In general, these automatic measures point to robust sex differences and sexual orientation differences in sexual attraction and arousal (Chivers, Rieger, Latty, & Bailey, 2004; Rieger et al., 2015; Rieger & Savin-Williams, 2012a). For this reason they are employed to examine these patterns in genetically identical twins with self-reported discordant sexual orientations.

Finally, because identical twins share 100% of their genes, factors other than genetics likely influence the discordant development of their sexual orientations. Such factors could include differing prenatal environments between twins, consisting of differences in exposure to androgens during early development. In order to examine possible exposure levels to prenatal androgens during early development that could have let to a discordant development of these twins, the ratios of finger lengths (a putative marker of prenatal androgen exposure; (Grimbos, Dawood, Burriss, Zucker, & Puts, 2010)) are examined in these twins.

The aforementioned studies are designed to independently assess the discordance of sexual orientation in identical twins by using measures that do not rely on self-report and by examining factors other than genetics. The present chapter introduces these three studies, which form the basis of this PhD thesis. The chapter provides an introduction to the literature, which motivated each study, in a way that demonstrates what insight can be gained from each study regarding the discordant development of sexual orientation in identical twins.

1.2 Gender Nonconformity of Identical Twins with Discordant Sexual Orientations: Evidence from Childhood Photographs

Masculinity and femininity refer to individual differences in gender-related traits and behaviours that exist within each sex. That is, those aspects of gender that vary among men and among women (Lippa, 2005). In this thesis, rather than using the words masculine and feminine, the terms gender nonconforming and gender conforming are used. Men who are more feminine, and less masculine, in their behaviours and interests than most men can be defined as gender nonconforming; similarly, gender nonconforming women are more masculine and less feminine than most women (Lippa, 2005). Gender nonconformity is more common in non-straight (gay men who are exclusively attracted to males, lesbian women who are exclusively attracted to females, and bisexual men and women who show equal attraction to males and females) than straight men and women, and this difference emerges in early childhood (Bailey & Zucker, 1995; Lippa, 2008; Rieger et al., 2008). In addition, across several studies and across men and women, genetic variations explain approximately 30% of the differences in sexual orientation (Bailey et al., 2016). Furthermore, between 10% and 50% of the co-development of sexual orientation with gender nonconformity is explained by genetic variations (Alanko et al., 2010; Bailey et al., 2000; Burri, Cherkas, Spector, & Rahman, 2011).

Because genetically identical twins can differ in both their sexual orientations and their degrees of gender nonconformity, sexual orientation and gender-related behaviour can not be fully determined by genetics. By self-report, twins with discordant sexual orientations differ in their gender nonconformity to a degree similar to unrelated straight and non-straight individuals (Bailey & Pillard, 1991). However, self-reports may distort actual differences in gender nonconformity (Gottschalk, 2003). The present study therefore examines whether identical twins with discordant sexual orientations differed in their observable gender nonconformity by evaluating photographs taken in childhood and adulthood. The present study further compares the differences in gender nonconformity between discordant twins to the difference between twins from concordant straight pairs and twins unrelated to these, from concordant non-straight pairs. Finally, this study also examines how social reactions from parents and peers during childhood related to the gender nonconformity of these twins, in order to understand whether some twins, more than others, may have been discouraged or encouraged to be gender nonconforming (and, eventually, non-straight).

1.2.1 Gender Nonconformity And Sexual Orientation

Research suggests that on average, pre-homosexual children (children who later become homosexual adults) show more gender-nonconforming behaviours than pre-heterosexual children (Bailey et al., 2000; Bailey & Zucker, 1995; Rieger et al., 2008). That is, it has been empirically shown that pre-homosexual children tend to display behaviour typically associated with the opposite sex, including more frequent play with toys aimed at the opposite sex, higher instances of cross-dressing, sex-atypical levels of aggression and identifying more closely with the opposite sex (Bailey & Zucker, 1995). These effects were large, $d = 1.31$, $0.45 < 95\%\ CI < 3.08$, and $d = 0.96$, $0.26 < 95\%\ CI < 1.66$, for boys and girls respectively.

This early-established pattern of gender behaviour during childhood persists into adulthood, with non-straight adults identified as being more gender nonconforming than their straight peers, on average. That is, non-straight men and women report more gender-nonconforming behaviors and interests, on average, than straight adults of their sex (Lippa, 2008; Rieger et al., 2010; Swift-Gallant, Coome, Monks, & VanderLaan, 2017). In one meta-analysis, gay men reported more feminine and less masculine interests and self-concepts in adulthood than straight men; lesbians reported more masculine and less feminine interests and self-concepts than straight women (Lippa, 2005). These effects were also large, $1.28 < d's < 1.46$, $1.18 < 95\%\ CI < 1.56$, and $0.60 < d's < 1.28$, $0.50 < 95\%\ CI < 1.38$, and, $.28 < d's < 1.46$, 1.18 < 95% CI < 1.56, respectively. It is worth noting that although gender nonconformity and a non-straight sexual orientation are reliably linked, on average, not all gender nonconforming children identify as same-sex oriented in adulthood (Green, 1987; Singh, 2012; Steensma, van der Ende, Verhulst, & Cohen-Kettenis, 2013). That is, the discussed effects only apply in general, and not to every individual.

However, the majority of previous research on gender nonconformity and sexual orientation has been conducted retrospectively, relying solely on the use of self-report measures to investigate the relationship between these traits. Self-report measures in the context of such research are likely to be subject to biases in recall and memory distortions (Baumrind, 1995; Gottschalk, 2003). For example, non-straight men and women may overstate the extent to which they were gender-nonconforming children, whereas straight men and women are likely to understate their childhood gender nonconformity; these biases could emerge because retrospective judgements may be prone to sexuality-specific social norms present in adulthood for or against reporting gender nonconformity during childhood (Ross, 1980).

One way to address the limitations of retrospective work is to conduct prospective research. Studies have followed children who were referred to clinics due to extreme levels of gender nonconformity and concern over their gender identity. In adolescence and adulthood, these groups were substantially more likely to identify as bisexual, gay or lesbian, compared with individuals who were not gender nonconforming in childhood (Drummond, Bradley, Peterson-Badali, & Zucker, 2008; Green, 1985; Singh, 2012). One needs to be cautious regarding whether findings from clinical samples represent the relationship between these variables for most people. However, two longitudinal studies, using data from the general population, confirm that early gender-nonconforming behaviours (as early as 3 to 4 years old) predict a same-sex orientation in adolescence or adulthood (Li, Kung, & Hines, 2017; Steensma, van der Ende, Verhulst, & Cohen-Kettenis, 2013). Such population-based, longitudinal work involves many logistical challenges. It takes years to conduct, needs hundreds of participants to capture enough with same-sex orientations, and can have substantial attrition rates. An alternative method is the assessment of gender nonconformity retroactively through the evaluation of behaviour depicted in visual images from childhood. This method is free of the limitations of self-report (because it does not rely on subjective accounts), clinical samples (because it draws from a wider population), and longitudinal studies (because it does not take years to conduct). Research using this method also suggests that differences in observable gender nonconformity are predictive of an adulthood sexual orientation from ages 3 to 4 years onwards (Rieger et al., 2008). That is, for both males and females, pre-homosexual children were rated as more gender nonconforming, on average, than pre-heterosexual children from age 3 or 4, and this difference carried into adulthood.

1.2.2 Identical Twins Discordant for Sexual Orientation

Identical twins are considered to be genetically identical (McGue, Osler, & Christensen, 2010). Despite their genetic similarity, identical twins can show phenotypic discordance with respect to a range of medical, physiological, and psychological conditions such as Type 2 diabetes (Vaag, Henriksen, Madsbad, Holm, & Beck-Nielsen, 1995), obesity (Marniemi et al., 2002; Ronnemaa, Karonen, Rissanen, Koskenvuo, & Koivisto, 1997) and schizophrenia (Hobson, 1964; Mosher, Pollin, & Stabenau, 1971). An additional trait for which twins can be discordant is sexual orientation (Bailey et al., 2000; Whitam, Diamond, & Martin, 1993).

Across several representative samples, and across men and women, this concordance rate was estimated as 24% (Bailey et al., 2016). That is, about 24% of non-straight twins had a twin who was non-straight too. In contrast, when considering the population as a whole, the occurrence of a non-straight sexual orientation is approximately 3.5% across both sexes, although other estimates suggest 7% in men, and 13% in women (Gates, 2011; Savin-Williams & Vrangalova, 2013). Thus, the chance of a same-sex orientation for co-twins of non-straight twins is higher than that reported for the general population. This suggests that familial factors, such as shared genetic factors, may be influential in the expression of homosexuality in related individuals (Sanders et al., 2014). In addition, because a substantial proportion (around 76%) of identical twins who are non-straight have straight co-twins (Bailey et al., 2000), factors other than their genes must account for their different sexual orientations. These factors could, in theory, also affect a correlate of sexual orientation, their degree of gender nonconformity.

1.2.3 Differences in Gender Nonconformity within Discordant Twin Pairs

Although differences in sexual orientation have been reported in identical twin pairs (Bailey et al., 2016), twins in these pairs have rarely been systematically compared with respect to their gender nonconformity. In one study of male pairs with discordant sexual orientations, non-straight twins reported more childhood gender nonconformity than their straight co-twins. The average difference was similar to that for unrelated non-straight and straight men, suggesting substantial environmental (unique, non-genetic) effects on the link of sexual orientation with gender nonconformity (Bailey & Pillard, 1991). However, this finding is subject to the potential limitations of retrospective self-reports. Self-reported childhood gender nonconformity was also assessed in female identical twins with discordant sexual orientations, but no information was given on their differences or how they compared to unrelated straight and non-straight women (Bailey, Pillard, Neale, & Agyei, 1993).

To avoid the drawbacks of self-report, the present study investigates possible differences in the childhood gender nonconformity of discordant twins by examining visual images from their childhoods. For unrelated individuals, sexual orientation differences in observable gender nonconformity, based on evaluations of visual images, emerge by ages 3 to 4 and carry into adulthood (Rieger et al., 2008). If twins with discordant sexual orientations are like unrelated straight and non-straight individuals, then differences in their observable gender nonconformity could emerge at ages 3 to 4, and remain present in adulthood (Hypothesis 2.1).

1.2.4 Correlation of Gender Nonconformity within Discordant Twin Pairs

Familial influences on the expression of a trait may include genetic or shared environmental factors. Of these two, the influences of genetic factors on gender behaviour and sexual orientation have received stronger support in most behavioural genetics studies (Bailey & Bell, 1993; Bailey et al., 2000; Burri et al., 2011; Kendler, Thornton, Gilman, & Kessler, 2000; Kirk, Bailey, & Martin, 2000; Långström, Rahman, Carlström, & Lichtenstein, 2010), although shared environmental influences on the expression of gender behaviour have been substantial in other research (Iervolino, Hines, Golombok, Rust, & Plomin, 2005; Knafo, Iervolino, & Plomin, 2005). Whichever the exact familial influences are (shared genes and/or shared environment), they could affect the co-development of gender nonconformity in related individuals. Further, both straight and non-straight individuals vary in their degree of gender nonconformity; some score high relative to others of their sexual orientation, others score low (Rieger et al., 2010). These variations could be linked in related individuals. That is, familial influences could contribute to related levels of gender nonconformity, even in twins with discordant sexual orientations. A non-straight twin who scores high on gender nonconformity, relative to other non-straight individuals, may have a straight co-twin who scores high on gender nonconformity, relative to other straight individuals. This would result in a correlation in the twins' gender nonconformity, even if they differed on average in their gender nonconformity.

Previous research has investigated the possibility that identical twins with discordant sexual orientations have related levels of gender nonconformity. Although a correlation in gender nonconformity was found in twins with concordant homosexual orientations, this hypothesis was not confirmed in discordant twins (Bailey & Pillard, 1991; Bailey et al., 1993). It is possible that there is, indeed, no relationship. Alternatively, failure to find evidence for this correlation in discordant twin pairs this may have been partly due to the sole reliance on self-report measures of gender behaviour, which are vulnerable to social desirability and other psychometric issues including recall biases and memory distortions (Baumrind, 1995; Gottschalk, 2003). Thus, in the present study, measures of gender nonconformity will include both self-report measures and observer ratings of gender behaviour from photos and videos from childhood and adulthood. By gaining potentially more reliable and valid indicators of gender nonconformity, it may become possible to detect a correlation in levels of gender nonconformity within discordant twin pairs (Hypothesis 2.2).

1.2.5 A Comparison of Discordant and Concordant Twin Pairs

A parsimonious model assumes that differences in gender nonconformity within twin pairs with discordant sexual orientations are as strong as those between unrelated individuals with different sexual orientations. Self-report measures support this hypothesis (Bailey & Pillard, 1991). However, it is also possible that due to shared influences between twins of a pair, differences in gender nonconformity within discordant twin pairs may be less distinct than those seen between unrelated straight and non-straight individuals. The present study examines this possibility by comparing discordant twins to identical twin pairs with concordant straight sexual orientations and identical twin pairs unrelated to them with concordant non-straight sexual orientations. Because being an identical twin is held constant across participants, any differences across twin types could be more easily interpreted with respect to the twins' similar or dissimilar sexual orientations. It is proposed that a difference in observable gender nonconformity within discordant twin pairs could be smaller than the analogous difference between concordant non-straight twins and unrelated concordant straight twins (Hypothesis 2.3). It is further examined whether, as previously reported based on self-reports, the correlation in gender nonconformity is weaker in discordant twin pairs than concordant twin pairs (Bailey & Pillard, 1991; Bailey et al., 1993). Unlike self-reports with their possible biases, it is predicted that observer ratings might show similar correlations in gender nonconformity across twin types (Hypothesis 2.4).

1.2.6 Responses to Gender Nonconformity

Several studies suggest that gender-nonconforming children experience negative reactions and rejection from others (Caldera, Huston, & O'Brien, 1989; Carter & McCloskey, 1983; Fagot, 1985; Langlois & Downs, 1980; Maccoby, 1998; Smith & Leaper, 2006). Peers can react negatively to gender nonconformity, and boys are especially critical about gender nonconformity in other boys (Wallien, Veenstra, Kreukels, & Cohen-Kettenis, 2010; Young & Sweeting, 2004). Similarly, parents can be more detached and unsupportive if their children are gender nonconforming (Alanko et al., 2009; Landolt et al., 2004). There are exceptions to these reactions. In one study on very feminine boys, who likely became non-straight, the most feminine boys had mothers who responded less negatively, if not more positively, to their sons' gender nonconformity (Green, 1987). However, the overall conclusion across research is that more gender-nonconforming children experience more negative reactions (Alanko et al., 2011). One possibility is that the child's gender nonconformity causes the parents' reactions. It is also possible that gender nonconformity and negative parental reactions reinforce each other; for example, parents may punish their child for such behaviours, and because punishment means attention, the child may feel encouraged to continue these behaviours (Alanko et al., 2011). That is, the child might further engage in gender nonconforming behaviours because of the attention they bring (Chance, 2009 as cited in Alanko et al., 2011). This notion is in line with the concept of learning as proposed by the social cognitive theory (Blakemore, Berenbaum, & Liben, 2009 as cited in Alanko et al., 2011).

Furthermore, because childhood gender nonconformity predicts adulthood gender nonconformity, adulthood gender nonconformity is also linked to past rejection from others (Rieger et al., 2008). It is therefore expected that in twins, higher levels of gender nonconformity in childhood and adulthood would relate to recall of increased rejection (Hypothesis 2.5).

1.2.7 Study 1

Study 1 investigates the development of gender behaviour in identical twins to illuminate possible non-genetic and familial influences on the expression of sexual orientation. Twin pairs with either discordant or concordant sexual orientations are recruited and their degree of observable gender nonconformity is examined via evaluations of photographs taken in childhood and adulthood. Twins also report their degree of gender nonconformity in childhood and adulthood and the reactions from others during childhood.

The following hypotheses are tested:

Hypothesis 2.1. Within discordant twin pairs, the non-straight twin is more gender nonconforming than the straight co-twin. Using observer ratings of their photographs, this difference might emerge by ages 3 or 4.

Hypothesis 2.2. For discordant twin pairs, the degree of observer-rated gender nonconformity of non-straight twins correlates with the degree of gender nonconformity of the straight co-twins.

Hypothesis 2.3. The difference in observer-rated gender nonconformity within discordant twin pairs is smaller than the analogous difference between concordant non-straight twins and unrelated concordant straight twins.

Hypothesis 2.4. The correlation of observer-rated gender nonconformity within discordant twin pairs is similar to this correlation within concordant twin pairs.

Hypothesis 2.5. Across discordant and concordant twins, higher levels of gender nonconformity relate to recall of increased rejection by others.

1.3 Physiological Sexual Arousal of Identical Twins with Discordant Sexual Orientations

The review above suggests that identical twins with discordant sexual orientations might be less different in their degree of gender nonconformity when using measures that do not rely on self-report. In a similar manner, measures of physiological sexual arousal could reveal more than self-report about how different identical individuals with different sexual orientations actually are. Physiological measures of an individuals' sexual response are based on automatic responses. Thus, the following study investigates patterns of sexual arousal within these pairs based on physiological measures of an individuals' sexual response, which include genital arousal and pupil dilation to sexual stimuli (Chivers et al., 2004; Rieger et al., 2015; Rieger & Savin-Williams, 2012a).

As indicators of sexual orientation, pupil dilation and genital response to sexually explicit stimuli have been assessed both independently (Chivers et al., 2004; Rieger & Savin-Williams, 2012a) and simultaneously (Rieger et al., 2015). On average, measures of genital arousal correspond with an individual's self-reported sexual orientation, particularly for men (Chivers, 2005; Chivers et al., 2004; Huberman & Chivers, 2015; Rieger et al., 2015; Suschinsky, Lalumiere, & Chivers, 2009). Likewise, in men more so than in women, pupils become more dilated in response to sexual stimuli corresponding with an individual's self-reported sexual orientation (Hess & Polt, 1960; Hess, Seltzer, & Shlien, 1965; Rieger et al., 2015; Rieger et al., 2013; Rieger & Savin-Williams, 2012a). In addition, measures of pupil dilation and genital response correspond with each other and with self-reported sexual attraction to stimuli, but more strongly so in men than in women (Rieger et al., 2015). The following sections include a review of research that has employed either or both of these measures and explain how these will be used within the context of the twin research design.

1.3.1 Sexual Orientation And Genital Arousal

Genital arousal is influenced by the activation of the autonomic nervous system that regulates vasocongestion in the genital regions and is unlikely to be in the conscious control of participants (Levin & Riley, 2007; Meston, 2000; ten Donkelaar, Němcová, Lammens, Overeem, & Keyser, 2011; Weiss, 1972). In their genital arousal, the majority of men display category-specific responses with exclusive arousal to the sex that corresponds with their self-reported sexual orientation (Chivers et al., 2004; Chivers, Seto, & Blanchard, 2007; Rieger et al., 2015; Rieger et al., 2013; Sakheim, Barlow, Beck, & Abrahamson, 1985). That is, straight men almost exclusively respond to females over males whereas gay men respond almost exclusively to males over females. Bisexual men show a more variable pattern of arousal, but respond on, average, more like gay men than straight men (Rieger et al., 2015; Rieger & Savin-Williams, 2012a).

In women, genital responses correspond less consistently with self-reported sexual orientation, compared with men. This is so because straight, bisexual and lesbian women show substantial genital arousal to both male and female sexual stimuli. One qualification of this pattern is that lesbians and bisexual women show marginally more genital response to females over males, whereas approximately equal arousal to both sexes is detected in straight women (Chivers et al., 2007; Rieger, Savin-Williams, Chivers, & Bailey, 2016). In this sense, non-straight women appear to be more male-typical than other women, responding in a way that is consistent with self-reported sexual orientation usually seen in men. However, independent of these differences between specific sexual orientation groups, the overall relationship between genital response and self-reported sexual orientation is much stronger in men than in women, because, as discussed, women are more likely to respond to both sexes (Chivers, 2005; Chivers et al., 2007; Rieger et al., 2015; Rieger & Savin-Williams, 2012a).

Sex-specific evolutionary selection pressures have been proposed to explain sex differences in patterns of sexual arousal (Baumeister, 2000; Suschinsky & Lalumière, 2011). For instance, Baumeister (2000) argued that the sexes evolved to differ in their sexual responsiveness as an adaptation to the sexual behaviour of the other sex. One hypothesis related to this proposal is that sexual response has different biological functions for men and women (Chivers et al., 2007). For men, an important function is to facilitate erection and penetration; for women, to facilitate lubrication and prevent genital injury in case of penetration. Indirect support for the hypothesised function of female arousal is derived from both cross-species and cross-cultural comparisons. Forced copulation in several species (Galdikas, 1985, McKinney et al., 1983; Thornhill, 1980) and in most human societies (Palmer, 1989; Sanday, 1981) indicate that it may have occurred throughout human evolution (Thornhill & Thornhill, 1983). Because forced copulation can lead to genital trauma (Slaughter et al., 1997), the female response to any sexual stimulus may have evolved in part to mitigate this risk. Anecdotally, several women who have been raped reported that they have experienced arousal and lubrication, even though intercourse was forced and without consent (Chivers et al., 2004; Chivers et al., 2007).

When proposing evolutionary hypotheses for sex differences in sexual arousal, the focus is on heterosexual men and women. The vast majority of people are heterosexual (Savin-Williams et al., 2012; Laumann et al., 1994), and a sexual orientation towards the other sex is likely promoted by evolutionary mechanisms; thus, a focus on heterosexual individuals is justified. From an evolutionary perspective, exclusive homosexuality as found in humans is a conundrum. Some research has suggested that, at least in men, the decreased fecundity of homosexual males is counter-balanced by the increased fecundity of their relatives (Rieger et al., 2012). Why such a balancing mechanism might exist and how it would relate to general sex differences in attraction and arousal is still unknown.

Research assessing genital arousal provides important insight into the different ways in which men and women respond to sexual stimuli. However, an alternative measure of arousal, which is considered less invasive, measures the response of another organ common to both men and women: the eye.

1.3.2 Sexual Orientation And Pupillary Response

Pupil dilation reflects activation of the autonomic nervous system (Bradley, Miccoli, Escrig, & Lang, 2008; Lang & Bradley, 2010). The autonomic nervous system is also linked with several automatic processes including blood pressure, heart rate, respiration, erection in men and vaginal vaso-congestion in women (Levin & Riley, 2007; Meston, 2000; ten Donkelaar et al., 2011; Weiss, 1972). In addition, pupil dilation reflects automatic responses that indicate an elevated interest in stimuli, including sexual stimuli (Hess & Polt, 1960; Hess et al., 1965; Rieger & Savin-Williams, 2012a).

Pupils become more dilated in response to sexual stimuli corresponding with an individual's self-reported sexual orientation (Hess & Polt, 1960; Hess et al., 1965; Rieger et al., 2015; Rieger et al., 2013; Rieger & Savin-Williams, 2012a); although this relationship pattern appears to be stronger in men than in women (Rieger et al., 2015; Rieger & Savin-Williams, 2012a). This sex difference in effect is comparable to the sex difference in genital response to sexual stimuli (Chivers et al., 2004; Huberman & Chivers, 2015; Rieger et al., 2015). Furthermore, within each sex, patterns of pupil dilation in response to sexual stimuli are comparable to those of genital response patterns (Rieger et al., 2015). In men, sexual orientation corresponds strongly with pupil dilation to sexual stimuli of the same sex or opposite sex;

straight men show increased pupillary response to stimuli showing the opposite sex as compared to non-straight men who show increased pupil dilation to the same sex (Rieger et al., 2015; Rieger & Savin-Williams, 2012a). In women, sexual orientation is weakly related to their sexual responses because women show increased pupillary response to both sexes (Rieger et al., 2015; Rieger & Savin-Williams, 2012a). One exception is that, similarly to their genital responses, lesbians show a slightly stronger pupil response to females over males. They are therefore displaying a more male-typical pattern of dilation, similar to what has been described for their genital arousal patterns (Rieger et al., 2015).

1.3.3 Sexual Arousal within Discordant Twin Pairs

To summarise the above, in their genital arousal and pupil dilation, the majority of men display arousal that is consistent with their self-reported sexual orientation (Chivers et al., 2004; Chivers et al., 2007; Rieger et al., 2015; Rieger et al., 2013; Sakheim et al., 1985). In women, these sexual responses (either genital arousal or pupil dilation) correspond less consistently with self-reported sexual orientation, compared with men. This is so because straight and non-straight (lesbian or bisexual) women show substantial sexual arousal to both male and female sexual stimuli. One qualification of this pattern is that non-straight women show marginally more genital response to females over males, whereas no preference is detected in straight women (Chivers et al., 2007; Rieger et al., 2016).

If the responses of identical twins with discordant sexual orientations are like those of unrelated individuals, then, within male twin pairs, straight male twins will show substantially stronger genital arousal and pupil dilation to female than male sexual stimuli and their non-straight co-twins will display stronger responses to male than female stimuli (Hypothesis 3.1). Within female twin pairs, it is hypothesised that straight and non-straight female twins will show similar response to male and female stimuli; however, lesbians may show somewhat stronger responses to females than males (Hypothesis 3.2).

1.3.4 Correlation of Sexual Arousal Within Discordant Twin Pairs

Based on previously observed patterns of sexual arousal in males and females, a simple model assumes that identical twins with discordant sexual orientations differ in their arousal in much the same way as unrelated individuals. However, there could be familial, if not genetic, effects on sexual arousal, which yield similar responses in sexual arousal across twins, even though they differ with respect to their sexual orientations. For instance, identical twins are more likely to be similar on several measures of autonomic response, including vasomotor persistence time and respiration than siblings who are not twins and unrelated individuals. Thus, an "autonomic constitution" may be partly accounted for by genetics (Jost & Warren Sontag, 1944; Piha, Rönnemaa, & Koskenvuo, 1994). Because pupillary and genital responses reflect activity of the autonomic nervous system (Bradley et al., 2008; Lang & Bradley, 2010; ten Donkelaar et al., 2011), and if such activity is partly driven by genetic influences (Piha et al., 1994), one might expect that within pairs of identical twins discordant for sexual orientation, average differences in sexual arousal may be reduced, compared with such arousal patterns of unrelated straight and non-straight individuals.

Further, regardless of any average differences between these twins, there may exist a correlation in the twins' sexual response. This could be conceptualised in two different ways. Firstly, the straight twin could show a correlation to his or her non-straight co-twin in the magnitude to his or her sexual response to their preferred sex. That is, straight twins who respond more strongly to the other sex than the same sex (as compared to other straight individuals) may have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals (Hypothesis 3.3). That pattern would create a correlation of arousal to the other sex over the same sex in straight twins with arousal to the same sex over the other sex in non-straight twins.

Alternatively, there may be a correlation in the twins' degree of sexual arousal to one sex over the other. That is, straight twins, who have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals), may also respond more strongly to the same sex than the other sex (as compared to other straight individuals (Hypothesis 3.4). That pattern would create a correlation of arousal to the same sex over the other sex in straight twins with arousal to the same sex over the other sex in non-straight twins. This could point to a predisposition for traits associated with homosexuality within the straight twin.

1.3.5 Study 2

Study 2 investigates differences in sexual arousal within identical twins with discordant sexual orientations by measuring their genital arousal and pupil dilation to explicit sexual stimuli. In sum, the following hypotheses are tested:

Hypothesis 3.1. In male pairs, straight twins will show substantially more sexual arousal to females over males, whereas their non-straight co-twin shows show substantially more sexual arousal to females over males.

Hypothesis 3.2. In female pairs, straight twins will be similar to their non-straight co-twins in their response to male and female sexual stimuli; however, lesbians may show somewhat stronger responses to females than males.

Hypothesis 3.3. Straight twins who respond more strongly to the other sex than the same sex (as compared to other straight individuals) may have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals).

Hypothesis 3.4. Alternatively, straight twins, who have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals), may also respond more strongly to the same sex than the other sex (as compared to other straight individuals).

1.4 Hormonal Influences on the Co-Development of Gender Non-Conformity And Sexual Orientation

Given that approximately one-third of identical twins rely on independent placentas (Patterson, 2007), and that the placenta may sometimes fail to inhibit the transfer of testosterone from mother to fetus (Hines et al., 2002), it is feasible that in those twin pairs, in which each sibling has an independent placenta, each twin is exposed to different levels of prenatal androgens from the maternal system. Such a factor could be relevant for the development of discordant sexual orientations in identical twins, given that exposure to atypical levels of prenatal androgen potentially increases the likelihood of a same-sex sexual orientation (Hines, 2011). Prenatal androgens, including testosterone, also affect the formation of female-typical and male-typical anatomy, including sex-specific finger length ratios (Zheng & Cohn, 2011). These finger length ratios, referred to as 2D:4D, are the length of the second finger divided by the length of the fourth finger and are putative biomarkers of exposure to prenatal androgens. Women have, on average, higher 2D:4D ratios than men, indicating that they are exposed to lower levels of testosterone in utero than men (see meta-analysis by Grimbos et al., 2010). Further, 2D:4D may be an indicator of early androgen exposure relating to future sexual orientation (Breedlove, 2010; Grimbos et al., 2010; Hall & Love, 2003; Hines, 2011; Pasterski et al., 2015; Putz, Gaulin, Sporter, & McBurney, 2004; Rahman & Wilson, 2003c; Williams et al., 2000). At least in females, the finger length ratios of non-straight (bisexual or lesbian) women are more male-typical than the finger length ratios of straight women (Grimbos et al., 2010). Moreover, identical female twins with discordant sexual orientations can differ in their 2D:4D in a manner consistent with the hypothesis that the non-straight twin was exposed to higher levels of prenatal androgens; for male pairs the hypothesised reversed pattern (whereby the non-straight twin is exposed to lower levels of prenatal androgens) is less clear (Hall & Love, 2003; Hiraishi, Sasaki, Shikishima, & Ando, 2012). In this study, it is investigated whether these patterns could be replicated in a much larger sample of identical twins than have been previously studied, to test the hypothesis that prenatal androgens influence the discordant development of sexual orientations in identical twins.

1.4.1 Prenatal Androgen Exposure

Research focus has been given to the possibility that sexual orientation for both men and women are predetermined during the intrauterine period, partly via exposure to prenatal androgens including testosterone (Balthazart, 2011; Bao & Swaab, 2011; Hines, 2011; Hines et al., 2002; Rahman, 2005; Rahman & Wilson, 2003a; Rahman & Wilson, 2003c). Fetal gonads, the structure of which may be at least partly determined by genetic factors, are the primary source of prenatal androgens in males. In comparison to the male fetus, the gonads of the female fetus produce only very small amounts of prenatal androgens, and the adrenal glands are the main source (Hines et al., 2002). A further source of prenatal testosterone for the fetus is the maternal system. That is, testosterone may be passed from the mother onto a developing fetus (Hines, 2011; Hines et al., 2002). The placenta normally provides some level of protection from this hormonal transfer, but there may be instances in which it fails (Hines et al., 2002). Such failure yields higher exposer of the fetus to maternal testosterone. In sum, there are different sources of testosterone, including gonads, adrenalin glands, and the mothers' hormonal system, all of which can contribute to the level of androgenisation of the fetus.

Given that approximately one-third of identical twins rely on independent placentas (Patterson, 2007), it is feasible that, in those twin pairs in which each sibling has an independent placenta, each twin is exposed to different levels of prenatal androgens from the maternal system. Furthermore, because identical twins share 100% of their genes, they are likely to share the same genetic factors that influence gonadal and adrenal androgen levels. Thus, any differences in the levels of exposure to prenatal androgens within pairs of identical twins possibly result from differences in the makeup of their placenta, which may fail to protect one or both foetuses from hormonal transfer from the maternal system. Together, these factors may impact the discordant development of sexual orientation in pairs of identical twins. In males, it is possible that those who were exposed to lower levels of testosterone in utero are more feminized and more likely to develop a non-straight sexual orientation as compared to those who are exposed to higher levels of prenatal testosterone. In females, it is possible that those who were exposed to higher levels of prenatal testosterone are also more masculinized and more likely to develop a non-straight sexual orientation.

1.4.2 Prenatal Hormones And Finger Length Ratios

The finger digits develop at a time in prenatal development during which the circulation of androgens affects the masculinization of morphology and tissue, including human genitals (Hines, 2011; van Anders, Vernon, & Wilbur, 2006). It is therefore likely that the presence of androgens has an impact on the development of the finger length (Hines, 2011; van Anders et al., 2006). All correlational studies in humans, experimental studies in animals, and theoretical reviews on this topic point to the possibility that finger length is sensitive to prenatal androgen exposure (Breedlove, 2010; Grimbos et al., 2010; Hines, 2011; Manning, Kilduff, Cook, Crewther, & Fink, 2014; Manning, 2011; Putz et al., 2004; Williams et al., 2000; Zheng & Cohn, 2011).

The mechanism for the action of androgens, including testosterone, on the growth of the finger digits and genitals is likely through the expression of Homeobox (Hox) genes (Cohen-Bendahan, van de Beek, & Berenbaum, 2005; Kondo, Zakany, Innis, & Duboule, 1997). Hox genes influence the formation of a number of body structures including the limbs during early embryonic development (Kondo et al., 1997). Testosterone regulates the expression of Hox genes, and in this way, has an indirect effect on the growth of finger digits (Kondo et al., 1997).

The ratio of the length of the second finger digit (index finger) to that of the fourth digit (ring finger), known as the 2D:4D ratio, provides one indication of exposure levels to prenatal testosterone. In particular, testosterone influences the growth of the fourth finger digit (Breedlove, 2010; Grimbos et al., 2010; Hines, 2011; Manning et al., 2014; Manning, 2011; Putz et al., 2004; Williams et al., 2000; Zheng & Cohn, 2011). Exposure to high levels of testosterone in utero, is likely to result in a longer fourth finger digit relative to the index finger, resulting in a lower ratio, which is calculated by dividing the length of the 2^{nd} digit by the length of the 4^{th} digit. Men have smaller 2D:4D ratios than women, indicating that men are exposed to higher levels of testosterone in utero than women (Breedlove, 2010; Grimbos et al., 2010; Hines, 2011; Putz et al., 2004; Williams et al., 2000).

As reviewed in the following sections, the 2D:4D ratio, and as such levels of prenatal androgen and prenatal estrogen, may not only differ by sex but can also be a significant predictor of sexual orientation (Breedlove, 2010; Grimbos et al., 2010; Hall & Love, 2003; Hines, 2011; Putz et al., 2004; Rahman & Wilson, 2003c; Williams et al., 2000).

1.4.3 2D:4D And Sexual Orientation

Women of a non-straight sexual orientation show a more masculinised or lower 2D:4D ratio in comparison to straight women (Grimbos et al., 2010; Hines, 2011; Putz et al., 2004; Rahman & Wilson, 2003c). In addition, lesbians who report themselves as "butch" tend to have lower or more masculinized finger length ratios than those describe themselves as "femme" (Brown, Finn, Cooke, & Breedlove, 2002a). A further example of the effect of exposure to elevated levels of testosterone in early foetal development comes from cases of Congenital Adrenal Hyperplasia (CAH) in women. Due to a genetic condition, women with CAH are exposed to unusually high levels of testosterone during their intrauterine period (Berenbaum, 1999; Berenbaum & Bailey, 2003). Females with CAH display a lower, or more masculinized, 2D:4D ratio on the right hand, than females without CAH (Brown, Hines, Fane, & Breedlove, 2002b). In adulthood, women with CAH are also more likely to identify as non-straight than typically developed women (Dittmann, Kappes, & Kappes, 1992). Thus, non-straight women may be exposed to elevated levels of prenatal androgens, including testosterone, as compared with straight women. In one meta-analysis, non-straight women had lower or more masculine 2D:4D in the right and left hand than unrelated straight women (Grimbos et al., 2010). These effects were small to medium, $.23 < \text{Hedges } g's < .29$, $.04 < 95\% \text{ CI} < .51$.

Compared to research on women, research investigating the relationship between the 2D:4D ratio and sexual orientation in men has been far less consistent (Grimbos et al., 2010), even when very large samples (over 200,000 individuals) were studied (Collaer, Reimers, & Manning, 2007). Although hypothesised relationships between 2D:4D ratios and sexual orientation in men have been confirmed in some studies (Lippa, 2003; McFadden & Shubel, 2002), other research has failed to replicate such findings or has even found the opposite pattern of results (Grimbos et al., 2010). For instance, samples of biological males with Complete

Androgen Insensitivity Syndrome (CAIS), which renders them completely unresponsive to androgens during development, are more likely to be attracted to men (Hines, Ahmed, & Hughes, 2003) and show a more female-typical 2D:4D ratio than control males (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009). In a meta-analysis, when comparing non-straight and straight men, no difference was detected in 2D:4D in the left or right hand, *g* for either hand = -.02, -17 < 95% CI < .13 (Grimbos et al., 2010). Thus, in general, the hypothesized link between 2D:4D and sexual orientation appears more inconsistent for males than females.

If a relationship between finger length ratios and sexual orientation in men exists at all, it may therefore be, smaller than the corresponding effect for women. Hence, compared with the effect for females, the effect for males might be more prone to measurement error. If so, this effect might be better detected in a more controlled research design than those previously employed, such as by comparing identical twins discordant for sexual orientation. By comparing genetically identical individuals who differ with respect to their sexual orientation, one might therefore have more statistical power to detect reliable effects, even if they are small in magnitude.

1.4.4 Differences in 2D:4D within Discordant Twin Pairs

Previous research supports the hypothesis that discordant female twins are exposed to different prenatal environments (Hall & Love, 2003; Hiraishi et al., 2012). In seven pairs of female identical twins with discordant sexual orientations, the non-straight female twins showed a significantly lower 2D:4D ratio than their straight co-twins on both hands (Hall & Love, 2003). This effect was partially replicated in another study with eight female pairs with discordant sexual orientation: The non-straight twins had a lower, or more masculinized, 2D:4D ratio on their left hand than their straight co-twins, suggesting stronger exposure to prenatal androgen for the non-straight twin than her straight co-twin; however, a similar effect was not found in the right hand (Hiraishi et al., 2012).

Within four male pairs, those who identified as non-straight had significantly higher or more feminized left hand 2D:4D ratios than their straight co-twins (Hiraishi et al., 2012). Because a higher 2D:4D ratio is more typical in females, a potential hypothesis is that the non-straight twin has been exposed to a lower level of prenatal testosterone than his straight co-twin.

If such effects can be replicated in the present sample of twins, then, within female pairs, the non-straight twin will display a lower 2D:4D ratio than her straight co-twin (Hypothesis 4.1). In male pairs, the non-straight twin will display a higher 2D:4D ratio than his straight co-twin (Hypothesis 4.2). It was further tested whether such effects were stronger in the left or right hand.

1.4.5 Sex Differences in 2D:4D

In addition to sexual orientation differences, the present research examined sex differences in 2D:4D. Because finger digits develop at a time in prenatal development during which the circulation of androgens affects the development of male-typical and female-typical anatomy, morphology and tissue it is possible that the presence of androgens has an impact on the development of male-typical and female-typical finger length (Hines, 2011; van Anders et al., 2006). Elevated testosterone exposure in utero influences the growth of the fourth finger digit. This should result in a longer fourth finger digit relative to the index finger, and a lower second to fourth finger length ratio in most males, as compared with most females (Breedlove, 2010; Grimbos et al., 2010; Hines, 2011; Manning et al., 2014; Manning, 2011). In fact, on average, women have higher 2D:4D ratios than men, in the left and right hand, $p < .001$, $g = .44$, and $p < .001$, $g = .55$, indicating that women are exposed to lower levels of testosterone in utero than men (Breedlove, 2010; Grimbos et al., 2010; Hines, 2011). In the present study on identical twins, it is predicted that females will have, on average, significantly higher finger length ratios than males (Hypothesis 4.3). If the aforementioned sexual orientation differences in 2D:4D are correct then sex differences should be stronger when straight males and females are compared.

1.4.6 Study 3

Study 3 investigates patterns of 2D:4D ratios of identical twins with discordant sexual orientations. In addition, raw data are available from two previous samples of discordant twin pairs (Hall & Love, 2003; Hiraishi et al., 2012), and these will be included in analyses as part of the present study. Because data on finger length ratios of identical twins with discordant sexual orientations are scarce and some previous samples were very small, a combination of present data with previous data will allow more powerful analyses than each individual study would, therefore yielding potentially more reliable results. In sum, the following hypotheses are tested:

Hypothesis 4.1. In female pairs, the non-straight twin will display a lower 2D:4D ratio than her straight co-twin.

Hypothesis 4.2. In male pairs, the non-straight twin will display a higher 2D:4D ratio than his straight co-twin.

Hypothesis 4.3. Females will have significantly higher finger length ratios than males.

1.5 Structure of Thesis

The goal of the present thesis is to examine how identical twins with discordant sexual orientations effectively differ in their degree of gender nonconformity and physiological sexual arousal and how differences between twins could be explained by variations of potential androgen exposure.

Chapter 2 presents Study 1, which investigates how gender nonconformity is expressed within 56 pairs (24 male pairs, 32 female pairs) of identical twins discordant for sexual orientation. This is investigated via observer ratings of photos from childhood and adulthood in order to overcome the potential limitations associated with the self-report method. In addition, Study 1 investigates social developmental factors that may have influenced the co-development of gender nonconformity and sexual orientation in discordant twin pairs. In particular, this study investigates how early responses from parents and peers during childhood are linked to gender nonconformity and sexual orientation in adulthood.

Chapter 3 presents Study 2, which assesses genital arousal and pupil dilation in response to sexual stimuli in order to objectively evaluate differences between identical twins with discordant sexual orientations on a physiological level. This study includes a sample of 15 pairs (6 male pairs, 9 female pairs) of identical twins.

Chapter 4 presents Study 3, which examines how prenatal androgen exposure affects the discordant development of sexual orientation in 32 pairs (14 male pairs, 18 female pairs) of identical twins. The ratio of the length of the index to ring finger is used as a marker for prenatal androgen exposure in this study. This finger length ratio is used to assess how prenatal androgen exposure in utero is linked to sexual orientation of these twins.

Finally, Chapter 5 provides a general discussion, which summarizes findings from Studies 1-3. In addition, this chapter highlights the implications of findings and directions for future research.

Chapter 2 Gender Nonconformity of Identical Twins with Discordant Sexual Orientations: Evidence from Childhood Photographs

2.1 Abstract

Childhood gender nonconformity (femininity in males, masculinity in females) predicts a non-straight (gay, lesbian, or bisexual) sexual orientation in adulthood. In previous work, non-straight twins reported more childhood gender nonconformity than their genetically identical, but straight, co-twins. However, self-reports could be biased. We therefore assessed gender nonconformity via ratings of photographs from childhood and adulthood. These ratings came from independent observers naïve to study hypotheses. Identical twins with discordant sexual orientations (24 male pairs, 32 female pairs) visibly differed in their gender nonconformity from mid-childhood, with higher levels of gender nonconformity for the non-straight twins. This difference was smaller than the analogous difference between identical twins who were concordant straight (4 male pairs, 11 female pairs) and identical twins unrelated to them who were concordant non-straight (19 male pairs, 8 female pairs). Further, twins in discordant pairs correlated in their observer-rated gender nonconformity. Non-genetic factors likely differentiated the discordant twins' gender-related characteristics in childhood, but shared influences made them similar in some respects. We further tested how recall of past rejection from others related to gender nonconformity. Rejection generally increased with gender nonconformity, but this effect varied by the twins' sexual orientation.

2.2 Introduction

Men who are more feminine, and less masculine, in their behaviours and interests than most other men can be defined as gender nonconforming; similarly, gender nonconforming women are more masculine and less feminine than other women (Lippa, 2005). Gender nonconformity is more common in non-straight (gay, lesbian, and bisexual) than straight men and women, and this difference emerges in early childhood (Bailey & Zucker, 1995; Lippa, 2008; Rieger et al., 2008). Another line of research suggests that across men and women, genetic variation explains approximately 30% of the differences in sexual orientation (Bailey et al., 2016). Furthermore, between 10% and 50% of the co-development of sexual orientation with gender nonconformity is explained by genetic variation (Alanko et al., 2010; Bailey et al., 2000; Burri et al., 2011).

Because sexual orientation and gender-related behaviour are not fully determined by genetics, genetically identical twins can differ in both their sexual orientations and their level of gender nonconformity. By self-report, twins with discordant sexual orientations differ in their gender nonconformity to a degree similar to unrelated straight and non-straight individuals (Bailey & Pillard, 1991). However, self-reports may distort actual differences in gender nonconformity (Gottschalk, 2003). We therefore examined whether identical twins with discordant sexual orientations differed in their observable gender nonconformity by evaluating photographs taken in childhood and adulthood. We further compared their difference in gender nonconformity to the difference between pairs where both twins were straight and pairs unrelated to them where both twins were non-straight. Finally, we examined how social reactions during childhood related to gender nonconformity of these twins.

2.2.1 Sexual Orientation and Gender Nonconformity

Gay men, lesbians, and bisexual men and women report more gender-nonconforming behaviours and interests, on average, than straight adults of their sex (Lippa, 2008; Rieger et al., 2010; Swift-Gallant et al., 2017). In one meta-analysis, gay men reported more feminine and less masculine interests and self-concepts in adulthood than straight men; lesbians reported more masculine and less feminine interests and self-concepts than straight women (Lippa, 2005). These effects were large, 1.28 < *d's* < 1.46, 1.18 < 95% CI < 1.56, and 0.60 < *d's* < 1.28, 0.50 < 95% CI < 1.38, and, .28 < *d's* < 1.46, 1.18 < 95% CI < 1.56, respectively. These differences emerge in childhood; in another meta-analysis, gay men recalled more gender nonconforming childhood behaviours and activities than straight men; the same was true for lesbians and straight women (Bailey & Zucker, 1995). These effects were also large, $d = 1.31$, 0.45 < 95% CI < 3.08, and $d = 0.96$, 0.26 < 95% CI < 1.66, respectively. Moreover, gender nonconformity in childhood predicts gender nonconformity in adulthood, suggesting some developmental stability of the trait (Rieger et al., 2008). In addition, non-straight men and women are more sex atypical than straight individuals in their neuroanatomical structures and cognitive functions, possibly due to differentiations of neural circuits during their early development (Savic, Garcia-Falgueras, & Swaab, 2010). This could lead to partially gender-reversed abilities in processing emotions or mental rotation tasks (Rahman & Wilson, 2003b; Rahman & Yusuf, 2015). Furthermore, same-sex-oriented individuals are more gender nonconforming across different societies, suggesting that non-cultural factors contribute to this link (Lippa, 2008; Semenyna, VanderLaan, & Vasey, 2017).

In adulthood, sexual orientation differences in gender nonconformity can be assessed by others based on motor behaviours, speech patterns, and physical appearances, even if displayed for only a few seconds (Johnson, Gill, Reichman, & Tassinary, 2007; Rieger et al., 2010).

Observer ratings of adults confirm, in general, the link of sexual orientation with self-reported gender nonconformity. However, unlike the research on adulthood gender nonconformity, the majority of previous research on sexual orientation and childhood gender nonconformity has relied on retrospective self-reports, asking adults to recall their own childhood behaviour. These self-reports may be subject to biases; for example, due to the internalization of societal norms, straight participants may underreport their actual childhood gender nonconformity (Baumrind, 1995; Gottschalk, 2003). Retrospective reports may also be problematic simply due to their reliance on a memory system that was not fully developed at the time during which gender behaviour emerged (Pillemer & White, 1989).

One way to address the limitations of retrospective work is to conduct prospective research. Studies have followed children who were referred to clinics due to extreme levels of gender nonconformity and concern over their gender identity. In adolescence and adulthood, these groups were substantially more likely to identify as bisexual, gay or lesbian, compared with individuals who were not gender nonconforming in childhood (Drummond et al., 2008; Green, 1985; Singh, 2012). One needs to be cautious regarding whether findings from clinical samples represent the relationship between these variables for most people. However, two longitudinal studies, using data from the general population, confirm that early gender-nonconforming behaviours (as early as 3 to 4 years old) predict a same-sex orientation in adolescence or adulthood (Li et al., 2017; Steensma et al., 2013). Such population-based, longitudinal work involves many logistical challenges. It takes years to conduct, needs hundreds of participants to capture enough with same-sex orientations, and can have substantial attrition rates. An alternative method is the assessment of gender nonconformity retroactively through the evaluation of behaviour depicted in visual images from childhood. This method is free of the limitations of self-report (because it does not rely on subjective accounts), clinical samples (because it draws from a wider population), and longitudinal studies (because it does not take years to conduct). Research using this method also suggests that differences in observable gender nonconformity are predictive of an adulthood sexual orientation from ages 3 to 4 years onwards (Rieger et al., 2008).

2.2.2 Sexual Orientation of Identical Twins

Although monozygotic twins are genetically identical, their sexual orientations are not always concordant (Alanko et al., 2010; Långström et al., 2010; Zietsch et al., 2012). In a nationally representative sample, and depending on the definition of sexual orientation, 20% to 38% of male identical twins who identified as non-straight had non-straight co-twins; similarly, for women, 24% to 30% of non-straight identical twins had non-straight co-twins (Bailey et al., 2000). Across several representative samples, and across men and women, this concordance rate was estimated as 24% (Bailey et al., 2016). That is, about 24% of non-straight twins had a twin who was non-straight too. In contrast, for the population as a whole, the occurrence of a non-straight sexual orientation may be as low as 3.5% across both sexes, although other estimates suggest 7% in men, and 13% in women (Gates, 2011; Savin-Williams & Vrangalova, 2013). Thus, the chance of a same-sex orientation for co-twins of non-straight twins is higher than that reported for the general population. Familial factors, perhaps including shared genetic factors, likely influence the development of a similar sexual orientation in these related individuals.

The aforementioned distributions of sexual orientations within identical twin pairs emphasize another pattern: A substantial proportion (62% to 80%) of identical twins who are non-straight have straight co-twins (Bailey et al., 2000). Because these twins are genetically identical, factors other than their genes must account for their different sexual orientations. These factors could, in theory, also affect a correlate of sexual orientation, their degree of gender nonconformity.

2.2.3 Differences in Gender Nonconformity within Discordant Twin Pairs

Although identical twins with discordant sexual orientations have been part of several behavioural-genetic studies (e.g., Alanko et al., 2010), individual twins in these pairs were rarely systematically compared with respect to their degree of gender nonconformity. In one study of male pairs with discordant sexual orientations, non-straight twins reported more childhood gender nonconformity than their straight co-twins. The average difference was similar to that for unrelated non-straight and straight men, suggesting substantial environmental (unique, non-genetic) effects on the link of sexual orientation with gender nonconformity (Bailey & Pillard, 1991). However, this finding is subject to the potential limitations of retrospective self-reports. Self-reported childhood gender nonconformity was also assessed in female identical twins with discordant sexual orientations, but no information was given on their differences or how they compared to unrelated straight and non-straight women (Bailey et al., 1993).

To avoid the drawbacks of self-report, the present study investigated possible differences in the childhood gender nonconformity of discordant twins by examining visual images from their childhoods. For unrelated individuals, sexual orientation differences in observable gender nonconformity, based on evaluations of visual images, emerge by ages 3 to 4 and carry into adulthood (Rieger et al., 2008). If twins with discordant sexual orientations are like unrelated straight and non-straight individuals, then differences in their observable gender nonconformity could emerge at ages 3 to 4, and remain present in adulthood (Hypothesis 2.1).

2.2.4 Correlation of Gender Nonconformity within Discordant Twin Pairs

In general, shared genes make individuals similar in their sexual orientation, gender behaviour, and their co-expression, whereas the shared environment (any shared influences other than shared genes) does not substantially affect similarity (Alanko et al., 2010; Burri et al., 2011; Kendler et al., 2000; Långström et al., 2010; Zietsch et al., 2012). However, some work points to the contribution of the shared environment to the expression of gender behaviour (Iervolino et al., 2005; Knafo et al., 2005). Whichever the exact familial influences are (shared genes and/or shared environment), they could affect a shared expression of gender nonconformity in related individuals. Further, both straight and non-straight individuals vary in their degree of gender nonconformity; some score high relative to others of their sexual orientation, others score low (Rieger et al., 2010). These variations could be linked in related individuals. That is, familial influences could contribute to related levels of gender nonconformity, even in twins with discordant sexual orientations. A non-straight twin who scores high on gender nonconformity, relative to other non-straight individuals, may have a straight co-twin who scores high on gender nonconformity, relative to other straight individuals. This would result in a correlation in the twins' gender nonconformity, even if they differed on average in their gender nonconformity.

Although a correlation in self-reported gender nonconformity has been found for twin pairs with concordant non-straight orientations, it was not confirmed for discordant pairs (Bailey & Pillard, 1991; Bailey et al., 1993). It is possible that there is, indeed, no relationship. Alternatively, failure to find such link in discordant pairs may have been due to the self-report measures with their aforementioned limitations. Examining gender nonconformity through the evaluation of childhood photographs may indicate a correlation between the degree of gender nonconformity in straight twins and their non-straight co-twins (Hypothesis 2.2).

2.2.5 A Comparison of Discordant and Concordant Twin Pairs

A parsimonious model assumes that differences in gender nonconformity within twin pairs with discordant sexual orientations are as strong as those between unrelated individuals with different sexual orientations. Self-report measures support this hypothesis (Bailey & Pillard, 1991). However, it is also possible that due to shared influences between twins of a pair, differences in gender nonconformity within discordant twin pairs may be less distinct than those seen between unrelated straight and non-straight individuals. The present study examined this possibility by comparing discordant twins to identical twin pairs with concordant straight sexual orientations and identical twin pairs unrelated to them with concordant non-straight sexual orientations. Because being an identical twin was held constant across participants, any differences across twin types could be more easily interpreted with respect to the twins' similar or dissimilar sexual orientations. To our knowledge, this is the first study that allowed such interpretation because it systematically compared the difference between twins with discordant sexual orientations to the difference between twins from concordant straight pairs and, unrelated to them, twins from concordant non-straight pairs. We proposed that a difference between straight and non-straight individuals in observable gender nonconformity could be smaller within discordant twin pairs than the analogous difference between concordant non-straight twins and, unrelated to them, concordant straight twins (Hypothesis 2.3).

We also examined whether, as previously seen in self-reports, the correlation in gender nonconformity is weaker in discordant twin pairs than concordant pairs (Bailey & Pillard, 1991;

Bailey et al., 1993). We postulated that, unlike self-reports with their possible biases, observer ratings may show similar correlations in gender nonconformity across twin types (Hypothesis 2.4).

2.2.6 Responses to Gender Nonconformity

Several studies suggest that gender-nonconforming children experience negative reactions and rejection from others (Caldera et al., 1989; Carter & McCloskey, 1983; Fagot, 1985; Langlois & Downs, 1980; Maccoby, 1998; Smith & Leaper, 2006). Peers can react negatively to gender nonconformity, and boys are especially critical about gender nonconformity in other boys (Wallien et al., 2010; Young & Sweeting, 2004). Similarly, parents can be more detached and unsupportive if their children are gender nonconforming (Alanko et al., 2009; Landolt et al., 2004). There are exceptions to these reactions. In one study on very feminine boys, who likely became non-straight, the most feminine boys had mothers who responded less negatively, if not more positively, to their sons' gender nonconformity (Green, 1987). However, the overall conclusion across research is that more gender-nonconforming children experience more negative reactions (Alanko et al., 2011). One possibility is that the child's gender nonconformity causes the parents' reactions. It is also possible that gender nonconformity and negative parental reactions reinforce each other; for example, parents may punish their child for such behaviours, and because punishment means attention, the child may feel encouraged to continue these behaviours (Alanko et al., 2011).

Furthermore, because childhood gender nonconformity predicts adulthood gender nonconformity, adulthood gender nonconformity is also linked to past rejection from others

(Rieger et al., 2008). We therefore expected that in twins, higher levels of gender nonconformity in childhood and adulthood would relate to recall of increased rejection (Hypothesis 2.5).

2.2.7 The Present Study

We investigated the development of gender behaviour in identical twins to illuminate possible non-genetic and familial influences on the expression of sexual orientation. We recruited twin pairs with either discordant or concordant sexual orientations and examined their degree of observable gender nonconformity via evaluations of photographs taken in childhood and adulthood. Twins also reported their degree of gender nonconformity in childhood and adulthood and the reactions from others during childhood.

The following hypotheses were tested:

Hypothesis 2.1. Within discordant twin pairs, the non-straight twin is more gender nonconforming than the straight co-twin. Using observer ratings of their photographs, this difference might emerge by ages 3 or 4.

Hypothesis 2.2. For discordant twin pairs, the degree of observer-rated gender nonconformity of non-straight twins correlates with the degree of gender nonconformity of the straight co-twins.

Hypothesis 2.3. The difference in observer-rated gender nonconformity within discordant twin pairs is smaller than the analogous difference between concordant non-straight twins and unrelated concordant straight twins.

Hypothesis 2.4. The correlation of observer-rated gender nonconformity within discordant twin pairs is similar to this correlation within concordant twin pairs.

Hypothesis 2.5. Across discordant and concordant twins, higher levels of gender nonconformity relate to recall of increased rejection by others.

2.3 Method

2.3.1 Recruitment and Participants

Twins. The University of Essex Ethics Committee approved this study. Advertisements for identical twins to participate in a study on sexual orientation were placed in the newsletter of the Department of Twin Research at Kings College London, on social media sites, and on three online news sites for gay men and lesbians (Gay Star News, Pink News, and Gay Times). We further recruited twins at three gay Pride festivals. Each twin who contacted us was encouraged to recruit the co-twin. Of the twins who registered their interest in the study online, 42% recruited their co-twins and took part. Forty-five percent of those who registered their interest at Pride festivals participated with their co-twins in the study. Twins self-identified as straight, bisexual, gay or lesbian. They were asked twice during the study about their sexual identities, and all responses were consistent. Twins were not asked to report on the sexual orientation of their co-twins, nor were they asked about the age at which they became aware of their co-twins' sexual orientation. The number of bisexual men and women (6 and 10 individuals) was low relative to the number of straight men and women (32 and 54) or gay men and lesbians (56 and 38). Furthermore, bisexual participants reported a stronger preference for the same sex than the other sex; thus they were grouped with gay men and lesbians into "non-straight".

Based on the available literature comparing straight and non-straight participants, sexual orientation differences in gender nonconformity have an effect size (*d*) exceeding 1.0. In this case a power of .8 can be obtained with 20 pairs, even if these measures are uncorrelated across twin pairs. If, due to familiarity, a small correlation of $r = .3$ is assumed across pairs, a sexual orientation difference with the effect $d = 1.0$ can be obtained with 15 pairs. Regarding social reactions to gender nonconformity, the most appropriate studies suggest an effect size $d = .7$. Depending on whether there is a small correlation ($r = .3$) or no correlation ($r = .0$) in measures across twins, 25 to 35 pairs are needed to achieve a power of .8. Hence, a minimum of 25 and a maximum of 35 discordant pairs were sought.

For twins with discordant sexual orientations, 24 pairs were male and 32 pairs were female. Four male pairs were concordant straight, 19 male pairs were concordant non-straight, 11 female pairs were concordant straight, and 8 were concordant non-straight. Distributions of age and ethnicity are shown in Table 2.1. The number of male pairs who were concordant straight was notably low in number compared to the other categories. Analyses indicated that these men differed significantly from concordant non-straight twins (and from discordant twins) in the hypothesized directions. In this sense, their smaller number did not appear to be problematic; however, in the limitations section, we speculate about why their numbers were so low.

Raters. Psychology students participated as raters of gender nonconformity for course credit. Twenty-five men self-identified as straight, 13 men as non-straight, 77 women as straight, and 17 women as non-straight. The higher proportion of women reflects that most psychology students at our institution are women. Ratings of gender behaviour are minimally affected by the raters' sex or sexual orientation (Rieger et al., 2010). This was also the case for present ratings (see below), and different sizes of rater groups did not appear to affect our findings.

2.3.2 Self-Report Measures

Twin zygosity. In addition to asking twins whether they were identical, five standardized items about physical and visual similarity were administered to establish zygosity (Kasriel & Eaves, 1976). An example question is "During childhood, could you ever have fooled friends by pretending to be your twin?" Each item was assessed on a scale ranging from 1 to 3, with lower scores reflecting higher similarity within twin pairs. For the majority of individual twins, their average scores were below 2, suggesting monozygosity. For the three individuals who scored above 2, on average, and thus indicated increased dissimilarity from the co-twin, zygosity was assessed by re-contacting twins and co-twins to confirm that they were identical and by examining photographs from childhood and adulthood to check similarity.

Gender nonconformity. The Childhood Gender Nonconformity Scale measured self-reported gender nonconformity during childhood (Rieger et al., 2008). Both men and women were given seven items. Example statements are "As a child I preferred playing with girls rather than boys" for males, and "As a child I often felt that I had more in common with boys than girls" for females. Gender nonconformity in adulthood was assessed with seven items for each sex with the Continuous Gender Identity Scale (Rieger et al., 2008). Example statements include "My mannerisms are more feminine than those for most men of my age" for men and "I assume most people see me as more masculine than other women" for women. For all measures, items were endorsed on 7-point scales, with higher scores representing higher levels of childhood or adulthood gender nonconformity. For both discordant and concordant twins of either sex, internal reliability (Cronbach's α) exceeded .84 for both childhood and adulthood measures.

Parental and peer rejections. Parental rejection was assessed using a 10-item version of the Recollection of Early Childrearing Scale (Ross, Campbell, & Clayer, 1982), measuring rejection versus acceptance by each parent during childhood. Example items include "My (mother/father) wished I had been like somebody else" and "I think my parent was mean and grudging toward me." Responses of peers during childhood were assessed using ten items from the Mother-Father-Peer Scale (Epstein, 1983). Sample statements include "When I was a child, other children were often unfair to me" and "When I was a child, other children often picked on me." Items were rated on 7-point scales, with higher scores representing higher levels of rejection. Cronbach's α for all three measures of rejection exceeded .72 for both discordant and concordant twins of either sex. Across twins, the three measures were correlated, *p's* < .0001, .40 < *r's* < .52, .30 < 95% CI's < .62. In addition to separate results for maternal, paternal, and peer rejection, we also report results for a composite of "overall rejection" across these measures.

2.3.3 Procedure

Collecting data and photographs. An online link to the self-report measures was emailed to each participant. It was stressed that answers should not be discussed with co-twins or others. Furthermore, participants were asked to arbitrarily select and send, at a minimum, one to three photographs from any available age from childhood into adulthood. This included photographs that were recently taken. There was no upper limit on the number of photographs. The context of the photographs varied. Most common were close-ups of the individual, followed by photographs taken at school, birthday events, and holidays. Twins were asked to identify themselves (and their co-twin, if depicted) in each photograph. The majority of photographs were cropped before they were rated by observers, so that only the individual twin was shown (without the co-twin or other people).

Twins as children. During childhood (defined as 0-15 years) the number of photographs of individuals within pairs was almost the same. Using pairs as units, the mean (SD) number of photographs was 14.86 (5.30), 12.06 (3.47), and 10.08 (3.47) for discordant males, concordant straight males, and concordant non-straight males, respectively. The mean (SD) age in years in these photographs was 7.63 (5.02), 6.34 (4.93), and 6.60 (5.02), respectively. The mean (SD) number of photographs of female twins was 12.37 (3.54), 14.15 (5.20), and 9.14 (2.11) for discordant pairs, concordant straight pairs, and concordant non-straight pairs. Mean (SD) ages in these photographs were 7.73 (5.01), 8.57 (4.76), and 6.89 (5.07), respectively.

Twins as adults. Ages in photographs from adulthood ranged from 16 (the legal age of consent in the UK) to 27. The number of photographs was almost exactly the same for twins within a pair. With pairs as units, the average (SD) number of photographs was 12.02 (4.95), 10.13 (4.22), and 9.85 (3.50) for discordant, concordant straight, and concordant non-straight males, respectively. Mean (SD) ages in the photographs were 21.86 (2.67), 22.50 (1.07), and 21.95 (1.99), respectively. For females, the average (SD) number of photographs was 12.92 (3.84), 14.80 (5.57), and 10.00 (1.87) for discordant, concordant straight, and concordant non-straight twins. Mean (SD) ages were 22.15 (2.31), 21.33 (1.72), and 21.62 (2.60), respectively.

Ratings. Photographs were shown in the stimulus presentation software Inquisit. We sorted the photographs into 8 sets: 2 sets showing female children, 2 with male children, 2 with adult females, and 2 with adult males. For both discordant and concordant pairs, photographs of each twin were in separate sets than those of the co-twin.

In the lab, each rater viewed 4 sets of photographs (males as children, females as children, males as adults, females as adults) showing the same individuals in childhood and adulthood, but never their co-twins. That is, raters never viewed twin pairs together. Raters were neither told that those shown in the photographs were twins, nor that they varied in sexual orientation. The order in which sets of photographs were presented was counterbalanced across raters. Within each set, photographs were presented for 3 seconds each and in random order.

We used rating procedures that were almost identical to those that have previously resulted in reliable sexual orientation differences in observer-rated gender nonconformity (Rieger et al., 2008). Raters of photographs were instructed to indicate their impression of each individual's appearance and demeanor in comparison to their impression of most people of this age and sex. For example, after each photograph of a boy, they were told to "rate whether this boy appeared or behaved in a more feminine or masculine way". Ratings were completed on 7-point scales. For photographs of males, the score of 1 was "more masculine", 4 was "average," and 7 was "more feminine." A reversed scale was used for ratings of females. Thus, for both sexes, 7 represented maximum gender nonconformity. Raters were told that they might see the same person more than once (because individuals provided several photos) and encouraged to judge photographs independently of each other. Ratings for each set took 10 to 20 minutes.

Straight male raters gave lower mean (SD) evaluations of gender nonconformity, 3.51 (0.98) than non-straight males, 3.65 (1.07); straight females, 3.60 (1.07), and non-straight females, 3.59 (1.09), were similar. Although a significant difference, its magnitude was minimal, $p = .004$, $R^2 = .002$, 95% CI [.000, .004]. Furthermore, average evaluations from the four groups of raters correlated strongly with each other, p's < .0001, .73 < r's < .89, .71 < 95% CI's < .90. Across all raters, inter-rater reliability (Cronbach's α) exceeded .93 for each combination of twin type (discordant or concordant), sex, and period (childhood or adulthood). Hence, for each photograph, an average rating of gender nonconformity was computed across all raters.

2.4 Results

2.4.1 Hypothesis 2.1

We hypothesized that within discordant pairs, non-straight twins are more gender nonconforming than their straight co-twins. This difference could emerge by ages 3 or 4.

A mixed factorial regression analysis was conducted for discordant twin pairs, separately by sex. The dependent variable was observer-rated gender nonconformity, across depicted ages from childhood into adulthood (0 to 27 years). Independent variables were twins' sexual orientation and the age at which a photograph was taken. Furthermore, an interaction between sexual orientation and age tested whether differences in observer-rated gender nonconformity between straight and non-straight twins changed with age. Twin pairs and individuals were included as random effects to account for dependency of data within pairs and repeated evaluations of each individual across ages.

For discordant male pairs, non-straight twins were rated as more gender nonconforming than their straight co-twins, on average, $p < .0001$, β [95% CI] = .23 [.15, .31]. Moreover, an interaction of sexual orientation with age indicated that differences in observer-rated gender nonconformity between straight and non-straight twins increased with age, $p < .0001$, $\beta = .16$ [.08, .24] (Figure 2.1). Similarly, for females pairs, non-straight twins were rated as more gender nonconforming than their straight co-twins, $p < .0001$, $\beta = .23$ [.15, .30], and the interaction with age indicated that differences in observer-rated gender nonconformity between straight and non-straight twins increased with age, $p < .0001$, $\beta = .16$ [.09, .24] (Figure 2.1).

Figure 2.1 further shows that for males, the confidence intervals for gender nonconformity of straight twins and their non-straight co-twins separated at approximately 8 years. For females, the separation was at age 6 (Figure 2.1). Hence, differences in observer-rated gender nonconformity between twins with discordant sexual orientations became significant at a later age than hypothesized.

In addition to these sexual orientation differences in observer-rated gender nonconformity as a function of age, we examined average differences in gender nonconformity for each measure (observer ratings and self-report). Two mixed factorial regression analyses were conducted separately for each sex and age period (childhood and adulthood). The dependent variable was observer-rated or self-reported gender nonconformity. The independent variable was sexual orientation as a fixed effect. Twin pairs were included as a random effect to account for dependency of data within pairs. For observer ratings, individuals were also included as a random effect to account for repeated evaluations of individuals.

In childhood, non-straight twins were, in general, rated as more gender nonconforming than their straight co-twins. This was found for males and females, $p = .006$, $\beta = .15$ [.04, .25], and $p = .0004$, $\beta = .16$ [.07, .24], respectively. These differences were stronger for their self-reported childhood gender nonconformity, $p = .0002$, $\beta = .58$ [.31, .85], and $p = .0001$, $\beta = .46$ [.24, .68], respectively. In adulthood, sexual orientation effects on observer ratings for males and females, $p < .0001$, $\beta = .40$ [.30, .50], and $p < .0001$, $\beta = .46$ [.32, .61], were similar to effects on self-reported gender nonconformity, $p = .004$, $\beta = .43$ [.16, .71], and $p < .0001$, $\beta = .40$ [.22, .59].

For a simple illustration of these effects, Figure 2.2 depicts these general differences between twins by measure, without controlling for dependency of data within pairs or individuals. Dots represent average gender nonconformity of individuals, averaged either across all ratings or across self-report scores. Differences in gender nonconformity between straight and non-straight twins were weaker with observer ratings than self-report. In fact, in these simple comparisons, differences in observer-rated childhood gender nonconformity were not significant.

Figure 2.2 further suggests that the differences between measures in the effect of sexual orientation on gender nonconformity were possibly due to straight twins reporting less gender nonconformity than what raters observed. Additional analyses (not shown here) confirmed that across childhood and adulthood, and across males and females, the difference between self-reports and ratings by observers was significant for straight twins, but not for their non-straight co-twins. We further address these discrepancies in the comparison below with concordant pairs.

In sum, these findings suggest that non-straight twins were more gender nonconforming than their straight co-twins, but this difference was less prominent with observer ratings than self-report, possibly because the straight twins rated themselves as less gender nonconforming (or, more gender conforming) than observers perceived them to be.

2.4.2 Hypothesis 2.2

We hypothesized that for twins with discordant sexual orientations, the siblings' degree of observer-rated gender nonconformity is correlated. A regression analysis was conducted separately for each sex, age period (childhood or adulthood) and measure (observer ratings or self-report). The dependent variable was gender nonconformity of the straight twin, and the independent variable was gender nonconformity of his or her non-straight co-twin. For observer ratings, random effects accounted for repeated entries of gender nonconformity within pairs and across ages. Table 2.2 shows the correlations (technically, regression coefficients) of gender nonconformity within discordant twin pairs. In the majority of cases, these correlations were stronger for observer ratings than self-report measures, thereby confirming the hypothesis that twins' related levels of gender nonconformity are detectable by others.

A mixed-factorial regression analysis, testing for differences by measure in the correlation of gender nonconformity within pairs, confirmed this pattern. Independent of sex and age period (childhood or adulthood), the correlation of gender nonconformity differed significantly by measure, $p < .0001$, $\beta = .15$ [.10, .21]. When broken down by measure, the correlations were stronger with observer ratings than for self-report, $p < .0001$, $\beta = .47$ [.36, .58], and $p = .06$, $\beta = .19$ [-.01, .38], respectively. However, this difference by measure was further moderated by sex and age period. Table 2.2 shows that in male discordant pairs, and across age periods, observer ratings of their gender nonconformity were more strongly correlated than their self-reports. In females, stronger correlations for observer ratings than self-reports were found in childhood, but the effect was reversed in adulthood.

2.4.3 Hypothesis 2.3

We hypothesized that the difference in observer-rated gender nonconformity within discordant twin pairs is smaller than the analogous difference between concordant straight twins and unrelated concordant non-straight twins. We first conducted analyses for concordant twins similar to those previously described for discordant twins (Figure 2.1). A mixed factorial regression analysis was computed separately for male and female concordant pairs. Observer-rated gender nonconformity was predicted by sexual orientation, age, and their interaction. Twin pairs and individuals were random effects.

For male twin pairs with concordant sexual orientations, non-straight twins were rated as significantly more gender nonconforming than unrelated straight twins, $p = .001$, $\beta = .58$ [.27, .89]. An interaction of sexual orientation with age suggested that this difference between straight twins and unrelated non-straight twins became stronger with age, $p = .0004$, $\beta = .14$ [.07, .23].

Figure 2.3 shows that this interaction was due to straight twins becoming less gender nonconforming with age, whereas non-straight twins remained on an intermediate level. Furthermore, this difference in gender behaviour became significant early, between 1 and 2 years.

Similarly, concordant female twins who were non-straight scored higher on observer-rated gender nonconformity than unrelated concordant female twins who were straight, $p = .006$, $\beta = .39$ [.14, .65]. In general, as age increased females became less gender nonconforming, $p < .0001$, $\beta = -.36$ [-.36, -.26]. However, the interaction between sexual orientation and age was marginally significant, $p = .06$, $\beta = .09$ [-.00, .18], indicating that differences between straight twins and unrelated non-straight twins in observer-rated gender nonconformity became somewhat stronger with age. These differences become significant at 3 years (Figure 2.3).

We then tested whether sexual orientation differences in observer-rated gender nonconformity further differed by pair type (discordant or concordant). A mixed factorial regression analysis, with twin pairs and individuals as random effects, predicted observer-rated gender nonconformity by sexual orientation, age, pair type, sex, and their interactions. Non-straight twins were rated as more gender nonconforming than straight twins, regardless of whether they were concordant or discordant, or male or female, $p < .0001$, $\beta = .33$ [.24, .43]. An interaction between sexual orientation and pair type indicated that the effect of sexual orientation on observer-rated gender nonconformity varied between twins with discordant or concordant sexual orientations, $p = .02$, $\beta = .13$ [.02, .24]. This interaction can be interpreted by comparing Figure 2.1 with Figure 2.3: The average difference in observer-rated gender nonconformity was stronger between concordant non-straight pairs and unrelated concordant straight pairs as compared to this difference within discordant pairs. In addition, this difference became distinct at a later age within discordant pairs than between concordant pairs.

Similar to the comparisons within discordant pairs, we then examined average differences in gender nonconformity for concordant pairs. A regression analysis for each sex and age period (childhood and adulthood) predicted gender nonconformity (observer-rated or self-reported) by sexual orientation. Twin pairs and, for observer ratings, individuals were random effects. For childhood, effects of sexual orientation on observer-rated gender nonconformity of males and females, $p = .004$, $\beta = .66$ [.26, 1.06], and $p = .03$, $\beta = .40$ [.05, .74], respectively, were similar to effects on self-reported gender nonconformity, $p = .006$, $\beta = .50$ [.16, .84], and $p = .0008$, $\beta = .61$ [.29, .92], respectively. For adulthood, the effects of sexual orientation on observer-rated gender nonconformity of males and females, $p = .005$, $\beta = .47$ [.17, .77], and $p = .005$, $\beta = .55$ [.20, .91], were somewhat stronger than those on self-reported gender nonconformity, $p = .04$, $\beta = .36$ [.02, .70], and $p = .02$, $\beta = .46$ [.09, .83]. Figure 2.4 is a simple representation of these findings (not accounting for data dependency within twin pairs). This pattern varied from that of identical twins with discordant sexual orientations, who were, at least in their childhood, less different in their observer-rated than self-reported gender nonconformity (Figure 2.2).

We then tested whether discrepancies between observer-rated and self-reported gender nonconformity differed by sexual orientation and across discordant and concordant pairs. Gender nonconformity was predicted by sexual orientation, pair type (discordant or concordant), sex, period (childhood or adulthood), measure (observer ratings or self-report), and their interactions. Twin pairs and individuals were random effects. The two-way interaction of sexual orientation with measure was significant, $p < .0001$, $\beta = .19$ [.16, .23]. This interaction was due to straight twins, in general, reporting less gender nonconformity, compared with how observers rated them,

$p < .0001$, $\beta = -.42$ [-.47, -.36], whereas for non-straight twins this difference was not as pronounced and in the opposite direction, $p = .02$, $\beta = .07$ [.01, .13]. The three-way interaction of sexual orientation, measure, and twin type was not significant, $p = .24$, $\beta = -.02$ [-.06, .02]. Hence, the stronger discrepancy between measures of gender nonconformity in straight twins than non-straight twins was not specific to those who were discordant or concordant.

2.4.4 Hypothesis 2.4

We hypothesized that the correlation of observer-rated gender nonconformity within discordant twin pairs could be similar to the correlation within concordant pairs (even if the average magnitude of the difference were greater for concordant pairs, which was Hypothesis 2.3). To test Hypothesis 2.4, we first computed correlations of gender nonconformity for concordant pairs, in a similar manner to those within discordant pairs (Table 2.2). For concordant pairs, we combined, for each sex, straight and non-straight pairs, because some pair numbers were low (**Error! Reference source not found.**). To ensure that correlations in gender nonconformity were not enhanced by grouping concordant straight and non-straight twins, we partialled out the effect of their sexual orientation (however, first order correlations were not much stronger than these partial correlations).

Within concordant pairs, the correlation of siblings' gender nonconformity was comparable in effect for observer ratings and self-report (Table 2.3). Thus, concordant pairs reported related levels of gender nonconformity, and others judged them as similarly related.

A mixed-factorial regression analysis tested whether discrepancies in the correlations of gender nonconformity depended on measure and twin type. The dependent variable was gender nonconformity of one twin. Independent variables included gender nonconformity of the other twin, measure (observer ratings or self-report), twin type (discordant or concordant), sex, age period, and their interactions. Twins were included as a random effect. Across measure, sex, and age period, the correlation between twins was weaker in discordant pairs than concordant pairs, $p < .0001$, $\beta = .36$ [.27, .46], and $p < .0001$, $\beta = .58$ [.47, .71], respectively. The interaction that tested for the difference in this effect was significant, $p = .005$, $\beta = .11$ [.03, .19]. Another interaction indicated that the correlation in gender nonconformity further depended on the combination of twin type and measure, $p = .004$, $\beta = .07$ [.02, .11]. For interpretation of this interaction see Table 2.2 and Table 2.3. For discordant pairs, correlations in gender nonconformity were, in general, stronger for observer ratings than for self-report (Table 2.2). For concordant pairs, correlations were similar across measures (Table 2.3).

2.4.5 Hypothesis 2.5

We hypothesized that across discordant and concordant twins, higher levels of gender nonconformity relate to recall of increased rejection by others.

Across individual twins of either type or sex, the four measures of gender nonconformity (observer ratings and self-report from childhood and adulthood) were significantly related, even after the effect of sexual orientation was partialled out, p's $\leq .0002$, $.13 < \beta$'s $< .54$, $.06 <$ 95% CI's $< .64$. Further, across measures of gender nonconformity, relationships with recalled rejection were similar in effect. For the sake of simplicity, we used for each twin a composite score of his or her gender nonconformity across measures in the following analyses. Higher scores represented greater gender nonconformity. For each sex and pair type, Cronbach's alpha exceeded .70 for the newly created composite score.

Across all twins, four mixed factorial regression analyses were conducted. Each of the measures of rejection (from mothers, fathers, peers, and overall) was predicted by the twins' gender nonconformity, sexual orientation, twin type, and their interactions. We further tested whether these effects differed by sex. Twin pairs were a random effect.

Table 2.4 shows that in general, greater gender nonconformity was associated with increased rejection. However, the interaction of gender nonconformity with sexual orientation was significant, or close to significant, for maternal rejection, peer rejection, and overall rejection. These interactions indicated that the relationship of gender nonconformity with rejection was stronger in magnitude for straight twins than non-straight twins, on average. For example, for overall rejection, the separate effects for straight and non-straight twins were p = .0003, β = .57 [.27, .87], and p = .02, β = .20 [.02, .38], respectively. There was a further tendency for an interaction among gender nonconformity, sexual orientation, and twin type: the relationship of gender nonconformity with rejection tended to be strongest for straight twins from concordant pairs. For example, for overall rejection the effect for concordant straight twins was stronger than for the combination of all other twins, p = .003, β = .73 [.28, 1.19], and p = .004, β = .26 [.08, .44], respectively. There were no significant sex differences in these effects.

2.5 Discussion

Based on evaluations of photographs, non-straight twins were rated as more gender nonconforming than their identical, but straight, co-twins. This difference emerged in childhood, but later than the difference between concordant non-straight twins and unrelated concordant straight twins, and it was also smaller in magnitude. Twin siblings' levels of observer-rated gender nonconformity were correlated, and the correlation was similar for pairs with discordant and concordant sexual orientations. These patterns were somewhat different for their self-reports, partly because straight twins reported less gender nonconformity than what was observed by raters. Finally, in general, gender nonconformity related to recall of past rejection from others.

2.5.1 Possible Reasons for Differences between Twins

Present findings suggest that identical twins with discordant sexual orientations visibly differ in their gender nonconformity from mid-childhood onwards (Figure 2.1). Factors other than genetics must account for their distinctions. It is possible that these factors are prenatal. Approximately 30% of identical twin pairs develop with different placentas, which could lead to varied hormonal exposure or to different inactivation of otherwise identical genes (Patterson, 2007). Both of these factors could be relevant for the development of different degrees of gender nonconformity and discordant sexual orientations. Regarding hormonal influences, prenatal androgen levels predict postnatal male-typical and female-typical behaviours (Auyeung et al., 2009), and exposure to atypical levels of prenatal androgen potentially increases the likelihood of a same-sex sexual orientation (Hines, 2011). Notably, in females, the digit ratios (a putative marker of prenatal androgen exposure, Grimbos et al., 2010) of non-straight twins appear more male-typical that the digit ratios of their identical, straight co-twins (Hall & Love, 2003; Hiraishi et al., 2012). This finding supports the hypothesis of early hormonal influences on gender-typed traits and sexual orientation.

Regarding the inactivation of genes, this can happen through methylation of specific DNA, which affects one twin but not the identical co-twin. Due to such epigenetic differences, identical twins can develop discordant morphological features or vulnerabilities to diseases (Fraga, 2005). Similarly, epigenetics could modify the genome of each twin differently during gestation, leading to discordant sexual orientations and gender behaviours. There is no full support for this hypothesis, although preliminary finings are consistent with it (Ngun & Vilain, 2014).

Different social influences could also lead to discordant sexual orientations and gender behaviours in identical twins. However, evidence for socialization effects on the origin of sexual orientation is weak, and because homosexuality and gender nonconformity are usually discouraged, it is unlikely that the social environment is causing them (Bailey et al., 2016; Rahman, 2005). Our data suggests that, across all twins, gender nonconformity related to self-reports of past rejection from others (Table 2.4). In this sense, it is unlikely that the social environment encouraged one twin over the other to become gender nonconforming (or, subsequently, non-straight). However, rejection from others was more strongly related to gender nonconformity of those who became straight than those who became non-straight. It is difficult to understand how this finding might relate to the development of discordant twins, since it seemed to be somewhat driven by the effect found in concordant straight twins. Yet, some speculation might be insightful. One previous study suggested that boys who were particularly gender nonconforming had mothers who were more tolerant of these behaviours (Green, 1987). Perhaps, in some cases, social reactions to gender nonconformity are less negative, for example, if parents conclude that they cannot change these behaviours because they sense that the child will become non-straight. In contrast, children who are expected to become straight and to follow traditional gender norms may experience harsher reactions if they exhibit gender nonconformity. Such differential reactions could foster gender nonconformity in children who become non-straight, whereas they might suppress it in children who become straight. The suggestion that reactions to gender nonconformity are more severe for those who become straight could also explain the finding that childhood gender nonconformity is a stronger predictor of depression in straight groups than non-straight groups (Roberts, Rosario, Slopen, Calzo, & Austin, 2013).

2.5.2 Possible Reasons for Similarity of Twins

Identical twins with discordant sexual orientations were in two respects similar in their observer-rated gender nonconformity. First, their differences were generally smaller, compared to the difference between concordant straight and unrelated concordant non-straight twins (Figure 2.1 & Figure 2.3). Secondly, discordant twins correlated, in general, in their level of observer-rated gender nonconformity (Table 2.2). That is, although non-straight twins were more gender nonconforming overall, there was variability in each group; those non-straight twins who were more gender nonconforming than other non-straight twins tended to have straight co-twins who were more gender nonconforming that other straight co-twins.

Variations in genes contribute modestly (but not fully) to the covariance of sexual orientation with gender nonconformity (Alanko et al., 2010; Burri et al., 2011). Thus, the twins' shared genes could make them similar in their gender nonconformity, but this must not result in identical sexual orientations. Another hypothesis is that atypical androgen levels during early gestation affect variations in sexual orientation, whereas atypical androgen levels during later gestation affect the degree of gender nonconformity (Bailey & Zucker, 1995). It is possible that different exposures to androgens during early development resulted in the twins' discordant sexual orientations, whereas similar exposure to androgen during later development, in addition to shared genes, yielded a similarity in their gender nonconformity.

Socialization could also contribute to similarity in gender behaviour. Identical twins possibly share environmental influences that are unique to their twinship (Iervolino et al., 2005). For example, parents may emphasize their similarity, or twins themselves may coordinate their appearances (e.g., by wearing similar clothing) and behaviours (Kendler & Gardner, 1998; Rose, Kaprio, Williams, Viken, & Obremski, 1990). Perhaps, then, one twin who was gender nonconforming in childhood, and became non-straight, influenced the straight co-twin to be more gender nonconforming; and vice versa, the straight twin affected the non-straight co-twin to express less gender nonconformity. Or, peers may treat the twins similarly in childhood, especially if they are unable tell them apart, and this similar treatment may elicit similar behaviours. All such influences could result in a reduced distinction between the twins and a stronger coordination of their gender behaviours.

Discordant twins differentiated significantly in their gender nonconformity between ages 6 and 8, and later than straight versus non-straight concordant pairs, who differentiated between ages 1 and 3 (Figure 2.1 & Figure 2.3). This is consistent with the hypothesis that identical twins with discordant sexual orientations are more similar to each other than unrelated individuals. Yet, one must be careful in drawing conclusions about the exact ages for these differentiations. First, in discordant pairs, the confidence intervals for these differentiations included the age of 3, the suggested age for such differentiation between unrelated straight and non-straight individuals (Li et al., 2017; Rieger et al., 2008). Second, for male concordant pairs, this differentiation in gender nonconformity was earlier than the suggested age of 3. Whether this unexpectedly early differentiation is robust is unclear, and any interpretation needs to take this into account.

2.5.3 Observer Ratings and Self-Report

The discussion thus far has focused on observer ratings of gender nonconformity. Findings were somewhat different for self-reported gender nonconformity, which showed larger differences between discordant twins, and were less correlated within discordant pairs, compared with observer ratings. These findings for self-report were comparable to previous results based on self-reports from discordant twins (Bailey & Pillard, 1991; Bailey et al., 1993). Within discordant pairs, average discrepancies between observer ratings and self-report were more pronounced in straight than non-straight twins (Figure 2.2). Because these straight twins have closely related siblings (i.e., twins) who are non-straight, it is tempting to suggest that, perhaps unaware to them, they have a predisposition to a same-sex orientation that has not fully developed, but is expressed in its correlate - their visible gender nonconformity. However, this discrepancy between observer ratings and self-report was also found among concordant straight twins. For straight twins in concordant pairs, this difference by measure cannot be reasonably explained based on an unaware predisposition to a co-twins' non-straight sexual orientation, since their co-twins identify as straight too. A more reasonable interpretation is that straight participants, independent of whether they had non-straight relatives, were less likely than non-straight participants to realize their visible degree of gender nonconformity (Gottschalk, 2003) or to acknowledge it. Another possible explanation for the discrepancy between observer ratings and self-report is that a twin might, in part, see the co-twin as a point of comparison. Thus, a straight twin in a discordant pair might see him- or herself as especially gender conforming because the co-twin's gender nonconformity provides a salient contrast. However, this explanation would not apply to concordant straight twins.

2.5.4 The Utility of Photographs

The present study used an uncommon method to assess gender nonconformity – via the evaluations of photographs. A concern may be that photographs do not encompass all facets of perceivable gender nonconformity, which include appearance, movements, voice patterns, interests, and activities (Rieger et al., 2010). The images used did not provide sound, and movements or activities, if noticeable, were static. For this reason, we had also asked twins to provide videos. However, only one pair provided one video from adulthood, whereas all twins readily provided photographs taken at several ages. We compensated for this potential loss of information in photographs by asking participants for several photographs across the ages. In fact, twins provided, on average, almost five times more photographs from their childhood (12.00 per participant, on average) than participants provided childhood videos in a previous study (2.50 per participant, on average, Rieger et al., 2008). Likewise, in adulthood, the average number of photographs (11.67) greatly exceeded the one video taken from each participant in the previous study. Hence, the several photographs of the same twin potentially increased the chance to detect reliable signs of his or her gender nonconformity.

Moreover, facets of gender nonconformity (appearance, movements, voice patterns, interests, and activities) are modestly related (Rieger et al., 2010). Perhaps for this reason (in addition to the several photographs per participant) present effects of sexual orientation on observer-rated gender nonconformity, using photographs, were similar to the previously reported effects using ratings of videos (which included sound and movements, Rieger et al., 2008). For example, for unrelated straight and non-straight individuals, the main effect of sexual orientation on observer-rated gender nonconformity from ages 0 to 15 was comparable, $p = .001$, $\beta = .37$ [.17, .59], and $p < .0001$, $\beta = .38$ [.23, .53], respectively.

Furthermore, the Internet made it easy to share photographs, without twins being required to visit the lab or use physical mail. This was a true benefit since this research focused on a rare group, with most twins living far from the lab, and we wanted to minimize attrition. In sum, we believe that evaluations of photographs offer an effective mechanism for studying the link of sexual orientation with observable gender behaviour.

2.5.5 Limitations

A limitation of the present study was the small number of male twins who were concordant straight (4 pairs). Statistically, several hypothesized effects involving these twins were detected. Moreover, comparisons of such twins with discordant twins have not been made before. In this respect, the inclusion of these twins was informative, despite their low numbers. However, one should consider why their numbers were low. In our experience, straight males are less interested in participating in research on sexual orientation, compared with other groups. Furthermore, with recruitment methods like ours (e.g., advertising for research on sexual orientation on gay news sites) straight twins are less easily reached than non-straight twins (Bailey et al., 2016). We aimed to compensate for this by further recruitment via a twin registry and social media sites, but here, too, the advertisement pointed to a study on sexual orientation. In discordant pairs, non-straight twins may have motivated the straight co-twins to participate. In concordant straight pairs, encouragement by a co-twin seems less likely. This limitation could be overcome with deception (e.g., by advertising broadly for research on twins without disclosing the nature of the study). Yet, this would likely yield small numbers of the rarer groups, concordant-non-straight and discordant twins. Ethically, this approach is also problematic, especially when participants share personal information, including photographs.

Another limitation is a potential participant bias in selecting photographs. In order to minimize the risk that such bias affected findings, we had encouraged participants to send us any photographs, regardless of quality and content, and we did not explicitly tell participants that this study is on the relationship of gender nonconformity with sexual orientation. Because the method confirmed the overall relationship of gender nonconformity with sexual orientation, similar to previous studies using self-report, cross-cultural comparisons, or prospective designs (Lippa, 2008; Steensma et al., 2013), we think that self-selection of photographs did not strongly affect general findings. Still, we cannot rule out that participants selected photographs that they preferred over others, and this could have affected results in some unknown ways.

2.6 Conclusion

Identical twins with discordant sexual orientations visibly differed in their gender nonconformity, starting in childhood. Moreover, the method used - the evaluation of gender-related characteristics seen in photographs - pointed to subtle similarities of these twins, possibly because of shared influences. With the collection gathering of data through twin registries, it may become possible to examine these patterns longitudinally in more detail and to identify factors that affected the twins' similar and dissimilar development. Thus, further studies of twins with discordant sexual orientations, which go beyond self-report, will provide a unique window into the developmental of sexual orientation.

Table 2.1 - Distribution of Numbers, Age, and Ethnicities by Twin Type (Twins with Discordant or Concordant Sexual Orientations) and Sex.

Males	Discordant	Concordant Straight	Concordant Non-Straight
Number of Pairs	24	4	19
Average Age	31.08 [26.21, 35.96]	23.00 [20.40, 25.60]	31.42 [26.71, 36.13]
Percentage Caucasian	85 [67.52, 94.08]	50 [15.00, 84.99]	82 [58.97, 93.81]
Females	**Discordant**	**Concordant Straight**	**Concordant Non-Straight**
Number of Pairs	32	11	8
Average Age	29.38 [26.10, 32.65]	28.45 [23.62, 33.29]	27.75 [20.60, 34.90]
Percentage Caucasian	91 [77.04, 96.95]	100 [75.75, 100.00]	70 [39.68, 89.22]

Note. Units are pairs. Numbers in brackets are 95% confidence intervals.

Table 2.2 - Correlations within Discordant Twin Pairs for Observer-Rated and Self-Reported Gender Nonconformity in Childhood and Adulthood

Measure	Discordant Males	Discordant Females
Observer-rated Childhood	.70*** [.52, .87]	.54*** [.37, .71]
Observer-rated Adulthood	.63* [.22, 1.03]	.17 [-.19, .54]
Self-Reported Childhood	-.18 [-.62, .26]	.12 [-.24, .49]
Self-Reported Adulthood	.00 [-.45, .46]	.42* [.08, .76]

Note. Numbers are standardized regression coefficients, *β's*, with 95% confidence intervals in brackets. For observer ratings, twins were included as a random effect to account for repeated measures within pairs. Higher scores indicate stronger correlations (technically, regression coefficients) of gender nonconformity between twins. *$p < .05$. ***$p < .0001$.

Table 2.3 - Correlations within Concordant Twin Pairs for Observer-Rated and Self-Reported Gender Nonconformity in Childhood and Adulthood

Measure	Concordant Males	Concordant Females
Observer-rated Childhood	.57*** [.38, .76]	.67*** [.50, .84]
Observer-rated Adulthood	.55** [.24, .89]	.71* [.21, 1.20]
Self-Reported Childhood	.56* [.15, .98]	.58** [.16, .99]
Self-Reported Adulthood	.41† [-.09, .91]	.46* [.01, .92]

Note. Numbers are standardized regression coefficients, *β's*, with 95% confidence intervals in brackets. Concordant straight and concordant non-straight pairs are combined and the effect of sexual orientation is partialled out. For observer ratings, twins were included as a random effect to account for repeated measures within pairs. Higher scores indicate stronger correlations (technically, regression coefficients) of gender nonconformity between twins. †$p < .10$. *$p < .05$. **$p < .001$. ***$p < .0001$.

Table 2.4 - Multiple Regression Analyses for Gender Nonconformity, Sexual Orientation, and Twin Type predicting Degree of Rejection across 196 Individuals

Measure	Maternal Rejection[1]	Paternal Rejection[1]	Peer Rejection[1]	Overall Rejection[1]
Gender Nonconformity (GN)[2]	.33 [.11, .55]*	.21 [.00, .43]*	.38 [.18, .59]***	.40 [.21, .60]***
Sexual Orientation (SO)[3]	-.08 [-.29, .13]	.04 [-.17, .26]	-.14 [-.34, .06]	-.08 [-.27, .12]
GN X SO	-.19 [-.42, .03]†	-.17 [-.39, .05]	-.29 [-.49, -.08]*	-.26 [-.45, -.06]*
Twin Type (TT)[4]	-.11 [-.33, .12]	.04 [-.2, .29]	.12 [-.1, .34]	.04 [-.18, .26]
GN X TT	.04 [-.18, .27]	.09 [-.14, .32]	.17 [-.05, .38]	.11 [-.09, .32]
SO X TT	-.10 [-.32, .12]	-.10 [-.33, .13]	-.10 [-.31, .11]	-.11 [-.31, .10]
GN X SO X TT	-.09 [-.33, .15]	-.14 [-.37, .09]	-.23 [-.45, -.01]*	-.20 [-.41, .01]†

Note. R^2*'s* for the four models are .12, .10, .18, and .16, respectively. Numbers are standardized regression coefficients, β*'s*, with 95% confidence intervals in brackets. [1]Higher scores indicate more rejection. [2]Higher scores indicate more gender nonconformity, derived from a composite across all measures. [3]A score of 0 indicates "straight," 1 indicates "non-straight". [4]A score of 0 indicates "discordant," 1 indicates "concordant." Moderations by sex were not significant and are not shown. Twin pairs were a random effect. †$p < .10$. *$p < .05$. ***$p < .0001$.

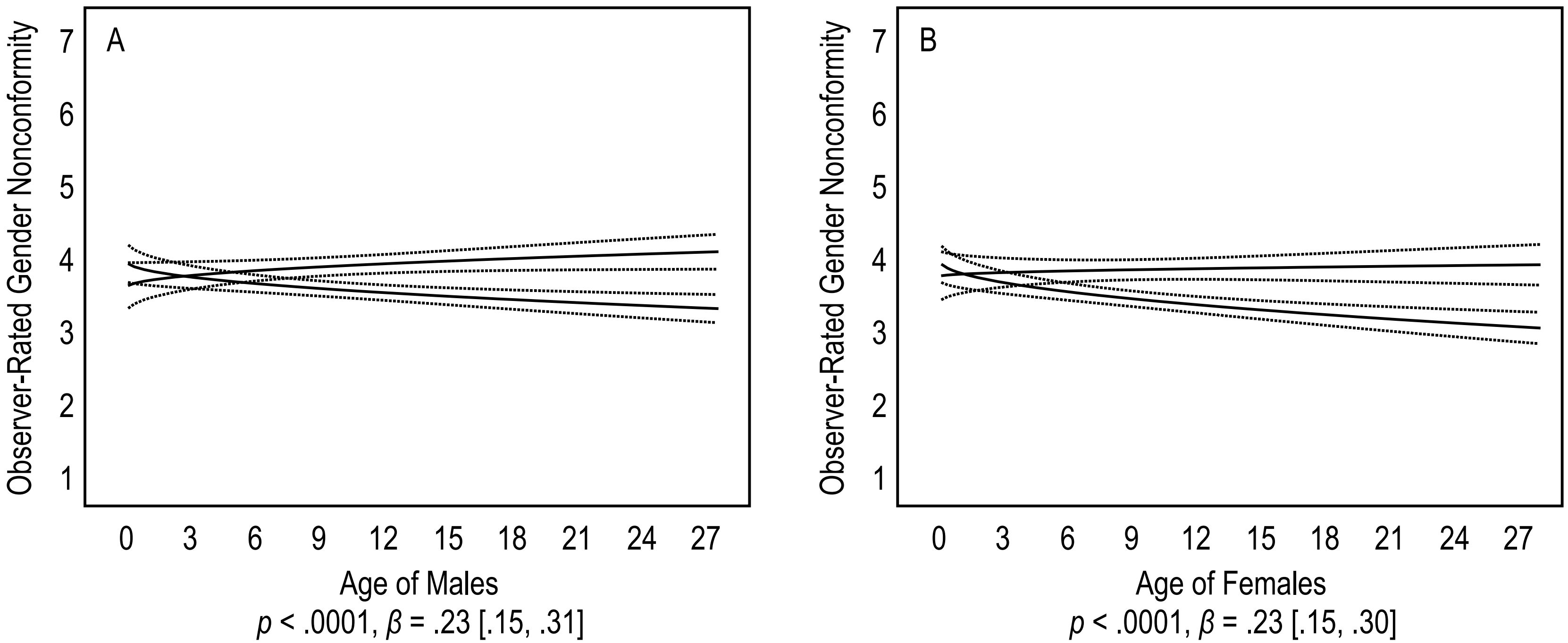

Figure 2.1 - Observer-rated gender nonconformity from photographs of A) 24 male twin pairs and B) 32 female twin pairs with discordant sexual orientations. For each photo, ratings of gender nonconformity were averaged across raters. Upper and lower triple-lines represent regression coefficients with 95% confidence intervals for non-straight twins and straight co-twins, respectively. The x-axis represents the twins' age. On the y-axis, 1 is the least gender-nonconforming score, and 7 is the most gender-nonconforming score. Estimates are restricted to the age of 0 or older. Statistics represent the main effect of sexual orientation on gender nonconformity.

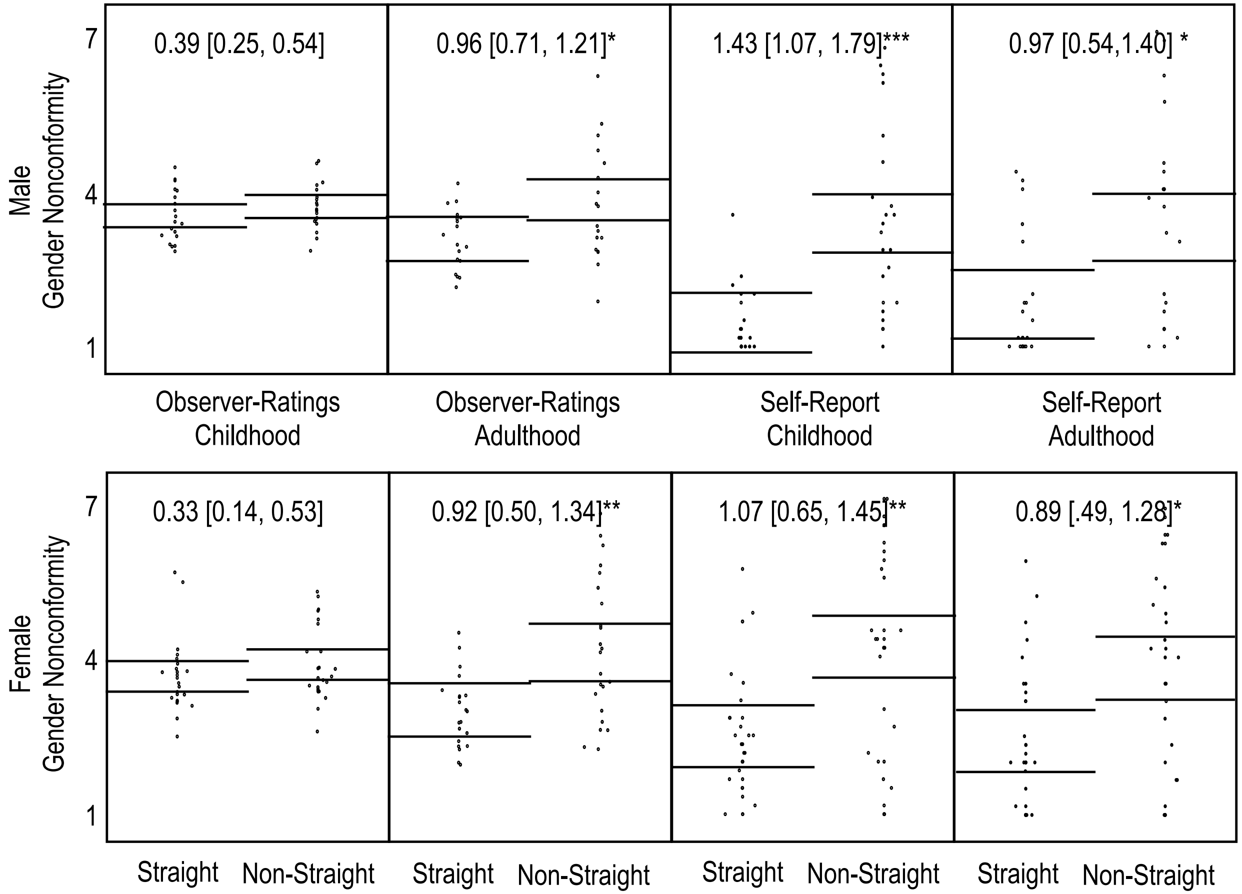

Figure 2.2 - Observer-rated and self-reported gender nonconformity of 24 male and 32 female twin pairs with discordant sexual orientations. Dots represent gender nonconformity of individual twins, averaged either across all ratings or across their self-report scores. Lines are the means' 95% confidence intervals. On the y-axis, 1 is the least gender-nonconforming and 7 the most gender-nonconforming score. Numbers represent Cohen's *ds* with their 95% confidence intervals. * $p < .05$. ** $p < .001$. *** $p < .0001$.

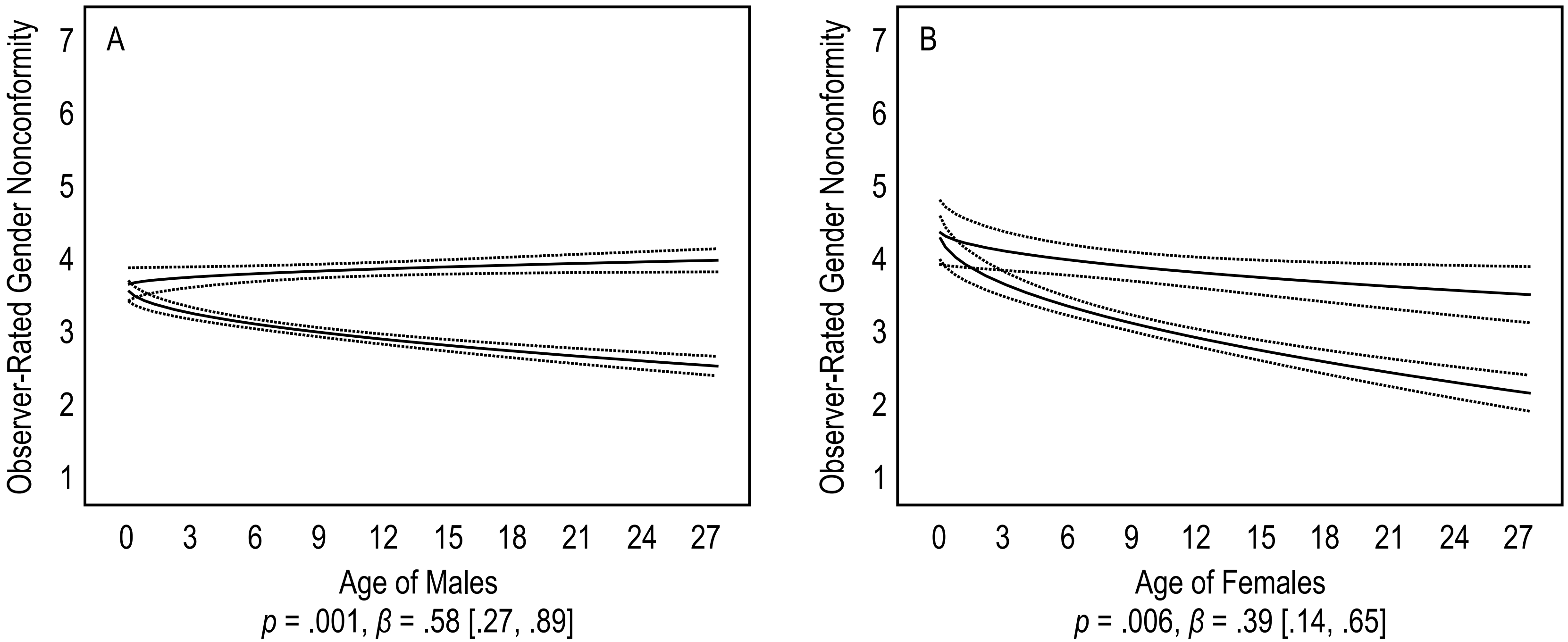

Figure 2.3 - Observer-rated gender nonconformity from photographs of A) 23 male twin pairs and B) 19 female twin pairs with concordant straight or concordant non-straight sexual orientations. For each photo, ratings of gender nonconformity were averaged across raters. Upper and lower triple-lines represent regression coefficients with 95% confidence intervals for non-straight twins and unrelated straight twins, respectively. The x-axis represents the twins' age. On the y-axis, 1 is the least gender-nonconforming score, and 7 is the most gender-nonconforming score. Estimates are restricted to the age of 0 or older. Statistics represent the main effect of sexual orientation on gender nonconformity.

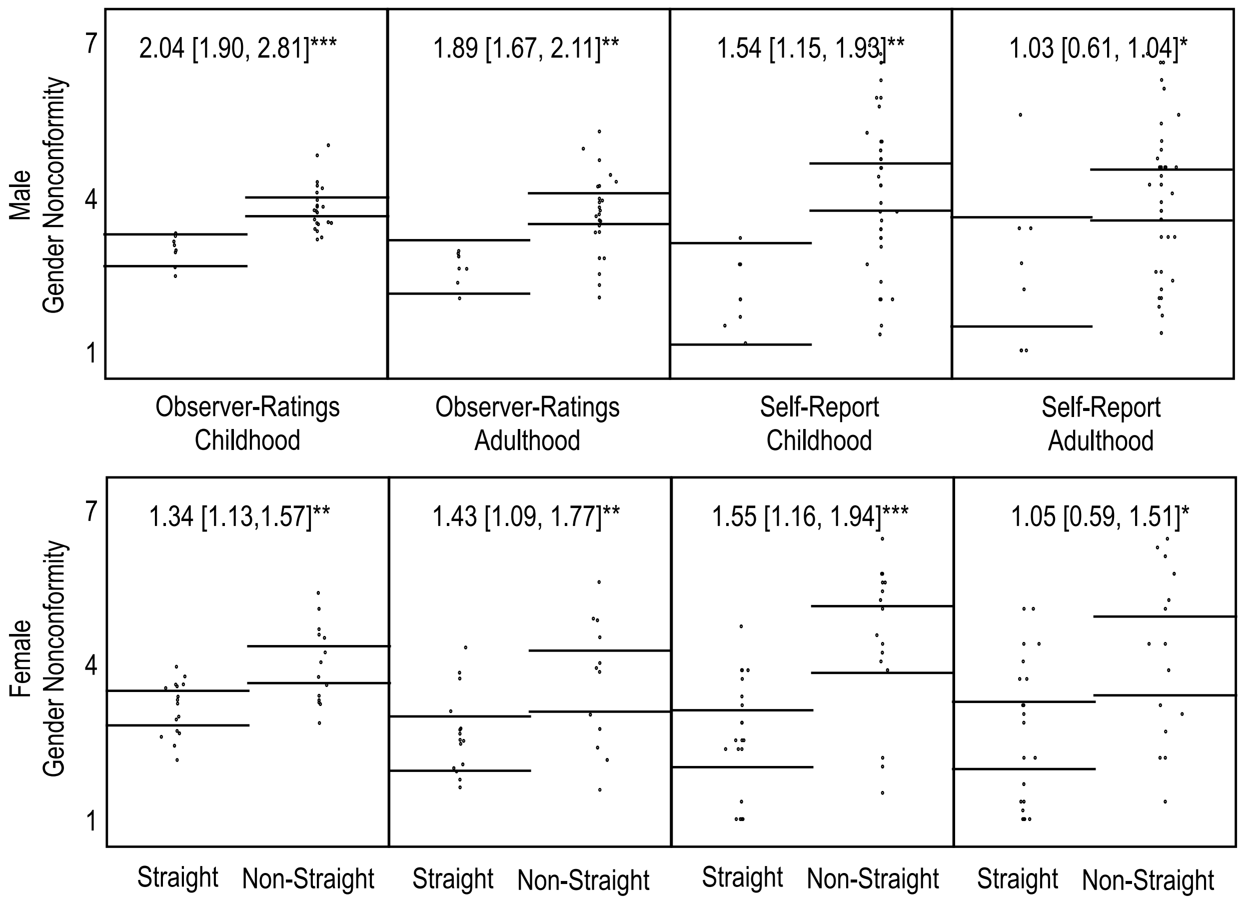

Figure 2.4 - Observer-rated and self-reported gender nonconformity of 23 male and 19 female twin pairs with concordant straight or concordant non-straight sexual orientations. Dots represent gender nonconformity of individual twins, averaged either across all ratings or across their self-report scores. Lines are the means' 95% confidence intervals. On the y-axis, 1 is the least gender-nonconforming and 7 the most gender-nonconforming score. Numbers represent Cohen's *ds* with their 95% confidence intervals. * $p < .05$. ** $p < .001$. *** $p < .0001$.

Chapter 3 Physiological Sexual Arousal of Identical Twins with Discordant Sexual Orientations

3.1 Abstract

Genetically identical twins can differ in their self-reported sexual orientations; however, it is unclear to what degree these self-assessments reflect observable differences between twins. Such differences include their genital arousal or pupil dilation patterns while viewing sexual stimuli. The present study investigated these responses in 6 male pairs and 9 female pairs with discordant sexual orientations. Straight males responded more strongly to females over males; their non-straight co-twins responded more strongly to males over females. Female twins responded approximately equally to both sexes, albeit lesbians somewhat more to females over males. These differences within twin pairs in their physiological sexual responses were similar in direction and magnitude to analogous differences previously assessed in unrelated straight and non-straight men and women. Furthermore, there were no consistent correlations between twins and their co-twins in either their genital arousal or pupil dilation to one sex over the other. Hence, factors other than genetics likely contribute to different physiological expressions of sexual orientation in identical twins.

3.2 Introduction

Physiological measures of an individuals' sexual response include genital arousal and pupil dilation to sexual stimuli (Chivers et al., 2004; Rieger et al., 2015; Rieger & Savin-Williams, 2012a). Both measures are based on automatic responses and do not rely on self-report that is prone to several biases in the context of sexuality research (Friedman et al., 2004; Ragins et al., 2007). For example, in previous work involving identical twins with discordant sexual orientations, straight twins appeared to under-report their degree of gender nonconformity (a correlate of a homosexual orientation) as compared with how others viewed them (Watts, Holmes, Raines, Orbell, & Rieger, 2017a). In a similar manner, measures of physiological sexual arousal may reveal more effectively than self-report how different identical twins with different sexual orientations actually are. Thus, the following study investigates patterns of sexual arousal within pairs based of identical twins, with one identifying as straight and the other identifying as non-straight (gay, lesbian or bisexual) by using measures of genital arousal and pupil response.

3.2.1 Differences in Sexual Arousal within Discordant Twin Pairs

The majority of men display genital arousal patterns that are consistent with their self-reported sexual orientation (Chivers et al., 2004; Chivers et al., 2007; Rieger et al., 2015; Rieger et al., 2013; Sakheim et al., 1985). That is, straight men almost exclusively respond to females over males whereas gay men respond almost exclusively to males over females. Similarly, straight men show increased pupillary response to stimuli showing females over males as compared to gay men who show increased pupil dilation to males over females. Bisexual men show a more variable pattern of arousal, but respond on, average, more like gay men than straight men (Rieger et al., 2015; Rieger & Savin-Williams, 2012a). In women, these sexual responses (either genital arousal or pupil dilation) correspond less consistently with self-reported sexual orientation, compared with men. This is so because straight, bisexual and lesbian women show substantial sexual arousal to both male and female sexual stimuli. One qualification of this pattern is that lesbians and bisexual women show marginally more sexual response to females over males, whereas approximately equal arousal to both sexes is detected in straight women (Chivers et al., 2007; Rieger et al., 2016). In this sense, non-straight women can sometimes be more male-typical than other women, because responding in a way that is consistent with self-reported sexual orientation is usually seen in men.

In the present study, both genital arousal and pupil dilation to sexual stimuli were assessed in discordant twin pairs. If the responses of these twins are like those of unrelated individuals, then, within pairs of male twins, straight male twins will show substantially stronger genital arousal and pupil dilation to female than male sexual stimuli and their non-straight co-twins will display stronger responses to male than female stimuli (Hypothesis 3.1). Within pairs of female twins, straight and non-straight female twins will show similar response to male and female stimuli; however, lesbians may show somewhat stronger responses to females than males (Hypothesis 3.2).

3.2.2 Correlation of Sexual Arousal Within Discordant Twin Pairs

Based on previously observed patterns of sexual arousal in males and females, a simple model assumes that identical twins with discordant sexual orientations differ in their arousal in much the same way as unrelated individuals. However, there could be familial, if not genetic, effects on sexual arousal, which yield similar responses in sexual arousal of twins, even if they have different sexual orientations. Identical twins are more likely to be similar on several measures of autonomic response, such as vasomotor persistence time, respiration and heart rate, compared with both siblings who are not twins and with unrelated individuals. Together these responses can be thought of as an individual's "autonomic constitution", which may be partly accounted for by genetics (Jost & Warren Sontag, 1944; Piha et al., 1994). Because pupillary and genital responses reflect activity of the autonomic nervous system (Bradley et al., 2008; Lang & Bradley, 2010; ten Donkelaar et al., 2011), and if such activity is partly driven by genetic influences (Piha et al., 1994), one could speculate that within pairs of identical twins discordant for sexual orientation, average differences in sexual arousal may be reduced, compared with such arousal patterns of unrelated straight and non-straight individuals.

Further, regardless of any average differences between these twins, there may exist a correlation in the twins' sexual response. This could be conceptualised in two different ways. Firstly, straight twins could resemble their non-straight co-twins with respect to the magnitude of their sexual response to their preferred sex. That is, straight twins who respond more strongly to the other sex than the same sex (as compared to other straight individuals) may have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals (Hypothesis 3.3). That pattern would create a correlation of arousal to the other sex over the same sex in straight twins with arousal to the same sex over the other sex in non-straight twins.

Alternatively, there may be a correlation in the twins' degree of sexual arousal to one sex over the other. That is, straight twins, who have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals), may also respond more strongly to the same sex than the other sex (as compared to other straight individuals; Hypothesis 3.4). That pattern would create a correlation of arousal to the same sex over the other sex in straight twins with arousal to the same sex over the other sex in non-straight twins. This could point to a predisposition for traits associated with homosexuality within the straight twin.

3.2.3 The Present Study

In the present study investigated differences in sexual arousal within identical twins with discordant sexual orientations by measuring their genital arousal and pupil dilation to explicit sexual stimuli. In sum, the following hypotheses were tested:

Hypothesis 3.1. In male pairs, straight twins will show substantially more sexual arousal to females over males, whereas their non-straight co-twin shows show substantially more sexual arousal to females over males.

Hypothesis 3.2. In female pairs, straight twins will be similar to their non-straight co-twins in their response to male and female sexual stimuli; however, lesbians may show somewhat stronger responses to females than males.

Hypothesis 3.3. Straight twins who respond more strongly to the other sex than the same sex (as compared to other straight individuals) may have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals).

Hypothesis 3.4. Alternatively, straight twins, who have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals), may also respond more strongly to the same sex than the other sex (as compared to other straight individuals).

3.3 Method

3.3.1 Recruitment and Participants

Twins. The University of Essex Ethics Committee approved this study. Advertisements for identical twins to participate in a study on sexual orientation were placed in the newsletter of the Department of Twin Research at Kings College London, on social media sites, and on three online sites for gay men and lesbians (Gay Star News, Pink News, and Gay Times). We further recruited twins at three Pride festivals. Each twin who contacted us was encouraged to recruit the co-twin. Recruited twins self-identified as straight, bisexual, gay or lesbian. They were asked twice during the study about their sexual identities, and all responses were consistent.

Based on the available literature comparing straight and non-straight participants, sexual orientation differences in sexual arousal (depending on measure and participant's sex) have an effect size d exceeding 1.0. In this case a power of .8 can be obtained with 20 pairs, even if these measures are uncorrelated across twin pairs. If, due to familiarity, a small correlation of $r = .3$ is assumed across pairs, a sexual orientation difference with the effect $d = 1.0$ can be obtained with 15 pairs.

Participants included 6 twin male pairs and 9 female twin pairs, yielding a total of 15 pairs of identical twins discordant for sexual orientation or 30 individuals. The number of bisexual women (2 individuals) was low relative to the number of straight and lesbian women (9 and 7). Furthermore, these bisexual women reported a stronger preference for females over males; thus they were grouped with lesbians into "non-straight". Although there were no bisexual males in the present sample, gay male twins were still labelled as "non-straight" for consistency with females. Distributions of age and ethnicity are shown in Table 3.1.

3.3.2 Measures

Twin zygosity. In addition to asking twins whether they were identical, five standardized items about physical and visual similarity were administered to establish zygosity (Kasriel & Eaves, 1976). An example question is "During childhood, could you ever have fooled friends by pretending to be your twin?" Each item was assessed on a scale ranging from 1 to 3, with lower scores reflecting higher similarity within twin pairs.

Stimuli. Three-minute videos of 3 male stimuli and 3 female stimuli were used for the study. Videos were audible and had similar content (i.e., a naked person in a bedroom) and showed either a male or female model masturbating. Six 2-minute videos were taken from a nature documentary. These were used for assessing baseline genital responses, facilitating participants' return to an unaroused level. Although these contained nonsexual content, the somewhat engaging nature of their content could elicit pupillary responses. For this reason, for pupil data, two 2-minute animations of clouds were used for assessing baseline. All videos were of similar luminance; furthermore, luminance was set to equal upper and lower thresholds across stimuli by using the programs MPEG Streamclip and Final Cut Pro. Videos had a resolution of 768 by 536 pixels, and were presented full screen.

Genital data. Genital responses were recorded every 5 milliseconds using a BIOPAC MP100 data acquisition unit and the program AcqKnowledge. An indium/gallium strain gauge measured changes in penile circumference while viewing stimuli. The signal was sampled at 200 Hz, low-pass filtered to 10 Hz and digitized with 16 bits resolution. Gauges were calibrated over six 5-mm steps before sessions and signals were transformed into millimetres of circumference. Women's genital arousal was assessed via change in vaginal pulse amplitude, using a vaginal photoplethysmograph. The amplitude signal was sampled at 200 Hz, and highpass filtered at 0.5 Hz with 16 bits resolution. Amplitude was measured as peak-to-trough amplitude for each vaginal pulse. Vaginal pulse amplitude signals exhibit both convergent and discriminant validity of female sexual response (Suschinsky et al., 2009).

Pupil data. Pupil dilation to sexual stimuli reflects sex and sexual orientation differences in genital arousal, suggesting it is a valid indicator of sexual response (Hess et al., 1965; Rieger et al., 2015; Rieger & Savin-Williams, 2012a). A SR Research Remote infrared gaze tracker recorded pupil data every millisecond with a 35 mm lens focused on participants' preferred eye. The program EyeLink computed pupil area as the number of the tracker's camera pixels occluded by the infrared light reflected by pupil. If pupils dilated while viewing stimuli, more pixels were occluded. The program Python was used for all data processing. Because raw pupil area data included "0's" that represented missing values, for example from blinks or head movements, these values were removed prior to further analyses.

The assessment of pupil dilation in addition to genital arousal allowed minimizing potential drop out rates. For instance, if participants opted out of the more intrusive measurement of genital arousal, they still had the option to have their responses assessed via pupil dilation. Of the 6 male pairs, 1 pair opted to provide pupil dilation measures only, the remaining 5 pairs agreed on both measures. Of the 9 females pairs, 3 pairs opted to provide pupil dilation measures only, 6 pairs agreed on both measures. Thus, the majority of twin pairs provided both measurements.

3.3.3 Procedure

Participants provided written informed consent and were seated in a dimly lit booth facing a screen with resolution of 1024 by 768 pixels. For calibration of their pupil data, participants fixated and re-fixated their gaze on 9 points that defined the outline of the screen. Next, in privacy, males placed the gauges midway around their penises and females inserted the photoplethysmograph. Eye movements were then remotely recalibrated from the control room. Participants were instructed to watch all videos carefully, regardless of whether they liked the content. While viewing stimuli they were free to watch whatever part of the video, as long as they kept their eyes on the screen. First, participants watched an animation of clouds followed, in random order, by presentations of sexual stimuli alternating with nature scenes. Ratings of subjective arousal followed after watching sexual stimuli. The final video was the second animation of clouds. The procedure took 45 minutes per twin.

Genital data and pupil data were analysed in a way that have previously yielded reliable results (Rieger et al., 2015). For each participant, these data were averaged for each stimulus. For genital data, average response to the 10 seconds preceding a stimulus (at which time baseline for sexual stimuli was established) was subtracted from the average response to this stimulus. For pupil data, average responses to the animations of clouds were subtracted from average responses to all other stimuli. Resulting change scores where standardized by computing z-scores within participants. We then computed, for each participant, average values reflecting genital response and pupil response, respectively, to male stimuli and female stimuli.

These averages were then used to create a contrast score for each participant, which represents the difference in sexual arousal to males over females. Positive scores represented more arousal to male stimuli and negative score represented more arousal to female stimuli.

3.4 Results

3.4.1 Hypothesis 3.1

Hypothesis 3.1 states that in male pairs, non-straight twins will show substantially more sexual arousal to males over females, whereas their straight co-twin shows show substantially more sexual arousal to females over males.

A mixed factorial regression analysis was conducted separately for each measure (genital arousal and pupil dilation), where the dependent variable was response to males over females and the independent variable was sexual orientation as a fixed effect. Twin pairs were included as random effects to account for dependency on the data. With respect to genital arousal, straight twins showed significantly more response to females over males than their non-straight co-twins, and vice versa, $p = .01$, β [95% CI]= .91 [.42, 1.68]. Similarly, with respect to pupil dilation, sexual orientation significantly predicted response to one sex over the other, $p = .02$, $\beta = .67$ [.13, 1.04], whereby straight twins showed significantly more pupil dilation to females over than their non-straight co-twins and vice versa. Figure 3.1 illustrates these differences between twins on a group level, and by relating the response of each twin to the response of the co-twin.

3.4.2 Hypothesis 3.2

It was hypothesized that in female pairs, straight twins will be similar to their non-straight co-twins in their response to male and female sexual stimuli; however, lesbians may show somewhat stronger responses to females than males. In the case of two individual female twins, their co-twins were not able to take part in the study. The responses of the missing twins within pairs were imputed based on the correlations of all other twins' sexual responses with their sexual orientations. Analyses were conducted twice, one time with excluding incomplete pairs and then again with imputed data for the missing twins. In both cases results were comparable in effect. The latter are reported throughout this paper.

A mixed factorial regression analysis was conducted separately for each measure measure (genital arousal and pupil dilation), where the dependent variable was response to males over females and the independent variable was sexual orientation as a fixed effect. Twin pairs were included as a random effect. With respect to genital arousal, both straight and non-straight females responded similarly to male and female stimuli, although lesbians somewhat more so to the females than males, and the difference was not trivial in effect, $p = .11$, $\beta = -.60$ [-1.35, .20]. With respect to pupil dilation, sexual orientation significantly predicted response, $p = .01$, $\beta = -.46$ [-.79, -.12], whereby non-straight twins showed significantly more pupil dilation to females over males than their straight co-twins.

Figure 3.2 illustrates these differences between twins on a group level, and by relating the response of each twin to the response of the co-twin. Across both measures, straight twins responded approximately equally to male and female stimuli, on average, whereas their non-straight co-twins responded more strongly to females than to males. These patterns are similar to those seen in previous work (Rieger et al., 2015). When data for the present sample were reanalyzed treating twins as unrelated individuals, in males, sexual orientation effects on genital arousal and pupil dilation, $p = .0003$, $\beta = .91$ [.66, 1.44,], and $p < .02$, $\beta = .67$ [.13, 1.04], were comparable with those previously found in unrelated straight and non-straight men, $p < .0001$, $\beta = .84$ [.74, .95], and $p < .0001$, $\beta = .74$ [.58, .90], respectively. For females, the effects of sexual orientation on genital arousal and pupil dilation, $p = .04$, $\beta = -.60$ [-1.11, -.04], and $p = .05$, $\beta = -.46$ [-.92, .01], were somewhat stronger than those previously found, although present confidence intervals still included those previously found effects, $p = .08$, $\beta = -.21$[-.44, .03], and $p = .05$, $\beta = -.23$ [-.43, .00].

3.4.3 Hypotheses 3.3 and 3.4

Hypothesis 3.3 states that straight twins who respond more strongly to the other sex other sex than the same sex (as compared to other straight individuals) may have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals. Alternatively, Hypothesis 3.4 states that straight twins, who have non-straight co-twins who respond more strongly to the same sex than the other sex (as compared to other non-straight individuals), may also respond more strongly to the same sex than the other sex (as compared to other straight individuals. Confirming Hypothesis 3.3 would result in a negative correlation of arousal to males versus females within pairs of twins. Confirming Hypothesis 3.4 would result in a positive correlation of arousal to males versus females within pairs of twins within pairs. Both hypotheses were tested simultaneously. For each measure (genital response or pupil dilation), the dependent variable included the arousal contrast scores of the non-straight twins, and the independent variable was the arousal contrast score of the straight co-twins.

Figure 3.3 and Figure 3.4 show that in general, there were inconsistent correlations between twins with respect to either pupil dilation or genital arousal. If anything, Hypothesis 3.3 was confirmed for males with respect to their genital arousal (but not their pupil dilation patterns). That is, within male pairs there was a correlation in the degree of sexual arousal to their preferred sex (females in straight twins and males in non-straight twins). However, for females, pupil dilation patterns (but not genital arousal patterns) tended to confirm Hypothesis 3.4. That is, within female pairs, there was a correlation in the degree of sexual arousal to males over females.

3.5 Discussion

Based on measures of pupil dilation and genital arousal, straight male twins showed significantly more sexual arousal to females over males than their non-straight co-twins and vice versa. Straight and non-straight female twins did not differ as strongly as males in their genital arousal or pupil dilation patterns to males or females, although non-straight twins showed somewhat more sexual responses to females than males.

Hence, even though the twins in the present study share all of their genes, the difference in their sexual arousal is comparable to the arousal patterns of unrelated straight and non-straight individuals. In addition, there was no significant correlation between twins of either sex with respect to either genital arousal or pupil dilation to the same sex or their preferred sex. Hence, factors other than genetics likely account for average differences in arousal found between twins, whereas familial factors (such as genetic factors) had little influence. The responsible factors could be prenatal - although these twins are developing at the same time prenatally, they may have been exposed to different intrauterine environments given that approximately 30% of identical twins develop with different placentas (Patterson, 2007). This could lead to varied hormonal exposure or to chemical modification of otherwise identical genes, both of which could be relevant for the development of twins with discordant sexual orientations.

Regarding hormonal exposure, research generally supports the hypothesis that levels of prenatal androgens influence sexual orientation (Bailey et al., 2016). For instance, previous work suggests that discordant twins can differ in their digit ratios, which are potential markers of individual prenatal influences unique to each twin (Hall & Love, 2003; Hiraishi et al., 2012). Thus, differences in the twins' prenatal environments could contribute to their discordant sexual orientations. Regarding the modification of an individual's genes, this can happen through the methylation of DNA, which affect the genome of one twin but not the identical co-twin during gestation. Such epigenetic processes could account for the discordant development of certain traits in identical twins, despite the fact that they share all of their genes (Petronis et al., 2003). Preliminary finings are consistent with this hypothesis, although it is still lacking full support (Ngun & Vilain, 2014).

Non-genetic factors on the development of sexual orientation and sexual attraction could also include social influences, although research generally does not support the notion that the social environment reinforces a non-heterosexual orientation (Bailey et al., 2016). In fact, it appears unlikely that the social environment encourages individuals to develop sexual arousal to the same sex (and traits associated with this) (Rieger et al., 2008; Watts et al., 2017a).

3.6 Limitations

In the present study, pupil dilation was included both as an additional measure of arousal, to verify results, and as an alternative for those uncomfortable with measures of genital arousal. Although this may have worked in some cases, as evident by those twins who opted to provide pupil dilation measures only, highly explicit sexual stimuli were employed (i.e. a male or female model masturbating) to elicit responses from participants. Given that such material could be considered highly intrusive in itself, the stimuli used may have limited the recruitment of twins. Although recruitment may have been limited, previous work has shown that using explicit versus non-explicit sexual stimuli yields similar patterns of arousal in males and females (Watts, Holmes, Savin-Williams, & Rieger, 2017c). Relatedly, numbers in the present study were admittedly small. However, the pattern of results found were in line with those previous found in direction and magnitude.

3.7 Conclusion and Future Directions

Identical twins with discordant sexual orientations differ in their sexual arousal in a same way similar to how unrelated straight and non-straight individuals do. In addition, these twins did not display any reliable correlations in their sexual response to the same sex or other sex. Hence, factors other than genetics likely contributed to the difference in the physiological expression of their sexual orientations. These other factors could include differing intrauterine environments during the early development of the twins, which could lead to varied hormonal exposure or epigenetic differences between twins. Future work could examine early hormonal exposure, for example by measuring the aforementioned finger length ratios. Thus, further studies of identical twins, employing such biological markers, could provide insight into the early developmental of their discordant sexual orientations.

Table 3.1 - Distribution of Numbers, Age and Ethnicities by Sex across 15 Discordant Twin Pairs

	Males	Females
Number of Pairs	6	9
Average Age	25.00	26.22
	[19.43, 30.57]	[24.04, 28.40]
Percentage Caucasian	100	89
	[75.75, 100]	[67.20, 88.89]

Note. Units are pairs. Numbers in brackets are 95% confidence intervals. The majority of these twin pairs (5 male and 6 female) provided genital arousal measures, whereas all of them provided pupil dilation measures.

Figure 3.1 - A) Genital arousal of 5 male twin pairs with discordant sexual orientations in response to males over females and B) pupil dilation of 6 male twin pairs with discordant sexual orientations in response to males over females. On each graph, the points represent the average responses of each twin and a line matches each twin within a pair. Horizontal lines represent the means' 95% confidence intervals. On the y-axis, positive numbers reflect stronger responses to males, and negative numbers reflect stronger responses to females, z-scored within participants. Numbers are effect sizes expressed as Cohen's *d* and, in parentheses, 95% confidence intervals. * $p < .05$. **. $p < .001$.

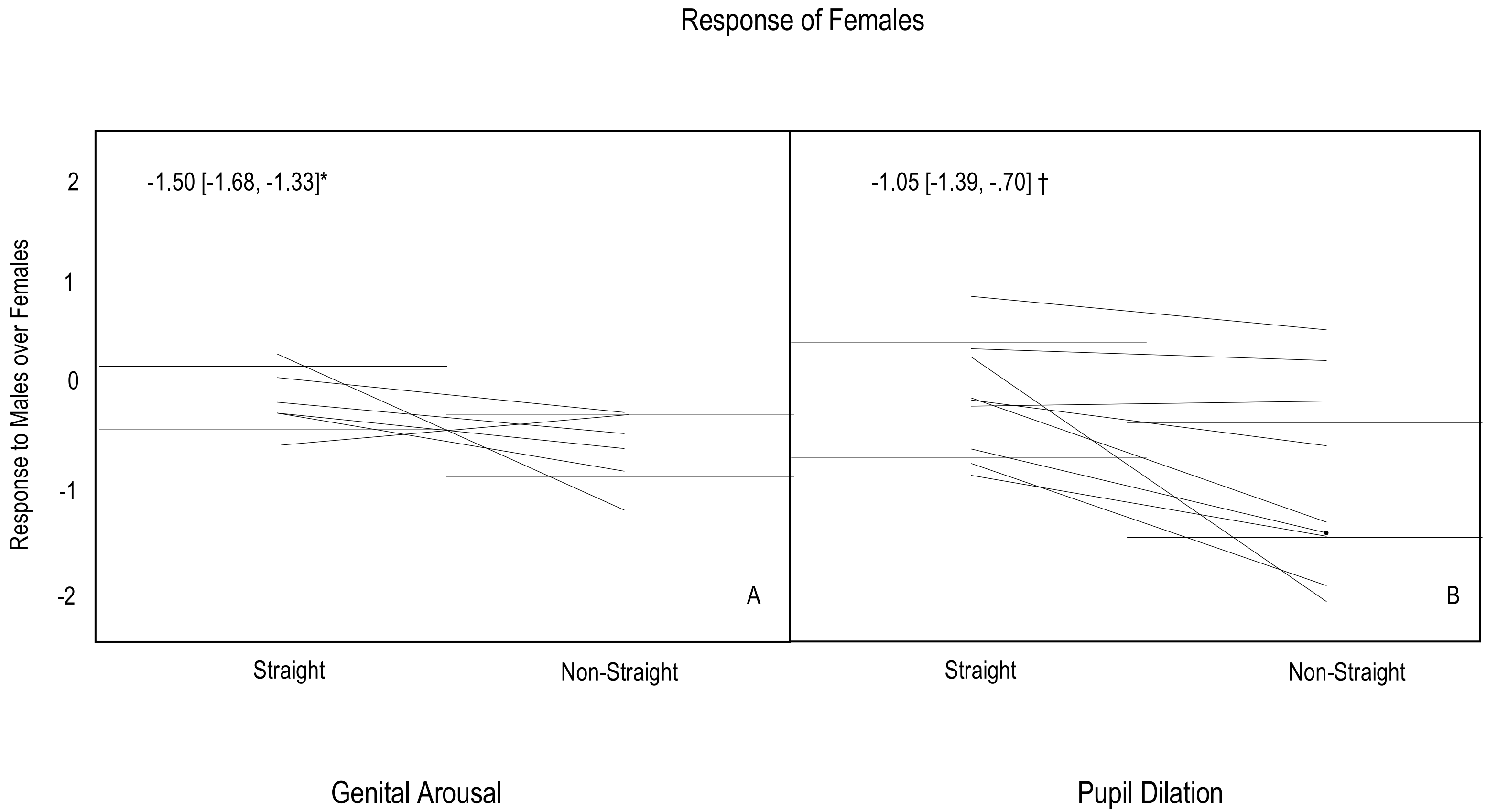

Figure 3.2 - A) Genital arousal of 6 female twin pairs with discordant sexual orientations in response to males over females and B) pupil dilation of 9 female twin pairs with discordant sexual orientations in response to males over females. On each graph, the points represent the responses of each twin and a line matches each twin within a pair. Horizontal lines represent the means' 95% confidence intervals. On the y-axis, positive numbers reflect stronger responses to males, and negative numbers reflect stronger responses to females, z-scored within participants. Numbers are effect sizes expressed as Cohen's *d* and, in parentheses, 95% confidence intervals. † $p < .10$. * $p < .05$

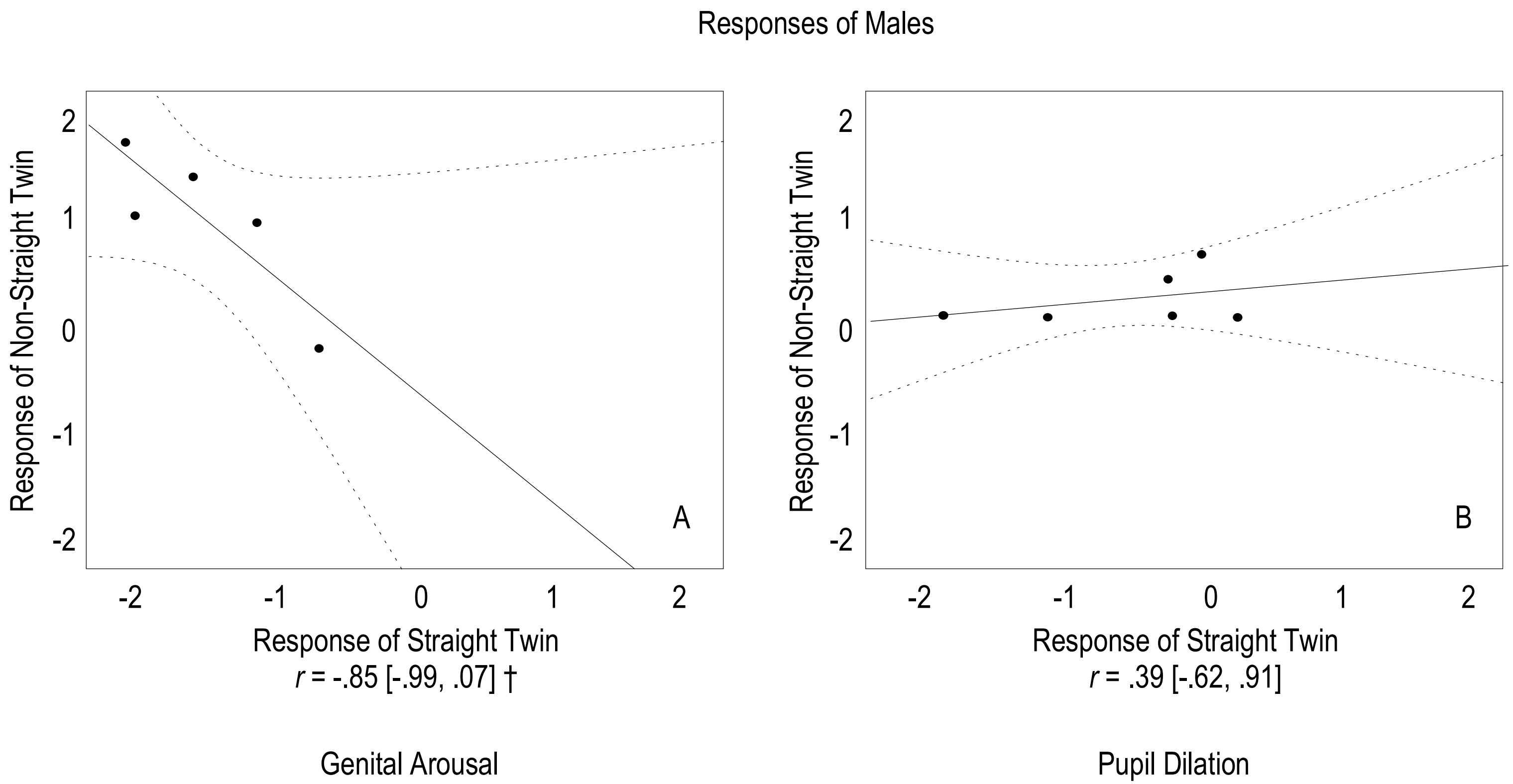

Figure 3.3 - A) Correlation in genital arousal to males over females within 5 male twin pairs with discordant sexual orientations and B) correlation in pupil dilation to males over females within 6 male twin pairs with discordant sexual orientations. On each graph, the points represent the responses within twin pairs. Horizontal lines represent the means' 95% confidence intervals. On each axis, positive numbers reflect stronger responses to males, and negative numbers reflect stronger responses to females, z-scored within participants. Numbers are within pair correlations expressed as Pearson's *r* and, in parentheses, 95% confidence intervals. † $p < .10$.

Responses in Females

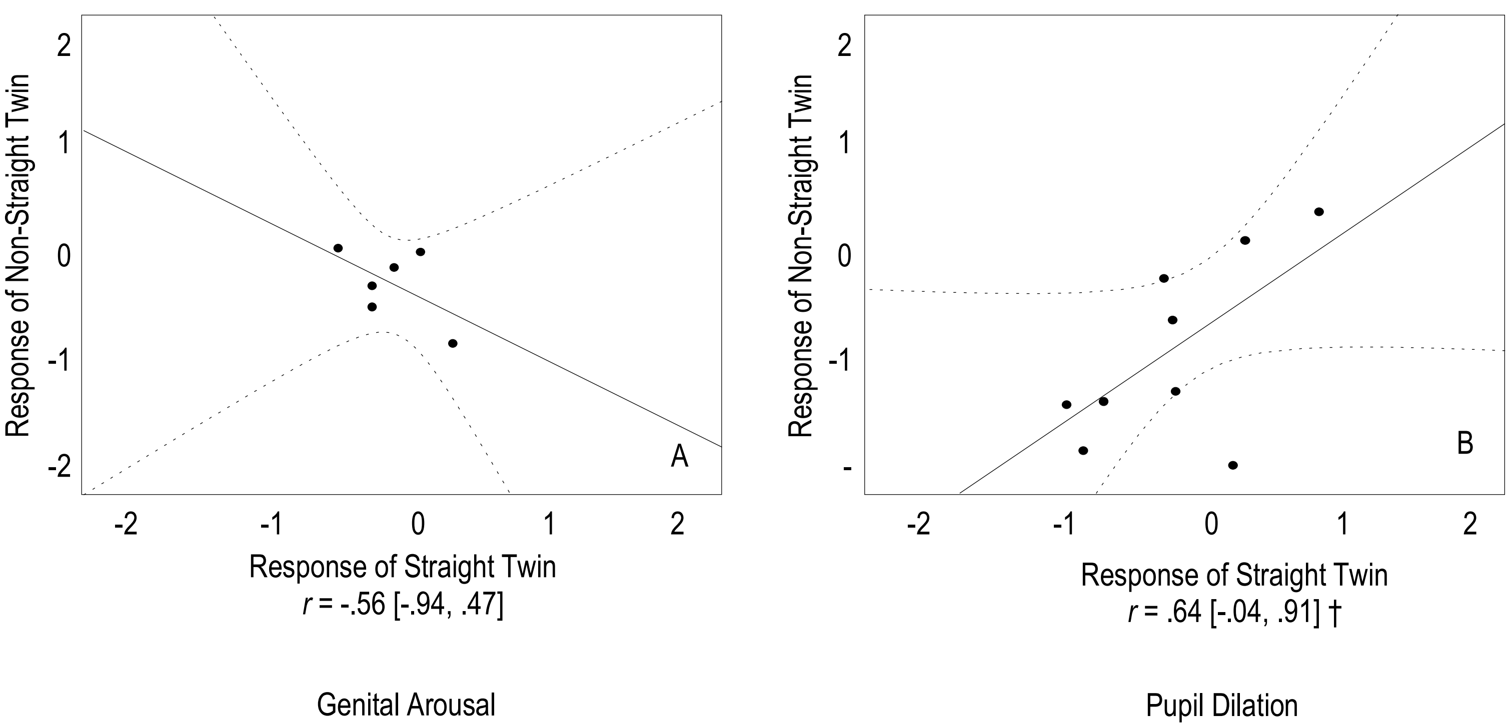

Figure 3.4 - A) Correlation in genital arousal to males over females within 9 female twin pairs with discordant sexual orientations and B) Correlation in pupil dilation to males over females within 9 female twin pairs with discordant sexual orientations. On each graph, the points represent the responses within twin pairs. Horizontal lines represent the means' 95% confidence intervals. On each axis, positive numbers reflect stronger responses to males, and negative numbers reflect stronger responses to females, z-scored within participants. Numbers are within pair correlations expressed as Pearson's *r* and, in parentheses, 95% confidence intervals. † $p < .10$

Chapter 4 Finger Length Ratios of Identical Twins with Discordant Sexual Orientations

4.1 Abstract

Varying prenatal hormonal influences may contribute to the development of discordant sexual orientations in genetically identical twins. A proposed marker of masculinization from prenatal androgens is the ratio of the index to ring finger, referred to as 2D:4D. This ratio is likely lower if an individual was exposed to higher androgens. In 32 identical twin pairs (18 female and 14 male) with discordant sexual orientations, each non-straight twin was compared to his or her straight co-twin for differences in 2D:4D. Within female twin pairs, non-straight (bisexual or lesbian) twins had significantly lower, or more masculinised, finger length ratios than their straight co-twins, but only in the left hand. For males, there was no significant difference between straight and non-straight twins orientation in the finger length ratios of either hand. However, hypothesized sex differences were confirmed with females having, on average, higher ratios than males in both hands, suggesting that females were exposed to lower levels of prenatal androgens than males. A reanalysis of present data in combination with previous data from twins (Hall & Love, 2003; Hiraishi et al., 2012) suggested that these results were reliable. However, not all findings were in line with hypotheses. For example, in the combined data, both non-straight males and non-straight females had more masculinized rations than straight males and females, and the hypothesised sex difference was not, as expected, stronger within straight individuals.

4.2 Introduction

Identical twins, who share 100% of their genes, can differ in their sexual orientations and in traits associated with sexual orientation, including their degree of observable gender nonconformity (Watts et al., 2017a), and their physiological sexual arousal (Watts, Holmes, Raines, Orbell, & Rieger, 2017b). Thus, factors other than genetics may account for differences in both. It is possible that these factors are prenatal. For example, approximately one-third of identical twins rely on independent placentas (Patterson, 2007), and placenta may sometimes fail to inhibit the transfer of testosterone from mother to fetus (Hines et al., 2002). It is feasible that, in those twin pairs, in which each sibling has an independent placenta, each twin is exposed to different levels of prenatal androgens from the maternal system. Such a factor could be relevant for the development of discordant sexual orientations in identical twins because exposure to atypical levels of prenatal androgen potentially increases the likelihood of a same-sex sexual orientation (Hines, 2011). Prenatal androgens, including testosterone, also affect the formation of female-typical and male-typical anatomy, including sex-specific finger length ratios (Zheng & Cohn, 2011). These finger length ratios, referred to as 2D:4D, are the length of the second finger divided by the length of the fourth finger and are, in humans, putative indicators of exposure to prenatal androgens. Women have, on average, higher 2D:4D ratios than men, suggesting that they may have been exposed to lower levels of testosterone in utero than men (see meta-analysis by Grimbos et al., 2010). Further, 2D:4D may be a predictor of sexual orientation (Breedlove, 2010; Grimbos et al., 2010; Hall & Love, 2003; Hines, 2011; Pasterski et al., 2015; Putz et al., 2004; Rahman & Wilson, 2003c; Williams et al., 2000). At least in females, the finger length ratios of non-straight (bisexual or lesbian) women are more male-typical than the finger length ratios of straight women (Grimbos et al., 2010). Moreover, identical female twins with discordant sexual orientations can differ in their 2D:4D, whereby non-straight female twins display male-typical finger length ratios as compared to straight female twins; for male pairs a hypothesized reversed pattern (non-straight male twins display female-typical finger length ratios as compared to straight male twins) is less clear (Hall & Love, 2003; Hiraishi et al., 2012). In this study, we investigated whether these patterns could be replicated in a much larger sample of identical twins than have been previously studied, to test the hypothesis that prenatal androgens influence the discordant development of sexual orientations in identical twins.

4.2.1 Sexual Orientation Differences in 2D:4D

Women of a non-straight sexual orientation show a more masculinised or lower 2D:4D ratio in comparison to straight women (Grimbos et al., 2010; Hines, 2011; Putz et al., 2004; Rahman & Wilson, 2003c). In addition, lesbians who report themselves as "Butch" tend to have lower or more masculinized finger length ratios than those describe themselves as "Femme" (Brown et al., 2002a). A further example of the effect of exposure to elevated levels of testosterone in early foetal development comes from cases of Congenital Adrenal Hyperplasia (CAH) in women. Due to a genetic condition, women with CAH are exposed to unusually high levels of testosterone during their intrauterine period (Berenbaum, 1999; Berenbaum & Bailey, 2003). Females with CAH display a lower 2D:4D ratio on the right hand, than females without CAH (Brown et al., 2002b). In adulthood, women with CAH are also more likely to identify as non-straight than typically developed women (Dittmann et al., 1992). Thus, non-straight women may be exposed to elevated levels of prenatal androgens, including testosterone, as compared with straight women. In one meta-analysis, non-straight women had lower or more masculine 2D:4D in the right and left hand than unrelated straight women (Grimbos et al., 2010). These effects were small to medium, .23 < Hedges *g's* < .29, .04 < 95% CI < .51.

Compared to research on women, research investigating the relationship between the 2D:4D ratio and sexual orientation in men has been far less consistent (Grimbos et al., 2010), even when very large samples (over 200,000 individuals) were studied (Collaer et al., 2007). Although hypothesised relationships between 2D:4D ratios and sexual orientation in men have been confirmed in some studies (Lippa, 2003; McFadden & Shubel, 2002), later research has failed to replicate such findings or has even found the opposite pattern of results (Grimbos et al., 2010). For instance, samples of biological males with Complete Androgen Insensitivity Syndrome (CAIS), which renders them completely unresponsive to androgens during development, are more likely to be attracted to the same (biological) sex (Hines et al., 2003) and show a more female-typical 2D:4D ratio than would be expected of control males (Berenbaum et al., 2009). In the aforementioned meta-analysis, when comparing non-straight and straight men, no difference was detected in 2D:4D in the left or right hand, g for either hand = -.02, -17 < 95% CI < .13. Thus, in general, the hypothesized link between 2D:4D and sexual orientation appears more inconsistent for males than females.

If a relationship between finger length ratios and sexual orientation in men exists at all, it may therefore be, smaller than the corresponding effect for women. Hence, compared with the effect for females, the effect for males might be more prone to measurement error. If so, this effect might be better detected in a more controlled research design than those previously employed, such as by comparing identical twins discordant for sexual orientation. By comparing genetically identical individuals who differ with respect to their sexual orientation, one might therefore have more statistical power to detect reliable effects, even if they are small in magnitude.

4.2.2 Differences in 2D:4D within Discordant Twin Pairs

Previous research supports the hypothesis that discordant female twins differ in their finger length ratios related to their sexual orientation (Hall & Love, 2003; Hiraishi et al., 2012). In seven pairs of female identical twins with discordant sexual orientations, the non-straight female twins showed a significantly lower 2D:4D ratio than their straight co-twins on both hands (Hall & Love, 2003). This effect was partially replicated in another study with eight female pairs with discordant sexual orientation: The non-straight twins had a lower, or more masculinized, 2D:4D ratio on their left hand than their straight co-twins, indicating stronger exposure to prenatal androgen for the non-straight twin than her straight co-twin; however, a similar effect was not found in the right hand (Hiraishi et al., 2012).

Within four male pairs, those who identified as non-straight had significantly higher or more feminized left hand 2D:4D ratios than their straight co-twins (Hiraishi et al., 2012). Because a higher 2D:4D ratio is more typical in females, a potential hypothesis is that the non-straight twin has been exposed to a lower level of prenatal testosterone than his straight co-twin.

If such effects can be replicated in the present sample of twins, then, within female pairs, the non-straight twin will display a lower 2D:4D ratio than her straight co-twin (Hypothesis 4.1). In male pairs, the non-straight twin will display a higher 2D:4D ratio than his straight co-twin (Hypothesis 4.2). We further tested whether such effects were stronger in the left or right hand.

4.2.3 Sex Differences in 2D:4D

In addition to sexual orientation differences, the present research examined sex differences in 2D:4D. Because finger digits develop at a time in prenatal development during which the circulation of androgens affects the development of male-typical and female-typical anatomy, morphology and tissue, it is possible that the presence of androgens has an impact on the development of male-typical and female-typical finger length (Hines, 2011; van Anders et al., 2006). Elevated testosterone exposure in utero influences the growth of the fourth finger digit. This should result in a longer fourth finger digit relative to the index finger, and a lower second to fourth finger length ratio in most males, as compared with most females (Breedlove, 2010; Grimbos et al., 2010; Hines, 2011; Manning et al., 2014; Manning, 2011). In fact, on average, women have higher 2D:4D ratios than men, in the left and right hand, $p < .001$, $g = .44$, and $p < .001$, $g = .55$, indicating that women are exposed to lower levels of testosterone in utero than men (Breedlove, 2010; Grimbos et al., 2010; Hines, 2011). In the present study on identical twins, it was predicted that females would have, on average, significantly higher finger length ratios than males (Hypothesis 4.3). If the aforementioned sexual orientation differences in 2D:4D are correct, then sex differences should be stronger when straight males and females are compared.

4.2.4 The Present Study

In the present study, we investigated patterns of 2D:4D ratios of identical twins with discordant sexual orientations. In addition, raw data were available from two previous samples of discordant twin pairs (Hall & Love, 2003; Hiraishi et al., 2012), and these were included in analyses as part of the present study. Because data on finger length ratios of identical twins with discordant sexual orientations are scarce and some previous samples were very small, a combination of present data with previous data allowed more powerful analyses than each individual study would, therefore yielded potentially more reliable results. In sum, the following hypotheses were tested:

Hypothesis 4.1. In female pairs, the non-straight twin will display a lower 2D:4D ratio than her straight co-twin.

Hypothesis 4.2. In male pairs, the non-straight twin will display a higher 2D:4D ratio than his straight co-twin.

Hypothesis 4.3. Females will have significantly higher finger length ratios than males.

4.3 Method

4.3.1 Recruitment and Participants

Twins. The University of Essex Ethics Committee approved this study. Advertisements for identical twins to participate in a study on sexual orientation were placed in the newsletter of the Department of Twin Research at Kings College London, on social media sites, and on three online sites for gay men and lesbians (Gay Star News, Pink News, and Gay Times). We further recruited twins at three Pride festivals. Each twin who contacted us was encouraged to recruit the co-twin. Recruited twins self-identified as straight, bisexual, gay or lesbian. They were asked twice during the study about their sexual identities, and all responses were consistent.

Power was estimated by examining effect sizes found in previous research (Hiraishi et al., 2012). With 12 pairs of discordant twins (4 male pairs, 8 female pairs), effect sizes (Cohen's *d's*) were d = .81 and d = .36 for the left and right hand of females and d = .43 and d = .23 for the left and right hand of males. Given that our sample size greatly exceeded this number, it was reasoned that such effects, if of similar magnitude, should be significant in the present research. Participants included 18 female twin pairs and 14 male twin pairs, yielding a total of 32 pairs of identical twins with discordant sexual orientations, or 64 individuals. The number of bisexual women and men (3 and 1 individuals) was low relative to the number of straight women and men (18 and 14) or lesbians and gay men (15 and 13). Further, bisexual participants reported a stronger preference for the same sex than the other sex; thus, they were grouped with lesbians and gay men into “non-straight”. Distributions of age and ethnicity are shown in Table 4.1.

Twin zygosity. In addition to asking twins whether they were identical, five standardized items about physical and visual similarity were administered to establish zygosity (Kasriel & Eaves, 1976). An example question is “During childhood, could you ever have fooled friends by pretending to be your twin?” Each item was assessed on a scale ranging from 1 to 3, with lower scores reflecting higher similarity within twin pairs. For the majority of individual twins, their average scores were below 2, suggesting monozygosity. For the one individual who scored above 2, on average, and thus indicated increased dissimilarity from their co-twin, zygosity was assessed by re-contacting the twin pair to confirm that they were identical.

Procedure. Twins were informed that finger lengths might reveal how prenatal influences shape the people we become, but exact hypotheses were not shared with twins. Those who agreed to take part were sent instructions on photographing their hands. Participants were asked to place their hands flat, with their palms facing upwards, on a flat surface with small gaps between their fingers, and to take these photos with the camera held 25 to 30cm directly above their palm. An example photograph was sent to twins to use as guidance. Participants who provided unsatisfactory photographs (i.e. poorly focused, poor resolution, taken at an angle) were asked to retake photographs.

Open-source vector graphics package Inkscape 0.91 was used for measuring finger lengths due to its ability to draw precise lines, which can snap to the midpoint of another drawn line, and measurement of lines are made within hundredths of a pixel. Such computer-assisted measurements, conducted by several experimenters, have resulted in the highest inter-rater reliability compared to other methods of 2D:4D measurement (Allaway, Bloski, Pierson, & Lujan, 2009).

Collected photographs were given to three independent and trained raters, who were blind to the sex and sexual orientation of the twins. Given previously reported procedures (Allaway et al., 2009), each rater drew a line as wide as the finger following the lowest crease at the base, between the metacarpal and proximal phalange. They then drew another line beginning at the tip of the finger down towards the base, using a function on the software to have this line automatically snap to the middle of the line at the base, which allowed raters to avoid guessing where the centre point of the finger base was. They then zoomed in and finely adjusted the top of the line to match the tip of the finger as closely as possible. The software was then used to measure the line in pixels to calculate the ratio. An example of a measured hand is also shown in Figure 4.1.

For each twin a total of 4 finger measurements were produced: the second (2D) and fourth (4D) finger digits on the left and right hands. Inter-rater reliability for all 4 measures was very high, with Cronbach's alpha exceeding .98 for males and females.

The measurements for each finger of each participant were then averaged across the three raters for each individual twin, and these averaged measures were used to calculate the 2D:4D ratio for both hands of each twin. This was achieved using the standard formula for a finger length ratio: length of the second finger divided by length of the fourth finger.

4.4 Results

4.4.1 Hypotheses 4.1 and 4.2

Hypothesis 4.1 stated that, within female pairs of identical twins with discordant sexual orientations, the non-straight twin will display a lower 2D:4D ratio than her straight co-twin. Hypothesis 4.2 stated that within male pairs of identical twins with discordant sexual orientations, the non-straight twin will display a higher 2D:4D ratio than his straight co-twin.

Using mixed-factorial regression analyses, 2D:4D ratios were predicted by the twins' sexual orientation as a fixed factor and twin pair as a random effect to account for repeated measures of finger length ratios within pairs.

For females, non-straight twins had significantly lower or more masculinised finger length ratios in the left hand than their straight co-twins, $p = .01$, $\beta = -.31$ [-.52, -.09]. This effect was not found for the right hand, $p = .92$, $\beta = -.02$ [-.40, .36]. For males, no significant differences were found. If anything, non-straight twins had lower (or more masculinised) finger length ratios in the left and right hand than their straight co-twins, $p = .28$, $\beta = -.17$ [-.49, .15], and, $p = .22$, $\beta = -.26$ [-.71, .19], respectively.

To visualize theses findings, we computed, within each twin pair and separately for each hand, a difference score by subtracting the finger length ratio of the straight twin from that of the non-straigh twin. This resulted in a negative score if the non-straight twin had a lower or more masculinized finger length ratio than the straight co-twin, and a positive score if the non-straight twin had a higher or more feminized finger length ratio than the straight co-twin. The distributions of the sexual orientation difference scores for both hands and both sexes are shown in Figure 4.2 and Figure 4.3. For females, non-straight twins had significantly more masculinized finger length ratios than their straight co-twins in the left hand only (Figure 4.2); for males, non-straight twins did not have significantly more feminized left or right hand digit ratios than their straight co-twins (Figure 4.3.). Although not significant, non-straight males had somewhat more masculinized finger length ratios than their straight co-twins.

4.4.2 Hypothesis 4.3

We predicted that females would have significantly higher (or more feminized) finger length ratios, in both hands, than males. Differences in left and right finger length ratios were predicted by biological sex as a fixed factor and twin pair as a random factor.

In the left hand, females had higher or more feminized finger length ratios than males, although this effect did not reach significance, $p = .07$, β [95% CI] = .28 [-.02, .58]. In the right hand, females had significantly higher or more feminized finger length ratios than males, $p = .03$, $\beta = .28$ [.02, .53].

An additional mixed-factorial regression analysis was computed, testing whether these effects differed by sexual orientation and hand. If sex differences in 2D:4D are reliable, in addition to hypothesized sexual orientation differences, then any difference between males and females should be stronger for straight participants as compared to non-straight participants. In addition, hand was included in the model given that previous work has sometimes found differences in the strength of effect by hand.

Independent of sexual orientation and hand, females had more feminized finger length ratios than males, $p = .002$, $\beta = .27$ [.10, .44]. There was also a significant main effect of sexual orientation, $p = .04$, $\beta = -.17$ [-.34, -.002], which indicated that regardless of sex and hand, those identifying as non-straight had significantly lower or more masculinised finger length ratios than those who identified as straight. The interaction of sex and sexual orientation was not significant, $p = .77$, $\beta = .03$ [-.14, .20]. Thus, there was no indication that the sex difference in 2D:4D was more pronounced in straight individuals than non-straight individuals, nor was there an indication that the sexual orientation difference (with non-straight individuals having more masculine ratios) was more pronounced in women than men.

To illustrate sex differences in finger length ratios between men and women of either sexual orientation, Figure 4.4 shows group averages and their 95% confidence intervals were calculated for both sexes across both hands.

4.4.3 A Comparison of Samples

Given that raw data were available from previous studies (Hall & Love, 2003; Hiraishi et al., 2012), these were combined with data in the present study before performing similar analyses as described above. From the previous studies, 15 pairs of discordant female twins (7 from Hall & Love, 2003; 8 from Hiraishi et al., 2012) and 4 pairs of discordant male twins (Hiraishi et al., 2012) were added to the current data set, resulting in a total of 32 female pairs and 18 male pairs. In Hall and Love (2003), inked prints of the twins' hands were taken. Their fingers were then measured using calipers. In Hiraishi et al. (2012), finger lengths were measured via photocopies of the twins' hands using calipers.

4.4.4 Hypotheses 4.1 and 4.2

In order to test the effects of sexual orientation on finger length ratios across all three studies, a mixed factorial regression was conducted separately for each sex. The dependent variable was left or right hand finger length ratio. Independent variables were sexual orientation and study sample as fixed effects. In addition, the model included an interaction between sexual orientation and study sample. This interaction was computed to test whether differences in finger length ratios between straight and non-straight twins were dependent on the study. Twin pairs were included as a random effect. Table 4.2. shows that controlling for study sample, non-straight female twins had more male-typical left hand finger length ratios than their straight co-twins, p = .002, β = -.27 [.44, -.09]. In the right hand of female twins, there was no significant effect of sexual orientation on finger length ratio, $p = .38$, $\beta = -.11$ [-.35, .13]. For males, the non-straight twins did not have significantly more feminized left or right hand finger length ratios than their straight co-twins, $p = .99$, $\beta = .002$ [-.29, .30], and, $p = .59$, $\beta = -.12$ [-.57, .33], controlling for study sample. Table 4.2. also shows that there were some significant main effects of study. Whether or not these differences by study on finger length ratios (which were independent of effects of sexual orientation) were meaningful, is unclear. There were no significant interactions of sexual orientation and study. To visualize these findings across studies, we computed, within each twin pair and separately for each hand, a difference score by deducting the finger length ratio of the straight twin from that of the non-straight twin (Figure 4.5. and Figure 4.6.). Across studies, non-straight female twins had significantly more masculinized finger length ratios than their straight co-twins in the left hand only (Figure 4.5.); finger length ratios did not differ between straight and non-straight male twins in either hand (Figure 4.6). Thus, across samples, findings suggest that within female twin pairs, non-straight twins tend to have more masculinized left hand finger length ratios than their straight co-twins. Within male twin pairs, non-straight twins do not have more feminized finger-length ratios than their straight co-twins.

4.4.5 Hypothesis 4.3

Differences in left and right finger length ratios were predicted by biological sex as a fixed factor and twin pair as a random factor. Including this random factor accounted for repeated measures of finger length ratios within pairs.

In the left hand, females had significantly higher or more feminized finger length ratios than males, $p = .03$, $\beta = .26$ [.03, .50]. In the right hand, females had somewhat higher or more feminized finger length ratios than males, although this effect was not significant, $p = .18$, $\beta = .14$ [-.07, .36]. The differences across sexes are further illustrated in Figure 4.

An additional mixed-factorial regression analysis was carried out, testing whether these effects differed by sexual orientation and hand. Independent of sexual orientation and hand, females had more feminized finger length ratios than males, $p = .01$, $\beta = .18$ [.04, .31]. There was also a significant main effect of sexual orientation, $p = .02$, $\beta = -.16$ [-.30, -.03], indicating that regardless of sex and hand, those identifying as non-straight had significantly lower or more masculinised left hand ratios than those who identified as straight. Interactions between sex, sexual orientation and hand were not significant in analyses. For example, the interaction of sex and sexual orientation was not significant, $p = .81$, $\beta = -.02$ [-.15, .12]. Thus, there was no indication that the sex difference in 2D:4D was more pronounced in straight individuals than non-straight individuals, nor was there an indication that the sexual orientation difference (with non-straight individuals having more masculine ratios) was more pronounced in women than men.

To illustrate average sex differences in finger length ratios, regardless of sexual orientation, averages and their 95% confidence intervals are displayed in Figure 4.7.

4.5 Discussion

Non-straight females had more male-typical left hand 2D:4D ratios than their straight co-twins. These differences were not found in the right hand. For males, no significant effect of sexual orientation was detected in either hand. In addition, sex differences in 2D:4D were found, with women displaying significantly higher or more feminized finger length ratios in both hands than men. A reanalysis of present data in combination with previous data from twins (Hall & Love, 2003; Hiraishi et al., 2012) suggested that these results were reliable, although unexpected findings also appeared to be robust: When data were combined, both non-straight men and women had more masculinized ratios than straight men and women, and a predicted sex difference, although confirmed, was not stronger in straight than non-straight individuals.

4.5.1 Sexual Orientation Differences

Results show that non-straight female twins showed lower or more masculinized finger length ratios than their straight co-twins, suggesting that the non-straight twins may have been exposed to higher levels of testosterone in utero. However, in men, no sexual orientation differences in 2D:4D were found. If anything, predicted effects (non-straight males would have higher or more feminized finger length ratios than their straight co-twins) went in the opposite direction. This becomes especially evident in the combined data, whereby across men and women, non-straight twins appear more masculinized (reflected in a lower 2D:4D) than their straight twins. In women, this pattern confirms Hypothesis 4.1 and supports existing literature. In men however, this finding was unexpected. Although, this reversed effect (as compared to Hypothesis 4.2), has been previously found in white male populations. That is, non-straight males tended to have more masculine (lower) 2D:4D ratios than straight males did; the opposite was true in studies with more non-white participants (Grimbos et al., 2010). Although our results for males were not statistically significant, they were in line with that previously unexpected finding: non-straight males had more masculinized ratios than straight males, and this in a predominantly white sample. The exact mechanisms underpinning this effect remain unknown but the hyper-masculinization of 2D:4D in non-straight males has been found in other work (Grimbos et al., 2010).

4.5.2 Sex Differences

Results confirm the hypothesis that females have higher or more feminized finger length ratios than males indicating that they were exposed to lower levels of prenatal androgens than males. If all hypotheses were confirmed, this sex difference should be stronger in straight participants as compared to non-straight participants. However, regression analyses, including an interaction between sex and sexual orientation, suggest that the hypothesized sex difference is similar across straight and non-straight men and women (given that the interaction was not significant). Perhaps this was due to a lack of power, but because the effect size of the interaction between sex and sexual orientation was so minimal, (p = .81, β = -.02 [-.15, .12]), it appears an unlikely explanation. Alternatively, given that the sexual orientation effect in males is generally unreliable (according to previous a meta analysis by and the present data) one cannot fully expect that a sex difference becomes more prominent if sexual orientation is controlled for.

4.5.3 Limitations and Future Directions

The method used in the present study - the evaluation of finger length ratios as seen in photographs – provided a useful means of collecting data from a larger sample than would have otherwise been available. However, this method potentially introduced some variability in the quality of the photographs provided. Particularly poor photographs were rejected until a successful attempt was made, however there remained variation in the sharpness and resolution of the photographs, and this could have introduced some error into the measurements taken.

Although 2D:4D as a marker of prenatal androgen exposure has been unreliable in some instances, the present work highlights robust sex differences, in addition to sexual orientation differences in females. Additional work assessing other indices of prenatal androgen exposure, including measurement of testosterone levels in amniotic fluid (Hines, 2011), anogenital distance (Pasterski et al., 2015) and oto-acoustic emissions (Rahman, 2005) could also provide further support for 2D:4D as a measure and provide important insight into how non-shared environmental factors impact the discordant development of sexual orientation in identical twins.

Table 4.1 - Distributions of Numbers, Age and Ethnicities across 32 Discordant Twin Pairs

	Females	Males
Number of Pairs	18	14
Average Age	28.22	32
	[25.95, 30.49]	[27.38, 36.62]
Percentage Caucasian	88.89	92.86
	[74.69, 95.59]	[77.35, 98.01]

Note. Units are pairs. Numbers in brackets are 95% confidence intervals

Table 4.2 - Multiple Regression Analyses for Sexual Orientation and Study predicting Left and Right Hand Ratios for 66 females and 36 males.

Measure	Females Left[1]	Females Right[1]	Males Left[1]	Males Right[1]
Sexual Orientation (SO)[2]	-.27 [-.44, .11]*	-.11 [-.35, .14]	.002 [-.29, .30]	-.12 [-.57, .34]
Hall & Love (2003)[3]	.43 [.12, .75]*	.16 [-.20, .53]	NA	NA
Hiraishi et al. (2012)[3]	-.77 [-1.07, -.46]***	-.72 [-1.07, -.37]***	-.48 [-.97, -.002] †	-.32 [-.69, .06] †
SO X Hall & Love (2003)[3]	-.02 [-.28, .24]	-.10 [-.47, .27]	NA	NA
SO X Hiraishi et al. (2012)[3]	.05 [-.20, .30]	.02 [-.34, .37]	.16 [-.13, .46]	.17 [-.28, .62]

Note. Numbers are standardized regression coefficients, β*'s*, with 95% confidence intervals in brackets. [1]Higher scores indicate higher or more feminine ratios. [2]A score of 0 indicates "straight," 1 indicates "non-straight". [3]Shown statistics reflect contrasts, comparing the listed effect or interaction to that of the present study. Twin pairs were a random effect. $^{\dagger}p < .10$. $^{*}p < .05$. $^{***}p < .0001$.

Figure 4.1 - An example of an accepted left hand picture with measurements shown.

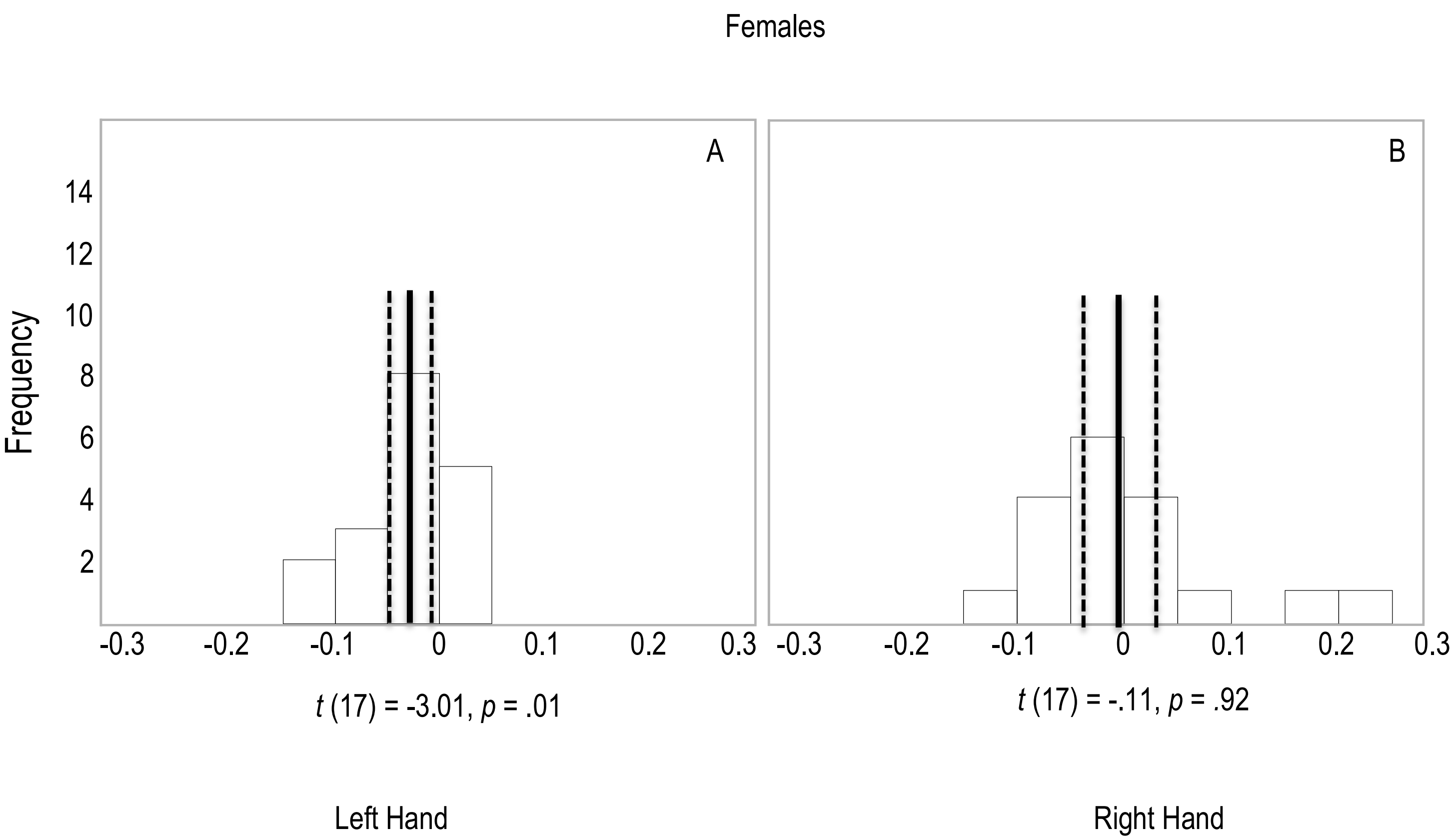

Figure 4.2 - Distributions of differences in 2D:4D between non-straight female twins and their straight co-twins. Solid black lines represent the mean of the distribution and the dashed lines represent the 95% confidence intervals of the mean. Statistics represent within-pair t-tests. In females, the non-straight twins had significantly more masculinised left hand finger length ratios than their straight co-twins (A). There was no significant difference in the right hand (B).

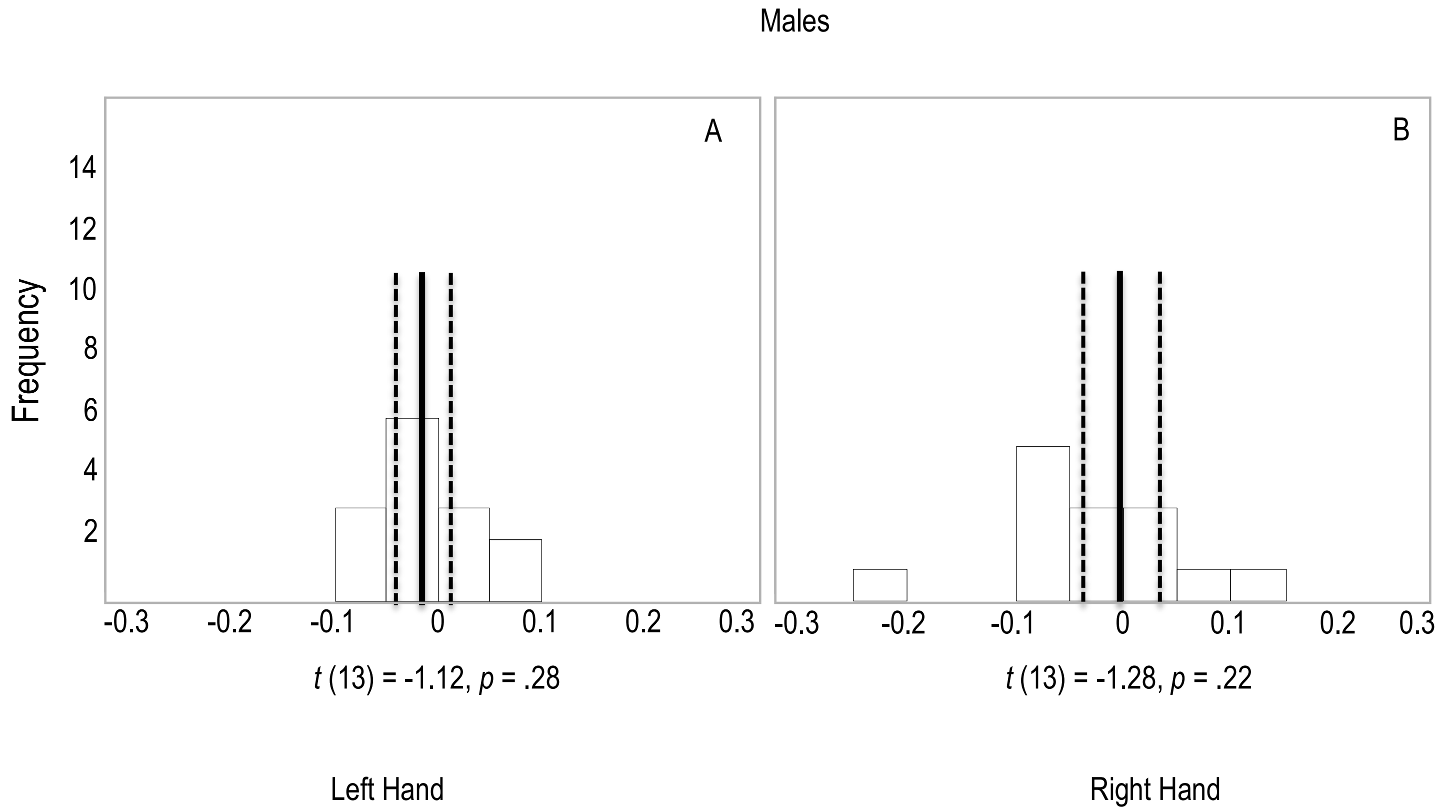

Figure 4.3 - Distributions of differences in 2D:4D between non-straight male twins and their straight co-twins. Solid black lines represent the mean of the distribution and the dashed lines represent the 95% confidence intervals of the mean. Statistics represent within-pair t-tests. Finger length ratios did not significantly differ between straight and non-straight twins in the left (A) or right (B) hand.

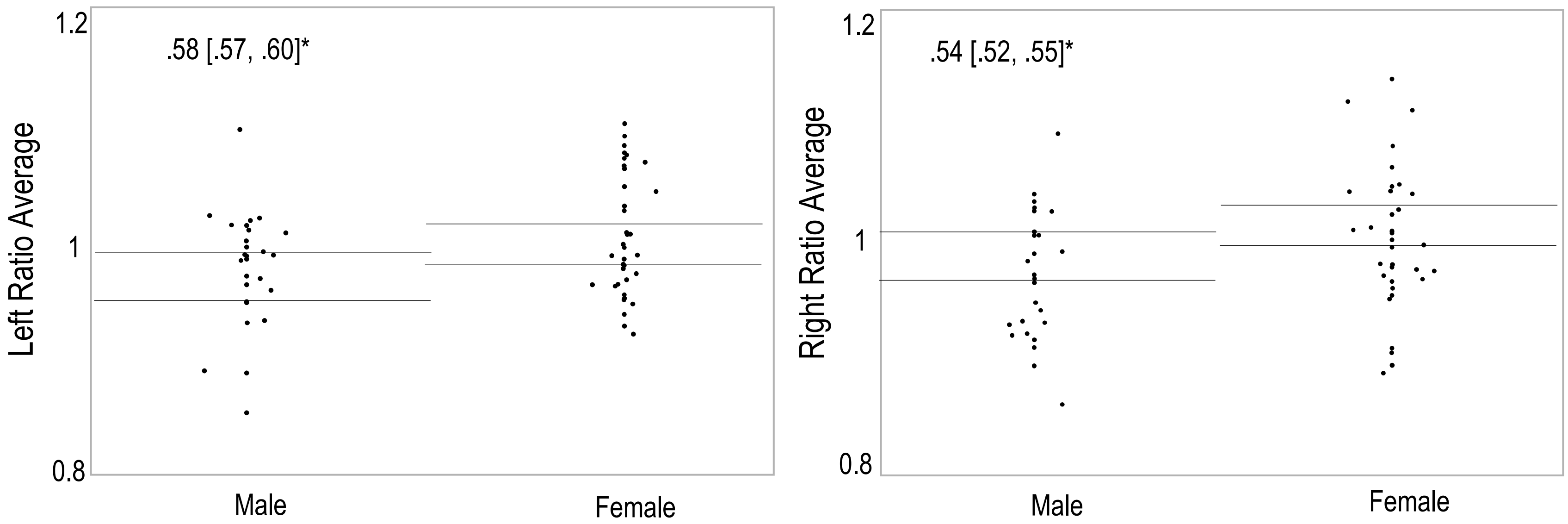

Figure 4.4 - Sex differences in 2D:4D across the left and right hands in 18 female and 14 male twin pairs with discordant sexual orientations. Dots represent finger length ratios of individual twins, averaged across all ratings. Lines are the means' 95% confidence intervals. On the y-axis, higher scores indicated a higher index to ring finger ratio. Numbers represent Cohen's *ds* with their 95% confidence intervals. * $p < .05$.

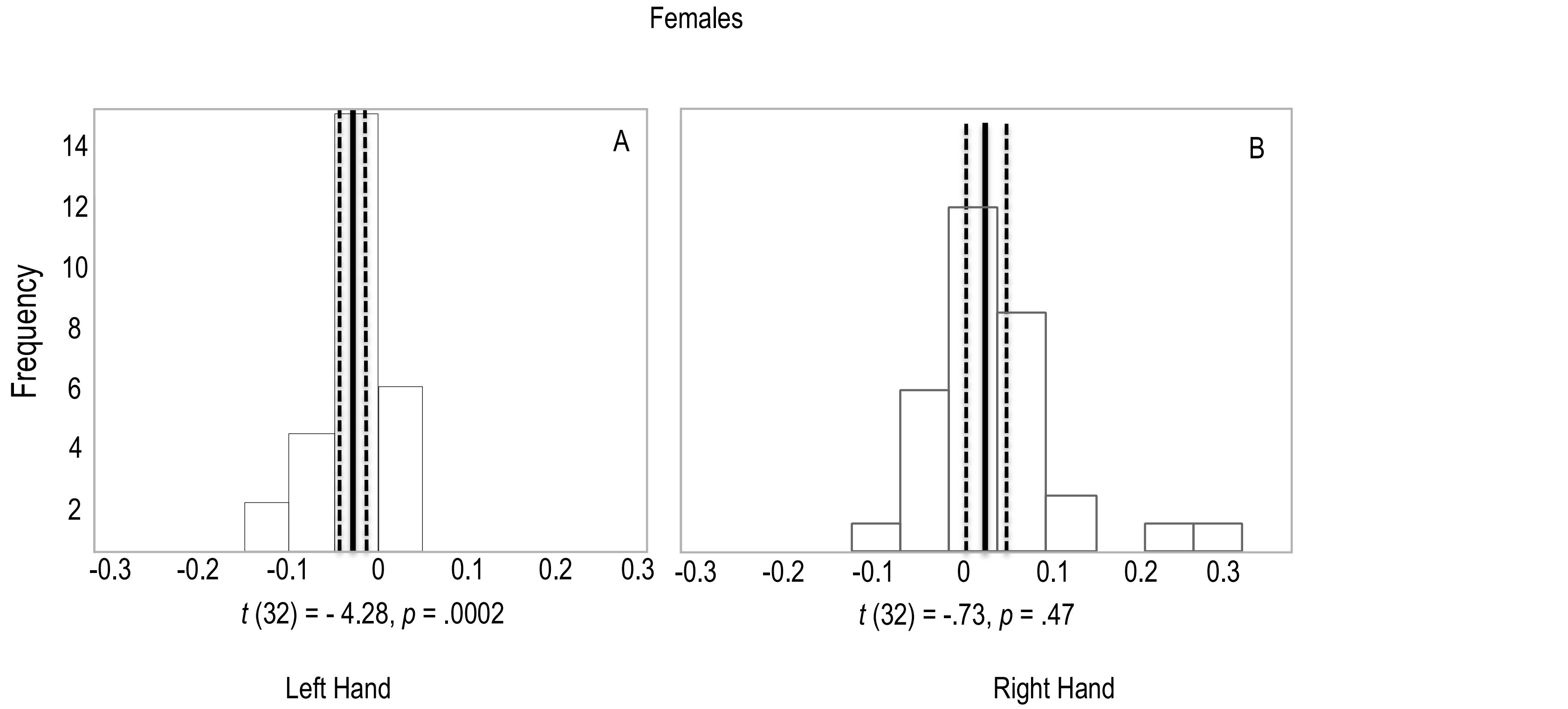

Figure 4.5 - Distributions of differences in 2D:4D between non-straight female twins and their straight co-twins. Solid black lines represent the mean of the distribution and the dashed lines represent the 95% confidence intervals of the mean. Statistics represent within-pair t-tests. In females, the non-straight twins had significantly more masculinised left hand finger length ratios than their straight co-twins (A). There was no significant difference in the right hand (B).

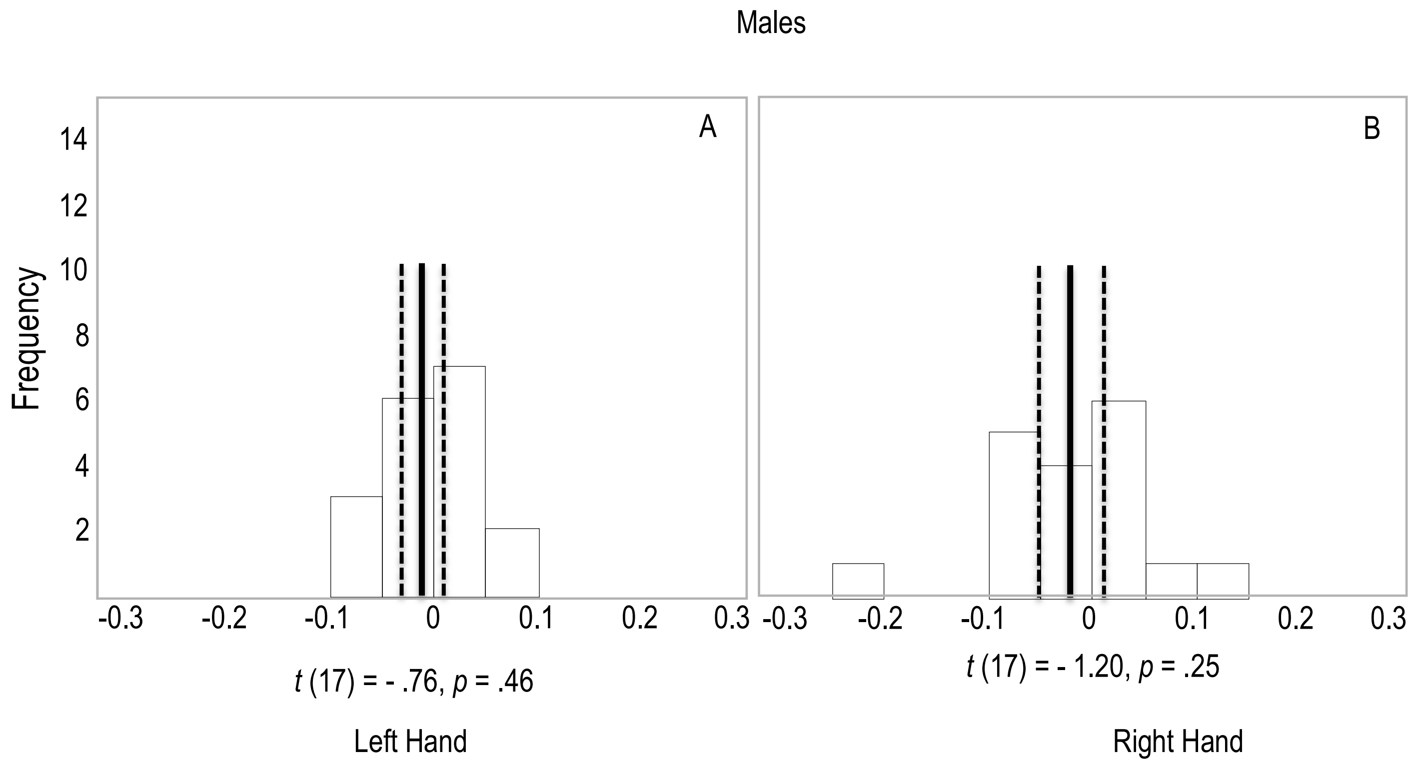

Figure 4.6 - Distributions of differences in 2D:4D between non-straight male twins and their straight co-twins. Solid black lines represent the mean of the distribution and the dashed lines represent the 95% confidence intervals of the mean. Statistics represent within-pair t-tests. Finger length ratios did not significantly differ between straight and non-straight twins in the left (A) or right (B) hand.

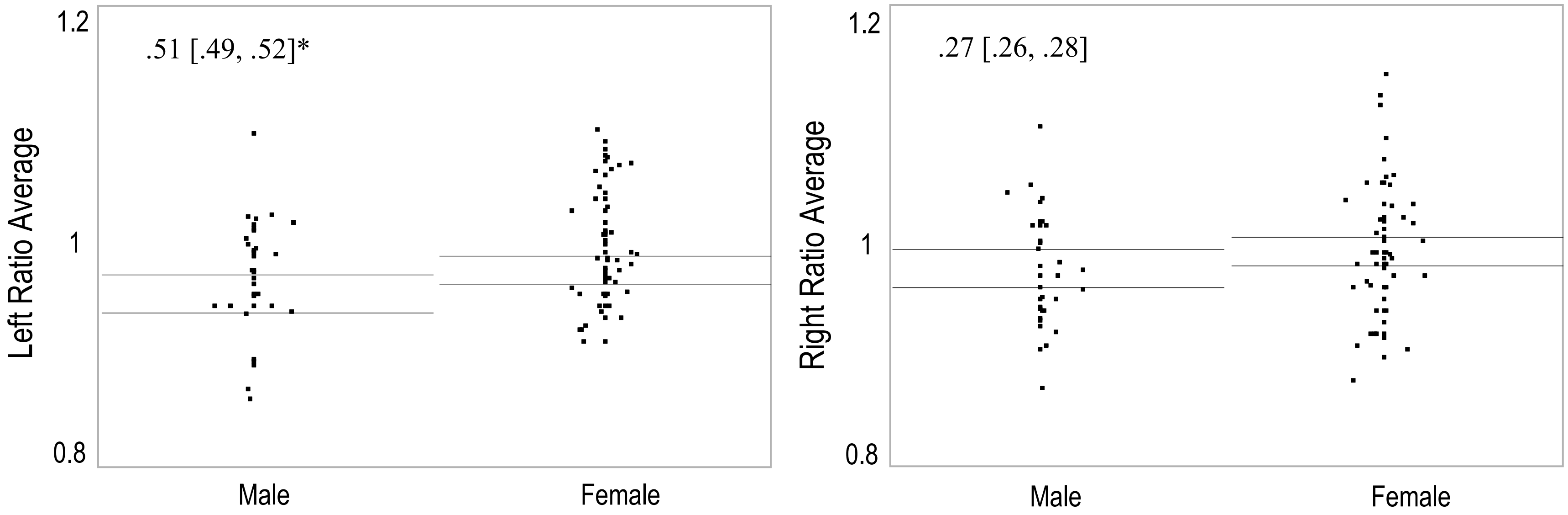

Figure 4.7 - Sex differences in 2D:4D across the left and right hands in 33 female and 18 male twin pairs with discordant sexual orientations. Dots represent finger length ratios of individual twins, averaged across all ratings. Lines are the means' 95% confidence intervals. On the y-axis, higher scores indicated a higher index to ring finger ratio. Numbers represent Cohen's *ds* with their 95% confidence intervals. * $p < .05$.

Chapter 5 General Discussion

The goal of this doctoral research was to assess how identical twins with discordant sexual orientations effectively differed in traits associated with sexual orientation. This was achieved by using measures that were free of the limitations of self-report, in addition to using self-report measures.

5.1 Summary of findings

The first study in this thesis, presented in Chapter 2, investigated the expression of gender nonconformity in identical twins in order to elucidate the potential non-genetic and familial influences on the development of sexual orientation. Twin pairs with either discordant or concordant sexual orientations were recruited and their degree of observable gender nonconformity was assessed via evaluations of photographs taken in childhood and adulthood. Twins also reported their degree of gender nonconformity in childhood and adulthood and the reactions from others during childhood. Based on observer ratings of photographs, identical twins with discordant sexual orientations visibly differed in their gender nonconformity from mid-childhood, with higher levels of gender nonconformity for the non-straight twins. This difference was smaller than the analogous difference between identical twins who were concordant straight and unrelated to them, identical twins who were concordant non-straight. In addition, twins in discordant pairs correlated in their observer-rated gender nonconformity. Thus, non-genetic factors likely differentiated the discordant twins' gender-related characteristics in childhood, but shared influences made them similar in some respects. Finally, rejection generally increased with gender nonconformity, but this effect varied by the twins' sexual orientation.

The second study of this thesis, presented in Chapter 3, investigated differences in physiological sexual arousal within identical twins with discordant sexual orientations by measuring their genital arousal and pupil dilation to explicit sexual stimuli. Based on these measures, straight males responded more strongly to females over males; their non-straight co-twins responded more strongly to males over females. Female twins responded approximately equally to both sexes, albeit lesbians somewhat more to females over males. These differences within twin pairs in their physiological sexual responses were similar in direction and magnitude to analogous differences previously assessed in unrelated straight and non-straight men and women. Furthermore, there were no consistent correlations between twins and their co-twins in either their genital arousal or pupil dilation to one sex over the other. Hence, factors other than genetics likely contribute to different physiological expressions of sexual orientation in identical twins.

An established marker of prenatal androgen exposure includes the ratio of the index to ring finger, referred to as 2D:4D. The third study of this thesis, presented in Chapter 4, investigated patterns of 2D:4D of identical twins with discordant sexual orientations. In addition, given that raw data was available from two previous samples of discordant twin pairs, this was included in Chapter 4 and reanalysed. Within female twin pairs, non-straight (bisexual or lesbian) twins had significantly lower, or more masculinised, finger length ratios than their straight co-twins, but only in the left hand. For males, there was no significant difference between straight and non-straight twins orientation in the finger length ratios of either hand. However, hypothesized sex differences were confirmed with females having, on average, higher ratios than males in both hands, suggesting that females were exposed to lower levels of prenatal androgens than males. A reanalysis of present data in combination with previous data from twins (Hall & Love, 2003; Hiraishi et al., 2012) suggested that these results were reliable. However, not all findings were in line with hypotheses. For example, in the combined data, both non-straight males and non-straight females had more masculinized rations than straight males and females, and the hypothesised sex difference was not, as expected, stronger within straight individuals.

5.2 Future Directions

The present work suggests that the social environment does not encourage gender nonconformity and subsequently, a non-straight sexual orientation in pairs of male or female identical twins. In addition, prenatal androgens, as assessed via finger length ratios, may affect sexual orientation in different ways in each sex. In the following subsections, social implications for males and females based on the aforementioned findings are highlighted. Then, separately for males and females, developmental implications derived from findings of the present work are discussed.

5.2.1 Reactions to Gender Nonconformity

The present work suggests that gender nonconformity of identical twins with discordant sexual orientation emerges early and despite negative responses from the social environment. This is generally consistent with the available literature on social reactions to gender nonconformity (Alanko et al., 2011). In future work, I would like to examine this pattern from another angle – that of the twins' parents. This could provide two benefits. Firstly, including parents in further work could enrich the overall impression of the twins' gender nonconformity during childhood. For instance, by providing further insight into the emergence of these behaviours. Secondly, I would be interested in gaining insight into adaptations of parenting styles and their overall responses to the gender nonconformity of each twin separately. Findings of Study 1 suggest that gender nonconformity may have been rejected during childhood more so in the twin who later became straight. Future work could further shed light on whether parents or peers actively discouraged gender nonconformity more so in those twins who later became straight adults than in those who became non-straight. In addition, further work could assess whether parental socialization of discordant twins occurred differently as a function of their levels of gender nonconformity during childhood. For example, past work has highlighted that mothers reinforced more proximity seeking behaviours in boys and a higher level of helping behaviours in girls in sets of opposite-sexed dizygotic twins (Goshen-Gottstein, 1981). Hence, gender-related behaviours, including proximity seeking and helping behaviours, among others, may have been reinforced differently as a function of levels of gender nonconformity in same-sexed pairs of twins discordant for sexual orientation. Consequently, the more gender nonconforming twin may have been reinforced to adopt additional behaviours usually encouraged in the other sex.

Apart from conducting future work with parents of identical twins with discordant sexual orientations, I am interested in teasing out exactly how the broader social environment responds to gender nonconformity. In particular, I am interested in identifying the behavioural characteristics and cues the social environment responds to when forming an overall impression of a person. Further, I would like to investigate these responses using measures that go beyond self-report. For instance, with implicit measures such as the Implicit Association Test (Greenwald, McGhee, & Schwartz, 1998) or the Function Acquisition Speed Test (FAST) (O'Reilly, Roche, Ruiz, Tyndall, & Gavin, 2012), I could investigate whether certain characteristics or traits, as seen in photographs, are responded to negatively or positively in general.

5.2.2 Protective Functions of Having a Twin

It has been reported that men and women with higher levels of gender nonconformity experience lower levels of wellbeing, regardless of their sexual orientation (Rieger & Savin-Williams, 2012b). I collected data on wellbeing in these discordant twins given that a comparison of such individuals, who were not only similar genetically but also in terms of their general upbringing, could give greater insight into how potentially negative effects work at the individual level. There were inconsistencies in the data however, whereby gender nonconformity and even recalled rejection did not always negatively relate to wellbeing. There have been other reported instances where negative experiences did not have detrimental effects on mental health outcomes for sexual minority individuals (Eisenberg & Resnick, 2006; Espelage, Aragon, Birkett, & Koenig, 2008; Kertzner, Meyer, Frost, & Stirratt, 2009; Moak & Agrawal, 2009; Murdock & Bolch, 2005). As such, a range of protective factors has been identified, which buffer against negative experiences and reduce the chances of poor mental health (Hawkins, Catalano, & Miller, 1992; Murdock & Bolch, 2005; Saewyc, 2011). Protective factors are described as confidence- and competence-promoting life experiences or environments, which could include, but are not limited to, nurturing family relationships and supportive friendships (Eisenberg & Resnick, 2006; Espelage et al., 2008; Kertzner et al., 2009; Moak & Agrawal, 2009; Murdock & Bolch, 2005).

In the present work, those twins who are gender nonconforming in their behaviours and interests also tended to report negative reactions from parents and friends to their gender nonconformity during childhood. However, in a few cases these negative experiences do not appear to have a substantial impact on wellbeing. It may be possible that having a very close and supportive relationship, such as a twin, serves as a protective factor that acts as a buffer against negative responses to gender nonconformity. It could be that a nurturing relationship between twins promotes good psychological adjustment and overall wellbeing in adulthood, despite any experienced rejection. In the present work, I recorded video interviews with twins, during which I ask several questions about their upbringing including whether there is something special about having or being a twin. Future work could include qualitative analyses of the twins' responses and an examination of the possibility that having or being a twin serves a protective function.

5.2.3 Placentation Status

A crucial determinant of the prenatal environment (and as such, fetal development) is placentation status within pairs of identical twins. Placentation status includes whether identical twins share a placenta during early development (monochorionic) or whether they develop independent placentas (dichorionic). Two-thirds of identical twins are monochorionic and the remaining third are dichorionic (Patterson, 2007). The placenta plays an important role during fetal development, serving as a barrier that inhibits the transfer of testosterone from mother to fetus; although the extent to which the placenta successfully prevents this hormonal transfer can vary (Hines et al., 2002).

It is feasible that for identical twins with discordant sexual orientations, each sibling has an independent placenta and as such, is exposed to different levels of prenatal androgens from the maternal system. Thus, any differences in the levels of exposure to prenatal androgens within pairs of identical twins possibly results from differences in the makeup of their placenta, which may fail to protect one or both fetuses from hormonal transfer from the maternal system. This could contribute to the discordant development of sexual orientation in pairs of identical twins. Findings of the present work suggest that variation in exposure to testosterone during early development could be relevant for the development of sexual orientation in females. Although findings regarding exposure to testosterone and sexual orientation are less clear in males, future work could include collecting data on placentation status in both female and male twin pairs. This can be done by interviewing the twins' mothers and if needed, studying hospital records. Such information could help provide insight into how any differences in the twins' prenatal environment may be linked to their discordant sexual orientations.

5.2.4 Developmental Implications for Males

Findings of the present work suggest that indicates that variance in prenatal androgen exposure may not account for variance in sexual orientation in men. Below I review evidence for one factor that might obscure such link and highlight the possibility that hormonal exposure could influence the expression of gender nonconformity.

5.2.5 Fraternal Birth Order

The factor most consistently linked with male sexual orientation in sexuality research to date is that of the fraternal-birth-order effect (Bailey et al., 2016). This includes the finding that non-straight males have a higher number of older brothers than straight males (Blanchard & Bogaert, 2004; Bogaert & Skorska, 2011). This effect, whereby each male child produced by the same mother has a progressively higher chance of being a non-straight, is substantial, increasing the odds of a male growing up to identify as non-straight by around 34% (Cantor, Blanchard, Paterson, & Bogaert, 2002; VanderLaan & Vasey, 2011). This is thought to be the result of immunological processes whereby the maternal immune system gradually protects itself against unique proteins found on the Y-chromosome in male foetuses, known as H-Y antigens, and which are, before conceiving her first son, not present in the maternal system (Blanchard, 2001). Antibodies produced by the maternal system prevent these antigens from functioning and may obstruct the typical sexual differentiation of particular brain structures. That is, antibodies from the maternal system are transferred across the placenta, which then target male-specific proteins found in the developing brain of the male foetus.

Because the prenatal environment of male foetuses varies as a function of the number of older brothers, future work could benefit from collecting data on the number of older brothers of male twins in addition to placentation status, to determine how and whether the both factors could provide insight into the discordant development of sexual orientation in identical male twins.

5.2.6 Hormonal Influences on Gender Nonconformity

Although a link between sexual orientation and prenatal androgenisation has not been consistently found, given that gender nonconformity is a correlate of sexual orientation (Bailey & Zucker, 1995; Lippa, 2008; Rieger et al., 2008), it is possible that in males there exists a link between gender nonconformity and exposure to prenatal hormones, as assessed via finger length ratios. In fact, in males, exposure to prenatal hormones, including testosterone, has been positively related to gender-typical play during childhood (Auyeung et al., 2009). However, just as that with sexual orientation, this relationship between prenatal androgen exposure and gender-typed behaviour has not been as consistently found across all available research (Hines, 2011; Hines et al., 2002). If such effect is small in magnitude, it is possible that the effect is more easily identifiable within pairs of male twins discordant for sexual orientation. Future work could investigate the link between gender nonconformity in males and exposure to prenatal androgens, as assessed via indices of testosterone exposure, by comparing the straight twin to his non-straight co-twin.

5.2.7 Developmental Implications for Females

As mentioned above, results suggest that altered androgen signalling may influence the development of sexual orientation in women. That is, non-straight female twins (bisexual or lesbian) may have been exposed to higher levels of testosterone during early development as compared to their genetically identical, but straight co-twins. Perhaps then, exposure to testosterone also influences correlates of sexual orientation in women, including gender nonconformity and physiological sexual arousal. Below I review evidence for both possibilities.

5.2.8 Hormonal Influences on Gender Nonconformity

A relationship between over exposure to prenatal androgens and gender nonconformity in females is mostly supported (Auyeung et al., 2009; Bao & Swaab, 2011; Brown et al., 2002b; Csathó et al., 2003; Hines, 2011; Hines et al., 2002). For example, fetal testosterone measured from amniotic fluid is positively associated with gender atypical play in girls (Auyeung et al., 2009). Further, women with Congenital Adrenal Hyperplasia (CAH), who are exposed to elevated levels of prenatal androgens, display increased signs of aggression and tend to be more gender nonconforming in their overall demeanor and in their preference for male-typical activities including working with engines and electronics, building models, yard work, hunting and fishing (Bao & Swaab, 2011; Berenbaum, 1999; Berenbaum & Bailey, 2003; Berenbaum & Beltz, 2011). With respect to finger length ratios, lesbians who report themselves as "Butch" tend to have lower 2D:4D ratios than those describe themselves as "Femme" (Brown et al., 2002a). Future work could examine whether gender nonconformity is related to 2D:4D and other indices of prenatal androgen exposure, including oto-acoustic emissions (Rahman, 2005). It is possible that within pairs of female twins discordant for sexual orientation, higher androgen exposure (as reflected by related markers) may be associated with gender nonconformity. Non-straight female twins, who are likely more gender nonconforming than their straight co-twins, may have been more masculinized during early development, as reflected in the aforementioned markers. This would suggest that prenatal androgenization contributes to the development of both sexual orientation and gender nonconformity in women. Further, because identical female twins with discordant sexual orientations correlate in their gender nonconformity, regardless of any average differences, they may also correlate in indices of prenatal androgenisation. This could further suggest that there may be some relationship between gender nonconformity and prenatal androgen exposure that is independent of sexual orientation.

5.2.9 Hormonal Influences on Physiological Arousal

Given that non-straight (lesbian and bisexual) women can show marginally more sexual response to females over males as compared to straight women (Chivers et al., 2007; Rieger et al., 2016), non-straight women can sometimes be more male-typical than other women, because responding in a way that is consistent with self-reported sexual orientation is usually seen in men. If some non-straight women are more male-typical in their sexual arousal, it is possible that the same women experienced more male-typical intrauterine environments during early development. As such, non-straight women who show greater sexual response to females over males may also have more masculinised markers of prenatal testosterone exposure than straight women. Future work could examine this hypothesised relationship in a larger sample of identical female twins with discordant sexual orientations. Within twin pairs, those who identify as non-straight will display more masculinized markers and will show stronger male-typical arousal patterns (stronger arousal to the females over males) as compared to their straight co-twins.

5.2.10 Physiological Arousal and Gender Nonconformity

Women are, on average, physiologically sexually aroused to both male and female sexual stimuli; although lesbians tend to be more aroused to females over males (Chivers et al., 2007; Rieger et al., 2016). In addition lesbians are, on average, more masculine than straight women in their nonsexual behaviour (Lippa, 2008; Rieger et al., 2010). A common influence could affect the expression of male-typical sexual and nonsexual traits (e.g. gender nonconformity) in some women, although previous work has failed to support this hypothesis (Rieger et al., 2016). However, one possibility is that gender nonconformity will be related to stronger arousal to females over males. In future work, the link between gender nonconformity and physiological sexual arousal could be tested in discordant twin pairs. One hypothesis is that within pairs, regardless of sexual orientation, females who are more gender-nonconforming will show stronger arousal to females over males.

5.3 Conclusions of Thesis

Identical twins with discordant sexual orientations differ in their degree of gender nonconformity, physiological sexual arousal, and to some extent, in indices of prenatal androgen exposure. These differences point to non-genetic influences during their development, even though some evidence for potential familial (e.g., genetic) factors was also found. Future work that includes parental perspectives on the emergence of gender nonconformity in identical twins and assesses their responses to specific gender-related behaviours and characteristics could provide important insight into the development of sexual orientation. Additional information on placentation status of the twins and fraternal birth order in male twin pairs could also provide an impression of the intrauterine environments that these twins were exposed to during their early development, which in turn, affected the formation of their discordant sexual orientations. Finally, an investigation of how correlates of sexual orientation, including gender nonconformity and physiological sexual response, are related in males and females could aid understanding of the expression and development of sexual orientation.

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