

Evidence for a Biological Influence in Male Homosexuality

Two pieces of evidence, a structure within the human brain and a genetic link, point to a biological component for male homosexuality

by Simon LeVay and Dean H. Hamer

Most men are sexually attracted to women, most women to men. To many people, this seems only the natural order of things—the appropriate manifestation of biological instinct, reinforced by education, religion and the law. Yet a significant minority of men and women—estimates range from 1 to 5 percent—are attracted exclusively to members of their own sex. Many others are drawn, in varying degrees, to both men and women.

How are we to understand such diversity in sexual orientation? Does it derive from variations in our genes or our physiology, from the intricacies of our personal history or from some confluence of these? Is it for that matter a choice rather than a compulsion?

Probably no one factor alone can elucidate so complex and variable a trait as sexual orientation. But recent laboratory studies, including our own, indicate that genes and brain development

play a significant role. How, we do not yet know. It may be that genes influence the sexual differentiation of the brain and its interaction with the outside world, thus diversifying its already vast range of responses to sexual stimuli.

The search for biological roots of sexual orientation has run along two broad lines. The first draws on observations made in yet another him—that for physical differences between men's and women's brains. As we shall see, "gay" and "straight" brains may be differentiated in curiously analogous fashion. The second approach is to scout out genes by studying the patterns in which homosexuality occurs in families and by directly examining the hereditary material, DNA.

Researchers have long sought within the human brain some manifestation of the most obvious classes into which we are divided—male and female. Such sex differentiation of the brain's structure, called sexual dimorphism, proved hard to establish. On average, a man's brain has a slightly larger size that goes along with his larger body; other than that, casual inspection does not reveal any obvious dissimilarity between the sexes. Even under a microscope, the architecture of men's and women's brains is very similar. Not surprisingly, the first significant observations of sexual dimorphism were made in laboratory animals.

Of particular importance is a study of rats conducted by Roger A. Gorski of the University of California at Los Angeles. In 1978 Gorski was inspecting the rat's hypothalamus, a region at the base of its brain that is involved in instinctive behaviors and the regulation of metabolism. He found that one group of cells near the front of the hypothalamus is several times larger in male

than in female rats. Although this cell group is very small, less than a millimeter across even in males, the difference between the sexes is quite visible in appropriately stained slices of tissue, even without the aid of a microscope.

Gorski's finding was especially interesting because the general region of the hypothalamus in which this cell group occurs, known as the medial preoptic area, has been implicated in the generation of sexual behavior—in particular, behaviors typically displayed by males. For example, male monkeys with damaged medial preoptic areas are apparently indifferent to sex with female monkeys, and electrical stimulation of this region can make an inactive male monkey approach and mount a female. It should be said, however, that we have yet to find in monkeys a cell group analogous to the sexually dimorphic one occurring in rats.

Nor is the exact function of the rat's sexually dimorphic cell group known. What is known, from a study by Gorski and his co-workers, is that androgens—typical male hormones—play a key role in bringing about the dimorphism during development. Neurons within the cell group are rich in receptors for sex hormones, both for androgens—testosterone is the main representative—and for female hormones known as estrogens. Although male and female rats initially have about the same numbers of neurons in the medial preoptic area, a surge of testosterone secreted by the testes of male fetuses around the time of birth acts to stabilize their neuronal population. In females the lack of such a surge allows many neurons in this cell group to die, leading to the typically smaller structure. Interestingly, it is only for a few days before and after birth that the medial preoptic neurons are sensitive to androgen; removing an-

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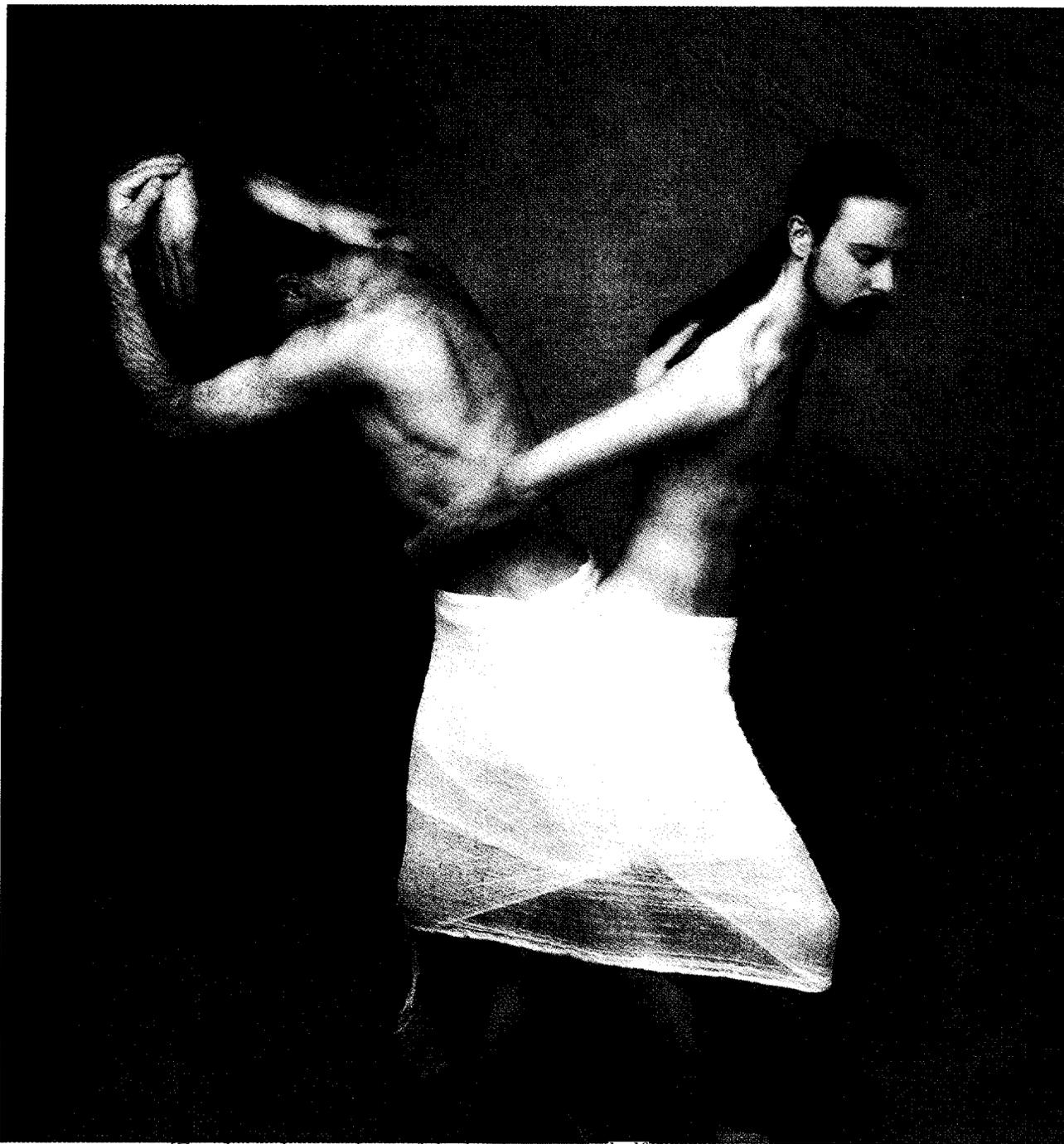
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Gorski and his colleagues at U.C.L.A., especially his student Laura S. Allen, have also found dimorphic structures in the human brain. A cell group named INAH3 (derived from "third interstitial nucleus of the anterior hypothalamus") in the medial preoptic region of the hypothalamus is about three times larger in men than in women. (Notably, however, size varies considerably even within one sex.)

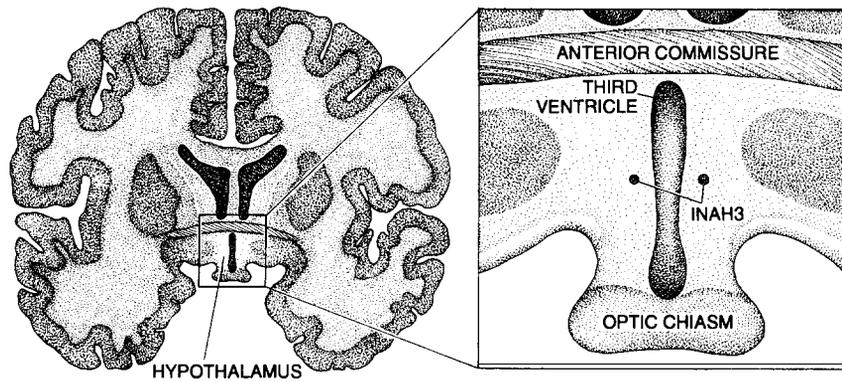
In 1990 one of us (LeVay) decided to check whether INAH3 or some other cell group in the medial preoptic area varies in size with sexual orientation as well as with sex. This hypothesis was something of a long shot, given the prevailing notion that sexual orientation is a "high-level" aspect of personality molded by environment and culture. Information from such elevated sources is thought to be processed primarily by the cerebral cortex and not by "lower" centers such as the hypothalamus.

LeVay examined the hypothalamus in autopsy specimens from 19 homosexual men, all of whom had died of complications of AIDS, and 16 heterosexual men, six of whom had also died of AIDS. (The sexual orientation of those who had died of non-AIDS causes was not determined. But assuming a distribution similar to that of the general populace, no more than one or two of them were likely to have been gay.) LeVay also included specimens from six women whose sexual orientation was unknown.



[Zeus] cut the members of the human race in half, like fruit that is to be dried and preserved, or like eggs that are cut with a hair. -Plato, Symposium

HYPOTHALAMUS of the human brain was examined for differences related to sexual orientation. The hypothalamus of each of the 41 subjects was stained to mark neuronal cell groups. The cell group termed INAH3 in the medial preoptic area was more than twice as large in the men as it was in the women. INAH3 also turned out to be two to three times larger in straight men than it was in gay men (*micrographs at far right*). This finding suggests a difference related to male sexual orientation about as great as that related to sex.



After encoding the specimens to eliminate subjective bias, LeVay cut each hypothalamus into serial slices, stained these to mark the neuronal cell groups and measured their cross-sectional areas under a microscope. Armed with information about the areas, plus the thickness of the slices, he could readily calculate the volumes of each cell group. In addition to Allen and Gorski's sexually dimorphic nucleus INAH3, LeVay examined three other nearby groups—INAH1, INAH2 and INAH4.

Like Allen and Gorski, LeVay observed that INAH3 was more than twice as large in the men as in the women. But INAH3 was also between two and three times larger in the straight men than in the gay men. In some gay men, as in the example shown at the top of the opposite page, the cell group was altogether absent. Statistical analysis indicated that the probability of this result's being attributed to chance was about one in 1,000. In fact, there was no significant difference between volumes of INAH3 in the gay men and in the women. So the investigation suggested a dimorphism related to male sexual orientation about as great as that related to sex.

A primary concern in such a study is whether the observed structural differences are caused by some variable other than the one of interest. A major suspect here was AIDS. The AIDS virus itself, as well as other infectious agents that take advantage of a weakened immune system, can cause serious damage to brain cells. Was this the reason for the small size of INAH3 in the gay men, all of whom had died of AIDS?

Several lines of evidence indicate otherwise. First, the heterosexual men who died of AIDS had INAH3 volumes no different from those who died of other causes. Second, the AIDS victims with small INAH3s did not have case histories distinct from those with large INAH3s; for instance, they had not been ill longer before they died. Third, the other three cell groups in the medial preoptic area—INAH1, INAH2 and INAH4—turned out to be no smaller in

the AIDS victims. If the disease were having a nonspecific destructive effect, one would have suspected otherwise. Finally, after completing the main study, LeVay obtained the hypothalamus of one gay man who had died of non-AIDS causes. This specimen, processed "blind" along with several specimens from heterosexual men of similar age, confirmed the main study: the volume of INAH3 in the gay man was less than half that of INAH3 in the heterosexual men.

One other feature in brains that is related to sexual orientation has been reported by Allen and Gorski. They found that the anterior commissure, a bundle of fibers running across the midline of the brain, is smallest in heterosexual men, larger in women and largest in gay men. After correcting for overall brain size, the anterior commissure in women and in gay men were comparable in size.

What might lie behind these apparent correlations between sexual orientation and brain structure? Logically, three possibilities exist. One is that the structural differ-

ences were present early in life—perhaps even before birth—and helped to establish the men's sexual orientation. The second is that the differences arose in adult life as a result of the men's sexual feelings or behavior. The third possibility is that there is no causal connection, but both sexual orientation and the brain structures in question are linked to some third variable, such as a developmental event during uterine or early postnatal life.

We cannot decide among these possibilities with any certainty. On the basis of animal research, however, we find the second scenario, that the structural differences came about in adulthood, unlikely. In rats, for example, the sexually dimorphic cell group in the medial preoptic area appears plastic in its response to androgens during early brain development but later is largely resistant to change. We favor the first possibility, that the structural differences arose during the period of brain development and consequently contributed to sexual behavior. Because the medial preoptic region of the hypothalamus is implicated in sexual behavior in monkeys, the size of INAH3 in men may in-

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Family Tree Studies and the X Chromosome

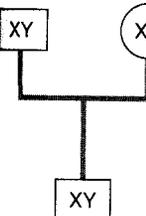
Family trees of male sexual orientation show the rates of homosexuality (*darker brown*) in maternally related males. Rates in paternal relatives were not significantly above the average population rate of 2 percent. This finding raised the possibility of involvement of the X chromosome (*shown below*). Males have two sex chromosomes—a Y inherited from the father and an X from the mother. Thus, a trait inherited through the mother's side might logically be influenced by a gene on one of her X chromosomes (*indicated here in red*). In fact, further experiments showed that one small area at the tip of the X chromosome—Xq28—was shared by a large percentage of gay brothers.

X CHROMOSOME

Xq28 (SHARED REGION)

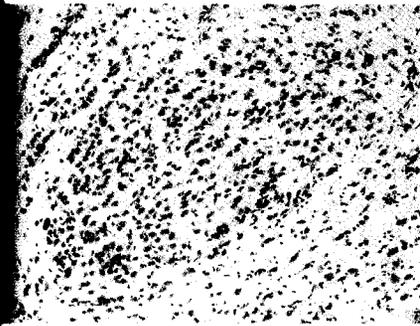


PATERN



HETEROSEXUAL MAN

HOMOSEXUAL MAN



deed influence sexual orientation. But such a causal connection is speculative at this point.

Assuming that some of the structural differences related to sexual orientation were present at birth in certain individuals, how did they arise? One candidate is the interaction between gonadal steroids and the developing brain; this interaction is responsible for differences in the structure of male and female brains. A number of scientists have speculated that atypical levels of circulating androgens in some fetuses cause them to grow into homosexual adults. Specifically, they suggest that androgen levels are unusually low in male fetuses that become gay and unusually high in female fetuses that become lesbian.

A more likely possibility is that there are intrinsic differences in the way individual brains respond to androgens during development, even when the hormone levels are themselves no different. This response requires a complex molecular machinery, starting with the androgen receptors but presumably including a variety of proteins and genes whose identity and roles are still unknown.

At first glance, the very notion of gay genes might seem absurd. How could genes that draw men or women to members of the same sex survive the Darwinian screening for reproductive fitness? Surely the parents of most gay men and lesbians are heterosexual? In view of such apparent incongruities, research focuses on genes that sway rather than determine sexual orientation. The two main approaches to seeking such genes are twin and family studies and DNA linkage analysis.

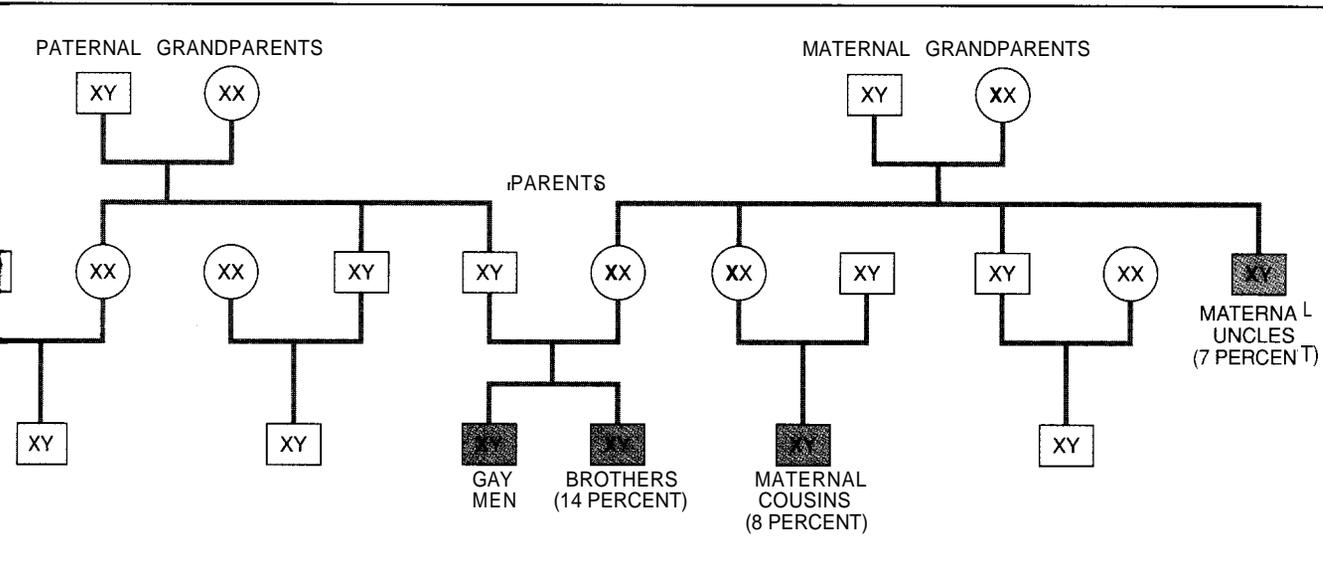
Twin and family tree studies are based on the principle that genetically influenced traits run in families. The first modern study on the patterns of homosexuality within families was published in 1985 by Richard C. Pillard and James D. Weinrich of Boston University. Since then, five other systematic studies on the twins and siblings of gay men and lesbians have been reported.

The pooled data for men show that about 57 percent of identical twins, 24 percent of fraternal twins and 13 percent of brothers of gay men are also gay. For women, approximately 50 per-

cent of identical twins, 16 percent of fraternal twins and 13 percent of sisters of lesbians are also lesbian. When these data are compared with baseline rates of homosexuality, a good amount of family clustering of sexual orientation becomes evident for both sexes. In fact, J. Michael Bailey of Northwestern University and his co-workers estimate that the overall heritability of sexual orientation—that proportion of the variance in a trait that comes from genes—is about 53 percent for men and 52 percent for women. (The family clustering is most obvious for relatives of the same sex, less so for male-female pairs.)

To evaluate the genetic component of sexual orientation and to clarify its mode of inheritance, we need a systematic survey of the extended families of gay men and lesbians. One of us (Hamer), Stella Hu, Victoria L. Magnuson, Nan Hu and Angela M.L. Pattatucci of the National Institutes of Health have initiated such a study. It is part of a larger one by the National Cancer Institute to investigate risk factors for certain cancers that are more frequent in some segments of the gay population.

Hamer and his colleagues' initial survey of males confirmed the sibling results of Pillard and Weinrich. A brother of a gay man had a 14 percent likelihood of being gay as compared with 2 percent for the men without gay brothers. (The study used an unusually stringent definition of homosexuality, leading to the low average rate.) Among more distant relatives, an unexpected pattern showed up: maternal uncles had a 7 percent chance of being gay, whereas sons of maternal aunts had an 8 percent chance. Fathers, paternal uncles and the three other types of cousins showed no correlation at all.



Although this study pointed to a genetic component, homosexuality occurred much less frequently than a single gene inherited in simple Mendelian fashion would suggest. One interpretation, that genes are more important in some families than in others, is borne out by looking at families having two gay brothers. Compared with randomly chosen families, rates of homosexuality in maternal uncles increased from 7 to 10 percent and in maternal cousins from 8 to 13 percent. This familial clustering, even in relatives outside the nuclear family, presents an additional argument for a genetic root to sexual orientation.

Why are most gay male relatives of gay men on the mother's side of the family? One possibility—that the subjects somehow knew more about their maternal relatives—seems unlikely because opposite-sex gay relatives of gay males and lesbians were equally distributed between both sides of the family. Another explanation is that homosexuality, while being transmitted by both parents, is expressed only in one sex—in this case, males. When expressed, the trait reduces the reproductive rate and must therefore be disproportionately passed on by the mother. Such an effect may partially account for the concentration of gay men's gay relatives on the maternal side of the family. But proof of this hypothesis will require finding an appropriate gene on an autosomal chromosome, which is inherited from either parent.

A third possibility is X chromosome linkage. A man has two sex chromosomes: a Y, inherited from his father, and an X, cut and pasted from the two X chromosomes carried by his mother. Therefore, any trait that is influenced by a gene on the X chromosome will tend to be inherited through the mother's side and will be preferentially observed in brothers, maternal uncles and maternal cousins, which is exactly the observed pattern.

To test this hypothesis, Hamer and his colleagues embarked on a linkage study of the X chromosome in gay men. Linkage analysis is based on two principles of genetics. If a trait is genetically influenced, then relatives who share the trait will share the gene more often than is expected by chance—this is true even if the gene plays only a small part. Also, genes that are close together on a chromosome are almost always inherited together. Therefore, if there is a gene that influences sexual orientation, it should be "linked" to a nearby DNA marker that tends to travel along with it in families. For traits affected by only

one gene, linkage can precisely locate the gene on a chromosome. But for complex traits such as sexual orientation, linkage also helps to determine whether a genetic component really exists.

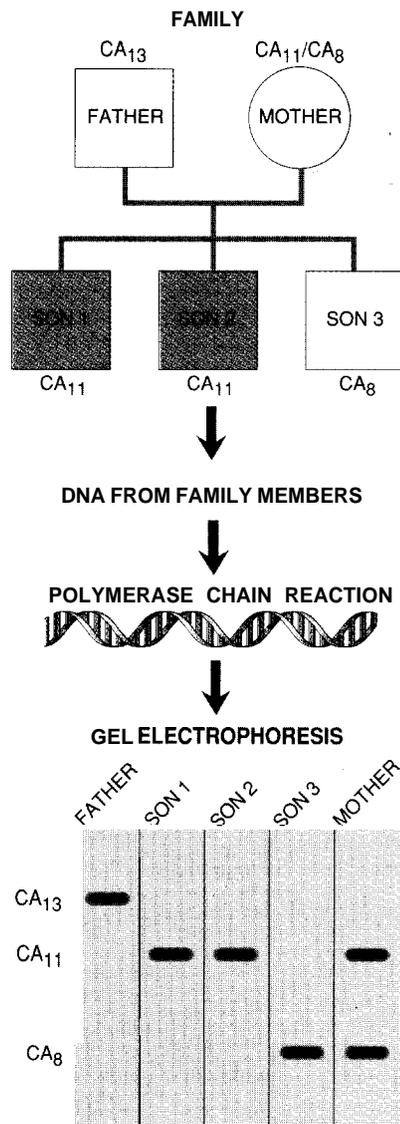
To initiate a linkage analysis of male sexual orientation, the first requirement was to find informative markers, segments of DNA that flag locations on a chromosome. Fortunately, the Human Genome Project has already generated a large catalogue of markers spanning all of the X chromosomes. The most

useful ones are short, repeated DNA sequences that have slightly different lengths in different persons. To detect the markers, the researchers used the polymerase chain reaction to make several billion copies of specific regions of the chromosome and then separated the different fragments by the method of gel electrophoresis.

The second step in the linkage analysis was to locate suitable families. When scientists study simple traits such as color blindness or sickle cell anemia—which involve a single gene—they tend to analyze large, multigenerational families in which each member clearly either has or does not have the trait. Such an approach was unsuited for studying sexual orientation. First, identifying someone as not homosexual is tricky; the person may be concealing his or her true orientation or may not be aware of it. Because homosexuality was even more stigmatized in the past, multigenerational families are especially problematic in this regard. Moreover, genetic modeling shows that for traits that involve several different genes expressed at varying levels, studying large families can actually decrease the chances of finding a linked gene: too many exceptions are included.

For these reasons, Hamer and his co-workers decided to focus on nuclear families with two gay sons. One advantage of this approach is that individuals who say they are homosexual are unlikely to be mistaken. Furthermore, the approach can detect a single linked gene even if other genes or noninherited factors are required for its expression. For instance, suppose that being gay requires an X chromosome gene together with another gene on an autosome, plus some set of environmental circumstances. Studying gay brothers would give a clear-cut result because both would have the X chromosome gene. In contrast, heterosexual brothers of gay men would sometimes share the X chromosome gene and sometimes not, leading to confusing results.

Genetic analysts now believe that studying siblings is the key to traits that are affected by many elements. Because Hamer and his colleagues were most interested in finding a gene that expresses itself only in men but is transmitted through women, they restricted their search to families with gay men but no gay father-gay son pairs. Forty such families were recruited. DNA samples were prepared from the gay brothers and, where possible, from their mothers or sisters. The samples were typed for 22 markers that span the X chromosome from the tip of the short arm to the end of the long arm.



PINPOINTING GENES shared by gay brothers (*darker brown*) first involved taking DNA from subjects. Several billion copies of specific regions of the X chromosome were then made using the polymerase chain reaction, and the different fragments were separated by gel electrophoresis. Gay brothers shared a marker, in this hypothetical example CA₁₁, in the Xq28 region at rates far greater than predicted by chance.

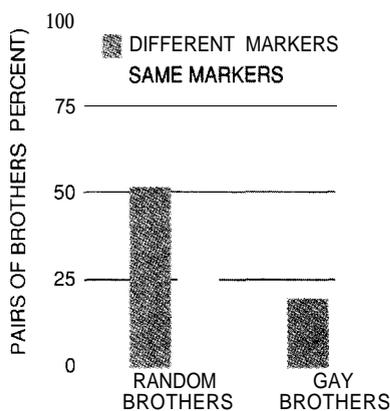
At each marker, a pair of gay brothers was scored as concordant if they inherited identical markers from their mother or as discordant if they inherited different ones. Fifty percent of the markers were expected to be identical by chance. Corrections were also made for the possibility of the mother's having two copies of the same marker.

The results of this study were striking. Over most of the X chromosome the markers were randomly distributed between the gay brothers. But at the tip of the long arm of the X chromosome, in a region known as Xq28, there was a considerable excess of concordant brothers: 33 pairs shared the same marker, whereas only seven pairs did not. Although the sample size was not large, the result was statistically significant; the probability of such a skewed ratio occurring by chance alone is less than one in 200. In a control group of 314 randomly selected pairs of brothers, most of whom can be presumed to be heterosexual, Xq28 markers were randomly distributed.

The most straightforward interpretation of the finding is that chromosomal region Xq28 contains a gene that influences male sexual orientation. The study provides the strongest evidence to date that human sexuality is influenced by heredity because it directly examines the genetic information, the DNA. But as with all initial studies, there are some caveats.

First, the result needs to be replicated; several other claims of finding genes related to personality traits have proved controversial. Second, the gene itself has not yet been isolated. The study locates it within a region of the X chromosome that is about four million base pairs in length. This region represents less than 0.2 percent of the total human genome, but it is still large enough to contain several hundred genes. Finding the needle in this haystack will require either large numbers of families or more complete information about the DNA sequence to identify all possible coding regions. As it happens, Xq28 is extraordinarily rich in genetic loci and will probably be one of the first regions of the human genome to be sequenced in its entirety.

A third caveat is that researchers do not know quantitatively how important a role Xq28 plays in male sexual orientation. Within the population of gay brothers studied, seven of 40 brothers did not share markers. Assuming that 20 siblings should inherit identical markers by chance alone, 36 percent of the gay brothers show no link between homosexuality and Xq28. Perhaps these men inherited different genes or were



GENE SHARING in the Xq28 region is significantly greater in gay brothers than in the general population. Of 40 pairs of gay brothers studied, 33 pairs shared the Xq28 region. In a control group of 314 randomly selected pairs of brothers, Xq28 markers were found to be almost equally distributed.

influenced by nongenetic physiological factors or by the environment. Among all gay men—most of whom do not have gay brothers—the influence of Xq28 is even less clear. Also unknown is the role of Xq28, and other genetic loci, in female sexual orientation.

How might a genetic locus at Xq28 affect sexuality? One idea is that the hypothetical gene affects hormone synthesis or metabolism. A candidate for such a gene was the androgen receptor locus, which encodes a protein essential for masculinization of the human brain and is, moreover, located on the X chromosome. To test this idea, Jeremy Nathans, Jennifer P. Macke, Van L. King and Terry R. Brown of Johns Hopkins University teamed up with Bailey of Northwestern and Hamer, Hu and Hu of the NIH. They compared the molecular structure of the androgen receptor gene in 197 homosexual men and 213 predominantly heterosexual men. But no significant variations in the protein coding sequences were found. Also, linkage studies showed no correlation between homosexuality in brothers and inheritance of the androgen receptor locus. Most significant of all, the locus turned out to be at Xq11, far from the Xq28 region. This study excludes the androgen receptor from playing a significant role in male sexual orientation.

A second idea is that the hypothetical gene acts indirectly, through personality or temperament, rather than directly on sexual-object choice. For example, people who are genetically self-reliant might be more likely to acknowledge and act on same-sex feelings than are people who are dependent on the approval of others.

Finally, the intriguing possibility arises that the Xq28 gene product bears directly on the development of sexually dimorphic brain regions such as INAH3. At the simplest level, such an agent could act autonomously, perhaps in the womb, by stimulating the survival of specific neurons in preheterosexual males or by promoting their death in females and prehomosexual men. In a more complex model, the gene product could change the sensitivity of a neuronal circuit in the hypothalamus to stimulation by environmental cues—perhaps in the first few years of life. Here the genes serve to predispose rather than to predetermine. Whether this fanciful notion contains a grain of truth remains to be seen. It is in fact experimentally testable, using current tools of molecular genetics and neurobiology.

Our research has attracted an extraordinary degree of public attention, not so much because of any conceptual breakthrough—the idea that genes and the brain are involved in human behavior is hardly new—but because it touches on a deep conflict in contemporary American society. We believe scientific research can help dispel some of the myths about homosexuality that in the past have clouded the image of lesbians and gay men. We also recognize, however, that increasing knowledge of biology may eventually bring with it the power to infringe on the natural rights of individuals and to impoverish the world of its human diversity. It is important that our society expand discussions of how new scientific information should be used to benefit the human race in its entirety.

FURTHER READING

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