Murine Chromosomal Regions Correlated With Longevity

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ABSTRACT

In this longevity analysis of 360 BXD recombinant inbred female mice (20 different strains), 2 strains had very significantly shorter survival and 1 strain had very significantly longer survival than the other 17 strains; 4 other strains had less significant lengthening of survival compared to the other 13 strains in a proportional hazards model of survival. Mean survival on the shortest lived strain was 479 days; on the longest lived strain the mean survival was almost double (904 days). Ranges of survival within strain were very large (averaging 642 days), and strain accounted for only 29% of the variation in survival, showing that there are important environmental and/or special developmental effects on longevity even in this colony housed in a single room. Each strain had been typed for markers of 141 regions on 15 chromosomes; 101 of these markers had distinguishable distributions on the 20 strains. The two shortest lived strains had the same alleles for 63% of the markers. The single region most significantly correlated with survival (marked by *P450, Coh, Xmmv-35* on chromosome *7*) divided the mice into two groups with survival medians which differed by 153 days (755 days for mice with a B genotype; 602 days for mice with a D genotype). Evaluated individually, 44% of the genetic markers (including some markers on 11 of 15 chromosomes with any markers typed) were found to be significantly correlated with survival ($P < 0.05$) although one would only expect 5% of the markers to be significant by chance. While studies of many markers should adjust for the multiple comparisons problem, one interpretation of these crude P values is that any experiment with only one of these "significant" markers typed would be likely to conclude that the marker was a significant predictor of survival. Two types of multiple regression models were used to examine the correlation with survival of groups of genes. When a proportional hazards model for survival was done in terms of genotype regions, a six genetic region model best correlated with survival: that marked by *P450, Coh, Xmmv-35* on chromosome *7* (B allele lives longer), *Ly-24* on chromosome *2* (B allele lives longer), *β2M* and *H-3* on chromosome *2* (D allele lives longer), *Lamb-2* on chromosome *1* (D allele lives longer), *Ltw-4* on chromosome *1* (B allele lives longer), and the *Igh* area of chromosome *12* (*Igh-Sa4, Igh-Sa2, Igh-Bgl, Igh-Nbp, Igh-Npid, Igh-Gte, Odc-8*, and *Ox-1*; D allele lives longer). A linear model that regressed mean survival (per strain) on genetic markers found a similar six region model to be best, but replaced *Coh* by *D12Nyu1* on chromosome *12*. It should be noted that in both types of regression, there were many other models almost as good as the best one. The total number of chromosomal regions marked by the genotype of the longer lived B parent (out of a possible 141) was not, in general, correlated with survival, although the two shortest lived strains had the most B genes. It appears that BXD recombinant inbred strains can vary widely in survival both within and between strains, that no single genetic marker which has yet been identified can account for much of this variance, (although groups of six or more markers may do so), and that it is not always those strains which inherit the most genes from the long-lived parent B that live longest. The large number of genetic markers found to be significantly correlated with survival raises questions of the reliability of conclusions based on survival studies of only one or two genetic regions.

MANY studies have shown that mice of different strains differ in life span. RUSSELL (1975) cited experiments which concluded that C57BL/6 mice lived longer than DBA/2 mice. The F_1 hybrid of these two strains outlived both parents, and females lived longer than males (RUSSELL 1966). Because some mouse strains differ at only a few genetic loci, it has been possible to study the role of individual loci in determining life span. For example, differences in life span were related to *H-2* haplotypes (or perhaps to linked genes), which were also associated with differences in immune responses (SMITH and WALFORD 1977; POPP 1978; WILLIAMS *et al.* 1981). Other investigators (RUSSELL 1966; YUNIS *et al.* 1984) have studied several genes and environmental factors and suggested that heterozygous genes are more likely to be associated with long survival than homozygous ones. Our previous work on [(C57BL/6 × DBA/2)F_1 × DBA/2] backcross mice (YUNIS *et al.* 1984) showed interactive effects on survival of month of birth,

regions of two chromosomes, and the sex chromosome. For the three loci studied, the genetic effect on longevity could be summarized by stating that greater heterozygousity was associated with longer life span. It should be noted that this previous experiment included only mice with genes which were either homozygous and from the DBA/2 parent or heterozygous. Hence we could not compare the effects of such genes with genes which were homozygous from the C57BL/6 parent.

In the present study we have used 20 recombinant inbred (RI) strains of the F_1 hybrids of C57BL/6 and DBA/2 mice, which have already been typed for 141 genetic markers, to analyse the correlation with longevity of a large number of genes in completely homozygous female mice. We hereafter refer to these BXD strains by number (*e.g.*, strain 2). For clarity, the usual genotype designations of the markers are replaced here by the symbol "B" if the marker was typed as being the same as the C57BL/6 parent and by the symbol "D" if the marker was typed as being the same as the DBA/2 parent.

MATERIALS AND METHODS

Experimental animals: The 395 female mice used in this study were obtained in six batches from the Jackson Laboratory: on 11/9/82, 12/1/82, 1/17/83, 2/1/83, 2/8/83 and on 3/22/83. At the time of receipt at the Michael Redstone Animal Facility of the Dana-Farber Cancer Institute, the mice were between 5 and 10 weeks old. The mice were born between September 1982 and February 1983, with the majority (355) born in 1982. Only female mice were used to avoid possible shortening of life span due to fighting among males. We do not have data on the life span of the parents of these mice.

Each mouse was labeled as belonging to one of 23 BXD recombinant inbred strains. These were, at the time, the only recombinant inbred strains of the F_1 hybrid of C57BL/6 and DBA/2 which were available. Seventeen of these strains included 19 to 21 mice, five included 9 or 10 mice, and one strain (13) included only 4 mice due to seizures during shipment. These four surviving mice of strain 13 are omitted from the analysis (all died before 226 days of age), as is one mouse (5749 of strain 27) which was missing a leg upon arrival at the Michael Redstone Animal Facility. Because of missing genotypic information, two other strains (31 and 32) are omitted from most analyses. (In addition, strain 32 differed from the others in that it was created by a DBA backcross, so that approximately $\frac{3}{4}$ of its alleles are typed as D and $\frac{1}{4}$ as B.)

Housing and surveillance: All mice were kept in one room of the animal facility. Animals were housed in polycarbonate cages of either size 12.5 × 9.25 × 6 inches which held 4 to 8 mice, or of size 11 × 7 × 5 inches which held 2 to 3 mice. The majority of the mice (83%) were housed 4 to 6 per cage. Cages remained on their original racks throughout the experiment but were occasionally rotated within the rack. All animals were maintained on standard Purina Chow and water *ad libitum*. Room temperature was kept at 74°F ± 2°F with alternate 12-hr light and dark periods. All cages were maintained with fiberglass filter bonnets. 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 bodies at least four times a week until 5/83, daily from then until 5/84, and after that three times a day on weekdays and once a day on holidays and weekends. At these times, animal care facility workers would bag and hold any bodies found during routine maintenance and cage cleaning. However, mice that were cannibalized and/or buried were occasionally missed. Full cage counts were done by the investigators once a month.

Six surveillance animals (DBA/2 females) were kept in the mouse room at all times. Two of these animals were taken once per month to test for infection by known pathogens. During the period of this study, serological evidence of exposure of the surveillance animals to Sendai virus occurred once in one surveillance mouse and there were three occasions when the surveillance mice showed serological evidence of exposure to mouse hepatitis virus. Despite these four reports of exposure in the surveillance mice, the RI strains did not exhibit signs of infection. However, the possibility could not be ruled out that these pathogens may affect longevity without gross clinical evidence of infection.

Pathology and life span: Most of the mice (264, or 67%) had exact birth and death dates recorded. However, 107 mice (27%) had birth dates known to occur on one of several consecutive days (all but three known to within four days) and 38 mice (10%) did not have exact death dates known (*i.e.*, dates known to within 1 to 64 days). Because complete cage counts were done once a month 27 of these animals had death dates known to within a month; the remaining 11 were victims of human error in cage counting. Hence, for 131 animals we do not know the exact survival time in days. For these mice both the longest and the shortest possible survival time were calculated; for 89, the difference between longest and shortest survival times was three days or less, and for 42 (11% of total) the difference between longest and shortest survival times was four days or more. All analyses were done twice, once using the longest possible survival time for each mouse and once using the shortest. Since the conclusions were the same in all cases, only the results based on shortest survival times are presented. There were no noticeable differences between survival curves based on the longest and shortest times, neither overall nor within strain.

Beginning in March 1983, dead mice that were not severely autolyzed nor destroyed by cannibalism were necropsied. Tissues were fixed in 10% neutral buffered formalin and examined histopathologically for significant lesions. Gross lesions were described at necropsy and representative sections of lesions and major organs were sampled for histopathological evaluation. The organs usually included were brain, heart, lung, liver, kidney, intestines, and reproductive organs; often the intestines and brain were too autolysed for evaluation. The last mouse died on 1/21/86.

Genetic typing: Published strain distribution patterns (Taylor 1987) were available for up to 141 genes (actually, markers of an area of a chromosome) which were identified as being from the B or D parent. Most of the strains were missing data on between one and six genes (mostly *Ly-22* on chromosome *4*, *Saac* on chromosome *9*, *Igh-Bgl*, *Igh-Npa* on chromosome *12*, and *Lyb-7* on an unknown chromosome). Strain 31 had unknown genotype for 34 genes (24% of total typed) and strain 32 had unknown genotypes for 38 genes (27% of total typed); these latter two strains were omitted from survival models because of the large number of unknown genotypes.

Statistical methods: Marginal comparisons of survival distributions (testing the difference between two or more

TABLE 1

Summary statistics by strain: days[a]

Strain	No. of animals	Mean	Median	SD	Range	Proportional hazards full models significance	Significantly better or worse survival
1	10	594	519	261	803	0.41	
2	20	479	490	128	531	<0.0001	Worse
5	10	528	452	317	920	0.91	
6	20	687	641	115	366	0.56	
8	20	617	637	170	818	0.18	
9	20	816	884	176	610	0.05	Better
11	20	750	847	266	950	0.04	Better
12	20	738	774	209	907	0.36	
14	19	493	529	138	548	0.0002	Worse
15	20	798	783	179	678	0.02	Better
16	20	642	677	191	862	0.38	
18	10	742	750	140	483	0.72	
19	20	904	939	151	647	0.003	Better
22	10	743	754	210	778	0.35	
23	21	763	750	133	456	0.31	
24	20	835	854	157	620	0.03	Better
27	19	711	746	153	592	0.76	
28	20	765	775	104	331	0.74	
29	21	703	704	114	349	0.64	
30	20	737	733	165	600	0.41	
Omitted strains:							
13	4	176	173	50	93	NA[b]	
31	10	577	501	136	390	0.06	
32	20	440	419	238	842	0.04	

[a] Taken from set of shortest possible survival times.
[b] Not applicable (mice not used in model).

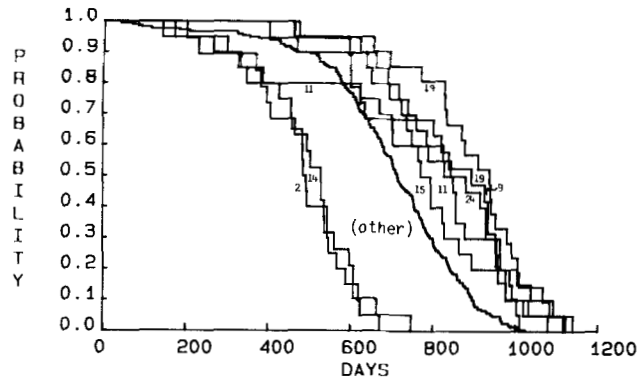
non-overlapping groups) used the log rank test (PETO and PETO 1972). Tests for the effects of other factors used the proportional hazards regression model (COX 1972) [see YUNIS *et al.* (1984) for a briefer explanation]. Correlation coefficients were also calculated by the proportional hazards model. The linear regression of survival on strain used ordinary least squares and analysis of variance (DRAPER and SMITH 1966). The linear regression of average survival in each strain on genes was based on the R-squared and Cp selection criteria and used the algorithm of FURNIVAL and WILSON (1974). (The Cp statistic is calculated as $\sigma^{-2}RSS - n + 2p$ where n is the number of observations and p is the number of regression coefficients.) The comparison of pathologic diagnoses by strain was based on the Exact Test (FISHER 1934).

RESULTS

Birth date and cage size: Month of birth was not significantly correlated with survival; medians were 667, 729, 678, 744, 718 and 802 days for the birth months September 1982 to February 1983 ($P = 0.22$, log rank test). Number of animals per cage was also not significant, with survival medians of 767, 694, 729, 732, 784, 657, and 748 days for animals housed 2, 3, 4, 5, 6, 7, and 8 per cage. Because each individual strain tended to consist of mice born over a span of only 2 or 3 months and because all but 2 strains had an average number of 5 mice per cage, the power of tests of the effect of these factors on longevity was very low. Also, it was not possible to use this data set to explore interactive effects on survival of strain, birth month, and cage population.

Differences in longevity between strains: Table 1 lists summary survival statistics by strain for the 20 strains included in survival models and for the three strains which had to be omitted from the models (strain 13 due to seizures during shipment, strains 31 and 32 due to large number of uncharacterized loci and strain 32 because it was a DBA backcross). Mean survival ranges from 479 days (strain 2) to 904 days (strain 19), a difference of 425 days; medians differ by 487 days. The range of age at death within a strain (for the strains included) varied from 331 days (strain 28) to 950 days (strain 11).

Proportional hazards model for strains: The significance levels in Table 1 are based on the full proportional hazards model. That is, when all the other strains were *separately* accounted for in the model, the addition of a variable for strain 1 did not improve the model much ($P = 0.41$). Two strains were found to be highly significantly associated with shorter survival times in the proportional hazards model including all other strains: 2 ($P < 0.0001$) and 14 ($P = 0.0002$). In this same model, five strains were found to be significantly associated with longer survival times than other strains: 19 ($P = 0.003$), 24 ($P = 0.03$), 9 ($P = 0.05$), 15 ($P = 0.02$), and 11 (P



STRAIN	DEAD	MEDIAN	75th PERCENTILE	MEAN
13 strains combined	221	714	819	698
2	20	490	561	479
14	19	529	597	493
15	20	783	887	798
24	20	854	957	835
11	20	847	964	750
9	19	884	952	816
19	21	939	998	904

FIGURE 1.—Survival of 20 recombinant inbred strains of the F_1 hybrids of C57BL/6 and DBA/2 mice; significant strains plotted separately.

= 0.04). However, there is a multiple comparisons problem. Since 20 strains were tested, one would expect at least one to have a significance level of $P < 0.05$ just by chance ($20 \times 0.05 = 1$). To eliminate this multiple testing problem, a conservative approach would be to only consider P values of 0.003 or less to be significant since $1 - (1 - 0.003)^{20} = 0.05$. Using this approach, strains 2 and 14 would be deemed to have significantly shorter survival, but only strain 19 would be deemed to have significantly longer survival.

Linear regression model for strains: Figure 1 shows separate survival curves using the shortest possible survival times for strains 2, 14, 19, 24, 9, 15, and 11 with one curve for all the remaining strains. Figure 2 shows separate survival curves for the 13 strains grouped together in Figure 1. The survival curves in Figure 1 are clearly different; however, strains did not account for much of the variation between mice in longevity. In a linear model for survival containing one variable for each of the 20 strains, strain accounted for only 29% of the variation in survival times. We calculated survival mean and standard deviation for each of the 74 cages of animals, but only for strain 23 was one cage (of four) associated with significantly less variation in survival than expected (in that cage all three animals died within a month of each other). Within strain, no cage was associated with significantly longer or shorter mean survival. So almost none of the observed variation in survival time could be associated with cage.

Genetic difference between strains: Figures 3 and 4 display a map of the available genetic markers. Forty genetic markers were not used in the analysis: the *Y* chromosome, because all mice were female,

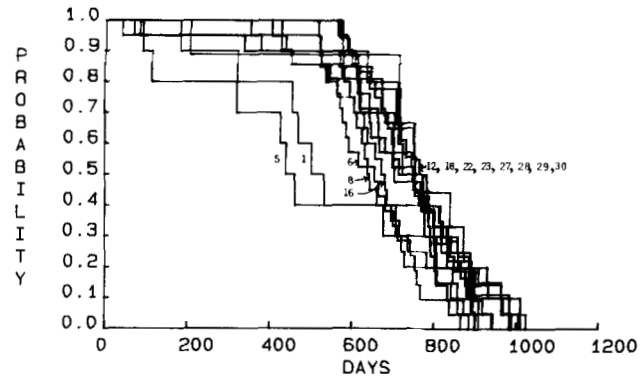


FIGURE 2.—Survival of 13 nonsignificant recombinant inbred strains.

and 39 markers which were statistically indistinguishable from some other markers which were used. We define two genetic markers to be statistically indistinguishable in this data set if their genotypes were not known to differ in any of the 20 included strains. For example, *Idh-1* and *Len-1* on chromosome *1* had the same genotype for all strains (D for strains 6, 8, 15, 16, 19, 27, 28, 29, 30 and B for the others), so they are statistically indistinguishable. Hence the analysis included 101 distinguishable markers: 15, 5, 4, 11, 5, 3, 11, 3, 13, 10, 1, 4, 1, 8 and 2, respectively, from chromosome *1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15, 17,* and *19*, and 5 markers not identified by chromosome. In Figures 3 and 4, indistinguishable markers are indicated by branching lines or by brackets.

Even in this set of 101 distinguishable genetic markers, there were many groups of markers which differed in genotype on only a few strains. If these few strains did not have unusually long or unusually short survival, it was very difficult to choose between such similar genetic markers in the models. For example, just within chromosome *1*, markers *Ltw-4*, "*E*," *Eph-1, Mtv-7, Mls,* and *Ly-9* were very similar. (Genotypes of *Ltw-4* and "*E*" agree for all strains but 23. So while a model might choose *Ltw-4* as a better predictor of survival than "*E*," it would not be better by much. Yet once *Ltw-4* was in a model, "*E*" would have a small chance of being added to the model because it does not differ much from *Ltw-4*.)

It is noteworthy that strains 2 and 14 (the two strains with significantly shorter life span) are known to be concordant in the alleles of 95 genes (68% of those typed; they are concordant on 64 or 63% of the distinguishable markers). The longer lived strains (9, 11, 15, 19, 24) do not share so many alleles; the most concordance is between strains 9 and 19: 53% of all markers typed and 44% of distinguishable markers. Some of the moderately long lived strains do share as many alleles as the shorter lived ones (*e.g.*, strains 28 and 30 share 66% if all markers typed and 64% of the distinguishable markers).

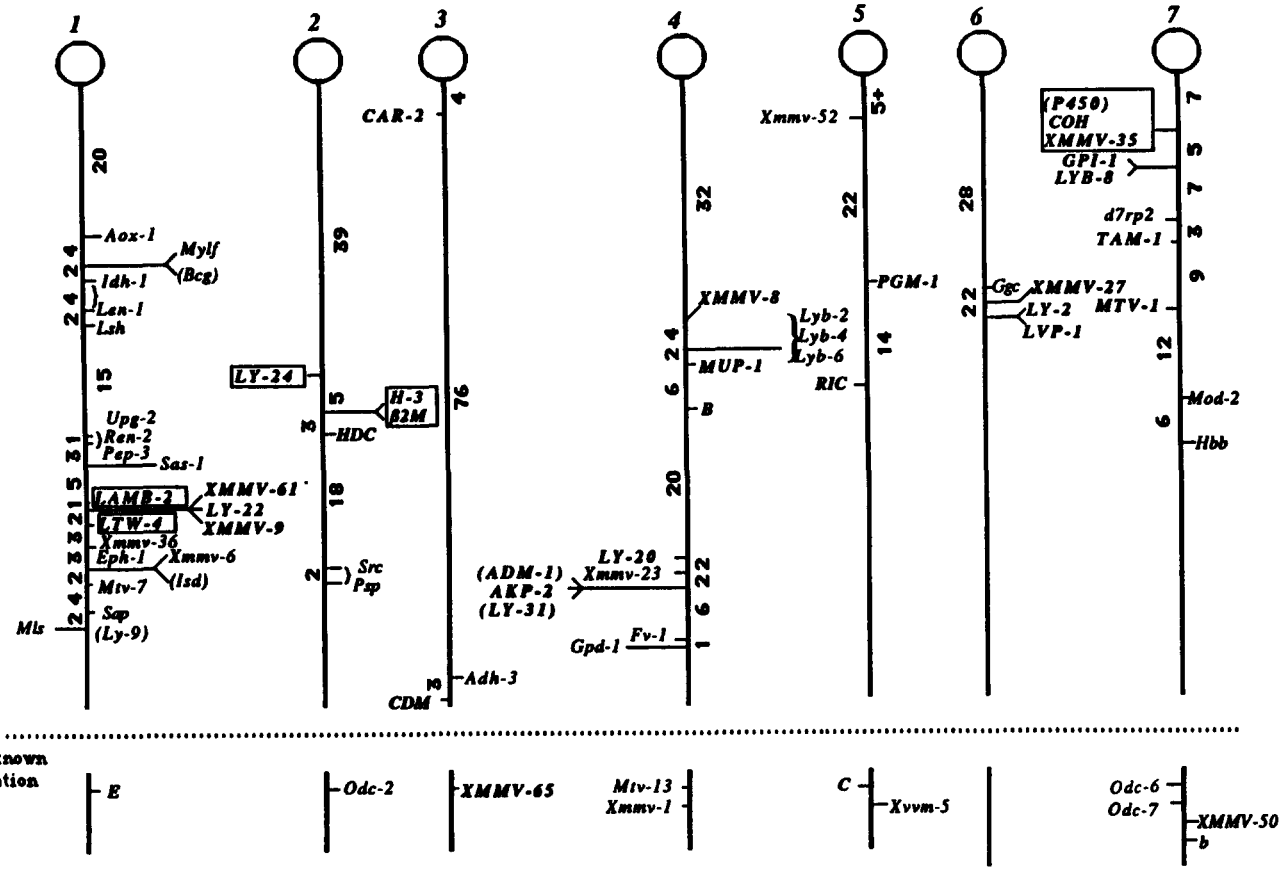


FIGURE 3.—Genetic map of the murine markers informative (polymorphic) in these strains; chromosomes *1*–*7*. Bold type markers were significantly ($P < 0.05$) correlated with survival when considered singly. Markers to the left of the chromosome line have B allele associated with longer survival. Markers to the right of the chromosomal line have D allele associated with longer survival. Markers in boxes were in the best proportional hazards model of survival. Branched lines or brackers represent markers which were indistinguishable in these mice. Markers in parentheses are not on the current murine gene map of the Jackson Laboratory but were indistinguishable in these mice from other markers on the gene map and hence were assumed to be located in the same area.

Differences in longevity associated with various genetic markers: Figures 3 and 4 display in bold type all genetic markers associated with marginal significance levels less than 0.05. These marginal tests were done by dividing the RI lines into two groups: those with a B typing in a particular location and those with a D typing. The survival of the two groups was then compared with a log rank test. Strains with unknown typing for that location were omitted from this analysis. (We also performed these tests assuming all unknown typings were B, and again assuming all unknown typings were D. There was not much difference in significance levels.) Each significance level represents a separate test done on a single genetic marker. For example, in the case of *Lamb-2* on chromosome 1, we compared animals from all strains with a B genotype for *Lamb-2* (strains 1, 2, 6, 8, 12, 14, 15, 16, 18) to all strains with a D genotype (strains 5, 9, 11, 19, 22, 23, 24, 27, 28, 29, 30) and found the D animals to live longer than the B animals ($P < 0.0001$).

In Figures 3 and 4, markers to the left of the chromosome line are those for which longer survival was associated with the B genotype. Markers to the right of the chromosome line are those for which long survival was associated with the D genotype. The different chromosomes showed rather different survival patterns. For example, on chromosome *1* all the markers except *Mls* associated the D genotype with longer survival; on chromosome 9, ten of the thirteen distinguishable markers associated the B genotype with longer survival. On chromosome *12*, nine of the ten distinguishable markers were associated with marginal significance levels less than 0.05; on chromosome *1* only 4 of the 15 distinguishable markers were associated with marginal significance levels less than 0.05. Only three chromosomes (*13*, *14*, and *19*) had no significant markers, and these three chromosomes had very few distinguishable markers typed (1, 4, and 2).

Since 101 markers were tested, one would expect about 5 to show significance levels of $P < 0.05$ just by chance ($101 \times 0.05 = 5.05$). In fact, 44 markers were associated with $P < 0.05$ (19 with the B genotype associated with longer survival; 25 with the D genotype associated with longer survival). In order to

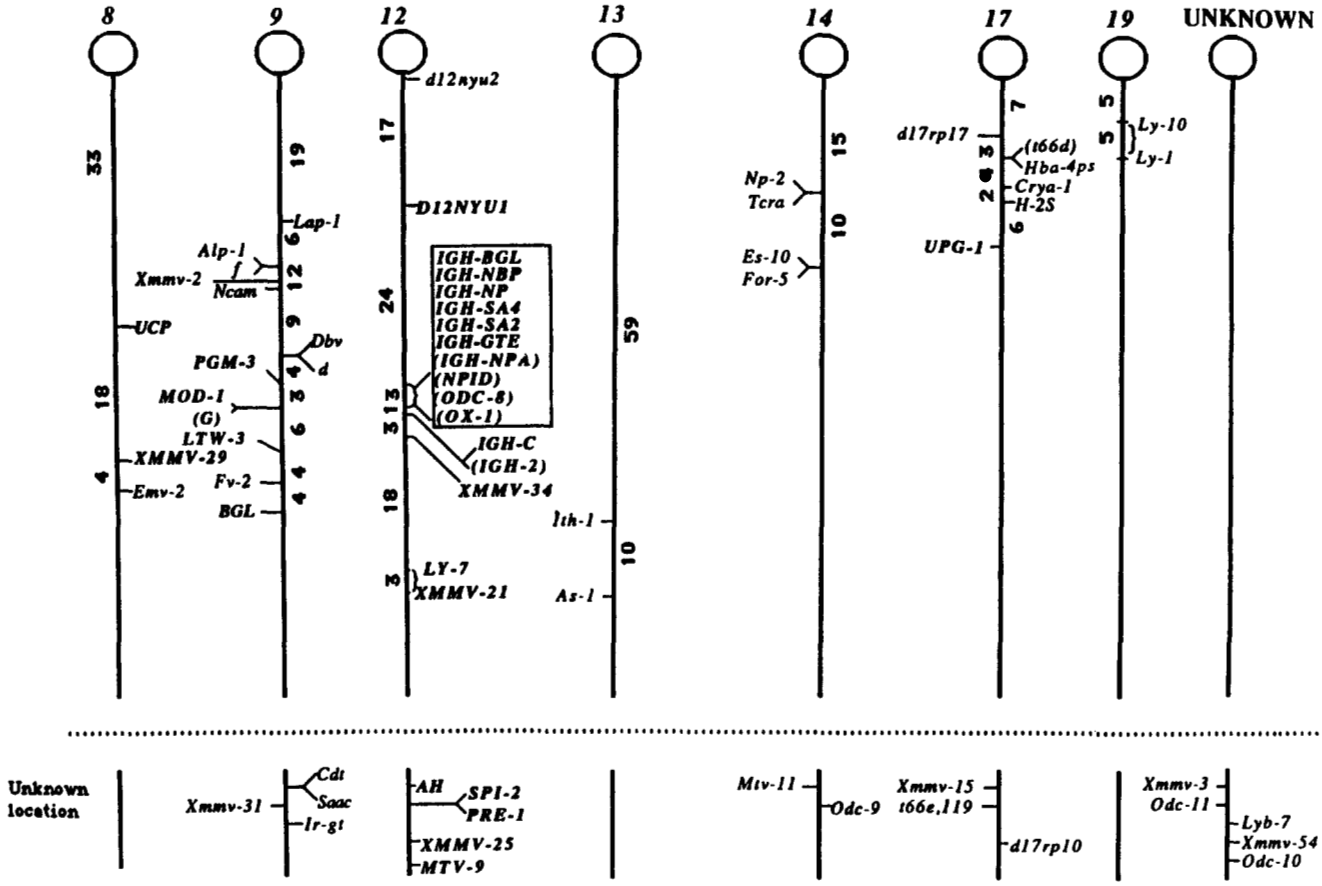


FIGURE 4.—Genetic map of the murine markers informative (polymorphic) in these strains; chromosomes *8–19* and unknown.

eliminate this multiple testing problem, a conservative approach would be to consider only P values less than 0.0005 to be significant since $1 - (1 - 0.0005)^{101} = 0.05$. There were 16 such markers: *Lamb-2* and *Ly-22* (indistinguishable from *Xmmv-61* and *Xmmv-9*) on chromosome *1; H-3* (indistinguishable from *β2M*) and *Hdc* on chromosome *2; Cdm* on chromosome *3; Xmmv-8, Fv-1,* and *Gpd-1* on chromosome *4; Coh* (indistinguishable from *P450* and *Xmmv-35*) and *Tam-1* on chromosome *7; Xmmv-29* on chromosome *8; Mod-1, Bgl,* and *Xmmv-31* on chromosome *9; D12Nyu1* and *Pre-1* on chromosome *12*.

Proportional hazards model for genes: These marginal tests have a disadvantage, in that a genetic marker may erroneously appear to be significantly correlated with survival, with, for example, the B allele living longer than the D, only because the strains with the B allele are more likely to have the longer lived allele of a more significant marker. For this reason, we used proportional hazards models in a step-up manner to identify markers associated with significantly longer or shorter survival. We could not use the full proportional hazards model for markers, as was done in Table 1 for strains. To use a full model with 101 markers one would need at least 103 strains. Therefore, we decided to apply the model in a step-up fashion. That is, we first identified the most significant single marker. Then we examined all 100 models involving the first marker and one other marker and picked the most significant. Then we examined all 99 models with that pair of markers plus one other, and so on. The most significant single marker model involved *Coh* (which was not statistically distinguishable from *P450* or *Xmmv-35*) on chromosome 7 (mice with the B genotype had a median survival of 755 days; mice with the D genotype had a median survival of 602 days). Table 2 lists the markers added to model on the first six steps together with the genes which were statistically indistinguishable from them. Further steps did not identify any genes with significance levels <0.05 when added to the model. In the model, the B allele of *Ltw-4* was associated with longer survival. As noted in Figure 2, when *Ltw-4* is examined alone, the D allele is associated with longer survival. We note that *Ltw-4* and *Lamb-2* have different alleles only on strain 19. So the effect of including both these genes in the model is to distinguish strain 19, which is unusually long-lived. In the same way, including *Coh* and *Ly-24* distinguishes strains 2 and 14 as unusually short-lived.

Linear regression model for genes: One problem with the proportional hazards model is that survival by strain does not appear to have proportional haz-

TABLE 2

Proportional hazards regression models and linear regression models on genetic markers

Chromosome	Statistically indistinguishable markers	Reference	No. of strains with unknown allele	Allele associated with longer survival	Significance level in final proportional hazards model	Significance level in final linear model
7	*P450*	SIMMONS and KASPER (1983)	0			
	Coh	WOOD and TAYLOR (1979)	0	B	0.0011	NA
	Xmmv-35	BLATT *et al.* (1983)	1			
2	*Ly-24*	COLOMBATTI *et al.* (1982)	0	B	<0.0001	0.0006
2	*β2m*	CHORNEY *et al.* (1982)	0			
	H-3	TADA *et al.* (1980)	0	D	0.0006	0.0012
1	*Lamb-2*	ELLIOTT, BARLOW and HOGAN (1985)	0	D	<0.0001	0.0006
1	*Ltw-4*	ELLIOTT, ROMEJKO and HOHMAN (1980)	0	B	<0.0001	<0.0001
12	*Igh-Sa4*	BEREK, TAYLOR and EICHMAN (1976)	0			
	Igh-Sa2	BEREK, TAYLOR and EICHMAN (1976)	0			
	Igh-Bgl	KIPPS and DORF (1979)	14			
	Igh-Nbp	MAKELA *et al.* (1981)	0	D	0.0018	<0.0001
	Igh-Npa	KARJALAINEN	5			
	Igh-Gte	JU and DORF (1980)	0			
	Odc-8	RICHARDS-SMITH and ELLIOTT (1984)	0			
	Ox-1	KARJALAINEN, MAKELA and TAYLOR (1982)	1			
	Npid		1			
12	*D12Nyul*	D'EUSTACHIO (1984)	0	D	NA[a]	0.0011

[a] Not applicable (gene not in final model).

ards. That is, the survival curves in Figure 1 do not differ enough in the tails (past about 730 days) to have proportional hazards. Hence, we also tried linear models, regressing mean survival of each strain on genetic markers. This necessarily decreased the amount of survival data used in the model, since the ranges of survival within strain were ignored, as was the fact that some strains had low survival early (compared to other strains) and higher survival later. Since this meant we had only 20 observations (20 strains), we could only include at most 18 markers in each model. We restricted the variable set to the 16 markers that were marginally most significantly correlated with survival (which included *Lamb-2, H-3*, and *Coh*) plus *Ltw-4, Ly-24*, and *Igh-Gte* (which were in the proportional hazards model). To decrease the variable set to 18, we did the analysis 19 times, each time omitting one marker. We based model selection on the R^2 and Cp selection criteria. Since there are computational shortcuts in calculating these values for all possible models, we did not use a step-up procedure. Instead, we identified the best regression model with one variable, the best regression model with two variables (even if neither one was in the best regression model with one variable), and so on. *Coh* was not the best model of size one, nor was it among the ten best models of size one. The Cp criterion indicated that a model of size seven was best overall; the R^2 criterion indicated that the model of size six was best. For both the R^2 and Cp statistic, the best model of size six included *Ly-24* and *Ltw-4* (B allele associated with longer survival), *H-3, Lamb-2, Igh-Gte*, and *D12Nyu1* (D allele associated with longer survival). Hence this model differed from the proportional hazards model only by substituting *D12Nyu1* for *Coh* (see Table 2 for a reference for *D12Nyu1*). *Coh* was not in the best model of size seven either, but it was in the third best model of size seven (out of a possible 50,388 models).

To further check which allele was associated with longer survival, the strains were divided according to their alleles for the *Coh, Ly-24, H-3, Lamb-2, Ltw-4*, and *Igh* markers (the proportional hazards model). Potentially, the six markers could be associated with $2^6 = 64$ different combinations of alleles; in this data set 13 of these combinations were represented among the 20 analyzed strains. We then compared the survival of groups of strains which differed in only one of the six genes (see Table 3). The gene "effect" in these small subgroups agreed well with the predictions from the model based on all 360 animals. Specifically, the B allele of *Ly-24* and *Ltw-4* was associated with longer survival in the three available comparisons and the D allele of *H-3, Lamb-2, Igh* and

TABLE 3

Strains which differ in only one of the six markers in model

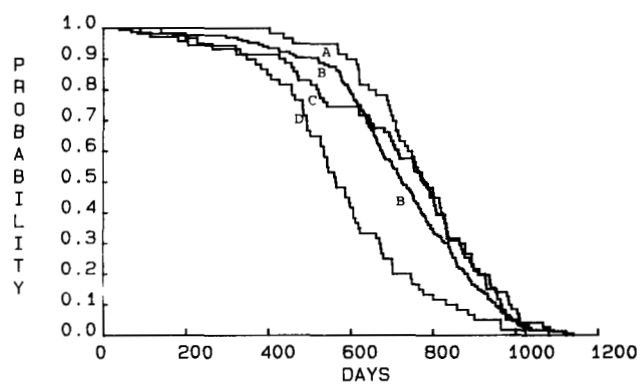
Marker	Strain(s) with B genotype	Strain(s) with D genotype	Difference in survival
Proportional Hazards Model			
**Coh*	15	12	Same
Ly-24	15	16, 18	B longer
Ly-24	22	5	B longer
H-3	5	23, 27, 29	D longer
**H-3*	6	12	D longer
**Lamb-2*	8	19	D longer
**Ltw-4*	19	23, 27, 29	B longer
Igh-Gte	23, 27, 29	8	D longer
Igh-Gte	8	16, 18	D longer
**Igh-Gte*	22	9	D longer
Linear Regression Model			
D12Nyu1	16	18	D longer
D12Nyu1	11, 30	24, 28	D longer
**D12Nyu1*	12	15	Same
Ly-24	12	16	B longer
**H-3*	6	12	D longer
**Lamb-2*	8	19	D longer
**Ltw-4*	19	23, 27, 29	B longer
Igh-Gte	8	18	D longer
**Igh-Gte*	22	9	D longer
Igh-Gte	23, 27, 29	24, 28	D longer

* Comparison applicable to both models.

D12Nyu1 was associated with longer survival in each of the six available comparisons. However, the single comparison available for *Coh* did not show much difference in survival. We did the same for the linear regression model, substituting *D12Nyu1* for *Coh*. In this set 15 of the possible 64 combinations were represented by at least one strain. Again, all the effects agreed well with the model, with the exception of one comparison for *D12Nyu1*.

Number of B Alleles, Mtv Genes and Xmmv Genes: Although the parent strain which has all *B* alleles lives longer than the parent strain which has all *D* alleles, the *D* allele was associated with longer survival for the β2*m* (or *H-3*), *Lamb-2*, and *Igh* markers in both the linear regression and the proportional hazards model. Figure 5 shows survival by number of *B* alleles among the 141 markers typed. The difference is in the opposite direction from what was expected a priori, since the category for the largest number of *B* alleles (89-97) includes the shortest lived strains (2 and 14). Strain 19, with 82 B markers, also had more than the average number of B markers.

We also examined the proportional hazards model based only on the five mammary tumor virus (*Mtv*) genetic markers. The associated loglikelihood was worse than that for the model in Table 2, in that the *Mtv* model did not account for as much of the variation. In particular, this model predicts that strain 14 (a short lived strain) and strain 24 (a long lived strain) would have similar survival. Adjusting for multiple comparisons, only *Mtv-9* would be considered significantly associated with survival. This marker divided the mice into groups that differed in medians by 87 days.



NUMBER OF B MARKERS	DEAD	MEDIAN	75th PERCENTILE	MEAN
A 46-56	60	775	896	779
B 62-71	169	725	856	721
C 76-85	71	779	891	725
D 89-97	60	564	697	583

FIGURE 5.—Survival by number of genes (of total of 141 markers typed) shared with longer lived "B" parent (C57BL/6).

Most of the genes in the model of Table 2 are mapped close to xenotropic virus genes (DAVISON 1986). *Coh* is indistinguishable from *Xmmv-35*. *H-3* is close to *Xmmv-71*, which was not typed in these mice. *Lamb-2* is close to *Xmmv-61*, which was typed in these

mice. The *Igh* markers are close to *Xmmv-50* (not typed in these mice) and *Xmmv-34* (which was typed). The current gene map lists 74 *Xmmv* genes primarily located on the chromosomes which we found to have significant genetic areas. With this large a number of *Xmmv* genes, the association in location of genes in our models with *Xmmv* might be due to chance. Altogether, sixteen xentropic viruses and two friend viruses were typed in these animals. The six most significant viruses in a step-up model were *Xmmv-35*, *Xmmv-34*, *Fv-1*, *Xmmv-31*, *Xmmv-8*, and *Xmmv-3* from chromosomes *7*, *12*, *4*, *9*, *4*, and unknown, respectively. For *Xmmv-35*, *Fv-1*, and *Xmmv-31* the B allele was associated with longer survival; for the other three markers, the D allele was associated with longer survival. While the log likelihood associated with the six xenotropic virus model showed better fit than the mammary virus model, it was still worse than the model of Table 2. Of course, this might not have been true if we had all the xenotropic viruses typed on these mice.

Pathology: Of the 360 mice analyzed in this report, 8 died before 5/83, when daily body checks were instituted, and 76 died between 5/83 and 5/84, when thrice daily body checks were instituted. Few of these short-lived mice were found sufficiently well preserved for necropsy. Even after 5/84, many dead mice were found to be too severely decomposed for adequate necropsy. Altogether, only 75 animals (21%) were necropsied. Ten mice were found to be at least partially autolyzed by the pathology laboratory, and seven (2%) had "no significant lesion." A total of 89 diagnoses were made on the 62 animals with some diagnosis; these included 33 lymphomas, 25 hepatomas/liver-spleen necrosis, 6 glomerulo-nephritis/hydronephrosis, 4 atrial thromboses, 4 lung carcinomas/lung hyperplasias, 4 other carcinomas, 2 sarcomas, 6 other tumors, 2 bile duct hyperplasia, 2 necrotizing metritis, 1 acute pneumonia, and 1 glomerular amyloidosis. There were no necropsy results available on strain 2; only one necropsy was available on each of strains 5, 6, 19, and 22. With the exception of strain 15 (3 hepatomas), all strains with more than one animal with a histologic diagnosis had more than one diagnosis. Among animals with any diagnosis, lymphomas (in 53% of the animals) and hepatomas (in 40% of the animals) were most common. While the animals diagnosed as having hepatoma died at slightly older ages than those diagnosed as having lymphoma (medians 2.2 and 1.9 yr), this difference was not significant ($P = 0.11$). Of the 4 necropsies available in strain 14, 3 had lymphomas. Among the 41 animals with necropsies from strains with intermediate survival (1, 5, 6, 8, 12, 16, 18, 22, 23, 27, 28, 29, 30) there were 23 diagnoses of lymphoma and 14 of hepatoma. Among the 17 animals with necropsies from strains with long survival (9, 11, 15, 19, 24) there were 7 diagnoses of lymphoma and 7 of hepatoma. So there was a tendency for the longer lived strains to have fewer lymphomas (41% *vs.* 56%), but this was not significant ($P = 0.39$). Nor was the difference in diagnosis of hepatomas (34% *vs.* 41%) significant ($P = 0.77$).

DISCUSSION

The genetic control of adult life span in any organism is a complex developmental function which is the product of the interaction between the genome and the internal and external environment. Although several attempts have been made to identify individual genes of life span in worms, fruit flies and mice, there is not a single report of an individual gene which completely explains differences in life span. In general, most studies have identified individual genetic regions that are associated with shortened life span.

Studies in the nematode *Caenorhabditis elegans* mutants demonstrated the complexity of life span genetics, suggesting that either the genes determining life span are rare or that life span is controlled by several genes (KLASS 1983). The genetic variability in lifespan of these nematodes led to heritability estimates of about 40%, using wild type strains (JOHNSON and WOOD 1982). Similarly, mosaics of *Drosophila melanogaster* have shown that life-shortening mutations could produce either organ or biochemical defects which correlated with life shortening (CLARK and GOULD 1970; GOULD and CLARK 1983). Also, using temperature-sensitive *X*-linked adult lethal mutations, it was possible to identify several markers of life span in *D. melanogaster* (BAIRD and LINZCZYNSYJ 1985). The suggestion that there are genes of life span was made by analyzing 12 different species of *D. melanogaster* representing different degrees of phylogenetic relatedness and various ecological backgrounds. The study demonstrated that greater similarity of life span exists between closely related taxa, but there was too much overlap of life spans to identify individual genes (SCHNEBEL and GROSSFIELD 1983). That environmental factors are important was shown by studies of the role of the population density in life span of short or long lived strains of fruit flies. It appears that genes with an environmental component to their expression control life span, and also that crosses of long and short lived strains result in increased longevity in fruit flies but not in nematodes (LUCKINGBILL and CLARE 1986; JOHNSON 1984; JOHNSON *et al.* 1984).

Several studies of murine life spans have established that both genetic and environmental factors contribute to the determination of life span. Mean survival time and gross pathology at death of mice of many inbred strains was related to the genetic background (STORER 1966; FESTING and BLACKMORE

1971; RUSSELL 1972; GOODRICH 1975; SMITH, WALFORD and MICKEY 1973; MYERS 1978; STORER 1978). Also, the mean life spans of some F_1 hybrid mice are longer than those of their parents (SMITH, WALFORD and MICKEY 1973; MYERS 1978). However, some long lived inbred strains have longer life spans than do some types of F_1 hybrid mice (MYERS 1978) which suggests that homozygosity per se does not necessarily decrease longevity.

The studies of different life spans in congenic mice differing only in *H-2* haplotypes led to the hope that a genetic effect on longevity would be relatively simple and based on one or a few selected genes. Since genes of the MHC (major histocompatibility complex) control the immune system it was anticipated that longevity may be associated with the expression of certain *H-2* phenotypes (BENACERRAF 1981; GREENBERG and YUNIS 1975, 1978; KATZ and BENACERRAF 1976). Several studies have shown that differences in the *H-2* region of chromosome *17* produces significant changes in the life span (MEREDITH and WALFORD 1977; POPP 1978; WILLIAMS *et al.* 1981). There was correlation between *H-2*, life span and maintenance of immune vigor (MEREDITH and WALFORD 1977; POPP 1978). Even in animals where most genes are identical (congenic strains) nonchromosomal effects were shown since the F_1 hybrids between $H\text{-}2^n$ and $H\text{-}2^b$ showed differences in life spans in mice produced by reversed breeding (POPP 1978). Although these are important experiments, they did not examine the role of other genetic factors alone or in combination in life span.

The work by RUSSELL on F_1 hybrids between C57BL/6 and DBA/2 and our work on backcross mice of these two strains [(C57BL/6 × DBA/2)F_1 × DBA/2] suggested that several genes and the environment act together to influence longevity in mice (RUSSELL 1966; YUNIS *et al.* 1984). Genes in the brown locus (*b*) segment of chromosome *4*, genes in a segment of the sex chromosomes and genes in a segment of chromosome *17* (*H-2*) influenced longevity, and there was a significant interaction of *H-2* and sex. Overall, it seemed as though the animals heterozygous for the most genes lived longer, but it should be pointed out that the only homozygous animals in that experiment had chromosomal regions marked by D alleles. That is, we did not have any homozygous mice with chromosomal regions marked with B alleles. Our findings on heterozygosity may represent hybrid vigor associated either with cominance of favorable alleles not held in common by the parental strains (RODERICK and SCHLAGER 1975) or with avoidance of deleterious effects of recessive genes which limit life span. In general, genetic interactions or additive effects of several genes and the environment are more important in conferring longer life span than the presence of specific individual alleles (YUNIS *et al.* 1984).

To study possible differences in longevity among homozygous mice, we studied 20 strains of BXD recombinant lines in which 101 genetic markers were informative. That the strains did differ in life span is demonstrated by the observed difference of 482 days in median survivals, a long time in a group of animals whose overall median survival was 704 days. However, since strain by itself (including those environmental variables which were identical within strain) accounted for only about 29% of the variance in life span, it is clear that environmental factors which are not well defined also significantly influence survival. This is also supported by the wide ranges of life span within individual strains (529 to 949 days) and by the large number of different diseases diagnosed at necropsy within most strains. Of course, we cannot rule out the possibility that some "within strain" genetic variation has occurred due to spontaneous mutation. However, the relative constancy of the median survival of the C57BL/6 parent strain for the last few decades is indirect evidence that such longevity-associated mutations are rare.

Several strains were very similar genetically. At the start of the experiment we expected that long-lived strains might share more alleles as a group than short-lived strains would share as a group. It seemed reasonable to assume that there might be many life-shortening diseases associated with many different markers, any one of which would be enough to shorten a strain's survival, and that long-lived strains would have to have "good" alleles for most of these disease markers. However, the 2 shortest-lived strains had more common alleles than any 2 long-lived strains (63% *vs.* 44% of the statistically distinguishable markers). This similarity of short-lived strains might partially explain why so many genetic markers (44% of those typed, including some on each chromosome with more than 4 markers typed) were individually associated with survival significance levels less than 0.05. Even after making a conservative adjustment for multiple comparisons, there were 16 markers on 8 chromosomes which were individually associated with significance levels less than 0.0005.

Although many chromosomal regions were individually associated with a significant difference in survival, no single region or even pair of regions could explain much of the life span differences associated with strain. This is not surprising, considering that many other traits are determined by multiple genes acting together (*e.g.*, body height, body weight and coat color). A proportional hazards model involving six regions did seem to predict survival fairly well in the sense that no other genes added significantly to this model. Although not all the

assumptions of this model are appropriate for this data set, it is comforting that a cruder linear model (based only on the average survival of each strain rather than the life spans of all the individual mice) chose a very similar model as being best (substituting *D12Nyu1* for *Coh*), References to these seven regions are given in Table 2. Future studies of the possible correlation of these seven genetic regions and survival in other strains of mice are necessary to decide if our findings are generalizable. Studies of sets of congenic mice that differ only on the seven genetic regions (or in some of the seven genetic regions) and not in other regions would be particularly useful in establishing whether these specific regions are influencing survival or whether any survival effects could be due to other regions highly correlated with those found significant in these recombinant strains.

Taken together, the observations of our present study and past work (YUNIS *et al.* 1984) suggest that several genes act individually or in groups to confer longer life span. In recombinant inbred mice of two genetic backgrounds (C57BL/6 and DBA/2), genes on chromosomes *1, 2, 7* and *12* are important in predicting survival, in the homozygous state. In mice from these same two backgrounds, heterozygous genes in chromosome *4* (*b* locus segment) and chromosome *17* (*H-2* segment) are important in predicting survival. (Perhaps the chromosome *4* and *17* regions failed to be significantly correlated with survival in the present experiment because we were studying only homozygous mice.) Our observations confirm and extend the suggestion by RUSSELL and our own studies that different genetic regions interact together and with environmental factors to influence longevity in mice. It is remarkable that even in this experiment minimizing environmental and genetic variability (within strain), we found complex survival and disease patterns. These results raise doubts as to the feasibility of studying the genetics of longevity in any outbred population of mice or humans.

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