

Quantitative and theoretical analyses of the relation between older brothers and homosexuality in men

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Abstract

Meta-analysis of aggregate data from 14 samples representing 10,143 male subjects shows that homosexuality in human males is predicted by higher numbers of older brothers, but not by higher numbers of older sisters, younger brothers, or younger sisters. The relation between number of older brothers and sexual orientation holds only for males. This phenomenon has therefore been called the fraternal birth order effect. Research on birth order, birth weight, and sexual orientation suggests that the developmental pathway to homosexuality initiated by older brothers operates during prenatal life. Calculations assuming a causal relation between older brothers and sexual orientation have estimated the proportion of homosexual men who owe their sexual orientation to fraternal birth order at 15% in one study and 29% in another. The maternal immune hypothesis proposes that the fraternal birth order effect reflects the progressive immunization of some mothers to male-specific antigens by each succeeding male fetus and the increasing effects of such immunization on sexual differentiation of the brain in each succeeding male fetus. There are at least three possible mechanisms by which the mother's immune response could influence the fetus: the transfer of anti-male antibodies across the placenta from the maternal into the fetal compartment, the transfer of maternal cytokines across the placenta, and maternal immune reactions affecting the placenta itself. This hypothesis is consistent with recent studies showing that the quantity of fetal cells that enter the maternal circulation is greater than previously thought, and that the number of male-specific proteins encoded by Y-chromosome genes is greater than previously thought.

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1. Introduction

Traditional biological theories of sexual orientation posit causal roles for inherited genes or prenatal sex hormones in the development of homosexuality. Such theories appear to have substantial merit—at least of a heuristic nature—because they have predicted various research findings over the past dozen years and plausibly explained several others after the fact (Mustanski et al., 2002; Rahman and Wilson, 2003). One of the most reliable findings on sexual orientation, however, arose outside the traditional lines of research and theory and does not appear explainable by genetic or hormonal

mechanisms alone. That is the fraternal birth order effect, the topic of the present article.

The fraternal birth order effect is the finding, based on epidemiological data, that older brothers increase the odds of homosexuality in later-born males. The only biological explanation of this effect that has been seriously proposed so far is the hypothesis that it reflects the progressive immunization of some mothers to male-specific antigens by each succeeding male fetus and the concomitantly increasing effects of anti-male antibodies on the sexual differentiation of the brain in each succeeding male fetus. This notion has been called the maternal immune hypothesis.

The basic epidemiological data and various lines of evidence bearing on the immunological interpretation of them have previously been reviewed by the present writer (Blanchard, 1997, 2001; Blanchard and Klassen, 1997) and, in abbreviated form, by others (Mustanski

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et al., 2002; Rahman and Wilson, 2003). These reviews were written for different purposes and with different emphases; the article by Blanchard (1997), for example, summarizes early research on birth order and sexual orientation going back to the 1930s. None of them, however, provides a complete overview of the topic as it stands today. The following article was therefore written to update these reviews with additional research that quantifies the statistical relation between older brothers and homosexuality, contributes to the theoretical interpretation of this relation, and addresses prior theoretical criticism. Its other purpose was to elaborate the maternal immune hypothesis beyond previous statements of it.

In the previous reviews, the basic findings about older brothers and homosexuality were introduced by summarizing the results of individual studies on birth order and sexual orientation. A different approach was used for this article. Instead of reviewing individual studies one more time, the writer combined the data from all the controlled studies in his research program on birth order and sexual orientation and reanalyzed these data using meta-analytic methods. The results of the meta-analyses (presented in the next section) provide a concise introduction to the findings of interest. They also demonstrate the reproducibility of these findings using a previously untried statistical approach.

2. Meta-analysis of the basic findings on birth order and sexual orientation

The core of the above-mentioned research program consists of 14 samples of homosexual and heterosexual males from 12 published studies (Table 1). These samples comprise 10,143 subjects (3181 homosexuals and 6962 heterosexuals) examined in Canada, the Netherlands, the UK, and the USA. The sources of information were archival data from several previous studies as well as data collected by the writer and his colleagues in Toronto. The combination of new and old data meant that some subjects were examined in recent years and others were examined decades ago; for similar reasons, their years of birth ranged from 1861 to 1989. The samples include psychiatric patients and non-patient volunteers, subjects examined in adulthood and subjects examined in childhood, men who wished to become women and men contented with their male role and anatomy, and men sexually attracted to adults as well as men attracted to children. Thus, with the exception of race, which was Caucasian for the great majority of subjects, they represent an extremely diverse collection of males.

The method that was used to combine the data from these samples for the purposes of the present article was to re-cast the original observations in the form of

Table 1
Studies on birth order and sexual orientation in males, from the Clarke research program on biodemographic correlates of sexual orientation

Authors	Description of the sample	N homosexual	N heterosexual
Blanchard and Bogaert (1996a)	American volunteers over age 18 interviewed by Alfred Kinsey and associates from 1938 to 1963 (see Gebhard and Johnson, 1979)	799	3,807
Blanchard and Bogaert (1996b)	Canadian volunteers	302	434
Blanchard and Bogaert (1998)	American sex offenders against adults, from earlier study by Gebhard et al. (1965)	156	173
Blanchard and Bogaert (1998)	American sex offenders against pubescents, from earlier study by Gebhard et al. (1965)	69	127
Blanchard and Bogaert (1998)	American sex offenders against children, from earlier study by Gebhard et al. (1965)	42	143
Blanchard et al. (2000)	Canadian pedophilic and hebephilic patients (men attracted to prepubescent and pubescent children, respectively)	65	152
Blanchard and Sheridan (1992)	Canadian outpatients referred for assessment of gender dysphoria (roughly, transsexualism)	193	273
Blanchard and Zucker (1994)	American volunteers from earlier study by Bell et al. (1981)	569	281
Blanchard et al. (1995)	Canadian child and adolescent outpatients, matched on age at presentation, sibship size, and year of birth	156	156
Blanchard et al. (1996)	Dutch gender-dysphoric patients, adult and adolescent samples combined	104	79
Blanchard et al. (1998)	British and American volunteers, from earlier studies by Siegelman (1972, 1973, 1974, 1978, 1981)	385	225
Bogaert et al. (1997)	Canadian pedophilic patients	68	57
Ellis and Blanchard (2001)	American and Canadian volunteers	175	971
Zucker and Blanchard (1994)	American psychoanalytic patients from earlier study by Bieber et al. (1962)	98	84

aggregate data. That means that a “case,” in statistical terms, was a group rather than a single individual.

The 14 samples were divided into 28 groups (14 heterosexual and 14 homosexual). The sample sizes of these groups are shown in Table 1. As explained below, however, sample sizes were not taken into account in the statistical analyses.

The data points were four measurements taken from each group: the number of older brothers per subject, older sisters per subject, younger brothers per subject, and younger sisters per subject. An example of a single data point would be the number of older brothers per subject for the homosexual males interviewed by Alfred Kinsey and his colleagues from 1938 to 1963 (see Table 1). Thus, the aggregate data consisted of 112 data points (28 groups × 4 measurements) rather than 40,572 data points (10,143 subjects × 4 measurements).

Fig. 1 represents the means of the aggregate data. These means are unweighted, that is, an aggregate value—such as the number of older brothers per subject in a given group—was treated the same whether that group consisted of 42 subjects (the smallest group) or of 3,807 subjects (the largest group). The figure shows that the average homosexual group had more older siblings and fewer younger siblings than did the average heterosexual group. In other words, the homosexuals were born later in their sibships. It is noteworthy that

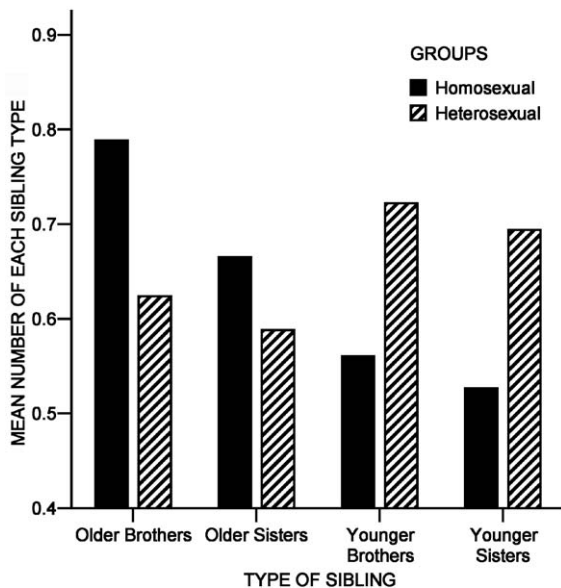


Fig. 1. Means of the aggregated data. Each bar represents an unweighted mean of means. Thus, for example, the leftmost solid bar indicates that the average for the 14 homosexual groups was 0.79 older brothers per subject. Because the groups were not weighted by sample size, the foregoing does not necessarily imply that the average for the 3181 homosexual individuals would also be exactly 0.79 older brothers. Similarly, the adjacent cross-hatched bar indicates that the average for the 14 heterosexual groups was 0.62 older brothers per subject, not that the average for the 6962 heterosexual individuals was 0.62 older brothers.

the average family sizes of the homosexual and heterosexual groups were practically identical: 2.61 (SD = 0.67) versus 2.70 (SD = 1.03) siblings per subject, respectively, $t(24) = 0.28$. (This comparison excluded the data of Blanchard et al., 1995, because their groups were individually matched on sibship size beforehand.)

Fig. 1 shows that the heterosexual and homosexual groups differed to some extent with regard to every type of sibling. It seems unlikely that all four differences arose independently; the mere fact that the total sibship sizes of the heterosexual and homosexual groups were practically identical implies that a relative excess for any one type of sibling had to be offset by relative deficits elsewhere, and vice versa. Moreover, numbers of older brothers and older sisters are inevitably correlated; an individual (or group) with more older brothers will also tend to have more older sisters (and vice versa). The same is true for numbers of younger brothers and younger sisters. The question therefore arises as to which parameter of sibship composition drives the observed differences between heterosexual and homosexual men.

One technique for investigating this question is logistic regression. In this type of analysis, sexual preference—dichotomously classified as heterosexual or homosexual—is the criterion variable, and numbers of older brothers, older sisters, younger brothers, and younger sisters are the predictor variables. Table 2 shows the results of logistic regression applied to the aggregate data.

The results indicated that the number of older brothers per subject carried some predictive value regarding a group’s sexual orientation, even after its numbers of older sisters, younger brothers, and younger sisters had been taken into account. In contrast, the amount of unique information carried by the number of older sisters per subject did not differ reliably from zero, when the numbers of the remaining three sibling-types were taken into account. Neither did the number of younger brothers or the number of younger sisters.

Table 2
Logistic regression of sexual orientation on numbers of siblings: model if term (predictor) removed

Predictor	Change in $-2 \log$ likelihood ^a	Significance of the change ^b
Older brothers	11.88	0.0006
Older sisters	1.40	0.2367
Younger brothers	3.70	0.0546
Younger sisters	0.54	0.4610

Note. The results show the effect of removing one predictor at a time from the regression equation, while leaving the remaining three predictors in the model. The removal of older brothers, and only older brothers, produced a statistically significant decrease in correct prediction of the groups’ sexual orientations.

^aDistributed as χ^2 with 1 degree of freedom.

^bTwo-tailed p .

Table 2 shows that the findings for younger brothers approached statistical significance. It will be recalled, however, that the homosexual groups had fewer younger brothers than did the heterosexual groups. This is apparent in Fig. 1. Thus, there was no overall tendency for the homosexual groups to have more brothers than the heterosexual groups. They were simply born later with regard to the brothers they had.

Another way of identifying the origin of the differences between the homosexual and heterosexual groups is to compare the sex ratios of their older and of their younger siblings with the expected Caucasian sex ratio of 106 male live births to 100 female live births (see Chahnazarian, 1988; James, 1987). In order to carry out these comparisons using unweighted aggregate data, the writer computed, for each of the 14 homosexual and 14 heterosexual groups, the ratio of older brothers to older sisters, and the ratio of younger brothers to younger sisters.

Fig. 2 shows the mean sex ratios of the older and younger siblings of the homosexual and heterosexual groups. For the homosexual groups, the mean ratio of older brothers to older sisters, 120.61 (SD = 19.57), differed significantly from the test value of 106 in a one-sample test, $t(13) = 2.79$, $p = 0.015$, two-tailed. Thus, the homosexual groups had an excess of older brothers in relation to the expected sex ratio of live births. In contrast, the homosexual groups did not have an excess of younger brothers, and the heterosexual groups did

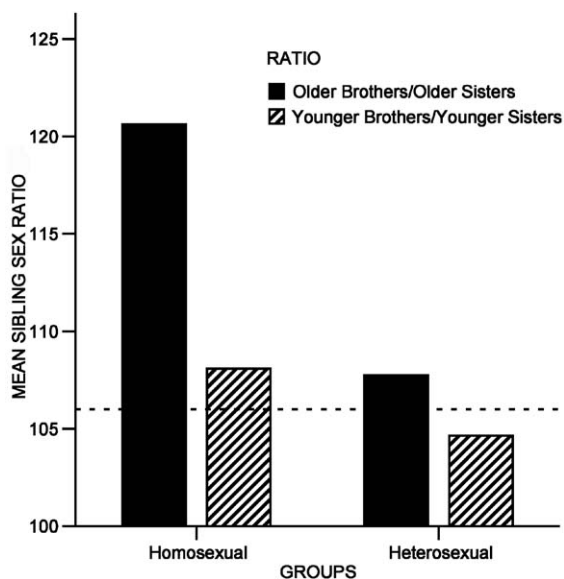


Fig. 2. Sex ratios of older and younger siblings. Each bar represents an unweighted mean of ratios. Thus, for example, the leftmost solid bar indicates that the average ratio of older brothers to older sisters for the 14 homosexual groups was about 121:100, and the adjacent cross-hatched bar indicates that the average ratio of younger brothers to younger sisters for these groups was about 108:100. The reference point for the observed values, which is indicated by the broken line, is the expected Caucasian sex ratio of 106 male live births per 100 female live births.

not have a significant excess (or deficiency) of older or younger brothers (all t values < 1.00).

In summary, the analyses of aggregate data undertaken here indicate that homosexual men have more older brothers than do heterosexual men, and that differences in other sibship parameters (older sisters, younger brothers, or younger sisters) are secondary consequences of the difference in older brothers. The same conclusions have been reached with analyses of individual rather than aggregate data (e.g., Blanchard and Bogaert, 1996b; Blanchard et al., 1998; Ellis and Blanchard, 2001), and by independent investigators (Green, 2000; Robinson and Manning, 2000; Williams et al., 2000). Similar conclusions were also reached by Jones and Blanchard (1998), who used a radically different statistical approach. In brief, Jones and Blanchard derived two theoretical equations for predicting a proband's birth order among his sisters from his observed birth order among his brothers. The first equation applies if sisters have no direct relation to a proband's sexual orientation but brothers do; the second applies if sisters have the same relation to a proband's sexual orientation as do brothers (including no relation). Comparisons with empirical data—a subset of the samples in the present Table 1—showed that the first equation held for homosexual men and the second for heterosexual men.

The main limitation to generalizing about the fraternal birth order effect is that most of the evidence for it comes from Western samples. The few data available, however, suggest that it also occurs in Asian and Polynesian populations (Poasa et al., 2004; Tsoi et al., 1977; Zucker and Blanchard, 2003).

3. Miscellaneous related findings

The positive findings for older brothers and homosexuality have been accompanied by various additional results, which, although negative, are crucial for interpreting the effect of older brothers on later-born males. A brief summary of these results suffices for the present review. The relation between number of older brothers and male homosexuality is not an artifact of higher maternal or paternal age (Blanchard and Bogaert, 1996a, b, 1997a, 1998; Blanchard and Sheridan, 1992; Bogaert et al., 1997) or of birth interval (Blanchard and Bogaert, 1997b).

Neither older brothers nor older sisters affect the odds of homosexuality in later-born females (Blanchard, 1997; Blanchard et al., 1998; Bogaert, 1997; Ellis and Blanchard, 2001). It therefore appears that females are invisible to the birth order phenomenon. Females do not influence their siblings' sexual orientation, and their siblings do not influence theirs. For this reason, the

relation between older brothers and male homosexuality was labeled the *fraternal* birth order effect.

4. Birth order, birth weight, and sexual orientation

None of the data presented so far addresses the timing of the fraternal birth order effect. During what phase of development does the mechanism linking older brothers and sexual orientation operate? Does this etiologic factor operate during a male's prenatal development, in his early childhood, or even later, in puberty or adolescence? One possible clue to the answer arose unexpectedly in a study on birth order, birth weight, and sexual orientation.

Blanchard and Ellis (2001) studied 3229 adult, homosexual and heterosexual, men and women, whose mothers knew the sex of every child (or fetus) that they carried prior to the subject. (This sample largely overlapped that of Ellis and Blanchard, 2001; see Table 1.) Information on birth weight, maternal gravidity, and other demographic variables was reported on questionnaires completed by the subjects' mothers.

This study yielded three main observations: (a) the heterosexual males with older brothers weighed less at birth than the heterosexual males with older sisters, (b) the homosexual males with older brothers weighed less than the heterosexual males with older brothers, and (c) the homosexual and heterosexual males with no older siblings, or older sisters only, did not differ in birth weight.

Each of Blanchard and Ellis's findings has since been confirmed once. Blanchard et al. (2002) studied 250 feminine boys referred to a child psychiatry service because of extreme cross-gender wishes or behavior and assumed, on the basis of previous research, to be prehomosexual (see Bailey and Zucker, 1995). The control subjects were 739 boys referred to the same service for reasons unrelated to sexual orientation or gender identity disorder and assumed, from base-rate probabilities, to be preheterosexual. The feminine boys with two or more older brothers weighed less at birth than did the control boys with two or more older brothers. In contrast, the feminine and control boys with fewer than two older brothers did not differ in birth weight. These results, therefore, essentially confirmed Blanchard and Ellis's second and third findings.

Blanchard et al. (2002) could not confirm Blanchard and Ellis's first finding. They found no difference in birth weight between the control boys with older brothers and the control boys with older sisters. The control boys also, however, failed to show the well-established increase in birth weight from first-born to second-born infants, which led Blanchard et al. (2002) to suggest that this and other discrepancies between their controls and subjects in previous investigations might

relate to the clinical disorders that prompted the referral of their controls to a psychiatric hospital.

Blanchard and Ellis's first finding was subsequently confirmed by Côté et al. (2003) in a nonclinical sample. Côté et al. studied birth weight in a series of 856 male and 862 female infants born in the province of Québec, Canada. Since these children were 5 months old when the data were collected, they were not—obviously—selected on the basis of their sexual orientation, and the sample may be regarded as overwhelmingly preheterosexual. Information on birth weight was collected from hospital records. The results showed that the male newborns with older brothers weighed less than the male newborns with older sisters.

The several implications of the birth weight data need to be considered separately. The finding that homosexual males with older brothers weigh less at birth than heterosexual males with older brothers, together with the finding that homosexual and heterosexual males without older brothers weigh the same, suggests that whatever mechanism links fraternal birth order and homosexuality operates during prenatal life. These findings imply, at the same time, that there are multiple developmental pathways to homosexuality and that certain characteristics (e.g. below-average birth weight) may be associated with only one of them. The statistical consequences of assuming that fraternal birth order is only one of several possible causes of male homosexuality are explored in the next section.

The foregoing evidence that the fraternal birth order effect reflects events during prenatal life is generally consistent with a lack of evidence that it reflects events during postnatal life. The most popular alternative to the maternal immune theory of this phenomenon is the notion that sexual interaction with older males increases a boy's probability of developing a homosexual orientation, and that a boy's chances of engaging in such interactions increase in proportion to his number of older brothers (e.g. Jones and Blanchard, 1998). Although this hypothesis may seem intuitively plausible, there are little empirical data to recommend it (see discussion in Purcell et al., 2000). On the contrary, the available data argue against such an explanation. Wellings et al. (1994, pp. 204–206) found that men who had attended all-male boarding schools were more likely than men who had not attended such schools to report some homosexual experience, but there was no difference between these groups in the amount of homosexual experience in later life. This suggests that homosexual sex-play in childhood is not an important determinant of sexual orientation in adulthood. In a related vein, Dawood et al. (2000) found no evidence that homosexuality was induced by incestuous sexual relations between brothers. Similarly, Bogaert (2000), in his reanalysis of the probability sample collected by Laumann et al. (1994), found no evidence that sex-play

with older brothers in childhood correlates with a man's sexual orientation in adulthood. Several writers have suggested that older brothers might influence the sexual orientation of later-born males via mechanisms other than sibling sex-play, for example, family dynamics, psychodynamics, or social learning processes. A number of these theories have been discussed elsewhere (Blanchard and Sheridan, 1992; Blanchard et al., 1995, 1996). None of these notions has any more empirical support than the sibling sex-play hypothesis.

Other potential implications of the birth weight data are less clear, partly because of conflicting results from different studies. One unresolved matter is whether the difference in birth weight between heterosexual males with older brothers and heterosexual males with older sisters has anything to do with the difference in birth weight between homosexual males with older brothers and heterosexual males with older brothers. Blanchard and Ellis (2001) attempted to tie these findings together with the hypothesis that a single mechanism is involved, and that the two findings simply represent dosage effects. According to this hypothesis, a mild fraternal birth order effect produces a slightly reduced birth weight but leaves sexual orientation unaffected. A stronger fraternal birth order effect produces a markedly reduced birth weight as well as an increased probability of homosexuality. Thus, heterosexual males with older brothers weigh less at birth than heterosexual males with older sisters, and homosexual males with older brothers weigh less yet.

If Blanchard and Ellis's single-mechanism hypothesis is correct, then one would expect that the effects of older brothers on birth weight would generally parallel the effects of older brothers on sexual orientation. Blanchard and Ellis (2001) and Côté et al. (2003) did, in fact, find that older brothers and older sisters lacked differential effects on birth weight in heterosexual females, which parallels the finding that older brothers and older sisters lack differential effects (or any effect) on sexual orientation in females. Different results, however, were obtained by Trotnow et al. (1976) and Magnus et al. (1985). These investigators found that newborn females as well as newborn males weighed less if they had older brothers than if they had older sisters. There is no obvious methodological reason for the discrepant results from Blanchard and Ellis (2001) and Côté et al. (2003) versus Trotnow et al. (1976) and Magnus et al. (1985) regarding birth weight in females. It is therefore unclear how well the data fit Blanchard and Ellis's single-mechanism hypothesis.

5. Estimates of the population attributable fraction

If one makes the assumption that older brothers correlate with homosexuality because older brothers are

a cause of homosexuality, then one can estimate the proportion of homosexual men who can attribute their sexual orientation to their fraternal birth order. This statistic, called the *population attributable fraction* (PAF) by epidemiologists, could be alternatively defined as the amount by which the prevalence of homosexuality would decrease in a population in which no one had any older brothers (e.g., in a country that effectively enforced a one-child policy). The latter form of definition is common among epidemiologists but is, of course, only of theoretical interest here.

The first attempt to estimate the PAF for homosexuality and fraternal birth order was published by Blanchard (2001). Blanchard's approach is easy to understand but computationally tedious. Blanchard's approach was refined and extended by Cantor et al. (2002). The main characteristics of the Blanchard–Cantor procedure are that it is applicable to case-control studies and that it uses information about the actual number of times an individual has been exposed to a causal agent (in this case, number of exposures equals number of older brothers). It requires an estimate of the population prevalence of homosexuality among men with no older brothers. Cantor et al. (2002) estimated this value as 2%. (This proved a close guess. In the study reported next, the observed prevalence of homosexuality among men with no older brothers was 2.18%.) Using this procedure, Cantor et al. (2002) estimated the PAF, the proportion of homosexual men whose sexual orientation is attributable to the fraternal birth order effect, at 15.1%. Cantor et al. (2002) did not attempt to develop any procedure for calculating confidence limits for the PAF computed by their method.

Blanchard and Bogaert (2004) recently produced a second estimate of the PAF, using fresh subjects, a different type of sample (probability or "random" rather than case-control), and a different method of computation. The subjects were a combined sample of 2256 heterosexual and 71 homosexual men examined in survey studies of sexual behavior in the USA and the UK. The original investigators were Laumann et al. (1994) and Wellings et al. (1994), respectively. The raw data from both surveys were archived for the potential use of other researchers; permission to use them was obtained by Anthony F. Bogaert. It should be noted that these data were not previously analysed in the writer's core research program (Table 1).

The PAF was calculated with the usual method for probability samples (see, for example, Rockhill et al., 1998, Table 1, Eq. (1)). The estimated value was 28.6%, which would mean that 28.6% of all homosexual men can attribute their sexual orientation to their fraternal birth order. The 95% confidence limits for this estimate, calculated with the logit method of Lachin (2000, p. 52), were 14.8% and 48.0%. Thus, although this second

estimate is nearly twice the 15.1% calculated by Cantor et al. (2002), the first estimate is still within the confidence limits of the second one. More importantly, the two values agree with regard to the essential point of the exercise: The proportion of homosexual men whose sexual orientation is attributable to fraternal birth order constitutes a minority, but not a negligible minority, of all homosexual men.

There are various reasons why attempts to estimate the PAF with greater and greater precision—aside from the enormous numbers of subjects needed to narrow the confidence limits substantially—would be misguided. One is that the real variable of interest might be the number of male fetuses carried by the subject's mother before she carried him, not the subject's number of live-born older brothers. There would be little reason to estimate the PAF for older brothers with utmost accuracy if older brothers are a less than perfect proxy for male fetuses carried a certain length of time. This point is related to the theoretical conjectures discussed in the next section.

6. The maternal immune hypothesis

The PAF calculations indicate that older brothers are, if not the most important cause of homosexuality, at least a cause important enough to warrant efforts at explanation. This assessment is reinforced by the number and diversity of subjects in which the fraternal birth order effect has been demonstrated. The most fully articulated explanation to date is the hypothesis of Blanchard and Bogaert (1996b) that this effect reflects the progressive immunization of some mothers to Y-linked antigens by each succeeding male fetus, and the concomitantly increasing effects of anti-male antibodies on the sexual differentiation of the brain in each succeeding male fetus. The remainder of this article concerns additional, mostly recent, data that bear on the plausibility of this notion.

Blanchard and Bogaert's (1996b) explanation, subsequently called the *maternal immune hypothesis* by Blanchard and Klassen (1997), runs as follows: The fraternal birth order effect may be triggered when fetal cells (or cell fragments) enter the maternal circulation, an event especially common during childbirth. If these cells are from a male fetus, they may include substances that only occur in, or on the surfaces of, male cells. The mother's immune system recognizes these male-specific molecules as foreign and starts producing antibodies to them. Following maternal immunization, maternal anti-male antibodies are available to cross the placental barrier and enter the brain of a male fetus. These antibodies somehow divert the sexual differentiation of the fetal brain from the male-typical pathway, so that the individual will later be attracted to men rather than

women. The probability—or strength—of maternal immunization increases with each male fetus, therefore the probability of homosexuality increases with each older brother.

The maternal immune hypothesis explains why older sisters do not affect the sexual orientation of later-born males, and why neither older brothers nor older sisters affect the sexual orientation of later-born females. That is because female fetuses do not produce male-specific substances and they would not be targets of anti-male antibodies. It similarly explains how the mother's body could ignore the number of female fetuses that she has carried while keeping some sort of running tally of the number of male fetuses. The maternal immune system would see female-specific substances from a female fetus as “self”—in effect, not see them at all—but it would see male-specific substances from a male fetus as “not-self,” that is, as foreign.

The maternal immune hypothesis might also explain the data on birth weight and sexual orientation. Animal studies of maternal immunization to paternal antigens have shown that such immunization can affect fetal weight, placental weight, or both. Both increases and decreases in conceptus weight have been obtained, depending on the experimental procedure (e.g. Gentile et al., 1992; Lu and Dawson, 1986; Saji et al., 1980). It is thus conceivable that anti-male antibodies produced by human mothers in response to immunization by male fetuses could decrease the birth weight of subsequent male fetuses as well as increase their odds of homosexuality. If that much is true, then the maternal immune hypothesis would simultaneously explain the absence of birth weight differences between homosexual and heterosexual males who lack older brothers. The hypothesis implies that homosexual males with no or few older brothers most likely acquired their sexual orientation from causes other than maternal immune responses. Their birth weights would not, therefore, show signs of maternal immune attack.

There is one epidemiological finding that appears, on first consideration, to be problematic for the maternal immune hypothesis. That is the fact that mothers can and do give birth to heterosexual sons after having given birth to homosexual sons. This was pointed out by Green (2000), who examined 14 homosexual male-to-female transsexuals who had at least one older brother and at least one younger brother. He found that 21 of the 22 younger brothers were heterosexual, and the remaining one was bisexual. Green argued that the maternal immune hypothesis implies that the younger brothers of a homosexual male should have a markedly elevated probability of homosexuality. This reading of the maternal immune hypothesis assumes that the birth of a homosexual boy indicates that the mother has been immunized by that point. Green concluded that his

findings disconfirmed the prediction of the maternal immune hypothesis.

Green himself noted that this prediction may be difficult to test adequately, especially if the testing is carried out on small samples, and if individual fetuses show differential vulnerabilities to the maternal immune response. The notion of differential vulnerabilities seems, in fact, much more likely than not. This can be illustrated with clinical examples from twin births. Twins exposed to teratogens of maternal origin can be affected to different degrees or in different ways (e.g., Reitnauer et al., 1997; Riikonen, 1994; Streissguth and Dehaene, 1993; Zemlickis et al., 1993), even though they receive the same dosage of teratogens in similar, if not identical, conditions. The difference in outcomes presumably relates, at least in part, to genetic differences between the exposed fetuses. It is even more likely that siblings gestated at separate times could be affected differently by maternal teratogens, because the outcomes in the case of singleton births might also be influenced by cofactors such as the mother's age, state of health, diet, and so on, during different pregnancies. In the present context, anti-male antibodies may be regarded as analogous to teratogens, although there is no intent to imply, by this analogy, that homosexuality should be considered a psychopathology.

There is a second problem with Green's prediction, a problem with the assumption that the birth of a homosexual boy indicates that the mother has been now immunized. The issue concerns a statistic called the *attributable fraction* (AF), which, despite the similarity in names, is quite different from the population attributable fraction (PAF). The AF may be defined, in the present context, as the proportion of homosexual men *with older brothers* who became homosexual because of their older brothers.

Blanchard's (2001) Table 2 gives the calculated AFs for homosexual men with 0–5 older brothers. Cantor et al.'s (2002) numbered equation 3 can be used to calculate the AF for homosexual men with any number of older brothers; this has been done for homosexual men with 0–9 older brothers in the present Fig. 3. Both sources show, for example, that only about 24% of homosexual men with one older brother and 43% of homosexual men with two older brothers can attribute their sexual orientation to the fraternal birth order effect. The remaining proportions owe their homosexuality to other causes such as polymorphic genes or atypical prenatal hormone levels (see Mustanski et al., 2002, for a review). There is therefore no reason to assume that the appearance of a homosexual male in a sibship means that maternal immunization (or any other birth-order-correlated factor) must have been established by that point.

In summary, Green's point is not without merit. It is unclear, however, how much of a jump in the odds of

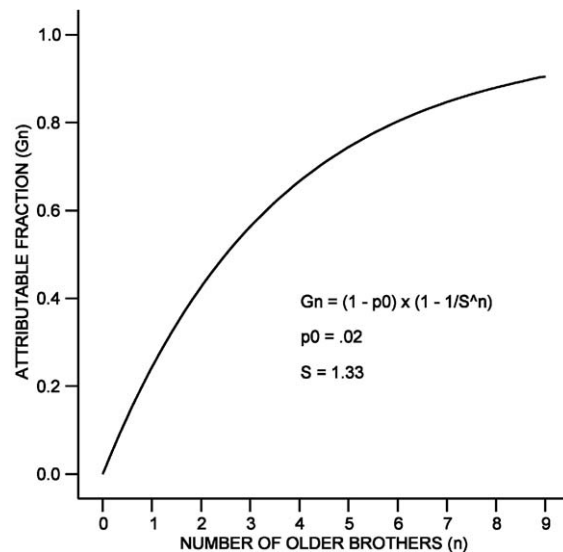


Fig. 3. Attributable fraction as a function of number of older brothers. The figure shows that 0% of homosexual men with no older brothers, 24% of homosexual men with one older brother, 43% of homosexual men with two older brothers, and so on, can attribute their sexual orientation to the fraternal birth order effect. The plotted values were calculated using numbered equation 3 from Cantor et al. (2002). The prevalence of homosexuality among men with zero older brothers (p_0) was estimated at 0.02 (see Blanchard and Bogaert, 2004), and the multiplicative change in the odds of homosexuality produced by each older brother (S) was assumed to be 1.33, based on the findings of Blanchard and Bogaert (1996b). The function was plotted as continuous although it is actually stepwise; this makes it easier to see that the effect of fraternal birth order would equal all other causes of homosexuality in a theoretical group of homosexual men with 2.5 older brothers (see numbered eq. (5) in Cantor et al., 2002).

homosexuality should be expected after the first homosexual son in a sibship, or how many subjects—probably a great many—would be needed to determine if the observed jump confirms the expectation.

The process envisioned by the maternal immune hypothesis may be thought of as one in which an earlier fetus influences a later fetus through the intermediary of the mother. The best known model for such a process is hemolytic disease of the newborn (HDN) caused by Rhesus (Rh) blood group incompatibility. HDN may develop when an Rh-negative woman is impregnated by an Rh-positive man and conceives an Rh-positive fetus. Red blood cells from the fetus cross the placenta and enter the mother's circulation throughout pregnancy (and especially at delivery) and stimulate maternal antibody production against the Rh factor. The antibodies reach the fetus via the placenta and destroy the fetal red blood cells, causing anemia. This phenomenon shows a distinct birth order effect (e.g. Adams et al., 1981). In the first pregnancy, the fetus is rarely affected; in the second pregnancy, the fetus may be mildly affected; and in subsequent pregnancies, the fetus may be so severely affected that it dies in utero. The obvious differences between the HDN model and the maternal

immune hypothesis are that HDN affects red blood cells rather than the brain, and that HDN is provoked by, and subsequently impacts, fetuses of both sexes. It is interesting to note, however, that the majority of studies to investigate the question have found that male fetuses are more likely than female fetuses to initiate maternal Rh-immunization (Renkonen and Seppälä, 1962; Renkonen and Timonen, 1967; Scott, 1976; Scott and Beer, 1973; Woodrow and Donohoe, 1968; versus Knox, 1968).

7. Candidate antigens

The maternal immune hypothesis presupposes the existence of substances that are produced only by males and that stimulate immune responses in females. Such substances would be antigenic from the standpoint of the female exposed to them but not from the standpoint of the male who produced them. Thus, commonly used phrases in the immunology literature, such as “male-specific antigen,” mean that the origin of the denoted substance is specific to males; the immunogenic effects of the substance would be specific to females.

Blanchard and Bogaert (1996b) suggested that one candidate for the hypothesized antigen is an H-Y antigen. The term *H-Y antigens* has been used historically to refer to the whole collection of molecules that are found on the surfaces of male cells but not on female cells, that stimulate immune reactions in females, and whose structural or regulatory genes reside on the Y-chromosome. H-Y antigens may be detected by transplantation tests, by assays that correlate with transplantation tests such as cytotoxic T-cell tests, or by serological tests. The antigens detected by transplantation tests are different from those detected by serological tests, and the antigens detected by serological tests appear to be heterogeneous in themselves (Wolf, 1998). The latter group of antigens are sometimes called serological H-Y or *serologically detected male antigens* (SDMA). A few genes coding for transplantation H-Y have been identified (e.g. Pierce et al., 1999; Vogt et al., 2002; Wang et al., 1995); however, the molecular nature and genetic basis of serological H-Y are presently unknown (Müller, 1996; Wolf, 1998). This knowledge gap is unfortunate, in the present context, because serological tests identify antibodies, and the maternal immune hypothesis postulates an antigen that stimulates antibody production, whether or not that antigen also provokes graft rejection or T-cell mediated cytolysis.

Various lines of indirect evidence supporting the hypothesis that maternal antibodies to some H-Y antigen might influence sexual orientation have previously been reviewed by Blanchard and Klassen (1997). Such evidence includes animal research showing that the immune systems of pregnant females recognize and react to fetal H-Y antigens, clinical evidence that male fetuses

are more antigenic to human mothers than are female fetuses and more likely to provoke maternal immune reactions, and research on tissue localization showing that H-Y antigens are strongly represented on the surfaces of brain cells. Blanchard and Klassen also noted the single published study on maternal immunization and filial sexual behavior, which found that male mice whose mothers were immunized to H-Y prior to pregnancy were much less likely to mate successfully with receptive females (Singh and Verma, 1987).

An alternative to seeking the candidate antigen among substances of proven antigenicity but unknown identity is to seek it among substances of known identity but unproven antigenicity. Eligible substances would have to comprise molecules of sufficient size and complexity to potentially stimulate the immune system, and they would have to be associated with the male sex. A class of molecules that fits this profile is male-specific proteins.

Recent research has shown that genes on the human Y-chromosome collectively encode at least 27 distinct male-specific proteins or protein families (Skaletsky et al., 2003). Three of these proteins are expressed in the fetal brain, and two of the three are relatively good examples of candidate antigens because they encode cell-surface proteins, which might be accessible to antibodies. The first is protocadherin 11 Y-linked (*PCDH11Y*, also known as *PCDH22* and *PCDHY*; Blanco et al., 2000), and the second is neuroligin 4 Y-linked (*NLGN4Y*; Jamain et al., 2003). Both the protocadherin and neuroligin families of proteins are cell adhesion molecules, thought to play an essential role in specific cell-cell interactions during embryonic brain development. It might be noted that another 12 of the 27 genes studied by Skaletsky et al. are ubiquitously expressed. It is possible that the brain might be more sensitive to reactions involving their products than are other tissues; thus, some of these ubiquitous proteins might also be candidate antigens. Blanco et al. (2000) suggested that *PCDH11Y* might have gained a male-specific function in brain morphogenesis, with behavioral consequences. Speaking in a similar but broader vein, Skaletsky et al. (2003) indicated that Y-chromosome genes (besides *SRY*, the testis-determining gene) could be related to sex-dimorphic differences in behavior and cognition as well as to differences in anatomy and physiology.

The search for the candidate antigen is broadened (and complicated) by an additional factor. Because H-Y antigens predominantly occur in the male sex of mammals, their genes were originally assumed to be on the Y-chromosome. There is evidence, however, that for at least one serological H-Y antigen, the structural gene may be autosomal and be controlled by genes on the sex chromosomes (Wolf, 1998). If this were the candidate antigen, then looking for it among Y-chromosome gene-products would be futile. Furthermore, the antigenicity of the molecules produced by its

Y-chromosome regulatory gene would be irrelevant. Thus, the search for the hypothesized male-specific antigen cannot necessarily be restricted to substances encoded by Y-chromosome genes.

There is, in summary, a variety of antigens or potential antigens that might play the role required by the maternal immune hypothesis. At the present time, however, there is no evidence that any specific one of them actually plays it.

8. Fetal-maternal cell traffic

It has long been known that fetal material enters the maternal circulation at parturition, as illustrated by the example of HDN. More recent research has shown that fetal cells of various types—nucleated erythrocytes, trophoblasts, lymphocytes, granulocytes, stem cells, and progenitor cells—enter the maternal circulation throughout normal pregnancies, and that fetal cells enter in much greater quantities during abnormal pregnancies (Bianchi and Lo, 2001; Lambert et al., 2001; Nelson, 2002; Pertl and Bianchi, 2001). The significance of this is that maternal exposure to male-specific antigens might take place much earlier in pregnancy than at delivery, depending on when such antigens are first expressed.

The available evidence indicates that serological H-Y is expressed quite early in development, at least in animals. Several studies have identified serological H-Y on preimplantation mouse and cow embryos at the 8-cell stage (e.g. Epstein et al., 1980; Krco and Goldberg, 1976; Shelton and Goldberg, 1984; White et al., 1983, 1987). This implies that miscarried or aborted male fetuses might also immunize the mother and thereby augment the probability of homosexuality in her subsequent male offspring. Thus, the real variable of interest might be the number of male fetuses carried by an individual's mother before she carried him, not the individual's number of live-born older brothers. In that instance, a PAF calculation based on the observed relation between a man's sexual orientation and his number of (live-born) older brothers would underestimate the proportion of homosexual men who owe their orientation to the fraternal birth order effect, or more precisely, to the mechanism underlying that effect. One might argue, on that basis, that the percentage of older-brother-type homosexual men is more likely to lie above than below the estimates previously presented in this paper.

9. Action of anti-male antibodies in the fetal brain

The notion that anti-male antibodies of maternal origin might influence sexual differentiation in the male

fetal brain has various precedents in theory and research that suggest that maternal antibodies can affect other aspects of neurodevelopment in the fetus. Adinolfi (1976) hypothesized that maternal antibodies against brain antigens could cause long-lasting damage to the fetal brain, resulting in mental retardation or selective neurological handicaps. Evidence supporting this hypothesis is exemplified by two recent studies that compared the effects of blood serum (a potential source of antibodies) from two types of women: mothers of normal children and mothers of children with neurodevelopmental disorders. The affected children of the latter had autism or severe specific language disorder in one study (Dalton et al., 2003) and dyslexia in the other (Vincent et al., 2002). Sera from these women were injected into pregnant mice. The sera from the mothers of affected children produced behavioral deficits in the mouse offspring. The authors of these studies concluded that the results were consistent with a role for maternal antibodies in the etiology of some forms of neurodevelopmental disorder.

Foster and Archer (1979) introduced the variable of birth order to this topic. They hypothesized that maternal antibodies against brain antigens could explain the common finding that later born children tend to have lower IQs than earlier born children. They argued that the probability or intensity of a maternal immune attack on the fetal brain would increase with increasing parity. A variant form of Foster and Archer's birth order hypothesis was advanced by Gualtieri and Hicks (1985). Gualtieri and Hicks were concerned to explain why males are more likely than females to suffer neurodevelopmental and psychiatric disorders of childhood. Their *immunoreactive theory of selective male affliction* proposed that the higher rates of disorders in males relate to the greater antigenicity of male fetuses, which makes them more likely to elicit immune reactions from gestating mothers. They speculated that the greater antigenicity of male fetuses might be accounted for by the presence of H-Y antigen, acting alone or in combination with other antigens. On the basis of this theory, Gualtieri and Hicks predicted that the deleterious effects of birth order on the neurodevelopment of later born boys should be greater if the preceding siblings are male.

A few studies have produced results that tend to support Gualtieri and Hicks's prediction. Ackerman et al. (1988) found that boys with primary cognitive disorders (learning disability or mental retardation) were more likely to have older brothers than were boys with emotional or behavioral problems. In contrast, older brothers did not discriminate between girls who had cognitive versus emotional-behavioral disorders. The authors did not investigate whether older sisters correlated with cognitive disorders in boys or girls. Their findings were confirmed by Flannery and Liederman

(1994), who showed that having older brothers correlated with mental retardation in boys, and that having older sisters did not. Neither older brothers nor older sisters correlated with mental retardation in girls.

There is a clear similarity between Gualtieri and Hicks's immunoreactive theory of selective male affliction and Blanchard and Bogaert's (1996b) maternal immune hypothesis of male homosexuality, in that both theories attempt to explain their phenomena in terms of maternal immune reactions to male fetuses. It is important to note, however, that both the targets and the biological effects of antibodies causing neurodevelopmental disorders might be quite different from the targets and actions of antibodies causing homosexuality. Speculations about the latter case are discussed in the remainder of this section.

The simplest and most obvious way that anti-male antibodies might produce homosexuality in males is by binding to, and thus inactivating, male-specific molecules (i.e., antigens) located within or, more likely, on the surface of fetal brain cells. This is based on the supposition that the phylogenetic purpose of such molecules includes the establishment of male-typical behavior, in particular, the focusing of sexual interest on women. It would not be necessary, for this theory, for anti-male antibodies to kill fetal neurons after interacting with them. (It would also not be inevitable—the binding of an antibody to an antigen often has no direct biological consequence.) It would merely be necessary for the antibodies to prevent male-specific molecules from performing their usual roles in the masculinization of sex-dimorphic brain structures, roles such as cell–cell recognition and interaction. The effect would be that relevant brain structures simply develop as if those molecules were absent, as in the female fetus. Thus, the theory does not imply that homosexual men should show other signs of a maternal immune attack on the brain, such as generalized cognitive deficits.

It may seem odd, given the overwhelming evidence that prenatal androgens masculinize the fetal brain, that the foregoing account is silent about the involvement of hormones. It is possible that maternal antibodies affect sexual orientation through some interaction with sex hormones, but it is also possible that antibodies affect sexual orientation by a completely separate route. The latter possibility is suggested by evidence that the normal causes of sex-dimorphic development include some factors that are independent of sex hormones. If there are factors that routinely contribute to sexual differentiation independently of hormones, then interference with those factors might produce homosexuality independently of hormones.

The evidence for nongonadal influences on sexual differentiation in the brain has been reviewed by other writers (e.g. Arnold, 2003; Vilain, 2000; see also Erickson, 1997). Two specific examples of such evidence

will be given here. The first example is the analysis of an individual, rare, gynandromorphic finch, in which the right half of the brain was genetically male and the left half was genetically female (Agate et al., 2003). The right half of the brain developed a more masculine phenotype. This difference could not be attributed to gonadal hormones, because both halves of the brain were exposed to the same hormonal environment. The second example is the recent identification of 51 genes that are differentially expressed in male and female mouse brain prior to gonadal formation (Dewing et al., 2003). Dewing et al. interpreted this finding as evidence that gonadal hormones may not be solely responsible for sex differences in brain development. They further hypothesized that these genes may play some role in the development of sex-dimorphic behavior. They did not, however, speculate on the mechanisms by which these genes could influence neural development.

It must be emphasized, to avoid any possible misunderstanding, that the maternal immune hypothesis is not meant to challenge the accepted view that prenatal androgens are responsible for most, or nearly all, of the routine masculinization of the human male fetal brain. The hypothesis simply asserts that some additional factor, which looks more immunologic than endocrinologic, appears to be involved in certain instances of male homosexuality.

10. Alternative mechanisms of action

The theoretical discussion in this article so far has basically elaborated the original suggestion of Blanchard and Bogaert (1996b) that the mechanism by which the mother's immune system affects the fetus is the transfer of anti-male antibodies across the placenta from the maternal into the fetal compartment. There are at least three other pathways, however, by which a mother's immune response could conceivably affect her fetus.

The first of these is the trans-placental transfer of a different product of the immune system, namely, cytokines. Cytokines are various proteins that are secreted by cells of the immune system and that serve to regulate the immune response. Th1 (T helper type 1) cells produce pro-inflammatory cytokines, which promote cell-mediated immunity, and Th2 (T helper type 2) cells produce anti-inflammatory cytokines, which promote humoral immunity (i.e. antibody production). There is some evidence that pro-inflammatory cytokines of maternal origin may cross the placental barrier and damage the development of the fetal brain (e.g. Gilstrap and Ramon, 2000; Kamenov et al., 1999; Urakubo et al., 2001). This raises the possibility that pro-inflammatory cytokines may be involved in the etiology of benign variations like homosexuality.

Th1- and Th2-type immune responses are mutually inhibitory (e.g. Elenkov and Chrousos, 1999; Petrovsky, 2001), so it seems unlikely that both pro-inflammatory cytokines and antibodies would be etiologic in the same atypical pregnancy outcomes. One might therefore consider the cytokine and antibody versions of the maternal immune hypothesis to be competing rather than complementary explanations of the fraternal birth order effect in male homosexuality. It is difficult to evaluate the relative merits of these two versions of the hypothesis when both are so highly speculative. One additional finding, however, tends to make the antibody version more attractive.

A few studies have yielded results suggesting that acute stress experienced by women during pregnancy increases the likelihood of homosexuality in their male offspring (Dörner et al., 1980; Dörner et al., 1983; Ellis et al., 1988; Ellis and Cole-Harding, 2001), or that chronic (i.e., characterological) maternal stress-proneness shifts other sex-dimorphic behaviors away from the typical masculine pattern (Bailey et al., 1991). A substantial body of evidence now indicates that stress does not simply suppress the immune system but rather shifts the Th1/Th2 balance away from cell-mediated immunity and toward humoral immunity, that is, toward antibody production (e.g., Elenkov and Chrousos, 1999; Kang and Fox, 2001; Marshall and Agarwal, 2000; Matalka, 2003). If maternal stress affects the sexual orientation of male offspring by facilitating antibody production, then the maternal stress effect and the fraternal birth order effect could be unified by the antibody version of the maternal immune hypothesis. The appeal of this theoretical simplification depends, however, on the reliability of the maternal stress effect, and this is still in question (Hines et al., 2002; Schmidt and Clement, 1990).

The second pathway by which a mother's immune response might affect her fetus is via changes in the placenta itself. Vernier (1975) found that newborn males with older brothers had higher placental weights than newborn males with older sisters, and that newborn males with multiple older brothers had higher placental weights than males with one older brother. The placental weights of newborn females, in contrast, were not affected by the sex of older siblings. The parallels in the effects of older brothers and older sisters on placental weight and on homosexuality are obvious. Vernier attributed his findings to a greater antigenicity of male fetuses compared with female fetuses.

The placenta is a major endocrinological organ of the fetus. Several studies have shown that placental size affects hormonal output (e.g., Chan and Leatham, 1977; Furuhashi et al., 1984; Liu et al., 1999; Spellacy et al., 1975; Vermeulen et al., 1982). It is therefore possible that maternal immune reactions to male-specific antigens alter the hormonal milieu of the fetus by enlarging

the placenta, and that this altered hormonal milieu predisposes the male fetus to homosexuality.

The third possible pathway begins, like previous versions, with hormonal or non-hormonal intrauterine factors that vary in strength with the number of prior male fetuses, but it ends with a somewhat different sequence of events. In this pathway, the hypothetical factors trigger epigenetic mechanisms in brain cells of the current fetus. Epigenetic mechanisms are processes that alter the phenotypic expression of genes by means of meiotically or mitotically heritable but potentially reversible changes in DNA methylation, chromatin structure, or both. In this scenario, it is epigenetically altered gene expression that affects the development of brain structures controlling sexual orientation. If this scenario were correct, the resulting homosexuality would represent an environmentally induced "epimutation" (Holliday, 1987). There is some evidence that the uterine environment can influence offspring's subsequent behavior through epigenetic mechanisms (e.g. Francis et al., 2003) although no obvious reason to link this phenomenon with maternal parity. What recommends this pathway for consideration is the array of evidence suggesting that epigenetic phenomena may underlie a variety of complex traits (e.g. Petronis, 2001).

11. Summary and conclusions

The finding that a man's sexual orientation relates to his number of older brothers is based entirely on epidemiological rather than experimental data. The evidence that this relation arises from events in prenatal life rather than experiences in childhood or adolescence is scanty but consistent. If the relation does arise in utero, then it most likely involves some kind of maternal immune response, whether or not any of the specific possibilities mentioned in this article is correct. That is because the fetus cannot "know" its own birth order—only the mother can "know" its birth order—and the only maternal organ system, apart from the nervous system, with the requisite memory capability is the immune system. Thus, a prenatal origin for the fraternal birth order effect—which may be accepted unless and until contrary evidence demonstrates otherwise—constrains the range of possible explanations considerably.

Males comprise roughly 50% of the human population. The prevalence of homosexuality in adult men is about 3%, and the fraternal birth order effect accounts for maybe 20% of them. One may therefore ask why a phenomenon that concerns perhaps 3 human beings in 1000 should be of general interest to biologists or behavioral scientists. The answer is that these individuals, like those with much rarer, intersex conditions, may provide clues to the processes involved in the development of typical (i.e. heterosexual) romantic and

sexual interests. The causes of heterosexuality may be self-evident from the evolutionary standpoint, but its origins are far from completely understood at the level of proximate causes. It is therefore to be hoped that the epidemiological evidence linking fraternal birth order and sexual orientation, which has now reached a volume that should be difficult to ignore, will stimulate research scientists to investigate possible underlying mechanisms in the laboratory.

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