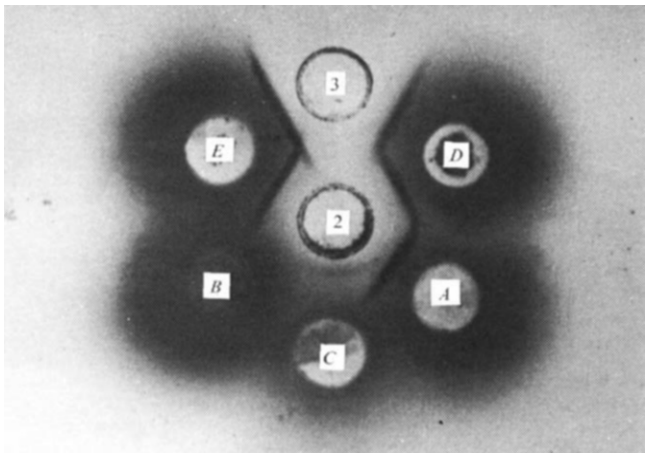

Fig. 3 Autoradiograph of double diffusion plates. Antisera: 2, rabbit anti-mouse κ chains and; 3, rabbit anti(rat)210. Radioactive products of clones prepared as described in Fig. 1. *A*, P1Bu1; *B*, 210.RCY3.Ag1; *C*, P1Bu2; *D*, hybrid clone HyIII-1; *E*, hybrid clone HyIII-2.

while ten spreads of the hybrid clone HyIII-1 had counts of 84, 84, 87, 77, 82, 84, 85, 82, 87 chromosomes after 6 months in culture. Only the hybrid lines can grow in the selective medium.

Figure 1 shows a comparison of the proteins synthesized by the different cell lines. In addition to the clones shown in Fig. 1 we have tested eighteen more. All synthesize immunoglobulin molecules of both parental types. One of them (HyIII-19) did not synthesize heavy chains (sample *k*, Fig. 1). The relative intensity of the different bands in the patterns is quite reproducible for each clone, but it differs from clone to clone. For example, clone HyIII-14 (sample *j*, Fig. 1) showed very little free mouse light chain. An important difference between parental and hybrid products is that hybrid cells produce an extra component (X) not seen in either parent. This component is not observed when the proteins produced by the mixture of the two parental lines are subjected to the same procedure (sample *f*, Fig. 1). Component X contains antigenic determinants recognized by both anti-mouse κ chains and anti-210 (rat) antibodies as shown by immunoisoelectric focusing studies (Fig. 2). The isoelectric point of X is intermediate between rat and mouse light chains and the band disappears if the material is reduced and carboxamidomethylated before isoelectric focusing. It seems likely, therefore, that it is a hybrid mouse–rat light chain dimer.

The region of the isoelectric focusing plate which contains the IgG molecules shows that they contain rat light chain determinants as well as mouse light chains. Although the anti-rat antiserum cross reacts with free mouse light chains it does not react with mouse light chains in IgG. Molecules made of mouse heavy chain and rat light chain are produced in good yields (Fig. 2). This results in shifts in the position of the major IgG bands (for example, HyIII-14 and 16 in Fig. 1). The occurrence of non-symmetrical molecules made up of one light chain of each parental type is indicated by the results shown in Fig. 3. These show that most of the IgG molecules are precipitated by both anti-mouse and anti-rat light chain antibodies.

Apart from component X which, as discussed above, is likely to be a dimer of one rat and one mouse light chain, no other band observed in the hybrids is absent from both parents. Scrambled molecules, such as would be expected if the integration of V and C sections took place at the mRNA level, were not observed. The possibility that band X contains such molecules is not excluded although it seems unlikely. One would have expected two new molecules banding in different positions and not necessarily recognizing both antisera. It seems, therefore, that the genes for the Ig chains of both parents are expressed in the hybrid clones. The presence of Ig hybrid molecules indicates that the expression of the two sets of genes is not attributable to the individual expression of segregants of the clones but that both parental genes are expressed in the same individual cell. This implies, therefore, that in a mouse–rat hybrid cell the expression of one light chain is not hindered by the expression of the other. It remains to be established that this is also true of hybrid cells of homologous species. The simplest explanation seems to be that the restricted expression of *V* and *C* genes is caused by the presence in the parental cells of single already integrated *V-C* genes. Such integration could be the result of a translocation event taking place in plasma cell precursors. A single translocation in a non-committed cell could start a chain of events leading to the activation of the integrated gene but not of other non-integrated genes for similar chains—including the sets in the other haploid chromosome. The integrated gene would in this way be active independently of the presence or absence of other integrated similar or homologous genes.

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

ALTHOUGH women are known to be more viable than men, 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

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

		Number of families		Livebirths		Stillbirths			Abortions		Accidental deaths		Natural deaths		Deaths of unknown 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	0	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 census[6,7]. 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 Japan[8]. 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.

female? The advantage accruing to the XX female through having this factor can be defined as X-heterosis[1]. Practically all 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 women 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. Even if the rates of foetal loss of males and females were identical, however, a significant X-heterotic effect could exist in XX foetuses but be balanced by relatively beneficial physiological factors primarily affecting male foetuses.

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 in view of the selective loss of heterozygous female foetuses of Xg^a negative mothers[2]. As yet, there is no evidence for a large beneficial effect of X-heterozygosity in any population for any trait, and there are no data 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 contributes 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 detail[1] 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

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, First Child by First Cousin Subset

	Socioeconomic status		Maternal age at birth, first child	
	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.

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-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. We also noted that the observed sex ratio in the X-outbred group is not necessarily what would be predicted in a completely outbred control population, as differences between the sexes in response 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) 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

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%

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 (137)	79.6%	0.7%	16.1%	0.7%
X-Inbred –4 (171)	83.3%	0.9%	12.3%	3.5%
X-Inbred total (308)	81.7%	1.1%	14.3%	2.9%

*A syncretic cult incorporating elements of Buddhism and Catholicism.

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%	10.9%	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 (307)	10.4%	19.8%	11.0%	15.6%	15.6%	5.5%	10.4%	11.7%

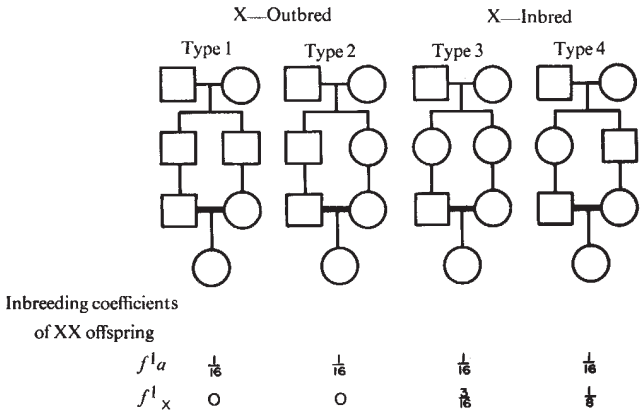

Fig. 1 Possible pedigrees of XX offspring of first cousin marriages. Note that XXs born to first cousins have identical inbreeding coefficients but may have different X chromosome inbreeding coefficients depending on the parental relationship. If heterozygosity for X-linked loci has an effect on a particular attribute, say, survival, this effect should be less for X-inbred females (that is, those for whom $f'_x > 0$) who will be more homozygous, on the average, than females for whom $f'_x = 0$, 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, thosei n 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.

females should have greater mortality and morbidity than X-outbred females, but there should be no effect on 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, maternal age at birth, 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 the first evidence consistent with a significant deleterious effect of X-inbreeding in any mammalian population.

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Photoperiodism and Adaptive Behaviour in a Small Mammal

SEASONAL changes in photoperiod have wide-ranging effects on the biology of most temperate zone vertebrates[1,2]. Although photoperiodic responses have been extensively studied in birds[3], research on photoperiodic effects in mammals has been confined largely to their influence on reproductive and pelage cycles[4,5]. Less well understood are the effects of seasonal differences in photoperiod on mammalian behaviour, although modification of behaviour is the principal means by which small mammals adjust to seasonal changes in their environment[6]. Furthermore, seasonal changes in photoperiod may be used as a cue by small mammals to alter behaviour before more critical changes in other environmental conditions, such as temperature. We describe here the effects of previous exposure to differences in both photoperiod and temperature on the expression of nesting, hoarding, daily activity, and food consumption in the white-footed mouse, *Peromyscus leucopus*.

Forty-eight male *P. leucopus* were individually caged and assigned at random to one of the following treatments: (1) warm acclimated (26° C) under a long-day photoperiod (16: 8 LD); (2) warm acclimated under a short-day photoperiod (9 : 15 LD); (3) cold acclimated (5° C) under a long-day photoperiod; or (4) cold acclimated under a short-day photoperiod. (All mice were 4–5 months old and reared in the laboratory on a 16 : 8 LD photoperiod at 26° C.) After 6 weeks of this treatment, individual mice were placed in a freshly cleaned 10 gallon aquarium for assessment of hoarding behaviour and daily activity. The aquarium was provided with a water bottle and a half pint, blackened jar which was used by the mouse as a harbourage. For three consecutive days, 40 g of soy beans was scattered on the floor of the aquarium, removed the following day, and the amount weighed. Daily activity was measured using an ultrasonic motion detector (Alton Electronics, Gainesville, Florida), which activated a 10 channel Esterline-Angus event recorder. Transmitting and receiving probes were centred and mounted 18 inches apart above the aquarium. After estimation of