Traits That Influence Longevity in Mice: A Second Look

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ABSTRACT

Analysis of genetic interactions in the F_2 of an intercross of (C57BL/6 × DBA/2) F_1J revealed influences of genetic factors on life span. Females lived longer than males. Dilute brown females died sooner than females of other colors. *H-2^b/H-2^b* males died sooner than *H-2^b/H-2^d* or *H-2^d/H-2^d* males, except that among dilute brown males those of type *H-2^b/H-2^d* died sooner. Cluster analysis suggested that male and female genotypes each fall into two groups, with female dilute brown mice having shorter lives than other females, and male *H-2^b/H-2^b* mice except dilute brown and dilute brown *H-2^b/H-2^d* mice having shorter lives than other males. The association of heterozygosity with life span was clearer in females than in males, yet the longest-lived female genotype was homozygous *H-2^d/H-2^d*, of dominant *Black* phenotype at the *Brown* locus of chromosome *4*, and homozygous *dd* at the *Dilute* locus of chromosome *9*. The shortest-lived females were dilute brown *H-2^b/H-2^b*. The longest-lived and shortest-lived male genotypes were dilute brown *H-2^d/H-2^d* and dilute brown *H-2^b/H-2^d*, respectively. Although histological findings at postmortem differed between the sexes, there was no association of particular disorders with other genetic markers. The importance of *H-2* in males was confirmed, but the allelic effects were perturbed, possibly by the absence of Sendai infection in this experiment. Overall our studies suggest that genetic influences on life span involve interactions between loci, and allelic interactions may change with viral infections or other environmental factors.

NUMEROUS studies have demonstrated that genetic and environmental factors play a pivotal role in determining life span in mice and other species. The role of genetics in life span is well documented in inbred strains of mice, since different strains differ in life span. In the last years the use of congenic strains of mice which differ only in a given chromosomal region has helped to elucidate the genes controlling life span in mice. For example, differences in *H-2* haplotype were associated with differences in immune responses (SMITH and WALFORD 1977; POPP 1978; WILLIAMS *et al.* 1981). Also it has been shown that the major histocompatability complex influences life span (GREENBERG and YUNIS 1975; SMITH and WALFORD 1977; MEYER, ARMSTRONG and WARNER 1989).

Our previous work on [(C57BL/6 × DBA/2) F_1J × DBA/2J] backcross mice showed that the interaction between genes in the *Brown* (*b*) locus of chromosome *4*, genes in the segment of chromosome *17* which contains the *H-2* haplotype, and genes in a segment of the sex chromosome, influenced longevity, and that increased heterozygosity at these loci was associated with longer life span (YUNIS *et al.* 1984). The current experimental protocol was different, in that the mice studied were F_2 hybrids, which included mice either homozygous *H-2^b/H-2^b* and *H-2^d/H-2^d* or heterozygous. In the backcross experiment, the animals were exposed to Sendai infection at an early age, but in the current experiment there was no exposure to Sendai infection.

Although the backcross method is more powerful than the intercross we chose the intercross in order to analyze *H-2^b/H-2^d* which would have required two different backcrosses for the comparison of *H-2^b/H-2^b*, *H-2^b/H-2^d* and *H-2^d/H-2^d*. However, the intercross method used could not distinguish *BB* from *Bb* of chromosome *4* nor *DD* from *Dd* of chromosome *9*, so we could only compare the *bb* genotype *vs.* the phenotype [*B.*] (*BB* plus *Bb*) and the *dd* genotype *vs.* the phenotype [*D.*] (*DD* plus *Dd*). The resulting F_2 produced more genetic combinations to test the hypothesis of heterozygote advantage.

There are 12 different groups of mice characterized by the interactions between sex, *H-2* haplotype and coat color. Although it is not possible to distinguish homozygosity from heterozygosity of *b* of chromosome *4* or *d* locus of chromosome *9*, the data confirmed our previous findings that the *H-2* haplotype influenced life span in males, the *b* locus chromosome influenced life span in females, and that heterozygosity is important in life span, although the genetic interactions vary depending on the experimental conditions.

MATERIALS AND METHODS

Inbred strains of [(C57BL/6 × DBA/2) F_1J] hybrid mice were obtained a 5–8 weeks of age from The Jackson Laboratory, Bar Harbor, Maine. The experimental [(C57BL/6 × DBA/2) F_1J × (C57BL/6 × DBA/2) F_1J] F_2 mice were bred and housed in the Michael Redstone animal facility of the Dana-Farber Cancer Institute (DFCI). The F_2 mice were born between June 12 and July 20, 1984. The control mice were inbred C57BL/6J, DBA/2J and (C57BL/6 × DBA/2) F_1J strains obtained in July and August 1984 from The Jackson Laboratory to be age-matched with the F_2 mice bred at DFCI.

All mice were housed in two rooms of the animal facility. The female littermates were separated at weaning and housed together in polycarbonate cages, ten mice per large cage (19 × 10.5 × 6 inches). Male mice were housed with their littermates in the same size cages, because fighting between males is reduced when they are housed with littermates. Litters were combined in cages so that a total of 9–11 males from two or more litters were housed together in each large cage. Males and females were distributed in both rooms. All animals were maintained on standard Purina Chow and water *ad libitum*. Room temperature was kept at 7°F ± 2° with alternate 12-hr light and 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. Maintaining the same cagemates, mice were moved to smaller cages, 4–6 mice per medium cage (12.5 × 9.25 × 6 inches) and 1–3 mice per small cage (11.5 × 7.5 × 5 inches) as the number of mice per cage decreased, to provide easier access to food and water. After 24 months of age, most mice were in small cages.

All mice were ear-tagged. The cages were labeled by color coding the cage cards and the shelves to be easily visible to all project and facility staff. The mice were checked 1–3 times per day, 7 days a week, to ensure accurate determinations of dates of death and to preserve bodies for later necropsy. The mice were inventoried weekly to maintain the cage counts.

Nine surveillance animals (DBA/2J females) were kept in each room at all times. Three or four of these animals were taken once per month to test for infection by known pathogens. Both rooms had persistent *Pasturella pneumotropica* infections during the study period. There was occasional serological evidence of exposure of the surveillance animals to mouse hepatitis virus and one instance each where one or two of the surveillance animals exhibited exposure to Klebsiella and Polyoma. There was no evidence of exposure to Sendai virus during the study and after April 1986 there was no evidence of exposure to pathogens. The study animals, which were inspected weekly, showed no sign of infection. 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 in one room on only one occasion.

Both male and female mice were phenotyped for *H-2* alloantigens beginning at 5 weeks of age and continuing until all mice had been typed. The *H-2* typing was performed on peripheral blood lymphocytes, obtained from the tail vein, using the anti-$H\text{-}2^b$ and anti-$H\text{-}2^d$ 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).

Because previous results showed a dependence of life span on month of birth, we used mice with birth dates spanning only a short range. The mice were kept in cages on 5-tier racks. Previous experiments have shown an effect of tier on life span, but no such effect was apparent in this study and it was not a source of variation in our results.

Statistical methods: Random segregation was tested using contingency-table chi-squared tests. Survival curves were drawn using the method of KAPLAN and MEIER (1958) and compared using log rank tests (PETO and PETO 1972). Confidence intervals around median life spans (see Table 4) were calculated using the method of LEHMANN (1975, p. 184). Proportional hazards regression models (COX 1972) were used to test the association of combinations of factors with life span and to model the effect of increasing heterozygosity on life span.

Clustering of genotype groups (these include some coat-color phenotypes: see the introduction) was performed using repeated log rank tests. A critical value of 0.1 was used to form clusters of genotypes as follows. Starting with all the

TABLE 1

Segregation of coat color markers in F_2 mice intercross (C75BL/6J×DBA2/J)F_1

Sex	*Brown* *Unknown*	*Brown* [*B.*]	*Brown* [*B.*]	*bb*	*bb*	
	Dilute *Unknown*	*Dilute* [*D.*]	*Dilute* *dd*	*Dilute* [*D.*]	*Dilute* *dd*	ALL
Females	1	659	227	224	76	1187
Males	3	686	221	214	64	1188
Total	4	1345	448	438	140	2375

At the *Brown* locus [*B.*] denotes the dominant black phenotype. The homozygote *BB* cannot be distinguished in the F_2 from the heterozygote *Bb*. Similarly at the *Dilute* locus, [*D.*] denotes either *DD* or *Dd* (the nondilute phenotype).

TABLE 2

Segregation of *H-2*

Sex	Missing	$H\text{-}2^b/H\text{-}2^b$	$H\text{-}2^b/H\text{-}2^d$	$H\text{-}2^d/H\text{-}2^d$	Total
Females	92	282	542	271	1187
Males	124	267	528	269	1188
Total	216	549	1070	540	2375

The totals include the mice for which the *H-2* type was not obtained.

TABLE 3

Life span percentiles (months)

Sex	No. of mice	Life span percentile 10%	25%	50%	75%	90%
B6 Females	32	23.2	25.6	28.1	31.4	31.9
B6 Males	38	18.8	23.1	28.2	30.8	33.6
D2 Females	28	20.0	20.6	23.7	27.4	29.4
D2 Males	17	13.8	22.2	23.3	25.6	27.1
F_1 Females	30	23.0	25.0	27.9	31.5	37.0
F_1 Males	30	22.1	26.3	28.9	32.2	37.7
F_2 Females	1187	17.9	22.4	26.9	31.1	34.7
F_2 Males	1188	14.7	20.9	25.6	29.7	33.0

The 10th percentile is the age in months by which 10% of mice had died. The 50th percentile is the median life span.

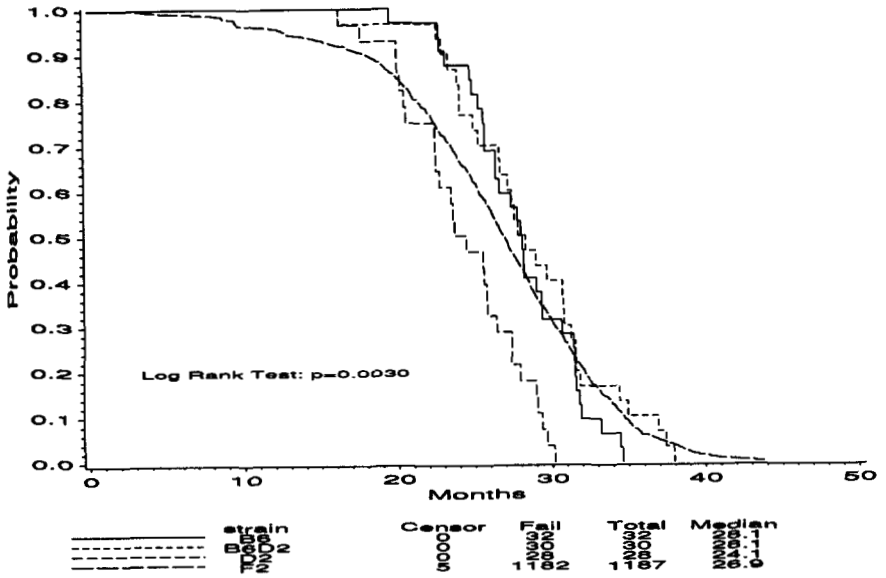

FIGURE 1.—Parental, F_1 and F_2 life spans: females. Censored mice were those few lost by drowning, for which a natural life span could not be determined. "Fail" denotes the remainder. Tabulated median life spans for the parents (B6, D2), F_1 (B6D2) and F_2 (F2) are in months.

genotypes in a single cluster, log rank tests were performed to test the significance of the difference of each genotype from the rest. The most significantly different genotype was removed from the cluster if its significance level was below 0.1. This was repeated until no further differences were found among the remaining genotypes. Next, the genotypes which had been removed one by one were formed into a new cluster, and the process repeated (in fact, no differences were found within these secondary clusters). In this way the genotypes were grouped into homogenous clusters. The purpose of this exercise was descriptive, and resulted in the clusters as depicted in Figure 10.

RESULTS

Segregation: The F_2 mice segregated for four markers: sex, brown color, dilute color and *H-2*. Of 2375 total mice, sex was known for all, coat color (the brown and dilute markers) was missing for 4 and *H-2* was missing for 216. There were 1187 male and 1188 female mice.

The segregation of the two color genes is shown in Table 1. The segregation is similar in the two sexes ($P = 0.60$), and in both sexes is similar to the expected 9:3:3:1 ratio (females $P = 0.97$; males $P = 0.72$).

The segregation of *H-2* by sex is shown in Table 2. The overall frequencies of *H-2* types is not significantly different from the expected 1:2:1 ratio ($P = 0.87$), and is similar in the two sexes ($P = 0.93$). *H-2* segregated independently of color ($P = 0.83$).

Parental types: For comparison, mice of the parental inbred and the F_1 types were also kept. Data were available on about 30 mice of each sex. Table 3 shows the times by which 10% and 90% had died, and the median. In each sex, life spans are substantially longer in parental C57BL/6J (B6) than parental DBA/2J (D2) mice. In females, the F_1 life spans were similar to the B6 parental type, except possibly beyond 32 months (Figure 1), while in males, the F_1 mice lived longer then either parental type (Figure 2). The meaning of this difference is unknown. The F_2 mice show a larger variance in life span, with the early 10th percentile being attained at approximately the same time as in the short-lived parent, while the later 90th percentile is delayed to approximately the same time as in the longer-lived parent. Although the numbers of F_2 mice were greater than the parent strains and the F_1, their overall survival was less than either the F_1 or B6.

Effects of sex, coat color and *H-2* genotype on life span: Females tended to live longer than males. Table 3 shows percentiles of the life span distribution in months, and the sex difference. From the 25th percentile on, the distributions differ by about 1.5 months (Figure 3).

The effects of differing genotypes on life span were distinctly different in the two sexes. In females, the situation was quite simple. Dilute brown mice, homozygous recessive at the *Brown* and *Dilute* loci, had significantly shorter lives than mice with the other three coat colors. Their median life span was 25.2 months compared with 26.8, and the 90th life span percentile lay at 32.0 months rather than 35.0. As Figure 4 shows, the difference did not appear until the mice were two years old, when about a third of the females had already died. Cox regression showed a marginal effect ($P = 0.065$) of *H-2*: $H\text{-}2^b/H\text{-}2^b$ females died sooner, with a median life span of 34.2 months compared with 35.0 for both $H\text{-}2^b/H\text{-}2^d$ and $H\text{-}2^d/H\text{-}2^d$. There was no interaction between color and *H-2*.

Among males, *H-2* was strongly associated with life span ($P = 0.0032$, Figure 5). $H\text{-}2^b/H\text{-}2^b$ males had distinctly shorter lives than $H\text{-}2^b/H\text{-}2^d$ or $H\text{-}2^d/H\text{-}2^d$ males ($P = 0.002$). However, there appeared to be an interaction with coat color markers, whereby dilute brown $H\text{-}2^b/H\text{-}2^b$ and $H\text{-}2^d/H\text{-}2^d$ males enjoyed moderately long lives and dilute brown $H\text{-}2^b/H\text{-}2^d$ males

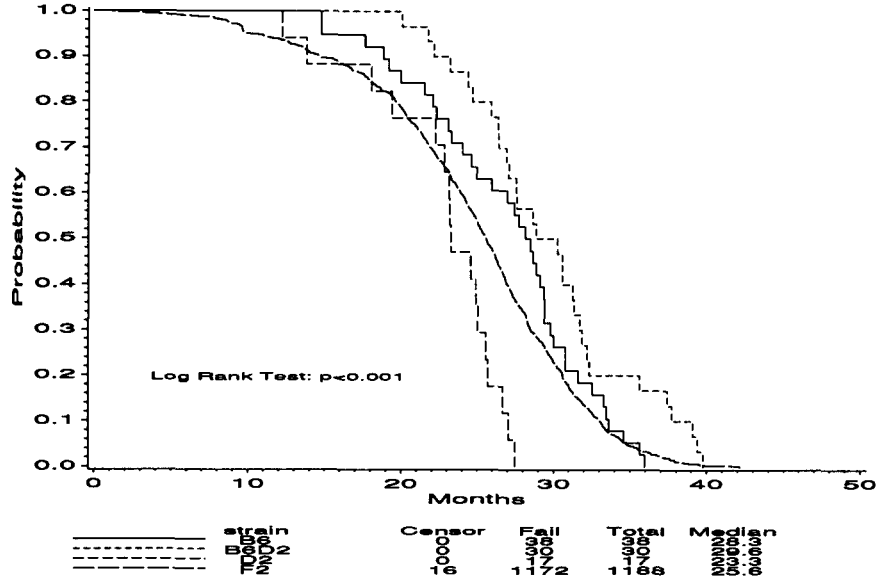

FIGURE 2.—Parental, F_1 and F_2 life spans: males.

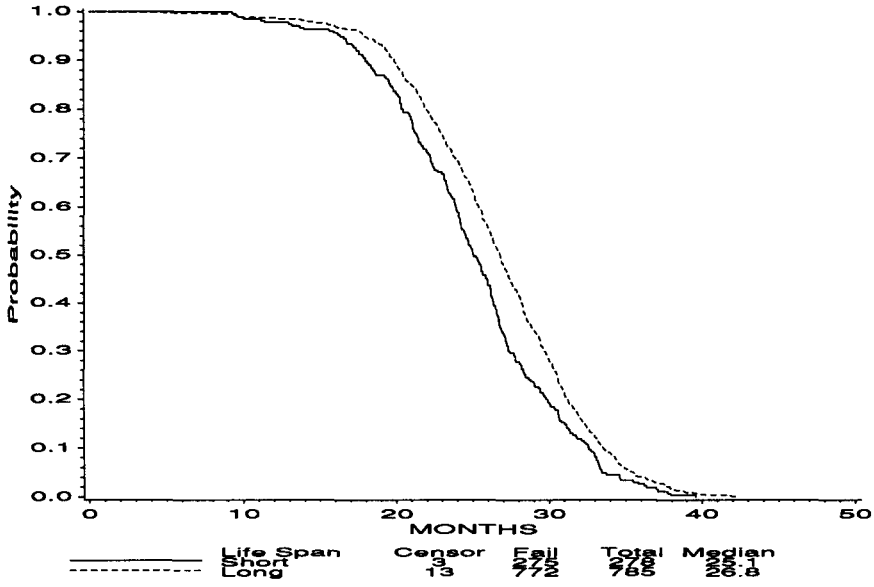

FIGURE 3.—F_2 longevity by sex.

died earlier than any other group. The different effect of *H-2* in male and female dilute brown mice is shown in Figures 6 and 7.

Sex differences within genotypes: Table 4 compares the two sexes within each of the 12 genotypes defined by *H-2* and coat color. The table shows the mean and median life span; the rankings of the genotypes within the sexes, based on the median; the numbers of mice in each group; and the significance level of a log rank test comparing the sexes. The strongest difference is found in $H\text{-}2^b/H\text{-}2^b$ mice of black or brown coat color.

Cluster analysis: Multiple log rank tests were performed within each sex, comparing the 12 genotypes defined by combinations of *H-2* and the two coat-color genes. Among females, the most significantly different genotype, with shorter life span than the rest, is $H\text{-}2^b/H\text{-}2^b$ *bbdd*, at $P = 0.0051$. After this, $H\text{-}2^d/H\text{-}2^d$ *bbdd* is different from the remaining genotypes at $P = 0.019$, and then $H\text{-}2^b/H\text{-}2^d$ *bbdd* is different at $P = 0.086$. The remaining genotypes are not distinguishable at a critical value of $P = 0.1$. The three extracted genotypes, comprising all dilute brown females, are not significantly different ($P = 0.37$). The females thus fall into two groups, as shown in Table 5. The survival experience of these groups is shown in Figure 8.

Among males, the most significantly different genotype, with short life span, is $H\text{-}2^b/H\text{-}2^b$ *bb*[*D.*], at $P = 0.0031$ (the word genotype is taken to include the phenotypes [*D.*] and [*B.*] at the dilute and brown loci). After this, $H\text{-}2^b/H\text{-}2^b$ [*B.*][*D.*], $H\text{-}2^b/H\text{-}2^b$ [*B.*]*dd* and $H\text{-}2^b/H\text{-}2^d$ *bbdd* are different from the remaining genotypes at $P = 0.053$, 0.042 and 0.048, respectively. The remaining genotypes are not distinguishable. The four extracted genotypes, when compared separately

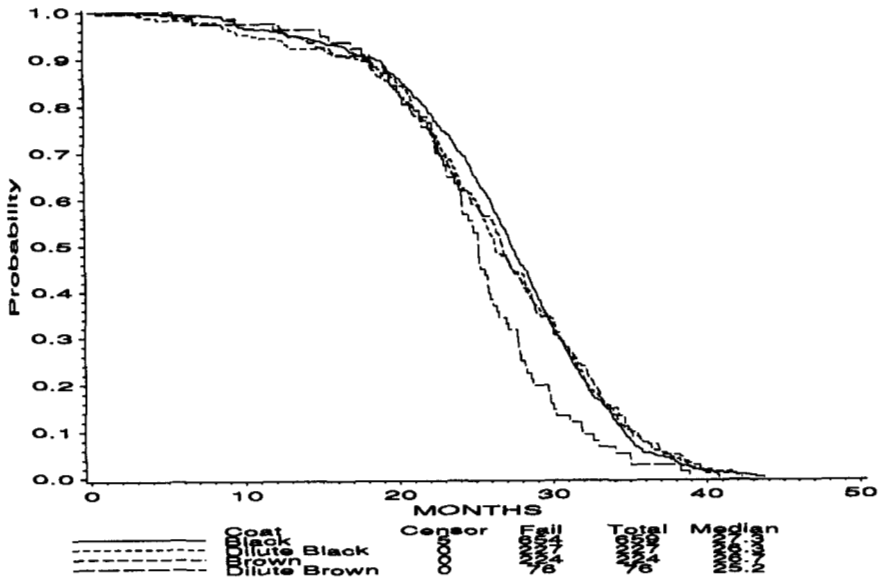

FIGURE 4.—Longevity by coat color markers in females. Combining the three *H-2* genotypes, this graph shows the rapid death rate of dilute brown females after 20 months.

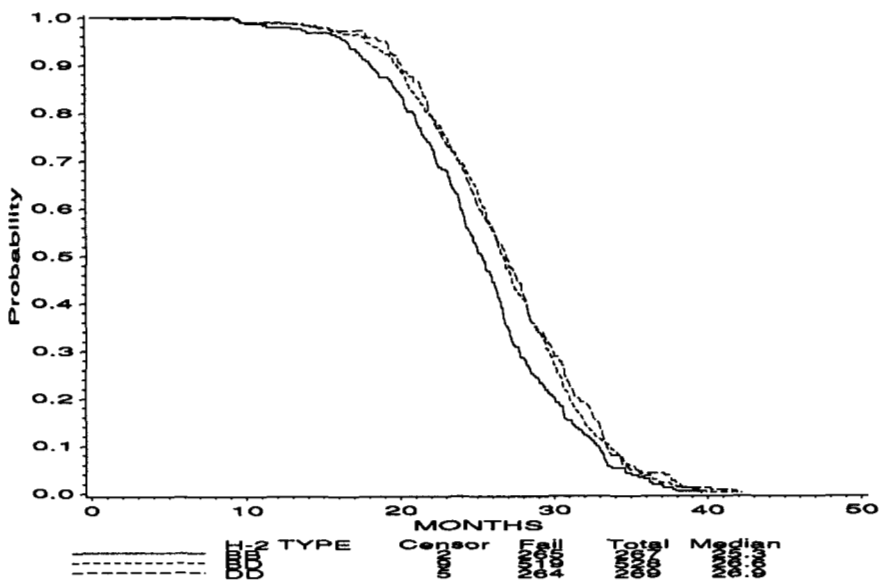

FIGURE 5.—Longevity by *H-2* in males. Combining all four coat colors, this graph illustrates that $H\text{-}2^b/H\text{-}2^b$ males tend to have shorter life spans that $H\text{-}2^d/H\text{-}2^d$ or $H\text{-}2^b/H\text{-}2^d$ males.

from the others, are not significantly different ($P = 0.33$), so the males also fall into two groups as shown (Figure 9). The median life spans for all the genotype are displayed in Figure 10 with the groups identified; note that the groups are defined by differences among the entire survival curves, not just the medians.

Heterozygosity index: To examine the overall association of degree of heterozygosity with life span, a heterozygosity index was formed as follows. One point was scored for females, one for mice of type $H\text{-}2^b/H\text{-}2^d$, two for black coat color and one for brown or dilute black coat color. Black mice scored twice for coat color, being potentially heterozygous at both the *Brown* and *Dilute* loci. The index thus ranged from 0 to 3 for males, 1 to 4 for females.

Considering mice of both sexes together, the heterozygosity index was highly significant ($P < 0.0001$), with greater heterozygosity being associated with longer life spans in a Cox (1972) regression model. After adjusting for sex, the significance was considerably lessened, to $P = 0.011$. It thus appears that sex is a major component of the apparent effect of heterozygosity.

The median life spans for the four heterozygosity groups in each sex are shown in Table 6, and the survival curves in Figures 11 and 12. These groups are significantly different in each sex: $P = 0.0076$ for females, $P = 0.037$ for males. With the exception of the dilute brown males (heterozygosity index 0), which had the greatest median life span among males, the group median life spans increased in order of increasing heterozygosity index.

As reported above, mice of both sexes with dilute brown (*bbdd*) markers had anomalous life spans, very short in females and rather long in males. If these mice, with heterozygosity indices of 0 for males and 1

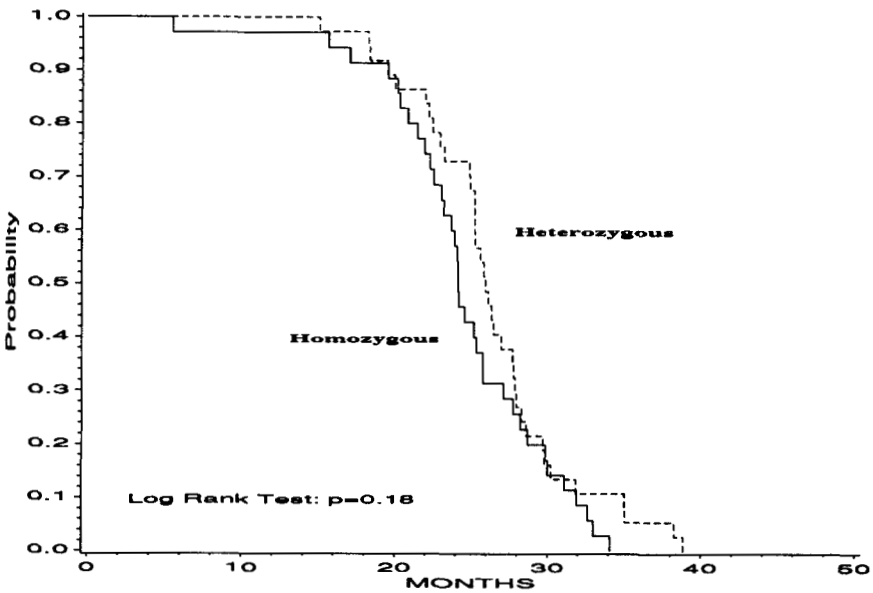

FIGURE 6.—Dilute brown mice: *H-2* effects: females. Considering only dilute brown mice, the two *H-2* homozygotes had similar life spans. With Figure 7, this illustrates the interaction of sex with *H-2*: in females $H\text{-}2^b/H\text{-}2^d$ mice did better than the homozygotes, while in males $H\text{-}2^b/H\text{-}2^d$ mice did worse.

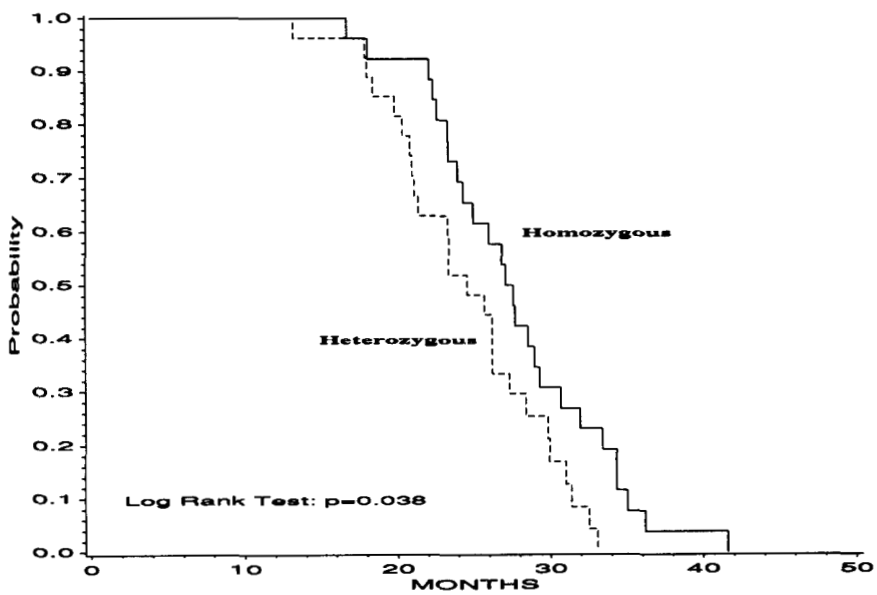

FIGURE 7.—Dilute brown mice: *H-2* effects: males.

for females, are excluded from the analysis, the remaining effect is different for the two sexes. In females, there is no residual association of heterozygosity with life span: $P = 1.0$ from a log rank test between the three groups, and $P = 0.83$ from a Cox regression testing for a linear effect of increasing heterozygosity. In males, however, the remaining groups are significantly different: $P = 0.095$ from the log rank test, and $P = 0.015$ from the Cox regression. According to this proportional hazards model, the hazard of death is reduced at any time by a multiplicative factor of 0.89 (95% confidence interval 0.81–0.98) for each unit increase in heterozygosity. The instantaneous risk of death for mice of heterozygosity index 3 is therefore estimated to be only about 0.70 (0.89^3) times the risk for mice of heterozygosity index 0.

Histopathology findings: Table 7 shows the occurrence of various postmortem pathologies. Lymphoma was the commonest finding, and was twice as common in females as in males. This agrees with our earlier findings in the backcross model. Hepatoma (parenchyma) was the next most common, appearing more often in males. The ratio of lymphoma to hepatoma was approximately 5:1 in females and 5:4 in males. Few hepatomas were found in the backcross. Among less common pathologies, lung carcinoma and pyelonephritis were more common in males, hydronephrosis in females. Except for hydronephrosis, these comparisons were not significant in the backcross. Due mainly to rapid lysis, postmortems were successful on only 25% of mice. The brain and pituitary were not studied.

There was no significant association in either sex of disease frequency with any of the genetic markers *H-2*, *Brown* and *Dilute*. Omitting genotypes with fewer than 10 necropsies, the associations of genotype with

TABLE 4

Group characteristics (life spans in months)

Genotype		No. of mice		Ranks of medians		Median life spans (95% confidence interval)		Log rank
H-2	Coat color	F	M	F	M	Females	Males	*P* value
bb	[*B.*][*D.*]	152	157	6	9	27.3 (26.3, 27.6)	25.6 (24.8, 26.0)	0.0030
bb	[*B.*]*dd*	46	58	8	11	26.5 (25.8, 28.0)	24.7 (24.2, 25.0)	0.157
bb	*bb*[*D.*]	65	36	7	10	26.7 (26.2, 27.2)	25.4 (23.9, 26.2)	0.0071
bb	*bbdd*	19	15	12	5	24.1 (23.9, 24.6)	26.8 (23.3, 28.5)	0.279
bd	[*B.*][*D.*]	301	294	2	4	28.4 (27.9, 28.7)	26.8 (26.4, 26.9)	0.0149
bd	[*B.*]*dd*	114	93	9	6	26.3 (25.7, 27.3)	26.4 (26.0, 27.3)	0.0923
bd	*bb*[*D.*]	90	114	3	2	28.1 (26.9, 28.5)	27.2 (25.8, 28.2)	0.0241
bd	*bbdd*	37	27	10	12	25.9 (25.3, 26.5)	24.6 (23.3, 26.2)	0.379
dd	[*B.*][*D.*]	158	162	4	3	27.9 (27.5, 28.4)	26.9 (26.6, 27.4)	0.113
dd	[*B.*]*dd*	50	53	1	7	29.3 (28.1, 30.2)	26.2 (25.3, 27.9)	0.0210
dd	*bb*[*D.*]	47	43	5	8	27.8 (27.2, 28.3)	26.1 (24.6, 27.9)	0.0658
dd	*bbdd*	16	11	11	1	24.7 (23.2, 27.1)	27.6 (26.0, 28.9)	0.0318

Coat colors: [*B.*][*D.*] = black; [*B.*]*dd* = dilute black; *bb*[*D.*] = brown; *bbdd* = dilute brown. The median life spans are ranked separately for each sex. Confidence intervals are wider for smaller groups of mice. The log rank tests compare entire survival curves, not just the medians. The medians are shown in Figure 10. Brackets indicate phenotypes.

TABLE 5

Genotype clusters by sex

Genotype	Females	Males
$H\text{-}2^b/H\text{-}2^b$		
[*B.*][*D.*] (black)	Long	Short
[*B.*]*dd* (dilute black)	Long	Short
bb[*D.*] (brown)	Long	Short
bbdd (dilute brown)	Short	Long
$H\text{-}2^b/H\text{-}2^d$		
[*B.*][*D.*] (black)	Long	Long
[*B.*]*dd* (dilute black)	Long	Long
bb[*D.*] (brown)	Long	Long
bbdd (dilute brown)	Short	Short
$H\text{-}2^d/H\text{-}2^d$		
[*B.*][*D.*] (black)	Long	Long
[*B.*]*dd* (dilute black)	Long	Long
bb[*D.*] (brown)	Long	Long
bbdd (dilute brown)	Short	Long

Genotypes (phenotypes in parentheses) were clustered separately for each sex into "Long" and "Short" life span groups using multiple log rank tests (see *Statistical Methods*). Figures 8, 9 show the corresponding survival curves. The groups are identified on Figure 10.

pathology are nonsignificant at $P = 0.36$ (females) and $P = 0.88$ (males). These tests were performed using a Monte Carlo algorithm (PATEFIELD 1981).

DISCUSSION

In the present experiment, as has been pointed out before in the experimental models of (C57BL/6 × DBA/2) (YUNIS *et al.* 1984; RUSSELL 1975), females lived longer than males (Figure 3). Similarly, the F_1 hybrid live longer than the parental types, at least in males (Figure 2). In this experiment, in females, the F_1 and B6 parental type were together longer-lived than the D2 parental type. The F_2 exceeded only the D2 parent in life span, in both sexes, being shorter-lived than either the B6 parent or the F_1.

In the previous report (YUNIS *et al.* 1984) it was found that backcross mice [(C57BL/6 × DBA/2) F_1J × DBA/2J] produce different genetic life span profiles in relation to coat color markers and *H-2* genotype. These backcross mice showed primarily two genetic effects, of the major histocompatibility complex in males and of the coat color markers in females. In the present report, the mice studied were the F_2 hybrid of the (C57BL/6 × DBA/2) F_1J, and the genetic markers studied were again *H-2* (chromosome *4*), *Brown* (chromosome *4*) and *Dilute* (chromosome *9*). However, it was possible to compare more homozygous and heterozgous combinations of genes than previously, due to the presence among the F_2 offspring of both homozygotes at each locus.

It is important to point out that influence of the *H-2* genes on life span can vary depending on the experimental model used, and also on the environmental conditions during the experiment. In two separate congenic experiments (SMITH and WALFORD 1977; GELMAN *et al.* 1990) it was observed that the $H\text{-}2^b$ haplotype was associated with longer life span than the $H\text{-}2^d$ haplotype; however, there was evidence of exposure to Sendai infection in both experiments.

In our present experiment we found that in the F_2 experimental model, mice typed as $H\text{-}2^d/H\text{-}2^d$ or $H\text{-}2^b/H\text{-}2^d$ lived longer than those typed $H\text{-}2^b/H\text{-}2^b$. Since the only important difference between the previously reported experiments was that the mice in the experiments reported before had been exposed to and possibly infected with Sendai virus, we suggest that exposure to infection can change the profile of life span. Since the susceptibility to this virus has been shown to vary between strains (STEWART and TUCKER 1978), with the highest susceptibility in $H\text{-}2^d/H\text{-}2^d$ mice (DBA/2) (PARKER, WHITMAN and RICHTER

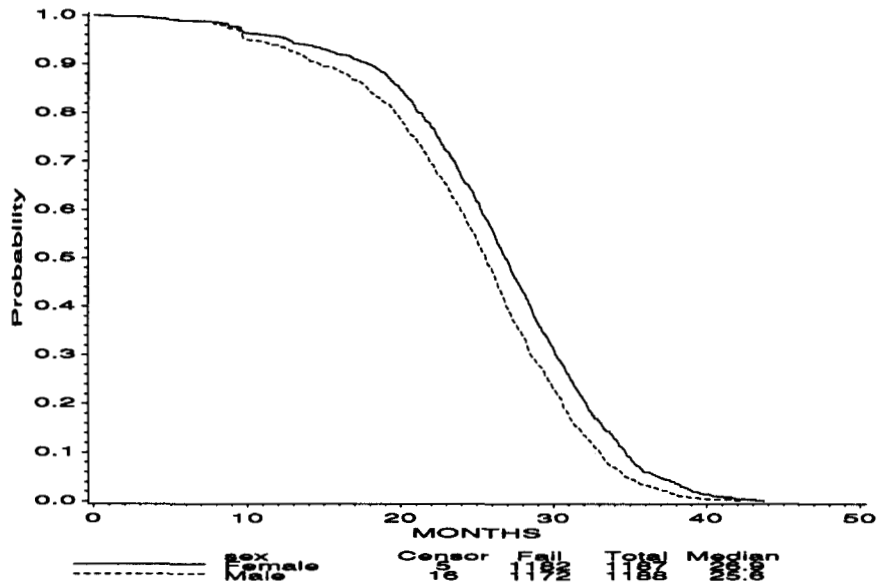

FIGURE 8.—Groupings by *H-2* and coat color markers: females. Genotypes groups are defined by nonsignificant log rank tests (see text). The group memberships are shown in Figure 10.

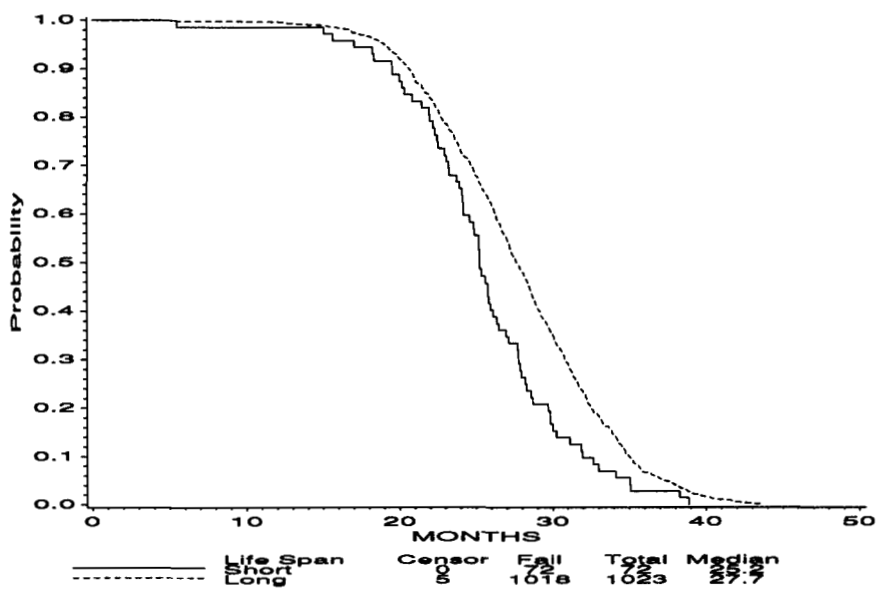

FIGURE 9.—Groupings by *H-2* and coat color: males.

1978), we believe that $H\text{-}2^d$ haplotype can confer susceptibility to that virus, but that in the absence of infection, the $H\text{-}2^d$ haplotype can confer longer life span than the $H\text{-}2^b$ haplotype. However, we corroborated the earlier finding that *H-2* significantly influences life span primarily in males (YUNIS *et al.* 1984). While the same loci remain important, it appears that environmental changes such as exposure to Sendai virus, and the segregation of other genetic systems in different experimental models, affect the details of genetic interactions. In this regard, in the backcross experiments reported before (YUNIS *et al.* 1984), the $H\text{-}2^b/H\text{-}2^d$ animals lived longer than the $H\text{-}2^d/H\text{-}2^d$, suggesting the influence of heterozygosity on life span, but in the F_2 experimental model reported here $H\text{-}2^d$ haplotype conferred long life span. Table 8 summarizes these findings.

In the previous paper it was reported that the heterozygosity index was associated significantly with life span. Life span was analyzed by ranking heterozygosity at each locus identified. The longest-lived mice in the backcross experiment 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 influence of $H\text{-}2^b/H\text{-}2^b$ was not assessed in the backcross experimental model. In the present experiment, however, we found that heterozygosity *per se* does not universally increase life span in mice. Furthermore, although the association of life span with heterozygosity was significant in females, the longest-lived mice were females predominantly heterozygous for the *Brown* locus of chromosome *4*, but homozygous for $H\text{-}2^d/H\text{-}2^d$ and for the *Dilute* locus of chromosome *9*.

Genes affecting life span have been identified in nematodes and fruit flies (GRIGLIATTI, RICHTER and

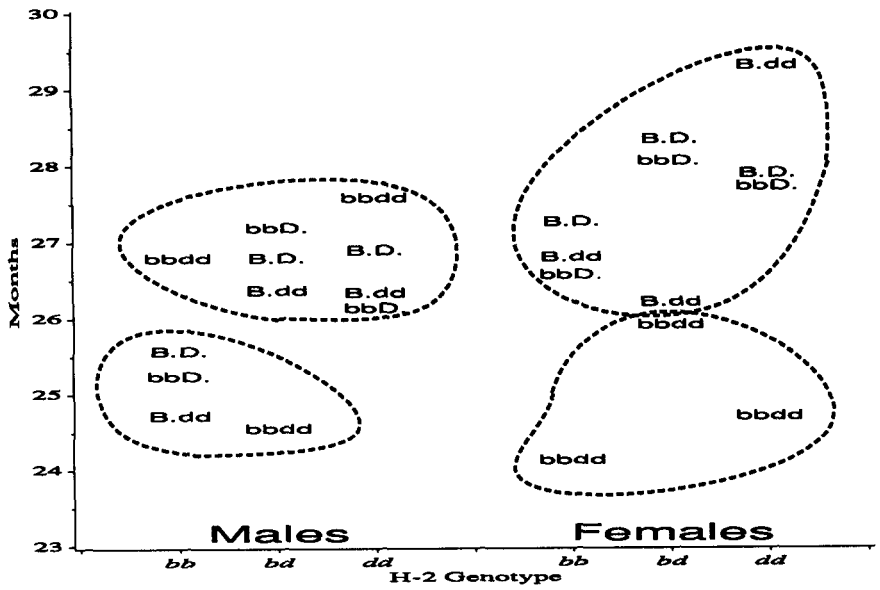

FIGURE 10.—Median life spans by genotype. The *x*-axis gives the *H-2* genotype. Codes plotted in the graph show the coat color marker phenotypes. Height up the graph shows median life span in months. Dashed lines group together genotypes/phenotypes for each sex with similar survival curves.

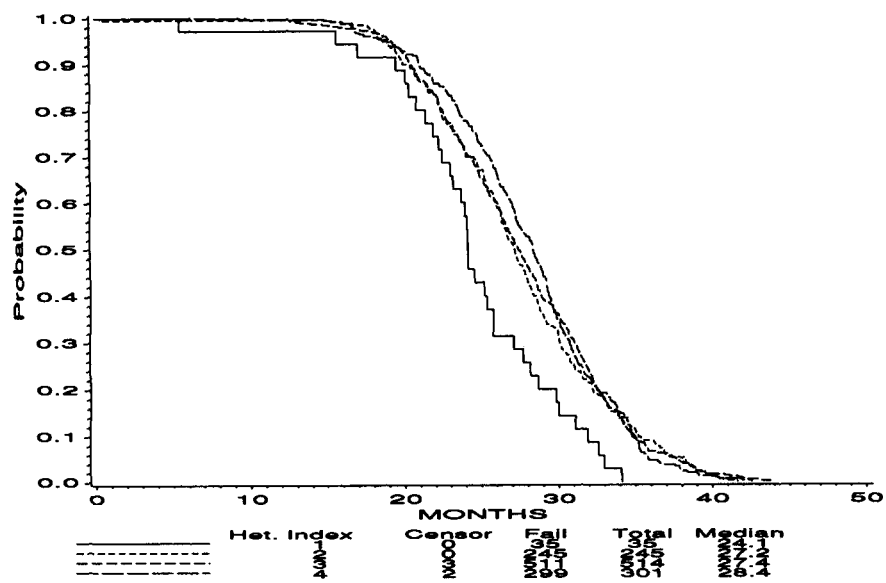

FIGURE 11.—Heterozygosity index: females. The heterozygosity index was formed as the number of heterozygous loci, counting females and mice phenotypically dominant at the *Brown* and *Dilute* loci as heterozygous.

TABLE 6

Median life spans by heterozygosity index

	Heterozygosity Index				
Sex	0	1	2	3	4
Males	27.3	25.4	26.4	26.8	
Females		24.1	27.2	27.4	28.4

Heterozygosity index: one point was given for each heterozygous locus, including the activated female *X* chromosome. For simplicity, the dominant types of the coat color markers were counted as heterozygous, even though approximately one-third of these mice would be homozygous for the dominant allele.

WHITEHEAD 1990; JOHNSON 1990; JOHNSON *et al.* 1990; GOULD and CLARK 1983) but it has not been possible to determine a single gene that can predict long life in outbred populations. Different life spans in various inbred strains of mice suggest genetic influences which have been associated with specific diseases; *e.g.*, leukemia in AKR mice and mammary tumors in C3H female mice. In inbred strains of mice it is possible to identify better life span with different *H-2* haplotypes using *H-2* congenic mice, and more recently to suggest that the *D* end of the *H-2* haplotype is a better predictor of life span than the *K* end (GELMAN *et al.* 1990). However, in experimental models where other genes are studied, such as in recombinant strains of mice, the genetics of life span demonstrated complex patterns. In recombinant strains of mice produced between C57BL/6J (B6) and DBA/2J (D2), with genes homozygous to either *B* or *D* and 141 identified polymorphic genes, two strains had a shorter life span and several chromosomal regions were found that best correlated with life span (GELMAN *et al.* 1988). One was in chromosome 7 (one *B* allele marked by Coh associated with longer life span), two in chromosome 2 (one *B* allele marked by Ly24 and one *D* allele marked by *H-3* associated with

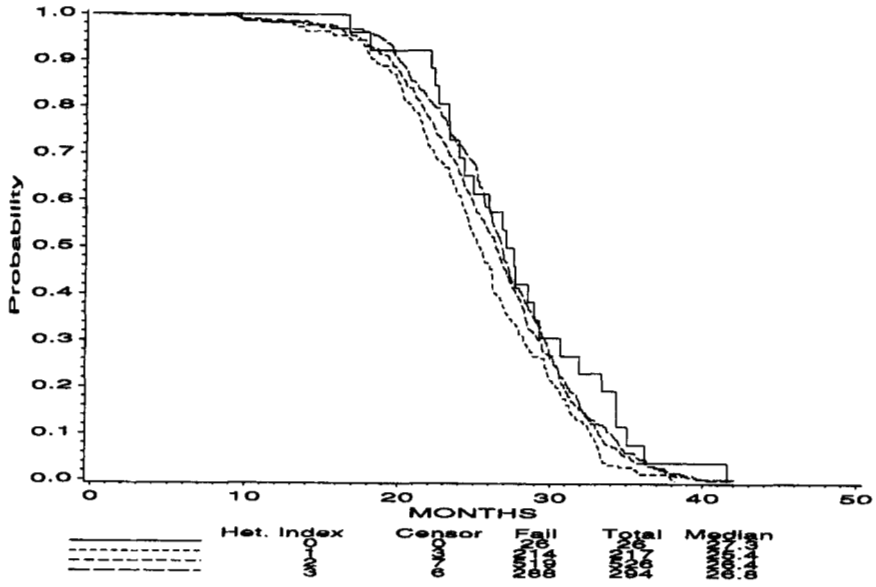

FIGURE 12.—Heterozygosity index: males.

TABLE 7

Necropsy findings

	Females	Males	Total	*P* value
Lymphoma	151	69	220	<0.001
Hepatoma	29	54	83	0.0057
Hemangiosarcoma	31	18	49	
Lung carcinoma	8	36	44	<0.001
Amyloidosis	16	17	33	
Glomerulonephritis	10	14	24	
Liver necrosis	13	7	20	
Pyelonephritis	3	15	18	0.0031
Hydronephrosis	13	4	17	0.025
Lipoma	10	7	17	
Gastric hyperplasia	5	9	14	
Fatty Liver	7	6	13	
Gastric ulcer	4	8	12	
Fibrosarcoma	9	2	11	0.028
Renal atrophy	8	2	10	0.050
Pneumonia	4	6	10	

A total of 237 necropsies were performed on females, 222 on males. Frequencies of disorders in the two sexes were compared using Fisher's exact test. Significance levels not shown are greater than 0.05.

longer life span), two in chromosome *1* (one *D* allele marked by Lamb-2 associated and one *B* allele marked by Ltw-4 associated with longer life span) and one in chromosome *12* (one *D* allele marked by Igh genes and Npid or D12Nyul associated with longer life span). Although DBA/2 has a shorter life span than C57BL/6 or the F_1 hybrid between the two, not all *B3* genes are beneficial for life span, since the two shortest lived recombinant strains of mice had more *B* genes then *D* genes analyzable among the 141 typed markers.

Gross pathology at death and mean life spans of many inbred mouse strains have been documented as differing by strain in some mice (BRONSON 1990). In other strains and hybrids the differences in life span could not be accounted for by specific diseases. The same is true in our present report, with significant differences in the profile of pathology only in female as compared with male mice.

Taken together our studies suggest that life span results from environmental factors interacting with several genes. Obviously the genetics of life span are very complex, and we anticipate that genes important in life span will need to be studied in many genetic backgrounds and environmental conditions. Our studies represent an attempt to identify some of the genes, or at least chromosome regions, that are involved. They emphasize the need to use several genetic models and different experimental conditions of housing of the animals, especially monitoring of animals for infections. It is remarkable that even after minimizing the genetic and environmental variations we have found complex patterns of disease and life span, which raise doubts as to the feasibility of studying genetic effects of life span in outbred populations. At the least, it would be necessary to identify genes that under all environmental conditions influence life span, a task difficult to undertake in the near future.

In summary the intercross genetic model (C57BL/6 × DBA2) F_1 was studies using sex, *H-2*, coat color genes in chromosomes *4* and *9* producing a total of 24 different genotypes. Primarily this model could study the $H\text{-}2^b/H\text{-}2^b$ homozygote, in addition to the $H\text{-}2^d/H\text{-}2^d$ homozygote and $H\text{-}2^b/H\text{-}2^d$ heterozygote which were studied before by the backcross model (YUNIS *et al.* 1984). In the previous study the mice were exposed to Sendai virus infection which was absent in the present experiment. We believe that this model produced perturbations of some of the relation analysis expected and previously found, primarily between life span and degree of heterozygosity. Indeed the longest lived type among those studied was homozygous at two of the three loci.

TABLE 8

Comparison between backcross and intercross life span studies

Parameter	Backcross (c57BL/6 × DBA2)F_1 × DBA/2	Intercross (C57BL/6 × DBA/2)F_1 × F_1
No. of genetic combinations	8 males, 8 females	12 males, 12 females
H-2 locus influence	Males	Males
$H\text{-}2^d$ short life in males	Yes	No
Sendai infection	Yes	No
b locus on chromosome *4*: *Bb* better than *bb* in females	Yes	Not studied
d locus on chromosome *9*: *Dd* not better than *dd*	Yes	Not studied
Longer life span	Females	Females
Genetic interactions	Dilute brown/brown coat color have shorter life span in females	Dilute brown has shorter life span in females
Heterozygosity effect	Strong	Relative
$H\text{-}2^b/H\text{-}2^d$	Disadvantaged in dilute brown males and brown males	Not disadvantaged

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