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Dual-gender macrochimeric tissue discordance is predicted to be a significant cause of human homosexuality and transgenderism

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Abstract

I present literature evidence that suggests that human chimerism may be quite common, occurring in between 5% and 15% of people. Chimerism has been believed to be rare because it usually presents without visible phenotype. In addition to the documented occurrence of dual gender macrochimeras with true hermaphrodite phenotype, there are reports of the occurrence of other natural human macrochimeras. The literature reviewed in this paper suggests that such macrochimerism is much more common than usually appreciated. Chimerism occurs in a patchy manner, with male cells outgrowing female in macrochimerism causing the majority to be phenotypically male. The literature also suggests that the sex of nervous system tissue is the primary determinant in higher animals of sexual attraction. From this, the existence of human macrochimeras in which large proportions of cells are male and female is predicted to have a correlation with homosexuality and transgender self-identification because in many such cases, the central nervous system, or crucial parts of it, will be of one sex and the gonads and body form will be of the opposite sex. I describe experiments to further clarify this hypothesis, which can also have potential benefit beyond this specific question.

Keywords: homosexuality; homosexual; transgenderism; transgender; chimerism; chimera; macrochimerism; macrochimera; tetragametic; dispermic; gender discordance

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Introduction

Chimerism

Chimerism is the condition of any single individual composed of cells derived genetically from more than one pair of gametes. It is known that chimerism stemming from dizygotes where at least one is male and another is female occurs in humans[1, 2]. These cases were detected due to investigation of the very rare phenotype of true hermaphroditism. There are very few instances where macrochimerism could be visible based on phenotype. The two that are known are first, true hermaphroditism, and second, apparent mosaicism. Every other chimera phenotype would present without any noticeable features.

The term tetragametic chimerism is often used to describe merging of fraternal twin embryos. However, the term macrochimerism will be used here since in theory more than two embryos could merge to become one chimeric organism and several different kinds of chimerism have been discovered.

Chimerism presents in quite a few forms, which derive from different causes. Perhaps the most common forms of chimerism are fetal microchimerism in mothers [3, 4] and maternal microchimerism that occurs in their children [5]. In the former type of microchimerism, cells from the fetus cross over the placenta and take up residence in the body of the mother. In the latter type, maternal cells cross over the placenta into the fetus and live on in the child. A somewhat less common form occurs in multiple births [6, 7] crossing from sibling to sibling. In all these forms of microchimerism, relatively small numbers of cells cross over and migrate to organs in the mother, child, or sibling where they may persist. In mothers, their offspring's cells have been shown to persist for decades after giving birth [3].

Transplanting of any organ or tissue, such as a kidney, bone marrow, and even whole blood transfusion is also a type of chimerism. Transplanted tissue can lead to migration of cells from the donor into the host, and this can be a concern when examining simple results, such as from blood tests, of possible chimerism. Chimerism results need to be evaluated for degree of relatedness as an early method of ruling out such a possibility. SCID mice can have humanized immune systems provided for them this way, a method that has become standardized today [8].

Methods for manipulating mammals to generate macrochimeras have been known for half a century [9, 10] and experimental examination of in-vitro created chimeras is well developed [11]. Bringing embryos together in the morula or early blastocyst stage can result in aggregation into a chimerical embryo [10, 12]. Experimental chimerism in animals shows a patchy pattern with most of one organ formed from one genotype, most of the next from another[13]. Natural chimerism also occurs with low level infiltration in organs[14]. As will be discussed below, these results provide insight into the potential behaviour of spontaneous chimeras.

Multi-species chimeras can be generated in the lab, which has generated some controversy. Most commonly this lab procedure is done by inserting stem cells from a foreign organism into an animal embryo, by injection[11]. Prior to registration of markers for self by the immune system, virtually any tissue will be accepted.

For the purposes of this discussion, a macrochimera is a single individual formed from cells originating in two or more separately fertilized embryos, leading to large proportions of the resulting organism being formed by each original participating embryo. It would not be correct to term the general case tetragametic because in theory, any number of embryos, each containing full gametes, could form a single chimera (e.g. fraternal triplet zygotes merging to a singleton) although more than two zygotes should be very rare from natural causes. Consequently, a more accurate term is dual-gender macrochimerism for the condition of cells of both sexes being present in large proportions. Male cells outgrow female cells in mouse model dual-gender merged embryos [15]. Consequently, it is reasonable to assume that male-female macrochimeras will be more likely to appear to be male.

Biological basis of homosexuality

A number of proposed biologically based mechanisms have been investigated regarding human homosexuality, all of them with degrees of controversy, and none have yet been conclusively proven. These ideas include exposure to prenatal androgens [16], hypothalamic dimorphism [17-19], birth order [20, 21], an hypothesized brain differentiation continuum [22], and antagonistic selection to increase female fecundity [23, 24]. The mystery of the role of biology in human homosexuality continues to be an area of strong interest and it is likely that a variety of biological causes have impact on human sexuality.

Hypothesis

It is proposed that when the sex of gonadal cells conflicts with the sex of central nervous system cells in whole or in part there will be nervous system development that can be discordant with the apparent sex as determined by gonads and visible phenotype. It is further proposed that dual-gender macrochimerism is one of the causes of homosexuality and transgender identification. This hypothesis includes transgender identification, but does not require it, as chimerism can generate a range of nerve gender proportions in the brain.

Hypothesis support

The following section provides three lines of evidence that my hypothesis is a reasonable explanation of some instances of homosexual or transgender behaviour.

First, the matter of rarity of human chimerism is discussed centered around Boklage's work in the field. There is considerable evidence that the dogma that chimerism is rare is an artifact resulting from causes ranging from systematically throwing out evidence to difficulty of diagnosis and lack of phenotype.

Second, a method is used to derive an absolute minimum lower bound estimate of the occurrence of male/female macrochimerism, and to describe other factors that should justify raising the estimate which are not readily quantifiable. The purpose of this lower bound estimate is primarily to prove that the condition described must occur and what the proportions should be in the population. Secondarily, it is intended to show

by deduction that the true value should be much higher due to difficult to quantify factors.

Third, evidence from literature regarding sexual behavior determination will be discussed that supports the hypothesis that a male/female macrochimera would be expected to display nervous system gender discordance with its gross sexual morphology.

Macrochimerism in humans is not rare

There are a number of major reasons for believing that macrochimerism in humans is not rare, which Boklage discusses and which I will summarize here.

Boklage states that spontaneous human chimerism is not rare [13, 15, 25]. He states, regarding chimerism, that it is:

> *"…impossible to differentiate from single-genotype people by ordinary observation and seriously difficult to identify even with the best of the newest biomedical technologies. Cases are discovered in the population with low frequency and high technical difficulty, creating the pervasive false impression that they are rare."*[13]

It has long been believed that dizygotic (fraternal) twinning is from dual ovulation. But in examining this idea over decades Boklage has found that there is no actual evidence to support this, it is instead a supposition handed down.

Boklage has assembled a large body of data on malformations of dizygotic and monozygotic (identical) twins. What he has shown is that while there is a variance between singletons and twins, there is not a significant variance between identical and fraternal twins. This makes the double ovulation hypothesis that has been assumed to be true for fraternal twins in his words "untenable"[25]. He states that all the evidence points to monozygotic and dizygotic twins sharing the same (still unknown) twinning mechanism. The significance of this is that knowing that both types of twins start from one egg-cell mass points to chimerism being much more likely.

He also discusses in the same paper that what appears as mosaicism cannot exclude chimerism and many cases of apparent mosaicism may well be chimerism.

Because data showing multiple individuals in a single sample is routinely thrown out as evidence of contamination, and the field dogmatically defines as evidence of poor lab technique any evidence that monochorionic fetuses are dizygotic, the fraction could be 5%-15% of the population, perhaps higher, although the natural mechanism is unknown [11, 13, 15]. Boklage relates the example of a conference presentation in 1986:

> *"A young physician from Glasgow tried to tell us about three monochorionic pairs among 12 in his sample, in whom he had found (with testing more extensive and more sensitive than the usual zygosity genotyping) discordant blood grouping markers suggesting dizygosity (Mortimer, 1987). The pillars of the Society came crashing down about his head. The tenor of the response from the floor was: '... of course, one must know, of course, that only monozygotic twins can be monochorionic. Results such as yours suggesting otherwise must have come from a very unreliable laboratory …"*[13]

There is considerable further discussion regarding chimeras that are well worth reading in these references to Boklage and I recommend them. In addition to this, cases of male-female chimerism have also been discovered on the basis of hermaphroditic phenotype [1, 2], so it is proven that the condition does occur. But generally speaking, chimeras would not be expected to show any obvious phenotypic signs.

Discussion of rare data for dual-gender macrochimerism in humans

The overall significance of the numbers worked out below should not be construed as an upper limit on occurrence. Instead, the exercise should primarily be considered a thought process that shows further proof of occurrence, proportions of occurrence and proceeds to an open-ended conclusion that provides a minimum.

In recent years two rare cases of women who are macrochimeras were identified because their children were identified by genetic testing as not being their own [15, 26, 27]. One of the tests was ordered by AFDC/TANF, the other was in preparation for an organ transplant. There are believed to be other cases, but since such records are not easily available and identification is complicated, documenting them has not been practical. In the case of the AFDC/TANF

ordered test, the white mother was threatened with having her children taken away and possible prosecution. This strongly motivated her and her extended family. In the other case, physicians decided to investigate her case when it was found by tissue typing for the transplant protocol.

For the purposes of the below estimate, these mothers are members of a population composed of all parentage tests conducted. However, there is good reason to believe that the rare event of diagnosis is the tip of a figurative iceberg, due to multiple factors: A. The sample size for the AFDC/TANF segment of mothers below is overstated due to inability to determine the exact number of tests ordered to prove parentage. The true sample size is probably orders of magnitude smaller than the pool used. B. Social factors result in poor and minority mothers not having the resources to prove a parentage genetic test is incorrect. C. Where parentage tests tell a mother that her child is not her own and the test is voluntary, there is generally little or no incentive to pursue an expensive course of research. D. The geometry of macrochimerism is not well understood, but evidence shows it is patchy, leading to the likely probability that the kind of female-female macrochimerism that has been detected will most likely have a 50% chance of having a different test result for the common buccal swab versus gonads in a female-female chimera.

Unquantifiable social disenfranchisement influencing reporting of macrochimerism

It should be noted that AFDC/TANF mothers were virtually destitute as the tests were administered to prevent welfare fraud. The majority of such recipients are without access to legal resources required. People of African ancestry have roughly double the dizygotic twinning rate of Caucasians, and yet the only documented macrochimera cases are Caucasian, while the twinning rates would lead one to believe that twice the number of macrochimeric mothers of African ancestry should be expected. This strongly suggests that where such mothers exist, these mothers were not properly diagnosed after testing negative for direct parentage. It is likely that most destitute mothers give up when confronted with a large government bureaucracy with the power to take their children and prosecute them. Thus it is highly unlikely that all occurrences of macrochimeric mothers have been properly recorded. The true numbers are unknown but there is good reason to believe that they are much higher.

Estimate of fraction of tests showing macrochimeric mothers

No data are available specifically for maternity testing or specific to the number of AFDC/TANF tests conducted. Testing figures are better termed parental "relatedness testing" [28]. In 2003, approximately 350,000 parental relatedness tests were conducted [29]. In discussion with experts in the field, the ratio of maternity tests was estimated in a range from 60% to 88% of tests conducted, with the balance paternity only.

Applying the 60% to 88% yields a range of maternity tests conducted per year of 210,000 to 308,000. While the number of relatedness tests rose between 2000 and 2005, for the purposes of this estimate it will be assumed that the number of maternity tests remained constant from 2000 to 2005 and that 2003 represents a rough average. Using the estimated fractions, the maternity test sample size is 1.05 million to 1.54 million over the 5 year period from 2000 to 2005. At least two macrochimeric mothers have been detected in that total population [26, 27] with other probable cases. Thus, on this basis alone female/female macrochimeras are roughly 2 in 1 million births. Applying the 50% detection ratio because of patchiness discussed above doubles that value to 4 in 1 million births.

Zygote sex ratio combinations define the ratio of male/female macrochimerism

Macrochimerism has three possible outcomes, shown in the style of a classic Punnet square in Table 1. A fertilized zygote is either XX or XY which denote genetically female and male zygotes respectively. This diagram is not actually a Punnet square, since it does not describe the mixing of genes in diploid cells. The situation is parallel, however, and the diagram shows the outcomes correctly. The point of this Punnet square is to determine the relative frequency of chimera types.

Just as in classical Mendelian genetics, the occurrence of female/female macrochimeras will be approximately 25% of the total, male/male macrochimeras will be 25% of the total, and male/female macrochimeras will be approximately 50% of the total since they can be formed two different

ways while the others can only be formed one way. Thus, there should be twice as many male/female macrochimeras as there are female/female chimeras. From above, the minimal number of female/female macrochimeric births is approximately 4 per million. Doubling that number results in a minimum of 8 male/female macrochimeric births per million as the next round minimum.

Table 1 – Punnet style square for dizygotic chimera formation showing the possible combinations of two fertilized zygotes that form one normal appearing fetus.

Zygotes	**XX**	**XY**
XX	XX/XX	XX/XY
XY	XY/XX	XY/XY

Dizygotic twinning rate differences effect on male/female macrochimera estimate

Dizygotic twinning rates have significant historical variance; for instance in Sweden in the 1960s the rate of dizygotic twinning was half what it was 200 years before [30] and dizygotic twinning is believed to be environmentally influenced. Accepted figures for current dizygotic twinning rates of Caucasians and those of Sub-Saharan African ancestry are 8 and 16 per thousand, respectively [30] although these can vary regionally by a few percentage points. The rates are for twinning rates prior to major use of in-vitro fertilization (IVF) procedures because twinning rates are higher today owing to IVF. Since the rate of macrochimerism in IVF procedures may be different from that in naturally conceived births, the higher IVF-influenced twinning rate will be ignored for purposes of estimation in this context.

For a macrochimera to appear requires dizygotic [31] twin embryos to occur. The figures above for dizygotic twinning rates show that Caucasians should be roughly 1/3 of such twins given an equal population distribution between the two groups (8/24). Within the AFDC/TANF federal system that ordered the tests, this equal population distribution is approximately true [32]. Other ethnic groups are ignored as they are either not significantly represented within the AFDC/TANF population, or else there are no specific dizygotic twinning data for them. Making the assumption that there should be equivalent rates of dizygotic twin merging for all ethnic groups would indicate that for the 4 Caucasian cases of female/female dizygotic chimerism developed above there should be at least 8 more, for a total of 12 female/female chimeras in both ethnic groups. As was previously discussed, Table 1 shows that there should be double that number of male/female chimeras, or 24 of them per million births, which is approximately 1 per 50,000 births.

Conclusion regarding rare event detection of chimeras

This 1 per 50,000 number should be viewed as an exercise in light of the major factors that lead to believing that this figure is probably off by orders of magnitude. Even for those most doubtful of this hypothesis, applying this rate of occurrence gives us a population minimum of 30,000 such people in the developed world. The question then becomes not, “Are there dual-gender macrochimeras?” but “How many dual-gender macrochimeras are really out there?” The true number could be quite large.

Nervous system sex and sexual orientation in humans and animals

The human brain is a large distributed system of approximately 100 billion cells divided into two hemispheres. In a mixed sex chimera, nerve cell sex ratios in the brain would be expected to be mixed in a continuum from all male to all female where the quantum element is a single cell among the approximately 100 billion cells. Chimerism occurs in a patchwork fashion and migration paths of cells can be complex in embryos[13, 33]. Along that continuum of patchwork composition of the brain the locations of patches and their interactions could vary a great deal. The distributed nature of the brain thus provides a logical basis for a range from transgender identification, to exclusive homosexuality and bisexuality.

A wild chimeric zebrafinch with half of its brain made up of male cells and the other half made up of female cells showed dimorphic sexual differentiation of the two sides of the brain but both sides of the brain had an identical hormonal environment [34]. In an experiment on quail, chimeric female brains in male quail bodies did not result in male behavior [35]. A large amount of work in humans shows sex-based neural differentiation [36] and the long term evidence

from the John's Hopkins experiment of boys raised as girls shows discordant sexual identity based on brain sex that is independent of nurture, parental beliefs, the child's formal beliefs or of hormones [37]. This discordance conflicts with the earlier belief that sex reassignment was a straightforward matter [38].

The John's Hopkins sex-reassignment experiment occurred at a time when it was practiced to raise boys with ablated or ambiguous genitalia as girls, treating them with hormones and transgender surgery. The belief was that hormones in humans determined sexual orientation entirely, as occurs in most fish and invertebrates such as shellfish, so all that was necessary was to bring physical morphology into coincidence with hormones. The fact that treatment of males with estrogen results in softening of skin, development of breasts and other female secondary characteristics helped create this view. Similarly, the masculinizing response of females to androgens was also thought indicative. An early review of the case of Joan provided support for this experimental treatment modality [38]. However, long term review of the case of Joan/John showed problems with this approach and it turns out that most genetically male children treated with female hormones self-identified as males, sometimes becoming suicidal owing to gender discordance [37, 39]. Thus, the idea that hormones alone activate the brain so as to determine sexual orientation is no longer credible.

Suggested Experiments

Without studies to examine this matter, it is impossible to determine beyond argument how prevalent dual-gender macrochimerism is even though it must be granted that it does occur. Such studies would be expensive to conduct and require many years. These would need to be large-scale studies examining multiple tissues for presence and proportions of cells using relatedness measures as well as valid population surveys.

Blood testing conducted through blood banks, or on subject volunteers, may be usable as an initial screening system, but validation should be attempted to ensure that it does not result in a low detection artifact since there is no guarantee that blood will always detect macrochimerism. In addition to blood studies, the determination of relatedness between cells within a single body by various kinds of biopsy is needed. Conducting such studies requires the determination of frequency of macrochimerism in the general population and determination of frequency in tissues of homosexual and transgender individuals. To do that reliably will require a large sample size both of subjects and of samples per subject.

It would be important to map the relatedness geometry of tissues in identified human macrochimeras to better understand the variability of distribution that naturally occurs since it may well differ significantly from experimental models. The Visible Human Project® [40] has done a great deal for anatomy. A "best case" chimera study would include large numbers of identified donated chimeras processed similarly, taking many relatedness samples of each section to map them. This could tell us if there are patterns to placement of chimeric cells, and if so, what those patterns are.

However, such studies could be justified on the basis of more than just answering this question if it could be practical to do full sequencing of those hundreds of thousands of section samples. If it were practical to do full sequencing, the study would have relevance to many areas: undiagnosed cancer incidence, precancerous conditions, persistent viral diseases, microbiological population distributions, mosaicism incidence, forensics and probably surprises we could not possibly predict.

Examination of chimeric brains would be required to properly examine this question. There are special problems examining brains, since living biopsies are out of the question, except where neurosurgery is already required to take place. Even then, results would be unlikely to mean a great deal because it wouldn't be possible to map enough of a living brain. This means that studies would need to occur on donated chimera brains, with many samples taken. Relatedness data would be available for brains as part of whole body studies, but it may be impractical to perform as many as desirable in toto. As a fallback, processing of donated brains in a similar manner to that proposed for a whole-body study would be important.

In summary, the set of experiments that should be done are extensive, expensive and the experiments and their results could be controversial. These facts should not prevent researchers from trying to move forward, but do explain a significant reason for writing up this hypothesis at this stage in its development. Depending on how such studies were done, they could provide benefits far beyond just this question. Such studies

would be cross-disciplinary, require collaboration between multiple labs, and would be best performed with data collection separated from analysis to provide the optimum utility for data discovered.

Conclusion

The true rate of occurrence for dual-gender macrochimerism is unknown, but evidence suggests strongly it may be high. Such chimerism has been identified, and so have same-sex chimeras. While the hard numbers indicate rarity, there are multiple reasons to believe that chimerism is not uncommon. It may in fact be the case that homosexuality and transgender identity are a primary indicator. If it is true that a sizeable percentage of humans are macrochimeras, then half of those are dual gender chimeras, and the majority of the dual-gender macrochimeras are male. That would suggest that such chimerism could be a significant mechanism in homosexuality and transgender identification.

Since discordance of sex between nervous system cells and gonads leads to apparent sexual identity discordance in many animal species, it follows that in humans it will express as a similar discordance when the gonadal cells are in conflict with the sex of the cells in the central nervous system. This tissue discordance hypothesis has not yet been considered as a probable cause of homosexuality or transgender identification in humans.

Sexual behaviour in humans is doubtless multi-factorial, with psychological, cultural and biological explanations all providing some contribution. Proposed biological causes are multiple, although none has yet proved conclusive. Dual-gender macrochimerism provides a compelling biological rationale for these phenomena.

For any specific individual, macrochimerism is clearly a biological accident that no one could control and is impossible to change after the fact. It is the author's hope that greater understanding of the biology of homosexual and transsexual behaviour will have a positive impact on the lives of homosexual and transsexual people, through explaining that (some of) the reasons for the differences between homosexual and heterosexual people reside in deep biological causes.

Glossary

AFDC – Aid to Families with Dependent Children
SCID – Severe Combined Immunodeficiency
TANF – Temporary Assistance for Needy Families

Author's Contributions

All work is that of Brian P. Hanley, PhD

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