

DNP-must-D-GAT gave equal haemolytic titres when assayed with guinea-pig sera produced against DNP-BSA. Thus, the DNP groups on the D- and L-polymers were similarly accessible to the products of B cells, if not their receptors.

Table 2 Inhibition by ABA-D-GAT of Stimulation *in vitro* of Lymphocytes from ABA-sensitive Guinea-pigs

Stimulating antigen	Inhibitor	Added at time	Stimulation index
Control	0	0	1.0
ABA-Tyr (100 µg)	0	0	9.9
ABA-Tyr (100 µg)	ABA-poly-D-GAT (25 µg)	-4 h	4.7
ABA-Tyr (100 µg)	ABA-poly-D-GAT (25 µg)	+4 h	7.8
None	ABA-poly-D-GAT (100 µg)	0	0.8

Recognition of ABA groups by receptors on T cells is more difficult to determine. We have long known that only ABA on L-polymers actively elicits all the *in vivo* or *in vitro* manifestations of cell mediated immunity. The failure of ABA conjugates of D-polymers to function cannot be attributed solely to structural features of the D-tyrosine to which the ABA group is attached, as an ABA-conjugate of D-tyrosine in an otherwise all L-amino acid polymer gives good reactions¹⁸.

To find out if ABA groups on the D-polymer can be "seen" by T cells, the possible competitive inhibition of stimulation of ABA sensitive T cells was studied. Lymph node cells from guinea-pigs sensitized with ABA-Tyr in CFA were cultured with the antigen ABA-Tyr and stimulation detected by uptake of ³H-thymidine. The results of one such experiment in which varied amounts of ABA-D-GAT were added 4 h before or 4 h after the stimulating dose of ABA-Tyr are shown in Table 2. While 100 µg of ABA-Tyr alone gave a stimulation index (SI) of 9.9, addition of as little as 25 µg ABA-D-GAT gave a reduction of the SI to 4.7 when given 4 h before. Addition 4 h after stimulation had much less effect (SI = 7.8).

Thus, the ABA groups on the L-polymer can be considered to be "seen" by receptors on T lymphocytes, as judged by reactivity, whereas the ABA groups on the D-polymer can only be inferred to combine with the same receptors as measured by inhibitive activity.

The failure of the ABA-DNP-must-D-GAT polymer to produce sufficient cooperation for antibody production could be resolved by assuming that mere physical coupling of T and B cells through the binding of their receptors to suitable determinants is not a sufficient condition for antibody formation. Other studies have indicated that T cells must be activated to synthesize RNA and protein before they can initiate antibody production by B cells¹⁴. As the ABA on D-polymers cannot activate the T cell, it cannot lead to cooperative antibody production. One possible reason for this may be a requirement for some sort of preliminary processing of antigen by macrophages before T cells are activated. Some recent studies^{15,16} have suggested that this may not occur with D-amino acid polymers.

L-GAT and D-GAT were a gift from Drs P. Maurer and T. Gill. DNP mustard was a gift from Dr A. Soloway. This work was supported in part by a grant from the Whitehall Foundation and the US Public Health Service.

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ON page 43 of the issue of July 6, 1973 we published a Letter by Dr Ernest B. Hook and Dr William J. Schull. In the course of sub-editing, the Letter was amended by us, but due to delays in the post the authors were unable to point out before publication that the sense of the Letter had been altered in two places. A correct version of the Letter is printed below.

The authors have also taken this opportunity to correct certain of the data in the tables which appeared in the original version.

Why is the XX Fitter? Evidence Consistent with an Effect of X-heterosis in the Human Female from Sex Ratio Data in Offspring of First Cousin Marriages

ALTHOUGH the human female is known to be more viable, on the average, than the human male, the biological mechanisms for this difference are poorly understood. A relevant question is, to what extent does heterozygosity for X-linked loci contribute to survival of the XX female? The advantage accruing to the XX female because of this factor may be defined as X-heterosis¹. Most instances of serious X-linked disorders such as haemophilia occur in males, but the total incidence of such severe diseases is relatively small and cannot account for the observed sex differences in age specific mortality rates. It is conceivable that X-heterosis is relatively insignificant in the total population, and that the bulk of the observed sex difference is attributable to physiological factors that are a consequence of sex differentiation (for example, the apparent oestrogen-sparing effect on coronary heart disease) and/or to psycho-cultural factors that may pertain to diminished exposure of woman to environmental hazards (for example, industrial pollutants).

Large sex differences in foetal death rates are not as well established as sex differences in postnatal death rates. But even if the rates of foetal loss of males and females were identical, a significant X-heterotic effect could exist in XX foetuses but be balanced by relatively beneficial physiological factors affecting primarily male foetuses.

Moreover, X-heterozygosity may not necessarily be beneficial. Evidence suggests that heterozygosity at the Xg^a blood group locus in one population at least has a deleterious effect during gestation because of a dearth of heterozygous female foetuses of Xg^a negative mothers². As yet, no evidence for a large beneficial effect of X-heterozygosity in any population for

Table 1 Livebirths, Foetal Loss and Death, by Marriage Subset*

		Number of families		Livebirths		Stillbirths			Abor-tions		Acci-dental deaths		Natural deaths		Deaths of un-known cause		Twin sets	
		Total	Fertile	M	F	M	F	?	Nat.	Th.	M	F	M	F	M	F	M	F
X-Outbred Type 1	Rural koseki	96	92	232½†	275	2½†	0	2	11	1	12	2	49	52	3	8	1‡	2
	Rural non-koseki	18	12	21	24	1	1	0	0	0	0	0	6	7	0	1	0	0
	Urban non-koseki	13	13	22	26	0	0	0	3	5	0	0	3	6	0	0	0	0
	Totals	127	117	275½†	325	3½†	1	2	14	6	12	2	58	65	3	9	1	2
X-Outbred Type 2	Rural koseki	113	107	255	273	3	3	5	8	16	6	0	61	49	0	1	1	2§
	Rural non-koseki	14	8	15	12	1	0	0	0	0	0	0	4	1	0	0	0	0
	Urban non-koseki	3	2	2	1	0	0	0	0	0	1	0	1	0	0	0	0	0
	Totals	130	117	272	286	4	3	5	8	16	7	0	66	50	0	1	1	2
X-Inbred Type 3	Rural koseki	111	104	290	258	6	2	2	5	3	8	2	63½†	58	8	5	1	1
	Rural non-koseki	12	9	19	20	0	0	0	1	0	0	0	8	6	0	1	0	0
	Urban non-koseki	14	12	22	20	0	0	1	1	0	0	0	3	0	1	1	0	0
	Totals	137	125	331	298	6	2	3	7	3	8	2	74½†	64	9	7	1	1
X-Inbred Type 4	Rural koseki	143	129	340	330	6	3	2	17	6	7	0	71	69½†	2	0	1	1
	Rural non-koseki	19	15	41	21	0	0	2	1	3	3	0	10	10	0	0	0	0
	Urban non-koseki	9	7	24	12	0	1	0	0	2	1	0	4	1	0	0	0	0
	Totals	171	151	405	363	6	4	4	18	11	11	0	85	80½†	2	0	1	1
X-Inbred all	Totals	308	276	736	661	12	6	7	25	14	19	2	159½†	144½†	11	7	2	2
X-Outbred all	Totals	257	234	547½†	611	7½†	4	7	22	22	19	2	124	115	3	10	2	4

* Pedigree and other data were collected in 1964 through a census directed at all marriages, legal or consensual, represented by at least one spouse alive and residing in Hirado at the time of the census⁶⁻⁷. Some 10,530 unions contracted subsequent to 1890 were ascertained, and their reproductive histories recorded. The information obtained at interview was routinely compared with the existing records of the public health office, the agricultural and fishing cooperatives, the tax office, and the kosekika, the office of custody of the koseki, the household censuses required by law in Japan⁸. All vital events which affect the composition of a family such as marriages, births and deaths must be reported to this office. Where there was a discrepancy between the interview and the koseki, the data from the latter were generally used. As reporting of livebirths is very complete whereas most abortions and many stillbirths go unrecorded, the observations here reported on abortions and stillbirths stem almost exclusively from interviews, whereas the livebirth data are a synthesis of interviews and koseki observations and are much more complete. Information on type of death was that obtained by interview. Most of the natural deaths were in early childhood years. About 20% of the deaths recorded were to those over 21 yr of age. Deaths of unknown cause were those which were recorded in the koseki but not recalled by those being interviewed. Almost all the deaths in this category were of individuals who had died in infancy. All twin pairs were concordant for sex, and in view of the very low rate of dizygotic twinning in Japan, each such set of twins was scored as one birth of that sex. One set of triplets resulted in two stillborn females and one liveborn male and was scored as one male livebirth and one female stillbirth (Type 2-Outbred). The following abbreviations are used: M, male; F, female; Nat., natural abortion; Th., therapeutic abortion. There was one child of unknown sex in the rural koseki group of Type 3 inbred marriage, and one natural death to a child of unspecified sex in the rural non-koseki group of Type 4 inbred marriage.

† Where one of the twins was liveborn, the other stillborn or only one died, the entry "½" is made in appropriate column (see above for rationale). ‡ This twin pair included one liveborn and one stillborn. § Both members of one twin set were stillborn.

any trait has been presented, and there are no published data available for an analysis of the net total effect of X-heterosis on differential survival in any population.

We present here data which suggest that, among the population of the Japanese Island of Hirado, X-heterosis contributed quite considerably to female survival during gestation. There is also a trend consistent with an X-heterotic effect on postnatal survival.

The theoretical basis for the approach used has been described in detail¹ and the discussion is limited here to first cousin offspring (see Fig. 1). Briefly, of the four types of first cousin marriages, two, types 1 and 2, may be said to be "X-outbred" as they result in XX daughters who are X-outbred and two, types 3 and 4, "X-inbred" as they result in XX daughters who are X-inbred. If X-heterosis is significant during gestation then a higher sex ratio at birth (that is of males to females) would be expected in the infants of X-inbred subsets of first cousin marriages than in those of the X-outbred subsets. And if X-heterosis is significant postnatally then X-outbred females should be relatively protected by this effect

compared with X-inbred females. The rationale for these considerations is presented in Fig. 1.

The data presented here were obtained from a large investigation of consanguineous marriages on the island of Hirado (Table 1). There were 565 unions of first cousins for which data were available; this was the largest category of consanguineous matings encountered and the only one on which a detailed analysis was undertaken. The sex ratios of livebirths, stillbirths, and postnatal deaths by first cousin subset appear in Table 1 along with details on the ascertainment and classification of the data.

The secondary sex ratios in both X-inbred subsets are higher than in both X-outbred subsets (Table 2) and the difference between the sex ratios of X-inbred subsets and X-outbred subsets is statistically significant (547.5/611=0.896 against 736/661=1.113, $\chi^2=7.24$, *d.f.*=1, $P<0.01$). If attention is restricted to the most homogenous groups within a subset, the rural, koseki checked pedigrees, the same effect is present (487.5/548 against 630/588, $\chi^2=4.65$, *d.f.*=1, $P<0.05$). The differences are thus consistent with a negative effect of X-

Table 2 Livebirth Sex Ratio and Death Rates by Cousin Subset

	Livebirth sex ratio	Non-accidental death rate*	
		Male	Female
X-Outbred			
Type 1	0.848	0.221	0.228
Type 2	0.951	0.243	0.178
All	0.896	0.232	0.205
X-Inbred			
Type 3	1.111	0.252	0.238
Type 4	1.116	0.215	0.222
All	1.113	0.232	0.229

* The non-accidental death rate is the sum of the natural deaths and deaths of "unknown cause" (which were almost all in the infant years) divided by the number of livebirths.

Table 3 Socioeconomic Status and Maternal Age at Birth of First Child by First Cousin Subset

	Socioeconomic status*		Maternal age at birth of first child (years)	
	Mean	Variance	Mean	Variance
X-Outbred				
Type 1	16.73	36.33	21.60	8.38
Type 2	18.28	86.98	22.22	11.61
X-Inbred				
Type 3	16.82	39.43	21.61	15.46
Type 4	17.36	71.43	21.92	13.82

* Socioeconomic status is represented here by a score which is the sum of the coded values associated with parental occupation, education, and related variables. See ref. 7 for details.

Table 4 Distribution of Families by Decade of Marriage

	-1899	1900-1909	1910-1919	1920-1929	1930-1939	1940-1949	1950-1959	1960-
X-Outbred Type 1 (127)	2.4%	8.7%	13.4%	18.1%	10.2%	30.0%	14.2%	3.1%
X-Outbred Type 2 (130)	0.8%	3.8%	14.6%	17.0%	13.8%	28.5%	16.9%	4.6%
Total X-Outbred (257)	1.6%	6.2%	14.0%	17.5%	12.1%	29.2%	15.6%	3.9%
X-Inbred type 3 (136)*	1.5%	8.1%	14.0%	22.8%	13.2%	26.5%	11.8%	2.2%
X-Inbred Type 4 (171)	2.9%	2.9%	18.7%	17.5%	16.4%	26.3%	14.6%	0.6%
Total X-inbred (307)	2.3%	5.2%	16.6%	19.9%	15.0%	26.4%	13.4%	1.3%

* In one family the date of marriage is not available.

inbreeding during gestation, which leads to a net loss of females in the X-inbred subsets. There is, however, no striking difference in foetal loss between X-inbred and X-outbred subsets. Although even late foetal wastage was probably very poorly ascertained, the lack of any difference here suggests that if X-heterozygosity is responsible for the sex ratio difference at birth, females lost because of X-inbreeding may be lost early in gestation, perhaps before awareness of pregnancy, and reproductive compensation readily occurs in view of the lack of any significant effect on total fertility.

The apparent magnitude of the effect is relatively large, and it may be specific to the population under investigation. In addition, it is present in spite of any influence polymorphism at the Xg^a locus might have had on sex ratio in those studied; but we have no data on the frequency of such alleles at this locus on the Island. Furthermore, the observed sex-ratio in

the X-outbred group is not necessarily what would be predicted in a completely outbred control population, as sex differential responses to autosomal inbreeding in both X-inbred and X-outbred subsets may also have had an effect on the sex ratios in those studied here.

Another trend of interest is the postnatal death rate. If X-heterosis is significant for postnatal survival then X-inbred females should have greater mortality and morbidity than X-outbred females, but there should be no effect in the males. What data are available are consistent with this hypothesis. Although deaths have probably been poorly ascertained because of migration and other factors, most of those recorded represent deaths in infancy and early childhood. The mortality rates for non-accidental deaths (as defined in Table 2) in males in the two groups are identical, 0.232. But the recorded death rate in X-inbred females, 0.229, is about 12% greater than that in X-outbred females, 0.205. With the number of individuals involved, however, the trend is not yet significant at the 5% level.

Most studies to date of offspring of consanguineous unions have compared them with outbred individuals, and conclusions may always be vitiated by possible systematic differences between the related and unrelated families. Our study, by making comparisons within the inbred group of first cousin offspring, avoids many of these pitfalls. It is still possible, however, that some systematic differences between the subsets might have contributed to the differences in sex ratios. Comparisons of X-inbred groups and X-outbred groups with regard to a number of possibly relevant factors are presented in Tables 3-6. There appear to be no major differences with regard to decade of marriage, geographical distribution on the island,

Table 5 Distribution of Families by Religion

	Buddhist	Catholic	Kakure*	Other
X-Outbred -1 (127)	81.1%	0.8%	17.3%	0.8%
X-Outbred -2 (130)	80.0%	0%	16.2%	3.8%
X-Outbred total (257)	80.5%	0.4%	16.7%	2.3%
X-Inbred -3 (135)	79.3%	1.5%	17.0%	2.2%
X-Inbred -4 (170)	83.5%	0.6%	12.4%	3.5%
X-Inbred total (305) †	81.6%	1.0%	14.3%	3.0%

* A syncretic cult incorporating elements of Buddhism and Catholicism.

† 2 intermarriages are excluded, and data are not available on one family.

Table 6 Distribution of Families by Geographic Area

	Hirado -1	Hirado -2	Nakano	Himosashi	Shishi	Nakatsura	Tsuyoshi	Shijiki
X-Outbred Type 1 (127)	9.4%	25.2%	13.4%	10.2%	20.5%	7.1%	6.3%	7.9%
X-Outbred Type 2 (130)	9.2%	17.7%	9.2%	22.3%	18.5%	10.0%	7.7%	5.4%
Total X-outbred (257)	9.3%	21.4%	11.3%	16.3%	19.5%	8.6%	7.0%	6.6%
X-Inbred Type 3 (137)	10.9%	22.6%	15.3%	12.4%	16.1%	3.6%	8.0%	10.9%
X-Inbred Type 4 (171)	9.9%	17.5%	7.6%	18.1%	15.1%	7.0%	12.3%	12.3%
Total X-inbred (308)	10.4%	19.8%	11.0%	15.6%	15.6%	5.5%	10.4%	11.7%

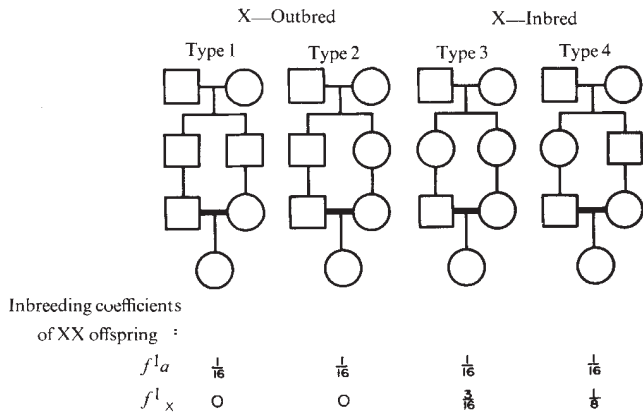


Fig. 1 Possible pedigrees of XX offspring of first cousin marriages. Note that XXs born to first cousins have identical autosomal inbreeding coefficients but may have different X chromosome inbreeding coefficients depending on the parental relationship. If heterozygosity for X-linked loci has an effect on a particular attribute, say, survival, this effect should be less for X-inbred females (that is, those for whom $f^1_x > 0$) who will be more homozygous for X-linked loci, on the average, than females for whom $f^1_x = 0$, that is, X-outbred females. If X-heterozygosity is beneficial, this advantage attributable to outbreeding should by definition be more significant for X-outbred than X-inbred females but would have no effect on XY males. Therefore, if X-heterozygosity contributes positively to survival to age n , the sex ratios (M/F) of X-outbred groups (that is, those in which X-outbred females occur, types 1 and 2 above) should be less than those of the X-inbred groups (those in which X-inbred females occur, types 3 and 4) and conversely if there is a negative effect. The secondary sex ratios, that is those noted at birth, should reflect the net effect of X-heterozygosity during gestation. The occurrence of XO females and males with more than one X-chromosome will confound the theoretical expectation, but the instances of such individuals in all populations studied to date appear to be less than 0.2% and are thus likely to be of trivial magnitude. See refs 1, 3 and 4 for further discussion.

maternal age at birth of the first child, socioeconomic status, or religion. This does not exclude the possibility of some other as yet unknown differences between these subsets that might be associated with altered sex ratio. At present, however, the only known systematic difference between these groups is the X-inbreeding coefficients of their female members, suggesting that this is the causal factor for the sex ratio data.

These observations provide, to our knowledge, the first evidence consistent with a significant deleterious effect of X-inbreeding in any mammalian population.

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Test of Selection and Neutrality in Natural Populations

MORE than a century after the publication of Darwin's *Origin of Species*, the question of the relative contribution of selected and nonselected ("neutral") genetic variation to evolution is still unresolved^{1,2}.

A test of the theory of selective neutrality of polymorphisms has recently been suggested by Lewontin and Krakawer³, on the basis of an idea first introduced by Cavalli-Sforza⁴ in analysing human evolution. The method is based on the contrasting effects of natural selection and the breeding structure on allele frequencies. Whereas natural selection operates differentially on each allele, inbreeding affects all alleles similarly. The inbreeding effect can be calculated in populations irrespective of their degree of isolation or their actual breeding structure, thereby avoiding manipulations with the parameters of population size, N , mutation rate, u , migration rate, m , and selection intensity, s , which are largely unknown in natural populations³.

Spatio-temporal variation in steady state gene frequencies, in the absence of selection, reflects the breeding system alone and can estimate a parameter \hat{F}_e the "effective inbreeding coefficient" across populations, or the "standardized variance of gene frequency"⁴. F_e is an equilibrium value reached after many generations and involves selection and random drift. The estimate is $\hat{F}_e = S^2_p / \bar{p}(1 - \bar{p})$ where \hat{F}_e is the estimate of effective inbreeding, S^2_p is the variance in the frequency of one one of two alternative alleles across populations³, and \bar{p} is the mean frequency of the allele across populations. Selectively neutral alleles will all have similar estimated \hat{F}_e values in spite of their individual variation in S^2_p and \bar{p} because effective inbreeding will be identical for all genes across populations. The average \hat{F}_e will thus estimate the true \hat{F}_e without significant heterogeneity among alleles. If, however, selection is operating on some or all of the loci, their \hat{F}_e values will be significantly heterogeneous and will not be estimates of the same \hat{F}_e . The test of homogeneity of \hat{F}_e estimates from different loci in steady state is thus a test for selection. The statistical properties of \hat{F}_e and a sampling theory for testing heterogeneity in \hat{F}_e are given in Lewontin and Krakawer³.

Here I describe a test by the \hat{F}_e method for selection and/or non-selective factors operating in natural populations of the fossorial rodent *Thomomys talpoides*, which belongs to the pocket gopher family *Geomyidae*. The basic data on gene frequencies are taken from ref. 5.

Alloenzymatic variation in proteins encoded by 31 loci was analysed electrophoretically in 276 specimens representing eleven populations and six chromosome forms of *Thomomys talpoides* complex of pocket gophers in the southern Rockies of the USA⁵. The eleven populations, their localities, abbreviations and respective diploid numbers are as follows. (1) Snowy Range Pass (SR), S.E. Wyoming, $2n=48$; (2) Willow Creek Pass (WC), N. Colorado, $2n=46$; (3) Dead Horse Creek (DH), head of White River, N. Colorado, $2n=44$; Three Forks Campsite (TF), N.W. Colorado, $2n=40$; (5) Kaibab Plateau (KP), N. Arizona, $2n=40$; (6) La Sal Mountains (LS), E. Utah, $2n=60$; (7) Molas Lake (ML), S.W. Colorado, $2n=60$; (8) Canjilon (CN), N. New Mexico, $2n=48$; (9) Redondo, Jemez Mountains (RD, JM), N. New Mexico, $2n=48$; (10) Las Conchas, Jemez Mountains (LC, JM),