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A Linkage Between DNA Markers on the X Chromosome and Male Sexual Orientation

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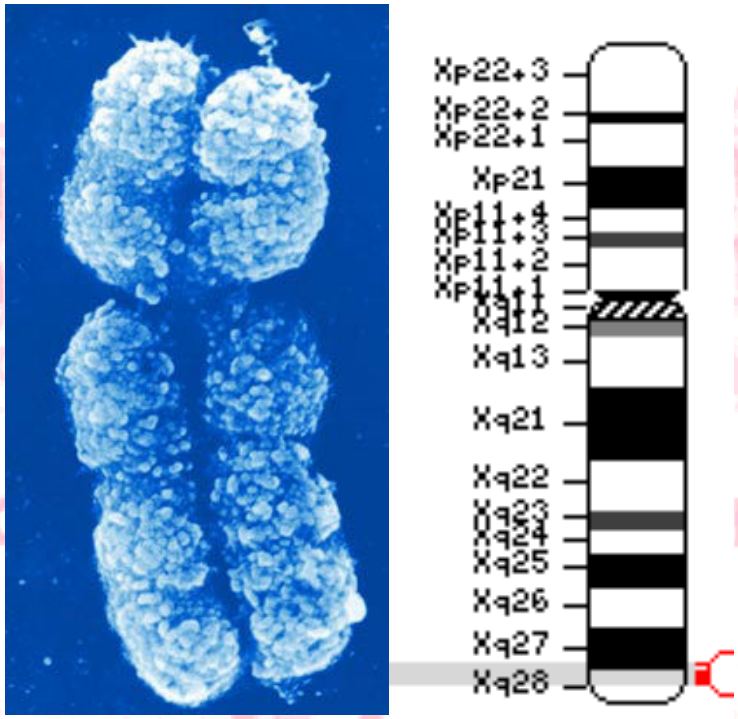
The role of genetics in male sexual orientation was investigated by pedigree and linkage analyses on 114 families of homosexual men. Increased rates of same-sex orientation were found in the maternal uncles and male cousins of these subjects, but not in their fathers or paternal relatives, suggesting the possibility of sex-linked transmission in a portion of the population. DNA linkage analysis of a selected group of 40 families in which there were two gay brothers and no indication of nonmaternal transmission revealed a correlation between homosexual orientation and the inheritance of polymorphic markers on the X chromosome in approximately 64 percent of the sib-pairs tested. The linkage to markers on Xq28, the subtelomeric region of the long arm of the sex chromosome, had a multipoint lod score of 4.0 ($P = 10^{-5}$), indicating a statistical confidence level of more than 99 percent that at least one subtype of male sexual orientation is genetically influenced.

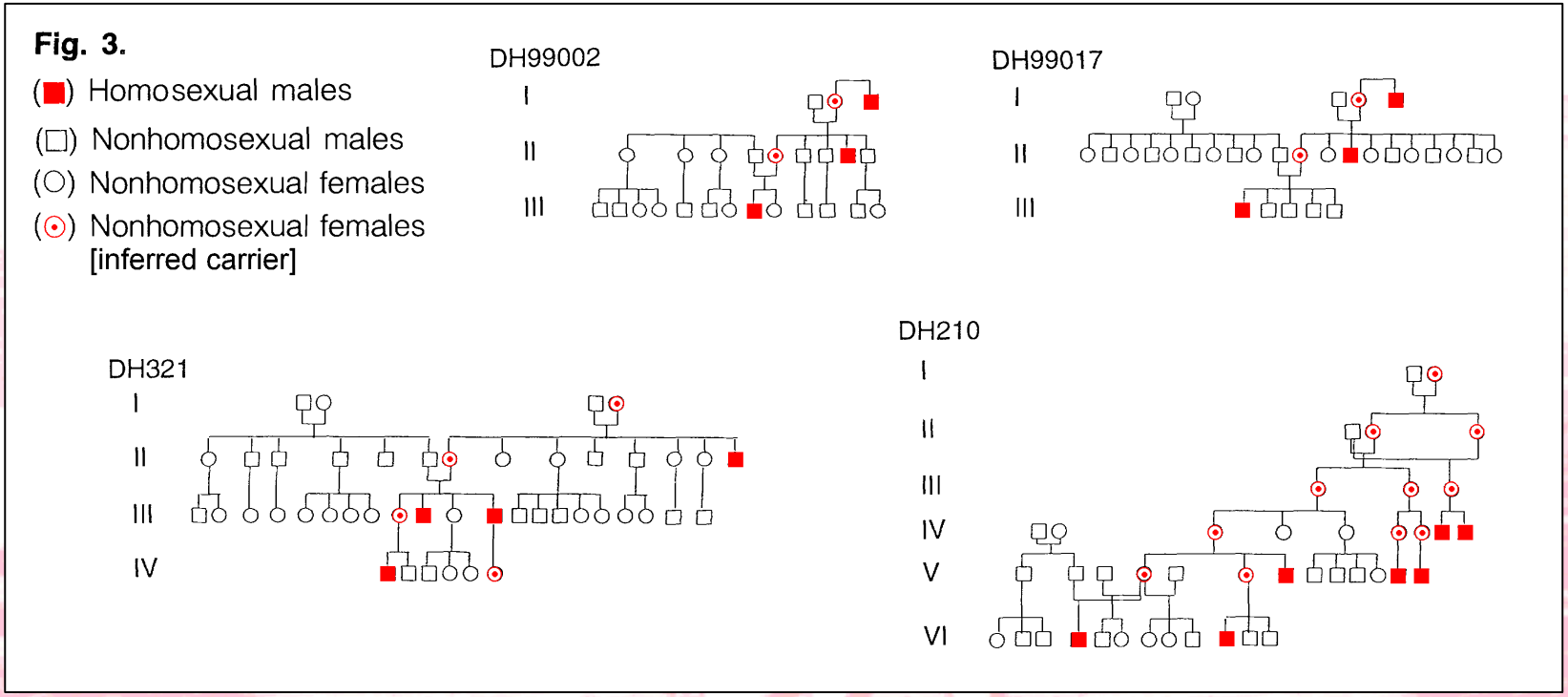
Study Design of Hamer *et al.* (1993)

- **QTL (Quantitative Trait Loci)** study:
  Possible *genetic influence* on (behavioral) *phenotype*
- **Linkage** between phenotype & genotype markers
- Same-Sex orientation of gay males [one subtype]
  - Behavior shared with gay cousin / uncle *but not* father
  - => **X-linked inheritance**
  - => simplifies search to **X chromosome**
- **Pedigree analysis** of pairs of brothers
  - Genetic **concordance** of **X** inherited from mother
    - Similarity by **S**tate [**S**]
    - Identity by **D**escent [**D**]: copies of *same allele*
    - Discordant [**n**]: brothers get *different* alleles
    - Uninformative [-]
- **Logarithm of Odds** (**LOD**) calculation
  - Z_1 = prob(**D**), given *observed* allele frequencies
  - **LOD** = *ln*[Z_1 / prob(*null*)], where p(*null*) = *random* expectation
  - Cf. Five-Card Stud

X chromosome: **Xq27~28** region



X-linked male homosexuality?



X-linkage is indicated where **uncles & nephews** share trait:
Mother (sister of **uncle**) is implicated as **carrier**

Identity by **(S)tate** & **(D)escent**

vs **discordant** (**-**) & **un-informative** (**n**) sites

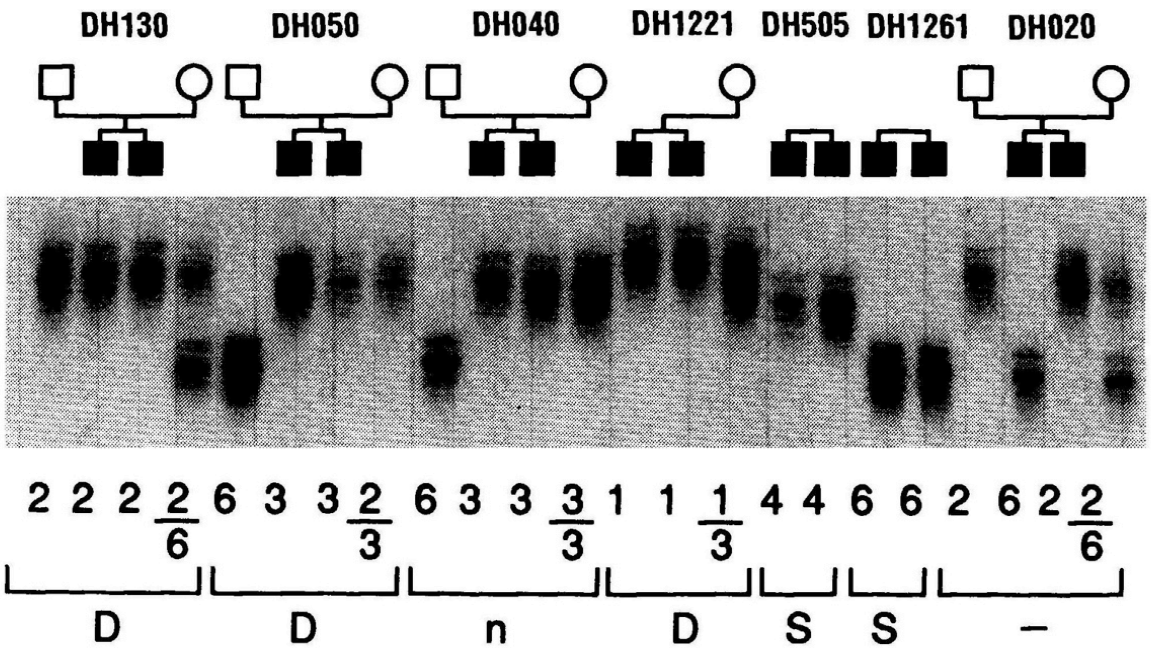
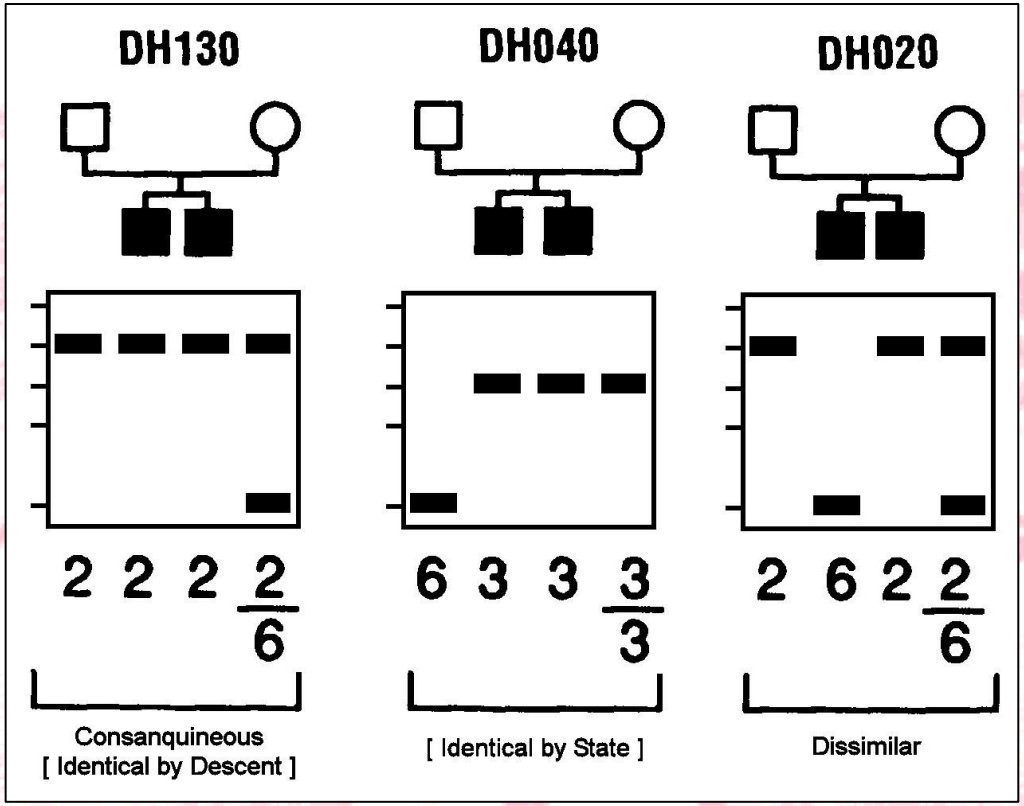


Fig. 4. Linkage analysis of the $(GT)_nGC(GT)_n$-repeat locus DXS1108.

Identity by **Descent** & **State:**

consanguineous (**IbD** or **IbS**) *vs* dissimilar **X** chromosomes



Trait mapping to **RS** region of **Xq28**

Proband	Marker Locus																				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	n	D	D	D	D	D	n	D	n	D	D	n	n	D	n	D	n	n	n	D	D	D
2			D		n		n	n	n	D	n	D	n	n	n	n	n	D	n	n	D	D
3	n			n	D	D	D	n	D	n	D	n	D	D	D	D	n	D	D	n	n	D
4	D	D			n		D	D	n	D	D	D	n	n	n	n	n	D	n	n	n	D
5						D			D	D	n	D	D	D	D	D	D	n	D	D	D	D
6	n	D	n							n		D	D	D	n	D	n	D	D	D	D	n
7	D	n	D	n	D	D	n						D	n	D	D	n	D	n	n	D	D
8	n					n	D	D	D	n	D	D	n			n	n	D	n	D	D	n
9	D	D				n			n	n	D		n			n	n	D	D	n	n	n
10	D	D	D	S	S	D	S	D	S	D	S						S	S	S	S	D	S
11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
12	S				S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13					S	S	S	S	S	S		S	S	S	S	S	S	S	S	S	S	S
14					S	S	S	S		S			S	S	S	S	S	S	S	S	S	S
15	S	S		S		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
16						S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
17	S	S	S			S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
18	S	S			S		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
19		S	S	S	S		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
20	S	S	S		S	S		S				S	S	S	S	S	S	S	S	S	S	S
21			S	S	S		S			S		S	S	S	S	S	S	S	S	S	S	S
22										S		S	S	S	S	S	S	S	S	S	S	S
23			S	S					S			S	S	S	S	S	S	S	S	S	S	S
24	S	S	S					S	S				S	S	S	S	S	S	S	S	S	S
25	S	S				S	S		S	S		S				S	S	S	S	S	S	S
26	S	S					S		S		S				S		S	S	S	S	S	S
27				S		S	S	S	S	S	S	S					S	S	S	S	S	S
28	S	S	S		S	S	S	S	S	S							S	S	S	S	S	S
29	S		S	S	S	S	S	S	S	S	S	S	S					S	S	S	S	S
30	n		n	n	n	n		n	n	D	D	D			n		n		n	n	n	n
31						n	D	D					n			n	n		n			n
32			D	D	n	D	D				D	D				n	n	n		n		
33	S	D	D	n	n	D	D	n	n	n	n		S			S			n	S	n	
34	S	S	S	S	S	S	S	S	S		S	S	S	S		S	S		S	S	S	
35			S	S	S	S	S	S	S	S	S	S	S	S		S	S					
36		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		S			

Linkage Analysis

over 22 X-chromosome molecular markers (**A-V**)

Table 2. Summary of linkage results. Linkage analysis was performed on 40 male homosexual sib-pairs; 22 X chromosome markers were used (*30*). The five marker loci on distal Xq28 are in boldface.

Locus	Location	AL*	HET†	Sib-pairs‡			z_1§	$2\ln L(z_1)$‖	P¶
				[D]	[S]	[–]			
A. .KAL	p22	6	0.77	5	16	14	0.51	0.01	ns
B. .DXS996	p22	11	0.84	7	14	18	≤.5	≤0	ns
C. .DXS992	p	8	0.87	6	13	19	≤.5	≤0	ns
D. .DMD1	p21	9	0.78	3	10	23	≤.5	≤0	ns
E. .DXS993	p11	6	0.80	3	14	17	≤.5	≤0	ns
F. .DXS991	p	8	0.77	8	14	14	0.57	0.61	ns
G. .DXS986	q	10	0.71	7	20	10	0.65	2.11	ns
H. .DXS990	q	7	0.76	4	19	13	0.55	0.25	ns
I. .DXS1105	q	5	0.48	3	20	9	≤.5	≤0	ns
J. .DXS456	q21	10	0.85	8	20	8	0.75	7.95	0.00241
K. .DXS1001	q26	10	0.82	8	16	13	0.60	1.09	ns
L. .DXS994	q26	5	0.75	7	17	13	0.55	0.26	ns
M. .DXS297	q27	5	0.70	5	21	8	0.71	4.25	0.01963
N. .FMR	q27	17	0.79	6	17	14	0.56	0.45	ns
O. .FRAXA	q27	8	0.72	4	17	13	0.56	0.38	ns
P. .DXS548	q27	6	0.67	7	20	7	0.73	5.21	0.01123
Q. .GABRA3	q28	4	0.35	2	23	3	0.74	2.39	ns
R. .DXS52	**q28**	**12**	**0.79**	**9**	**22**	**6**	**0.81**	**11.83**	**0.00029**
S. .G6PD	**q28**	**2**	**0.36**	**4**	**24**	**2**	**0.85**	**6.38**	**0.00577**
T. .F8C	**q28**	**2**	**0.41**	**5**	**24**	**3**	**0.82**	**6.56**	**0.00522**
U. .DXS1108	**q28**	**6**	**0.71**	**8**	**22**	**4**	**0.85**	**12.87**	**0.00017**
V. .DXYS154#	**q28**	**10**	**0.71**	**8**	**22**	**5**	**0.83**	**12.84**	**0.00017**
R/S/T/U/V	**q28**		**0.99**	**12**	**21**	**7**	**0.82**	**18.14**	**0.00001**

*AL is the number of different alleles observed in 62 to 150 independent chromosomes. †HET is the calculated heterozygosity; HET = $1 - \Sigma f_i^2$, where f_i = frequency of the ith allele. ‡[D] is the observed number of concordant-by-descent pairs; [S] is the observed number of concordant-by-state pairs; [–] is the observed number of discordant pairs; noninformative pairs are not included in this analysis. §z_1 is the estimated probability that two homosexual brothers share the marker locus by-descent (*31*). ‖$L(z_1)$ is the ratio of the likelihoods of the observed data at z_1 versus the null hypothesis of $z_1 = 1/2$ (*31*). ¶P (one-sided) was calculated by taking $2\ln L(z_1)$ to be distributed as a chi-squared statistic at one degree of freedom; ns· $P > 0.05$. # Only the maternal, X-linked contribution was considered for this sex-linked locus (*23*).

LOD score analysis (simplified)

Consider 21 *possible* concordance ratios: ***What odds***?
Consider 21 successive markers with *observed* ratios: ***Is there Linkage***?

Concordant	non-Concordant	ratio	odds ratio (0.8)	LOD	Chi Sq
0	40	0.00	0.00	-15.9	40.00
2	38	0.05	0.00	-14.7	32.40
4	36	0.11	0.00	-13.5	25.60
6	34	0.18	0.00	-12.3	19.60
8	32	0.25	0.00	-11.1	14.40
10	30	0.33	0.00	-9.9	10.00
12	28	0.43	0.00	-8.7	6.40
14	26	0.54	0.00	-7.5	3.60
16	24	0.67	0.00	-6.3	1.60
18	22	0.82	0.00	-5.1	0.40
20	20	1.00	0.00	-3.9	0.00
22	18	1.22	0.00	-2.7	0.40
24	16	1.50	0.03	-1.5	1.60
26	14	1.86	0.54	-0.3	3.60
28	12	2.33	8.71	0.9	6.40
30	10	3.00	139.38	2.1	10.00
32	8	4.00	2230.07	**3.3**	14.40
34	6	5.67	35681.19	**4.6**	19.60
36	4	9.00	570899.08	**5.8**	25.60
38	2	19.00	9134385.23	**7.0**	32.40
40	0	—	146150163.73	**8.2**	40.00

Marker	Concordant	non-Concordant	odds ratio (0.8)	LOD
A	16	24	0.00	-6.3
B	16	24	0.00	-6.3
C	20	20	0.00	-3.9
D	14	26	0.00	-7.5
E	20	20	0.00	-3.9
F	22	18	0.00	-2.7
G	24	16	0.03	-1.5
H	26	14	0.54	-0.3
I	28	12	8.71	0.9
J	30	10	139.38	2.1
K	32	8	2230.07	3.3
L	34	6	35681.19	4.6
M	36	4	570899.08	5.8
N	38	2	9134385.23	**7.0**
O	34	6	35681.19	4.6
P	30	10	139.38	2.1
Q	26	14	0.54	-0.3
R	22	18	0.00	-2.7
S	20	20	0.00	-3.9
T	16	24	0.00	-6.3
U	20	20	0.00	-3.9

LOD scores (long calculation)

Let z_1 = the probability that a pair of brothers share a marker allele by-descent (z_1 is an unknown parameter that is estimated from the data),

$\Gamma(z_1)$ = the likelihood of the observed data at z_1

$\Gamma(1/2)$ = the likelihood of the observed data under the null hypothesis of $z_1 = 1/2$

$L(z_1) = \Gamma(z_1)/\Gamma(1/2)$ = The ratio of the unlikely ***observed*** event to the unlikely ***random*** event, (where brothers share any allele with $p = z_1 = ½$)

$$2\sum_{J=1}^{N} \ln[L_J(z_1)] =$$

$$2\left(\sum^{G} \ln[(1 - z_1)/(1/2)] + \sum^{D} \ln[(z_1)/(1/2)] + \sum_{J=1}^{S} \ln\{f_J(z_1 + f_J - f_J z_1)/[f_J(1 + f_J)/2]\}\right) =$$

$$2\left(G\ln(2 - 2z_1) + D\ln(2z_1) + \sum_{J=1}^{S} \ln[2z_1 + 2f_J - 2f_J z_1)/(1 + f_J)]\right)$$

LOD score assignments over the X:

high scores (linkage) in **RS region** of **Xq28**