TRAITS THAT INFLUENCE LONGEVITY IN MICE

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ABSTRACT

Analysis of genetic interactions in the segregating backcross [(C57BL/6 × DBA/2)F_1 × DBA/2] mice revealed influences of genetic and environmental factors on life span. Using determinants of coat color (brown locus of chromosome *4* and dilute locus of chromosome *9*), serologically determined *H-2* antigens (chromosome *17*) and sex as genetic markers, we studied the effects of these genes on longevity. The results suggested that genes in the brown locus (*b*) segment of chromosome *4*, genes in a segment of the sex chromosomes and, to a more limited extent, genes in the segment of chromosome *17* which contains the *H-2* haplotype all influenced longevity. The coat color (*b* locus) segment of chromosome *4* was associated with life span predominantly in females, whereas the chromosome *17* (*H-2* haplotype) segment was associated with longer life primarily in males. The dilute locus *d* segment on chromosome *9* did not affect life span. Longevity appears to be influenced by interactions between genes in the chromosomal segment carrying *H-2*, those in the *b* segment, gender and the month of birth. Greater heterozygosity at the loci studied was associated with longer life span. Histopathological findings on mice that died at or after 28 months of age were comparable for all genetic combinations except that there was an increased frequency of lymphoma in females and an increased frequency of amyloidosis in males. Our analysis emphasizes the need for comprehensive studies of aging and longevity that would simultaneously determine the effects of several genetic regions and their interactions with the environment with respect to possible causes of death.

NUMEROUS studies have shown that mice of different inbred strains differ in survival rate. RUSSELL (1975), for example, cited experiments concluding that C57BL/6 mice lived longer than DBA/2 mice. The F_1 hybrid outlived parents of either sex, whereas females lived longer than males (RUSSELL 1975). Given that mouse strains differ at many genetic loci, it has been helpful to study congenic mice, distinguishable from each other only by the inclusion of a chromosomal segment from one strain in the background provided by another. Since it was anticipated that genes of the *H-2* region confer resistance to environmental insults, and thus influence survival (KATZ and BENACERRAF 1976; MEREDITH and WALFORD 1977; GREENBERG and YUNIS 1975, 1978), several investigators (SMITH and WALFORD 1977; POPP 1978; WILLIAMS *et al.* 1981) have used congenic strains differing with respect to *H-2* haplotypes.

These studies, however, did not take into account the influence on longevity of other genetic systems.

Here, we have selected an experimental model in which it was possible to study the effect on life span of interaction of genes in segments of the sex chromosome, genes in segments of chromosomes carrying *H-2* and other inheritable factors, such as coat color. Backcross matings were produced between C57BL/6 × DBA/2)F_1 females, and DBA/2 males were used to study the effect of genes not shared by the parents. Specifically, these matings were informative with respect to effects of chromosome segments that determine coat color (*i.e.*, effects of the brown (*b*) locus of chromosome *4* and the dilute (*d*) locus of chromosome *9*), of traits associated with the *H-2*^{b} and *H-2*^{d} haplotypes of chromosome *17* and of sex. With respect to the determination of coat color, it may be noted that interactions occur between the two alleles of the *b* locus (*B* and *b*) and two alleles of the *d* locus (*D* and *d*) resulting in four different coat colors: black (*B*, *D*); brown (*b*, *D*); dilute black (*B*, *d*) and dilute brown (*b*, *d*). Effects on life span of traits associated with the regions of chromosome *17* (the *H-2*^{b} and *H-2*^{d} haplotypes) and sex were also studied.

The experiments suggested that in addition to direct effects of the genes interaction between these genetic systems also significantly influences life span. For example, traits associated with the *H-2* genotype influenced life span much more in females than in males. Furthermore, it is evident that greater heterozygosity at the loci studied is associated with longer life span.

MATERIALS AND METHODS

All mice used in this study were bred and housed in the Michael Redstone animal facility of the Dana-Farber Cancer Institute. Inbred strains of mice [DBA/2J males and (C57BL/6 × DBA/2)F_1J hybrid females] were obtained at 5–8 wk of age from The Jackson Laboratory, Bar Harbor, Maine. Breeding for this study was begun in early 1979, and the mice studied were born within 7 days (±3 days) of 5/29/79 and within 7 days (±3 days) of 8/8/79.

All breeders and backcross mice were kept in two adjoining rooms of the animal facility. Animals of the same sex were housed together in polycarbonate cages of three different sizes; small cages (11.5 × 7.5 × 5 inches) held three to four mice, medium cages (12.5 × 9.25 × 6 inches) held five to seven mice and large cages (19 × 10.5 × 6 inches) held eight to 12 mice. All animals were maintained on standard Purina Chow and water *ad libitum*. Room temperature was kept at 72°F ± 2° with alternate 12-hour light and 12-hour dark periods. Initially, cages were cleaned once per week and bedding was changed once per week. After 14 months, the frequency of cage cleaning was increased to four times per week. Cages were checked for dead animals on alternate days. Mice older than 28 months were moved into small cages (one to three mice per cage) for easier access to food and water and were checked daily for deaths. Dead mice without evidence of autolysis were autopsied for histopathological examination. The last mouse died during the 36th month.

Nine surveillance animals (DBA/2J females) were kept in each room at all times. Three of these animals were taken once per month to test for infection by known pathogens. Despite occasional serological evidence of exposure of the surveillance animals to pneumonia virus of mice, Sendai, mouse hepatitis virus or *Pasteurella pneumotropica*, the backcross animals, which were inspected weekly, showed no signs of these infections. However, the possibility could not be ruled out that these pathogens may affect longevity without gross clinical evidence of infection. In routine screening for pinworm, infection was observed on three occasions. Mice were then given piperazine through water for 2 wk; on the 3rd wk, treatment was discontinued and the room thoroughly cleaned. Piperazine treatment was resumed for another 2 wk, and mice subsequently retested were found to be free of pinworm infection.

Both male and female mice were phenotyped for H-2 alloantigens and coat color at 8–10 wk of age. The H-2 typing was performed on peripheral blood lymphocytes, obtained from the tail vein, using the anti-*H-2*^{b} and anti-*H-2*^{d} cytotoxic antibodies (a gift from L. FLAHERTY, New York Department of Health, Albany, New York). The microcytotoxicity technique used 72-well Terasaki plates and guinea pig complement (WATSON *et al.* 1984).

Statistical analysis: For analysis of the difference between the expected and observed numbers of mice born with each combination of sex and the chromosomes *4*, *9* and *17* markers, and analysis of autopsy findings, the Fisher exact test for contingency tables was used (COX 1970).

The effects of genetic traits, birth cohort and heterozygosity score on life span distributions were assessed, one at a time, using the log rank test (PETO and PETO 1972). This test is based on the difference between the entire survival curves, not on differences at one specific time point or in means. To compare four survival curves (*e.g.*, coat color), a 2 × 4 contingency table may be formed at each time of death (on any of the four curves) giving, for each group, the number of deaths that have occurred by that time and the number of animals still alive. The sums over all tables of the observed number of deaths, minus the expected number of deaths for each of the four groups, form a four-dimensional vector, **w**. If the appropriate variance-covariance matrix for **w** is **V**, then $\mathbf{w}'\ \mathbf{V}^{-1}\mathbf{w}$ is distributed approximately as a chi-squared statistic with 3 degrees of freedom. Here, $\mathbf{w}'$ is the transpose of **w**, and $\mathbf{V}^{-1}$ is a generalized inverse of **V**. To compare only two survival curves 2 × 2 contingency tables are summed to obtain a statistic that is distributed approximately as a chi square with 1 d.f.

Tests for the effects of each of the three genetic markers, sex, and birth cohort while controlling for the effects of other factors, and also tests of interactions of these factors, used the Cox proportional hazards regression model (COX 1972). If $S(t)$ is a survival function; that is, if $S(t)$ is, for each time t, the probability that an animal lives longer than t, then the associated hazard function is defined as

$$h(t) = \frac{\frac{dS(t)}{dt}}{S(t)} = \frac{-d \log S(t)}{dt}$$

where d is the differentiation operator. There is a 1:1 relationship between hazard functions and survival functions. The function is sometimes called "the instantaneous death rate" since it represents the instantaneous chance of death at time t, conditional upon the animal surviving until t. This function could, of course, depend on covariates $z_1, z_2, \ldots, z_p$ (*e.g.*, z_1 could represent sex by letting $z_1 = 1$ if male and $z_1 = 0$ if female). Some of the z's could represent interaction terms. Thus, if z_1 represents sex and z_2 represents the relevant gene on chromosome *4*, then $z_3 = z_1z_2$ represents the interaction of sex and chromosome *4*. The proportional hazards model expresses this dependence in terms of equations:

$$h(t, z_1, z_2, \ldots, z_p) = h_0(t)e^{\beta_0+\beta_1z_1+\beta_2z_2+\ldots+\beta_pz_p},$$

where $h_0(t)$ is an underlying, unspecified, hazard function, dependent only on t and not on the covariates. The model uses the maximum likelihood approach to estimate the coefficients $\beta_0, \beta_1, \ldots, \beta_p$ and their variances. Note that a value of β_i not significantly different from 0 indicates that the factor (or interaction) represented by z_i has no significant effect on survival. In this analysis, the first model fit contained all five main effects terms (one each for chromosome *4*, chromosome *9*, chromosome *17*, sex and birth cohort), all ten two-way interaction terms, all ten three-way interaction terms, all five four-way interaction terms, the five-way interaction term and the constant term (β_0). The best set of covariates to use in the model was then chosen via a step-down procedure, using the likelihood ratio test. The latter is based on the fact that values of $-2 \log (M_{r-1}/M_r)$ are approximately distributed as a chi-squared statistic with 1 d.f., where M_r is the maximum likelihood associated with a specific model with r covariates, and M_{r-1} is the maximum likelihood associated with a specific model formed by dropping one of the covariates from a model of r terms (KALBFLEISCH and PRENTICE 1980). After the best set of covariates was chosen, the significance levels associated with each term (specifically, the P values for the interaction terms as shown in Tables 2 and 4: survival rates and histological findings) were calculated, using the asymptotic normality of the coefficients $\beta_1, \beta_2, \ldots, \beta_p$. The P values in Table 2 come from a Cox model with only main effect terms (no interactions) (KALBFLEISCH and PRENTICE 1980).

Survival curves were calculated using the Kaplan-Meier method (KAPLAN and MEIER 1958). Nonparametric confidence intervals on the median life span were calculated using the order statistic method (LEHMANN 1975).

RATIONALE

Studies of genetic influence on life span have been few and difficult to design. They have, however, suggested that longevity in animals, and, more specifically, in mice, is determined by interactions between the environment and many genes (RUSSELL 1975). Obstacles have been the need to delineate a number of genetic systems that could be investigated separately and to determine causes of death. Ideally, large groups of mice with different genetic combinations should be studied, in order to reduce the variability of environmental effects. Also, several genes should be studied simultaneously. Unfortunately, the feasibility of such experiments is limited by costs of maintenance for colonies of sufficient size.

Here, an experimental protocol using [(C57BL/6 × DBA/2)F_1 × DBA/2] backcross mice (Table 1) has been selected that permits analysis of the possible effects on longevity of segments of chromosomes *4*, *9* and *17* and of sex, in groups of mice born 3 months apart. This approach was useful in that it permitted study of interactions between traits marking chromosomal regions of chromosomes *4* and *9* that confer coat color (the *b* locus and *d* locus, respectively) and also analysis of the effect of heterozygosity. However, since our design did not include animals homozygous at $H\text{-}2^b$ (chromosome *17*), *B* at locus *b* (chromosome *4*) or *D* at locus *d* (chromosome *9*) the question of whether the animals heterozygous in regions of *H-2*, chromosome *4* and chromosome *9* live longer than all homozygotes could not be addressed. Also, in the present studies only genes differing between the C57BL/6 and DBA/2 strains were studied.

RESULTS

Eight to 10 wk after weaning, all mice were phenotyped for H-2 and coat color. The latter indicated genotypes associated with segments of both chromosome *4* (*b* locus) and chromosome *9* (*d* locus); *i.e.*, *B/b,D/d* for black; *B/b,d/d* for dilute black; *b/b,D/d* for brown and *b/b,d/d* for dilute brown.

Segregation of H-2, *sex and coat color*: In both sexes, segregation of the factors studied in backcross animals was approximately 50%; *i.e.*, 52% $H\text{-}2^b/H\text{-}2^d$ and 48% $H\text{-}2^d/H\text{-}2^d$. As expected, frequencies of each of the four coat color genotypes were approximately 25% (*i.e.*, 25% brown, 24% dilute brown, 28% black and 23% dilute black). Correspondingly, there are eight different genotypes grouped with respect to coat color and H-2; these, however, differed from the expected 12.5% segregation. The progeny frequency of dilute black $H\text{-}2^d/H\text{-}2^d$ was the lowest (9.9%) and that of black $H\text{-}2^d/H\text{-}2^d$ the highest (15.7%). The frequency of H-2 heterozygous $H\text{-}2^b/H\text{-}2^d$ dilute black males was twice that of homozygous $H\text{-}2^d/H\text{-}2^d$ males (Table 1). Conversely, in the dilute brown group, the frequency of the heterozygous $H\text{-}2^b/H\text{-}2^d$ males was lower than the homozygous $H\text{-}2^d/H\text{-}2^d$. In females, however, no such differences were observed.

TABLE 1

Genotypes of backcross progeny [(C57BL/6 × DBA/2)F_1 × DBA/2]

	Chromosome no.				No. of mice		
	17[a]	*4*[b]	*9*[c]	Coat color	Female	Male	Total no. of mice (%)
DBA/2 (males)	*d/d*	*b/b*	*d/d*	Dilute brown			
(C57BL/6 × DBA/2)F_1 (females)	*b/d*	*B/b*	*D/d*	Black			
Backcross mice (males and females)	*b/d*	*B/b*	*D/d*	Black	23	26	49 (12.6)
	d/d	*B/b*	*D/d*	Black	29	32	61 (15.7)
	b/d	*B/b*	*d/d*	Dilute black	21	30	51 (13.2)
	d/d	*B/b*	*d/d*	Dilute black	23	15	38 (9.9)
	b/d	*b/b*	*D/d*	Brown	29	27	56 (14.4)
	d/d	*b/b*	*D/d*	Brown	22	18	40 (10.3)
	b/d	*b/b*	*d/d*	Dilute brown	28	16	44 (11.3)
	d/d	*b/b*	*d/d*	Dilute brown	19	30	49 (12.6)
Total					194	194	388 (100.0)

[a] *H-2* genotype: *H-2*^{b} = *b* and *H-2*^{d} = *d*.
[b] Brown locus alleles: *b* or *B*.
[c] Dilute locus alleles: *d* or *D*.

Survival rates according to sex, coat color and H-2 *genotypes*: As shown in Table 2, the two variables most highly correlated with survival rate were the traits located on chromosome *4* ($P = 0.006$) and sex ($P = 0.006$). *H-2* haplotypes also showed correlation with life span ($P = 0.02$). Combinations of the traits for coat color (chromosomes *4* and *9*) yielded four groups with different survival rates ($P = 0.01$). However, no difference was found between the survival rates of the total group of mice carrying the *D/d* or *d/d* genotypes of chromosome *9* nor between the survival rates of mice born in two different months (5/29/79 or 8/8/79). Table 3 also shows the effect on survival rate of interaction between different genotypes and environmental factors. The Cox model, used to test for interactions between the different variables revealed two significant three-way interactions. *H-2*^{b}/*H-2*^{d} mice differed significantly in life span from the *H-2*^{d}/*H-2*^{d}, both in animals born on 5/29/79 and heterozygous for *b* (*B/b*) (median survival in months: 29.3 *vs.* 27.9) and in mice born on 8/8/79 and homozygous for *b* (*b/b*) (median survival in months: 27.7 *vs.* 23.8) ($P = 0.04$) (Figure 1). There was also an interaction between sex, *H-2* and the *b* locus of chromosome *4*. The *H-2*^{b}/*H-2*^{d} mice differed significantly in life span from the *H-2*^{d}/*H-2*^{d} in males homozygous for (*b/b*) at the brown locus (median survival in months: 26.2 *vs.* 24.1) and in females heterozygous for (*B/b*) of the brown locus (median survival in months: 30.5 *vs.* 28.1) (Figure 2) ($P = 0.05$). No significant effect on survival was found to be associated with the *d* locus (Table 2).

Effect of heterozygosity in longevity: Longevity was also analyzed by ranking the mice according to the number of heterozygotes at each locus studied. The median survival (Figure 3) was ranked in the same order as the number of heterozygous markers studied. Although there was a significant difference between the five groups ($P = 0.0004$ using the log rank test), the survival rates were not equally spread. The heterozygosity score was calculated as the sum

TABLE 2

Comparisons of life span according to sex, coat color and H-2 *genotypes*

	All mice			Females			Males		
	Median survival in months (95% C.I.)	90th percentile	Significance level (*P* values)	Median survival in months (95% C.I.)	90th percentile	Significance level (*P* values)	Median survival in months (95% C.I.)	90th percentile	Significance level (*P* values)
Sex									
Females	26.9 (25.9, 28.4)	33.5	0.006						
Males	25.7 (25.2, 26.8)	32.2							
Chromosome *17*									
H-2^d/H-2^d	25.5 (24.7, 27.1)	32.7	0.02	26.0 (24.7, 28.2)	33.2	0.38	25.3 (23.8, 27.1)	30.8	0.01
H-2^b/H-2^d	26.8 (26.2, 28.1)	34.1		28.1 (26.5, 29.5)	34.0		26.2 (25.2, 27.5)	32.9	
Chromosome *4*									
b/b	25.3 (24.8, 26.0)	33.1	0.006	25.5 (24.3, 26.9)	33.1	0.008	25.3 (24.4, 26.2)	32.2	0.30
B/b	27.8 (26.7, 29.1)	33.2		28.9 (27.1, 30.4)	34.5		26.6 (25.2, 28.2)	32.0	
Chromosome *9*									
d/d	26.1 (25.2, 27.1)	33.2	0.38	28.1 (26.1, 29.4)	33.3	0.23	25.2 (24.0, 26.2)	31.5	0.08
D/d	26.7 (25.8, 27.8)	33.1		26.6 (24.7, 28.2)	33.5		26.8 (25.4, 28.2)	32.3	
Color									
b/b d/d	25.3 (24.0, 26.6)	32.6		25.4 (23.3, 28.4)	33.5		25.2 (21.5, 26.4)	31.0	
B/b d/d	27.1 (25.6, 28.9)	33.2	0.01	30.1 (28.0, 31.2)	33.2	0.06	25.1 (23.6, 26.6)	31.3	0.10
b/b D/d	25.4 (24.6, 26.8)	33.1		25.4 (23.4, 27.2)	33.1		25.3 (23.8, 27.6)	32.3	
B/b D/d	28.2 (26.7, 29.6)	32.8		27.5 (24.8, 30.5)	35.5		28.2 (26.8, 29.9)	32.1	
Birthdate									
8/8/79	26.7 (25.7, 27.7)	32.8	0.91	26.9 (25.7, 28.9)	33.2	0.71	26.2 (24.2, 27.6)	32.3	0.60
5/29/79	26.1 (25.3, 27.5)	33.2		26.9 (25.2, 29.6)	34.1		25.5 (25.0, 26.7)	31.5	

C.I. = Confidence intervals. Significance levels from Cox model without interactions. 90th percentile = the time at which 90% of mice were dead and 10% were alive given in months.

TABLE 3

Genetic interactions between H-2, *brown locus, sex and date of birth*

Genotype	No. of mice	95% of confidence intervals	Median survival (months)
Females			
B/b;H-2^b/H-2^d	44	27.7, 31.2	30.5
B/b;H-2^d/H-2^d	52	24.8, 30.0	28.1
b/b;H-2^b/H-2^d	57	23.5, 28.2	25.8
b/b;H-2^d/H-2^d	41	23.3, 27.1	25.0
Males			
B/b;H-2^b/H-2^d	56	24.8, 28.2	26.4
B/b;H-2^d/H-2^d	47	24.2, 29.3	27.8
b/b;H-2^b/H-2^d	43	25.2, 28.9	26.2
b/b;H-2^d/H-2^d	48	21.7, 25.4	24.1
Birth date: 5/29/79			
B/b;H-2^b/H-2^d	58	26.5, 31.0	29.1
B/b;H-2^d/H-2^d	48	23.5, 29.5	27.8
b/b;H-2^b/H-2^d	52	23.3, 26.7	25.3
b/b;H-2^d/H-2^d	48	23.8, 26.5	25.0
Birth date: 8/8/79			
B/b;H-2^b/H-2^d	42	25.0, 29.1	26.8
B/b;H-2^d/H-2^d	51	25.2, 29.6	26.9
b/b;H-2^b/H-2^d	48	25.7, 28.9	27.6
b/b;H-2^d/H-2^d	41	21.3, 25.8	23.7

1 (if *H-2* = *H-2^b/H-2^d*) + 1 (if the coat color was black or dilute black) + 1 (if female) + 1 (if black or brown).

Histological findings: Important histopathological findings are summarized in Table 4, with *P* values for the difference between males and females. The incidence of lymphomas and glomerulosclerosis was higher in females than in males (50 *vs.* 18% and 28 *vs.* 11%, respectively). Hydronephrosis was also higher in females (21 *vs.* 2.8%). Amyloidosis, however, was higher in males (44.4%) than in females (12.5%). Surprisingly, 11 of 18 female mice of *B/b* genotype (chromosome *4*, brown locus), as compared to zero of 14 *b/b* female mice, died with a diagnosis of either sarcoma or adenocarcinoma. Lymphomas were found in spleen, mediastinal and mesenteric lymph nodes and liver, whereas amyloidosis was primarily found in the intestines, renal glomeruli or liver. The sarcomas included three leiomyosarcomas of the uterus, four fibrosarcomas of the soft tissues of the hip, one of the right kidney and one osteosarcoma metastatic to the liver. The adenocarcinomas included two of the uterus, whereas the remainder were of the lung. Hydronephrosis was associated with the tumors of the uterus, with invasion of the lower urinary tract.

DISCUSSION

Interactions between genotype and environment occur at every level of the life of an animal. Consequently, studies of the genetic control of longevity must analyze the extremely complex interactions between purely hereditary factors and environmental influences.

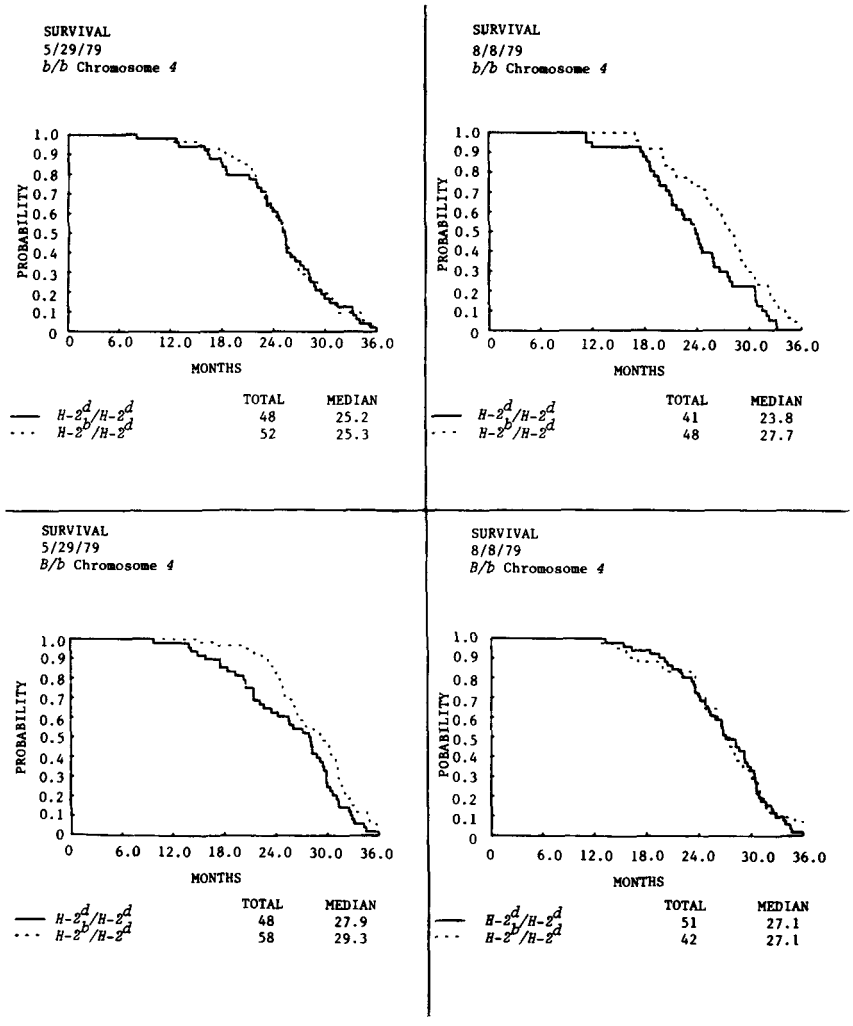


FIGURE 1.—The total refers to the number of mice per group and the median is given in months.

The experiments reported here suggest that genes in the segment of chromosome *17* which carries *H-2*, genes in the segment of chromosome *4* which carries the *b* locus for coat color and also genes in the sex chromosomes significantly influence life span in the genetic combinations differing between the C57BL/6 and DBA/2 strains. The influence of *H-2* determinants and sex on life span have been reported previously by others (RUSSELL 1975; SMITH and WALFORD 1977; POPP 1978; WILLIAMS 1981). Here, however, an effect of the *H-2* determinants on prolongation of life span was observed primarily in males. Other important findings included (1) effects of a segment of genes in chromosome *4* (*b* locus), as studied by coat color, which was more significant in females than in males; and (2) an influence of the simultaneous presence of certain alleles of the sex chromosome, of chromosome *17* (*H-2*) and chromo-

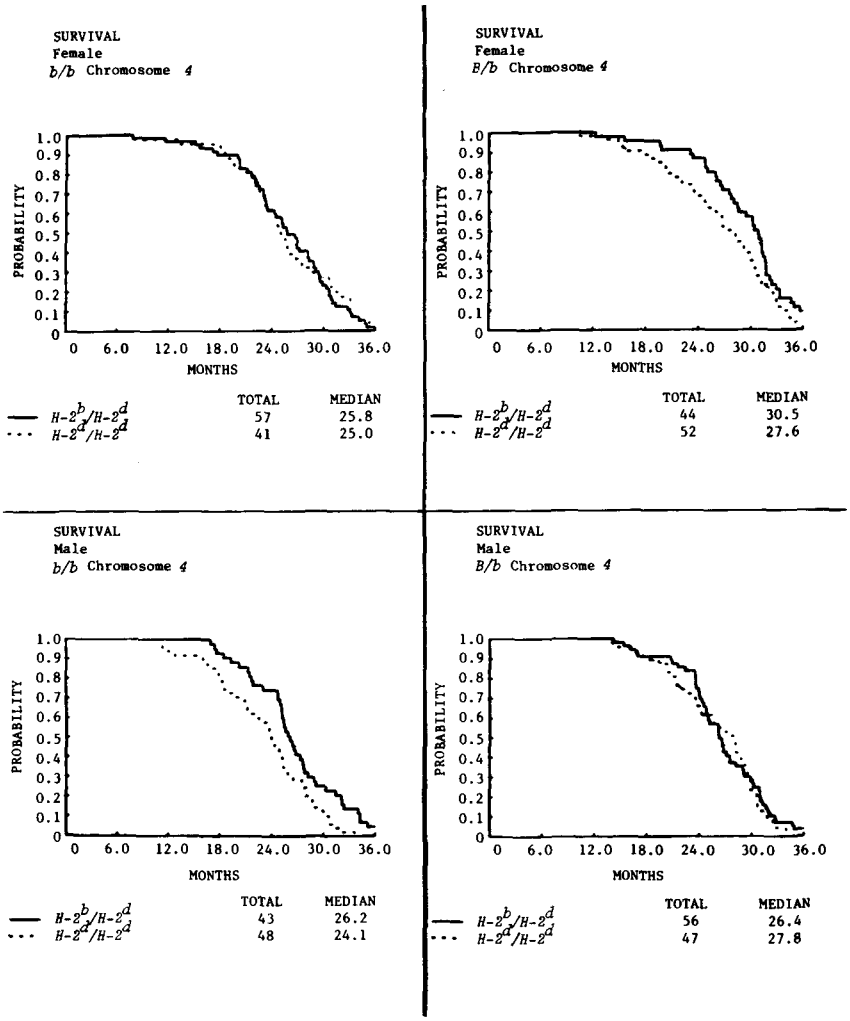
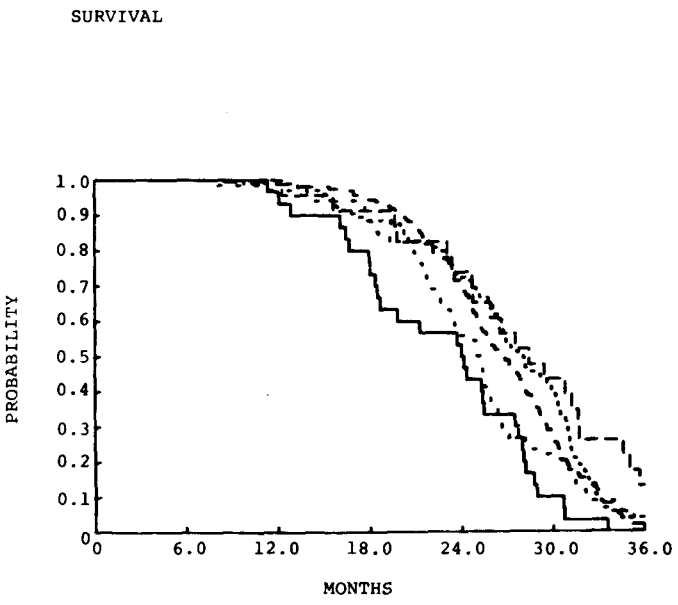


FIGURE 2.—Same as Figure 1.

some *4* (*b* locus) on life span. For example, among the eight different genotypes: $B/b;H\text{-}2^b/H\text{-}2^d$, $B/b;H\text{-}2^d/H\text{-}2^d$, $b/b;H\text{-}2^b/H\text{-}2^d$ and $b/b;H\text{-}2^d/H\text{-}2^d$ males and females, we found the longest lived group to be the females $B/b;H\text{-}2^b/H\text{-}2^d$ (median 30.5 months) and the shortest-lived group to be the males $b/b;H\text{-}2^d/H\text{-}2^d$ (median 24.1 months). Within these eight genotypes, the observed spread, 6 months, represents a 25% increase in median survival rate.

An important result of our study was the finding that increased heterozygosity directly correlates with longer life span. For example, the longest lived mice were females (in which one or the other of the two *X* chromosomes was activated), heterozygous at the *H-2* and brown (*b*) loci. The shortest lived group were males (with only one *X* chromosome), homozygous at the *H-2* and brown (*b*) loci. This effect may represent a case of hybrid vigor (heterosis), associated either with dominance of favorable alleles not held in common by the parental



HETEROZYGOSITY SCORE	TOTAL	MEDIAN
0	30	24.1
1	68	25.1
2	162	26.8
3	105	28.1
4	23	28.4

FIGURE 3.—The total refers to the number of mice per group and the median is given in months. The heterozygosity score was calculated giving a value of 1 to each of the heterozygotes examined: *H-2*^{b}/*H-2*^{d}, *B/b* of chromosome *4*, *D/d* of chromosome *9* and female sex chromosome and 0 to the group of homozygous males.

strains (RODERICK and SCHLAGER 1975) or with avoidance of the deleterious effects of recessive genes which limit life span (RUSSELL 1975).

Another finding, the biological significance of which is presently not clear, was the distortion of segregation in *H-2*^{d}/*H-2*^{d} males, giving fewer (18 of 194) brown (*b/b;D/d*) and more (30 of 194) dilute brown color (*b/b;d/d*) than expected. This observation might perhaps be explained, however, by effects on either fetal wastage or on fertilization and of interactions between *H-2*^{b} in males and the *B* allele of the *b* locus of chromosome *4*.

In this study, the season of birth is shown to influence life span, *i.e.*, when analyzed in relation to the *H-2* determinants and determinants of *b* locus as studied by coat color. This puzzling finding may be related to complex environmental effects (HALBERG 1980).

Although there was no significant difference in the incidence of pneumonia, kidney disease, hepatoma, lymphoma or amyloidosis in mice in relation to *H-2* or coat color traits, a finding of interest was the higher incidence, beyond 28 months of age, of lymphomas in females and of amyloidosis in males. A similarly increased incidence of lymphomas in females was reported previously

TABLE 4

Autopsy findings of mice of different genotypes

Genotype	*N*	Lymphoma	Sarcoma	Adenocarcinoma	Hepatoma	Glomerulosclerosis	Pneumonia	Amyloidosis
Female								
B/b;H-2^b/H-2^d	9	4	3	2	–	–	1	–
B/b;H-2^d/H-2^d	9	5	3	3	–	4	1	1
b/b;H-2^b/H-2^d	8	5	–	–	2	3	3	–
b/b;H-2^d/H-2^d	6	2	–	–	–	2	1	3
Total	32	16	6	5	2	9	6	4
Male								
B/b;H-2^b/H-2^d	7	1	1	1	–	1	2	3
B/b;H-2^d/H-2^d	8	1	2	–	2	1	2	5
b/b;H-2^b/H-2^d	8	2	–	–	1	1	1	3
b/b;H-2^d/H-2^d	4	1	1	1	1	–	1	1
Total	27	5	4	2	4	3	6	12
P values		0.02	0.74	0.44	0.40	0.19	0.76	0.008

–, None found.

among a few of the strains studied by SMITH and WALFORD (1978). These authors have also reported influences of the *H-2* region on the frequency of appearance of various forms of tumors; *e.g.*, hepatomas, adenocarcinomas, lymphomas and sarcomas. Here, among [(C57BL/6 × DBA/2)F_1 × DBA/2] mice of age >28 months, there appears to be an increased incidence of sarcomas and adenocarcinoma in female mice of black or brown coat color (*B/b* of chromosome *4*). More work is needed to determine whether this is due to a genetic influence or is secondary to the longer life span of *B/b* females (Table 4).

The observed influence on longevity of a gene (or genes) in the segment of chromosome *4* which carries the *b* locus for coat color is of particular interest in view of the recent identification of several genetic markers on that chromosome. If, for example, the DNA repair enzyme (PIN-FANG and RUDDLE 1981) (which maps at an unknown distance from the brown locus) affects survival, it should be possible to show that levels are different in the C57BL/6 (*H-2^b*) and DBA/2 (*H-2^d*) genotypes. Cell surface antigens such as Lyb.4 (HOWE *et al.* 1979), Ly22.2 (MERUELO, OFFER and FLIEGER 1983), the xenotropic gp70 antigen, Xen CSA (MORSE *et al.* 1979), G_{1x} (STOCKERT *et al.* 1976) and *Jt* (HAYES *et al.* 1984) have also been mapped in chromosome *4*. Of particular interest is *Jt*, which has been mapped between *Mup-RF* (4 cM to the left of *b*) and *Gpd-1* (33 cM to the right of *b*), I-J molecules important in immunoregulation resulted from the interaction of *Jt* with one gene within the *H-2* region. Although effects on longevity could only be studied when each of these factors would be present in one parent only, their presence on chromosome *4*, together with the results obtained here, suggests that genetic control of longevity may act via immunoregulatory mechanisms. However, this conclusion is valid only if the loci described differ between C57BL/6 and DBA/2.

In summary, this work suggests that interaction between a number of genes and the environment is more important in conferring longer life span than the presence of specific individual genes.

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LITERATURE CITED

COX, D. R., 1970 *Analysis of Binary Data.* pp. 48–52. Methuen and Company, Ltd., London.

COX, D. R., 1972 Regression models and life tables. J. R. Stat. Soc. (Ser. B) **34:** 187–202.

GREENBERG, L. J. and E. J. YUNIS, 1975 Immunopathology of aging. Hum. Pathol. **5:** 122–124.

GREENBERG, L. J. and E. J. YUNIS, 1978 Genetic control of autoimmune disease and immune responsiveness and the relationship to aging. pp. 249–260. In: *Genetic Effects of Aging*, Edited by D. BERGSMA and D. HARRISON. Alan R. Liss, New York.

HALBERG, F., 1980 Closing remarks: chronobiology and health. pp. 541–562. In: *Chronobiology: Principles and Applications to Shifts in Schedules*, Edited by L. E. SCHEVING and F. HALBERG. Sijthoff and Noordhoff, Rockville, Maryland.

HAYES, C. E., K. K. KLYCZEK, D. P. KRUN, R. M. WHITCOMB, D. A. HULLETT and H. CANTOR, 1984 Chromosome 4 Jt gene controls murine T cell surface I-J expression. Science **223:** 559–563.

HOWE, R. C., A. AHMED, T. J. FALDETTA, J. E. BYRNES, K. M. ROGAN, M. E. DORF, B. A. TAYLOR and R. E. HUMPHREYS, 1979 Mapping of the Lyb-4 gene to different chromosomes in DBA/2J and C_3H/HeJ mice. Immunogenetics **9:** 221–232.

KALFFLEISCH, J. D. and R. L. PRENTICE, 1980 *The Statistical Analyses of Fracture Time Data.* pp. 70–116. John Wiley and Sons, New York.

KAPLAN, E. L. and P. MEIER, 1958 Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. **53:** 457–481.

KATZ, D. H. and B. BENACERRAF (Editors), 1976 *The Role of the Products of the Histocompatibility Gene Complex in Immune Responses.* Academic Press, New York.

LEHMANN, E. L., 1975 *Non-parametrics.* pp. 182–184. Holden-Day, San Francisco.

MEREDITH, P. J. and R. L. WALFORD, 1977 Effect of age on response to T- and B-cell mitogens in mice congenic at the H-2 locus. Immunogenetics **5:** 109–128.

MERUELO, D., M. OFFER and N. FLIEGER, 1983 A new lymphocyte cell surface antigen, Ly-22.2, controlled by a locus on chromosome 4 and a second unlinked locus. J. Immunol. **130:** 946–950.

MORSE, H. C., T. M. CHUSED, J. W. HARLEY, B. J. MATHIESON, S. O. SHARROW and B. A. TAYLOR, 1979 Expression of xenotropic murine leukemia virus as cell-surface gp70 in genetic crosses between strains DBA/2 and C57BL/6. J. Exp. Med. **149:** 1183–1196.

PETO, R. and J. PETO, 1972 Asymptotically efficient rank invariant test procedures. J. R. Stat. Soc. (Ser. A) **135:** 185–198.

PIN-FANG, L. and F. H. RUDDLE, 1981 Murine DNA repair gene located on chromosome 4. Nature **289:** 191–195.

POPP, D. M., 1978 Use of congenic mice to study the genetic basis of degenerative disease. pp. 261–279. In: *Genetic Effects of Aging*, Vol. XIV, Edited by D. BERGSMA and D. HARRISON. Alan R. Liss, New York.

RODERICK, T. H. and G. SCHLAGER, 1975 Multiple factor inheritance. pp. 151–185. In: *Biology of the Laboratory Mouse*, Ed. 2. Dover Publications, Inc., New York.

RUSSELL, E. S., 1975 Lifespan and aging patterns. pp. 511–519. In: *Biology of the Laboratory Mouse*, Ed. 2. Dover Publications, Inc., New York.

SMITH, G. W. and R. L. WALFORD. 1977 Influence of the main histocompatibility complex on aging in mice. Nature **270:** 727–729.

SMITH, G. W. and R. L. WALFORD, 1978 Influence of the H-2 and H-1 histocompatibility systems upon life span and spontaneous cancer incidences in congenic mice. pp. 281–312. In: *Genetic Effects of Aging*, Edited by D. BERGSMA and D. HARRISON. Alan R. Liss, New York.

STOCKERT, E., E. A. BOYSE, H. SATO and K. ITAKURA, 1976 Heredity of the G_{1x} thymocyte antigen associated with murine leukemia virus: Segregation data simulating genetic linkage. Proc. Natl. Acad. Sci. USA **73:** 2077–2081.

WATSON, A. L. M., R. J. MAHONEY, S. A. D'AMICO, L. J. COVIELLO, L. FLAHERTY and E. J. YUNIS, 1984 Utilization of murine peripheral blood lymphocytes for H-2 typing. Tissue Antigens. In press.

WILIAMS, R. M., L. J. KRAUS, P. T. LAVIN, L. L. STEELE and E. J. YUNIS, 1981 Genetics of survival in mice: localization of dominant effects to subregions of the major histocompatibility complex. p. 247. In: *Immunological Aspects of Aging*, Edited by D. SEGRE and L. SMITH. Marcel Dekker Inc., New York.

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