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Heritability of life span in mice and its implication for direct and indirect selection for longevity

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Abstract

We found high narrow-sense heritability of life span based on the regression of offspring on average parental (midparent) life spans. In two mouse populations prepared using the 4-way-cross design, mean ± SE heritabilities were $62 \pm 11\%$ ($P < 0.001$) and $44 \pm 15\%$ ($P < 0.01$). To reflect inherited rates of aging, rather than resistance to early disease, data from the first 25% to die were deleted, so that only about 40% of families were used for offspring-midparent regressions. Heritabilities still remained high, 38% and 55%, for the same two populations, respectively. Populations studied in two other experiments did not show nearly as high heritabilities; in one case probably due to environmental stress, and in the other probably because the strains used did not have sufficient additive variance in genes regulating longevity. Significant heritabilities occurred only when a wild derived inbred strain was included in the 4-way cross. The age when a female ceased to reproduce appeared to be related to the life spans of her offspring, but only weakly, not approaching significance for any individual experiment. The age when a female became infertile was related to her life span, but the relationship disappeared when short-lived mice were excluded from the analysis. Our findings indicate that, in sufficiently diverse mouse populations, selection for increased longevity should be possible and that the direct selection for parental life span will be a more efficient strategy than selection for female reproductive life span.

Introduction

On the species level, differences in basic mechanisms of aging are obvious, as in the facts that dogs live six times less than human beings, and mice live five times less than dogs. However, genes causing differences in aging mechanisms between species are difficult to study.

Within a species, a powerful approach to study genetic mechanisms of aging is to identify genes whose allele frequency distributions are altered in populations selectively bred for increased longevity. There are, however, no reports in the literature about successful direct selection for increased life span in mammals.

Two breeding strategies have been successfully applied to *Drosophila* to select for increased longevity:

(1) direct, breeding based on parental life span (Zwaan, Bulsma & Hoekstra, 1995); and

(2) indirect, breeding for increased female reproductive life span (Luckinbill et al., 1984; Rose, 1984).

This paper analyzes which of the strategies would have the better chance to succeed in mouse populations.

The degree of narrow-sense heritability determines the extent of response of a population to selection. To assess the feasibility of selecting mice directly for increased longevity, we determined the narrow-sense heritability of life span in mouse populations not yet subjected to selection, the same populations that we intend to selectively breed for increased maximum life spans. The heritability of life span may be affected by both genes increasing and decreasing life span. Genes decreasing life span could, however, act through different mechanisms than those increasing life span. Death early in life can result from any early disease or defect, since life span is determined by

the first essential biological system to malfunction. In an organism as complex as a mouse, there are many essential biological systems, resulting in so many genes capable of reducing life span, that genes that alter basic mechanisms of aging are lost among them. Extending life span, on the other hand, requires maintaining functions in all essential biological systems, so that genes that increase life span must retard aging in many different biological systems; such genes may lead to underlying mechanisms of aging. For this reason, besides assessing total life span heritability, we also estimated heritability of life span in the longest-lived animals.

A gene increasing longevity cannot be found unless alleles that cause a significant difference in longevity segregate in the population. Thus, heritability can only reflect effects on life span of genes that are polymorphic and segregating in the population used. In order to maximize the potential heritability of life span in two of the experiments reported here, we produced segregating populations from some of the most diverse mouse strains that can interbreed.

Besides evaluating the direct strategy, we also analyzed an indirect one, selection for female's reproductive life span. Evolutionary theories of aging suggest that the evolution of longevity is an indirect result of selection for the optimal reproductive schedule (Rose, 1991). Thus, the reproductive life span should be directly related to the total life span. Since the female reproductive life span in mice is about one half of the total life span, an indirect strategy, breeding for increased female reproductive life span, may produce longer lived animals more rapidly than the direct selection. In this study, we tested whether indeed a female's reproductive life span was a predictor of her total life span and whether her reproductive life span correlated with the life spans of her offspring. If these were the case, selection for reproductive life span would increase longevity in our mouse populations.

Materials and methods

Mouse populations

Mice were produced using 12 genetically diverse inbred mouse strains in three independent 4-way crosses, a convenient type of mating that results in a reproducible yet highly genetically diverse population (Roderick, 1963; Harrison, Roderick & Paigen, 1995; Chrisp et al., 1996). One cross was duplicated, giving a total of four experiments: Experiments 1 and 1r – (LP/J × MOLD/Rk) F1 × (NZW/LacJ × BALB/cByJ) F1; Experiment 2 – (ST/bJ × C57BL/6J) F1 × (CAST/Ei × DBA/2J) F1; Experiment 3 – (SJL/J × YBR/Ei) F1 × (RIII/DmMob × CE/J) F1. Animals thus produced were the first segregating generation (S0). In each experiment, 40 S0 mated pairs were used after about 3 months of age to produce two mice of each gender, giving a total of 160 second generation (S1) mice. These were housed as virgins, in groups of four mice of the same gender. Here we focus on life spans of the first and second generations of the 4-way-cross animals.

All mice were introduced into the research colony when weaned at 4 weeks of age and housed in filter-hooded, double-sided plastic cages. The mice were kept in an isolated animal colony under positive air pressure, with filtered air, room temperature of 22 $\pm$ 2°C and light from 7 AM to 7 PM. All mice except Experiment 1r were kept in quiet conventional animal rooms. Experiment 1r was kept in a separate room that happened to have an unusually noisy air handling system. Quarterly, at least 10 mice from each room were used for routine animal health assessment by The Jackson Laboratory's diagnostic laboratory. In addition, pathologists screened any mice that appeared ill without explainable cause, such as extreme age or experimental treatment. Details of the testing procedures are available from The Jackson Laboratory's Quarterly Animal Health Reports.

Mice consumed a pasteurized component and composition defined diet (NIH 31, 4% fat); they received ad libitum food and chlorinated water, acidified to prevent growth of Pseudomonas. The mice were handled only to measure weights and tail lengths at 1, 3, 7 and 20 months after weaning and to collect tail tip samples for DNA analysis, except for Experiment 1r in which a number of physiological tests were performed. These included anesthesia followed by bone density measures two or three times and tail collagen and blood removal two times during the life span.

Heritability estimation

Life span narrow-sense heritabilities were assessed using uncensored and censored data. Initially, we excluded only life spans of animals, which were recorded as mishandled (escaped or killed accidentally). Total life span heritabilities were calculated for those uncensored life span sets. For different levels of cutoff (0–25%), we excluded (in each cross) a given

percentage of the shortest-lived mice from each of the three populations – fathers, mothers and offspring. Life spans of sons and daughters did not differ in any of the crosses and therefore were combined. Each family of mice consisted of a father, a mother, two sons and two daughters. Only families in which life spans of both parents and at least three offspring were above the cut-off were used in estimating heritability. Life span heritabilities were determined as the slope of linear regressions of the average of the offspring on midparent (average of father's and mother's) life span values, with the significance level determined by the one-tailed t-test (since we only tested whether slopes were positive). Using the average, rather than individual offspring life span values does not alter estimates of heritability (Lynch & Walsh, 1998, pp. 538–539).

Age at the last litter as a predictor of total life span

Female reproductive life span, her age at the last litter, was tested as a predictor of her total life span. A trivial correlation between the age of cessation of reproduction and life span exists because the information about the age at the last litter also carries the message that the female was alive at this age. To alleviate this problem, we calculated each mother's remaining life expectancy at the age of the last litter from the survival curve of the 40 breeding females in her population. At the age when reproduction stops, the survival curve provides information about the average remaining life span of females surviving to that age; this is the life expectancy for a female at that age. We tested whether life expectancy for females with early cessation of reproduction overestimated their actual life span, while life expectancy for females with late cessation of reproduction underestimated their actual life span. The key point was to test whether the age at the last litter predicted female longevity better than just the knowledge that the female was alive at that age. For example, if late reproduction predicts long life span, we expect a positive correlation between the age at the last litter and the difference between the actual longevity and the life expectancy at the age at the last litter.

The analysis was performed on both censored and uncensored data for each experiment separately. For different levels of cut-off, we excluded (in each cross) 0–25% of the shortest-lived mice and the same percentage of mice with early cessation of reproduction. We also performed the analysis of covariance on the combined data (censored and uncensored) with the dependent variable being the difference between the actual life span and the life expectancy at the age at the last litter. The covariate was the age at the last litter, and the factor was the experiment number. This analysis was followed by a simple regression on the combined data with the significance level determined by the one-tailed t-test (since we only tested whether slopes were positive).

Age at the last litter as a predictor of offspring life spans

Life spans of offspring may be predicted by the female reproductive life span, and selection for late female reproduction was successfully used in selective breeding for increased life span in *Drosophila* (reviewed in Rose, 1991). To assess whether a female's reproductive life span predicted her offspring total life span, we used a simple regression between the female's age at the last litter and the life spans of her offspring. For different levels of cut-off we excluded (in each cross) 0–25% of the shortest-lived mothers and offspring. Only families in which mother's life span and life spans of at least three of her offspring were above the cut-off were used in the regression analysis. Analysis of covariance on the combined data from all four experiments was done with the dependent variable being the average life span of offspring, covariate being the age at the last litter and the factor being the experiment number. This analysis was followed by a simple regression on the combined data with the significance level determined by the one-tailed t-test (since we only tested whether slopes were positive).

Results

Patterns of life spans in these studies

In all four cases, S0 breeding males lived longest and S0 breeding females least long, probably due to damage from bearing repeated litters. Male and female S1 virgin offspring had similar life spans, intermediate between life spans of the S0 males and females. Median and mean life spans were not as long as those of long lived inbred strains (Figure 1). However, it is important to note that the most long-lived mice seemed to have gene combinations retarding aging, as they lived longer than any other mice in our experience fed ad lib. Our previous record for life span was 1330 days from a population of about 2000 long-lived F1 hybrids in

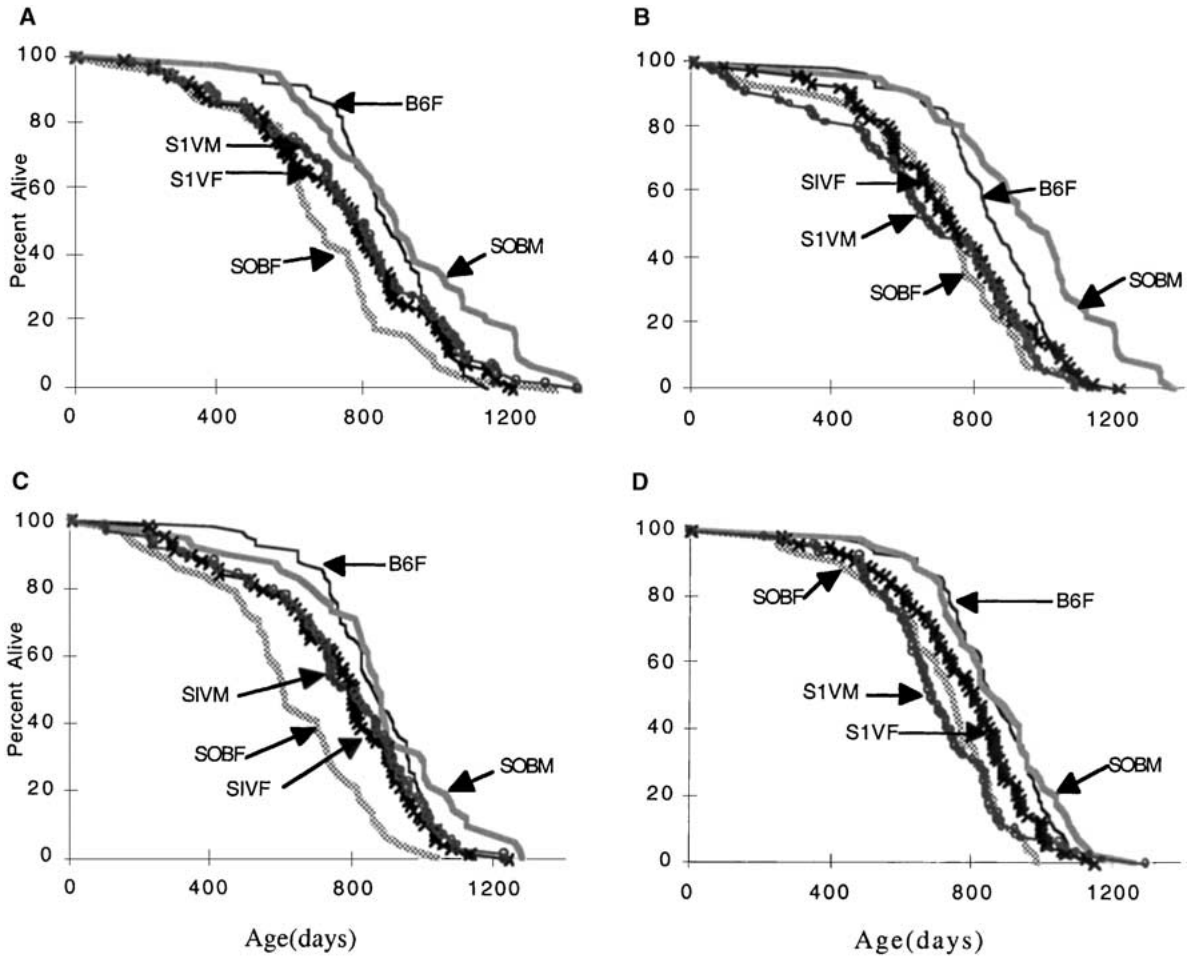

Figure 1. Life spans of the initial, S0, and next, S1, generations in four experiments with S0 mice produced by crossing two different F1 hybrids. The *X*-axes give life spans in days, and the *Y*-axes the percentages remaining alive. S0 breeding females (S0BF) were always the shortest lived, and S0 breeding males (S0BM) the longest lived, shown in thick light and dark lines, respectively. S1 virgin males (S1VM) and females (S1VF) had similar life spans. The B6 female standard (B6F) is a thin dark line at the same location on all plots. Results from Experiments 1, 2, 1r, and 3 are shown in panels A, B, C and D, respectively.

many studies. Maximums from about 250 mice each in Experiments 1 and 2 were 1392 and 1376 days.

Heritabilities of life spans

Life span narrow-sense heritabilities were determined as the slope of linear regressions of the offspring on midparent life span values, as illustrated in Figure 2. The slopes were significant in Experiments 1 (Figure 2A) and 2 (Figure 2B), 0.62 ± 0.11 ($P < 0.001$) and 0.44 ± 0.15 ($P < 0.01$) respectively, so that life span heritabilities were 62 and 44% (Table 1). The other two studies, Experiments 1r (Figure 2C) and 3 (Figure 2D), showed no significant heritabilities. To test whether heritabilities differed significantly among the crosses, we applied one-factor (cross) analysis of covariance to combined data. We still found significant heritability, but also a significant heterogeneity in slopes, indicating that some of the heritabilities might be significantly different from each other. When we applied the same analysis of covariance to all possible pair-wise combinations of our data (total of six), using the Bonferroni correction for multiple comparisons, the only statistically significant results were that the heritability in Experiment 3 was less than heritabilities in both Experiments 1 and 2.

To avoid the influence of genes causing early death which probably are not related to basic mechanisms of aging, we tested effects of excluding from the analysis the shortest-lived 5–25% mothers, fathers

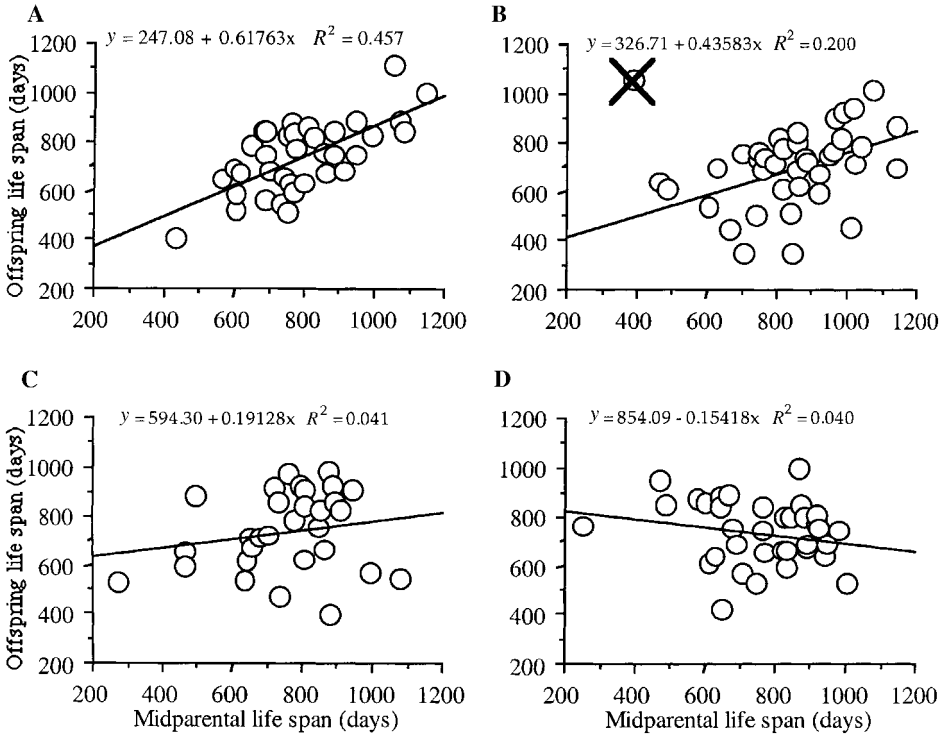

Figure 2. The regressions of average offspring on midparent life spans in Experiments 1 (A), 2 (B), 1r (C) and 3 (D) are given without excluding any data. Regression equations and R^2 values are given in each panel. A single outlier (shown crossed out in panel B) was omitted as there was less than 1 chance in 1000 that this was part of the same data set (this data point was outside 0.99999 density ellipse based on the bivariate normal distribution for the data). For this family, midparent life span of 391 days was probably underestimated because of the very short-lived breeder female, whose death at less than 170 days was most likely attributable to birth related complications.

Table 1. Heritability of life spans

Cut-off\cross (%)	Experiment 1	Experiment 1r	Experiment 2	Experiment 3
0	$0.62 \pm 0.11(36)^{c}$	$0.19 \pm 0.17(30)$	$0.44 \pm 0.15(38)^{b}$	$-0.15 \pm 0.13(35)$
5	$0.52 \pm 0.14(33)^{c}$	$0.12 \pm 0.19(26)$	$0.37 \pm 0.15(34)^{b}$	$-0.07 \pm 16(32)$
10	$0.41 \pm 0.15(29)^{b}$	$0.39 \pm 0.21(24)^{a}$	$0.27 \pm 0.17(32)$	$0.04 \pm 0.16(27)$
15	$0.30 \pm 0.17(25)^{a}$	$0.29 \pm 0.22(21)$	$0.30 \pm 0.19(24)$	$0.18 \pm 0.20(21)$
20	$0.27 \pm 0.17(20)$	$0.06 \pm 0.25(18)$	$0.51 \pm 0.17(18)^{b}$	$0.14 \pm 0.23(19)$
25	$0.38 \pm 0.20(15)^{a}$	$0.09 \pm 0.30(14)$	$0.55 \pm 0.20(15)^{b}$	$0.08 \pm 0.34(15)$

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$ statistically significant.
Heritability was estimated as the slope of the linear regression of offspring on midparent life spans. Different cut off levels refer to which fraction of the shortest-lived mice were excluded from each of the three populations, fathers, mothers and offspring, when heritability was estimated. A single outlier was excluded (details in Figure 2 legend).

and offspring. Excluding the shortest-lived 25% reduced the total number of families used in the analysis to about 40% of the initial number. Even after that, heritabilities of life span remained significant in Experiments 1 and 2, 0.38 ± 0.20 ($P < 0.05$) and 0.55 ± 0.20 ($P < 0.01$), respectively (Table 1, Figure 3A and B). Values of R^2 in Experiments 1 and 2 were 0.46 and 0.20 with 0% cut-off (Figure 2A and B) and 0.21 and 0.35 with 25% cut off (Figure 3A and B). They are the fraction of the total variation in the offspring life spans that was explained by midparent life spans.

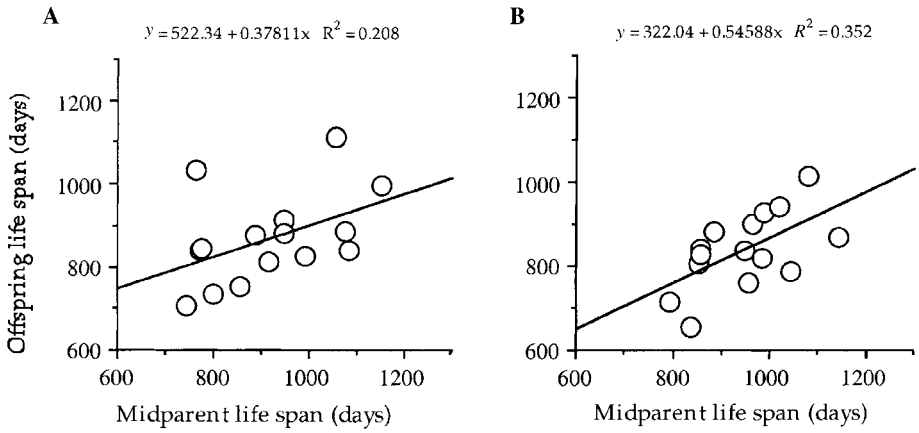

Figure 3. The regressions of average offspring on midparent life spans in Experiments 1 (A) and 2 (B) show results where the shortest lived 25% are excluded.

Female reproductive life span as a predictor of her total life span

The slopes of regressions between the age at the last litter and the difference between the actual longevity and the life expectancy at the age at the last litter were positive and highly significant in each cross, when uncensored data were used (0% cut-off in Table 2). These relationships, however, lost significance after the shortest-lived 15–25% were excluded. Since the analysis of covariance did not indicate any effect of the cross or heterogeneity in the slopes among crosses, we did a simple regression analysis on the combined data. The regression was also significant only up to a 10% cut-off level (for 0 and 5% cut-off, $P < 0.001$; for 10% cut-off, $P < 0.01$) and not significant when a higher percentage of short lived mice were excluded.

Female reproductive life span as a predictor of the life spans of her offspring

To evaluate how well female reproductive life span can be used to selectively breed for increased life span of the offspring, we estimated the correlation between the female's age at the last litter and the average life span of her offspring. This correlation was not significant in any experiment, but it was positive in all (data not shown). The analysis of covariance did not indicate any effect of the cross or heterogeneity in the slopes among crosses. We, therefore, did a simple regression analysis on the combined data. The slopes were marginally significant when the shortest lived 0–20% were excluded ($0.01 < P < 0.06$), but R^2 were low, explaining not more than 4% of the total variability (Table 3).

Discussion

A high degree of life span heritability was observed in this study in two crosses, Experiments 1 and 2. While a high degree of life span heritability suggests the segregation of alleles regulating aging, it may also result partially from segregation of deleterious alleles randomly fixed in inbred strains making up the crosses. Since life span is determined by the first system to fail, short life spans usually result from early diseases caused by such alleles. This phenomenon might inflate estimates of life span heritability for reasons unrelated to aging. In fact, alleles causing early death may mask alleles that retard aging. Even after removing almost 60% of the families containing the shortest lived 25% of mice, heritability estimates were still significant: 38 and 55% in Experiments 1 and 2 (Table 1).

Our heritability estimates of life span cannot be directly compared to those in the literature because only the broad sense and not the narrow sense life span heritability has been reported for mice. Generally, the broad sense heritability includes additive, dominance and epistatic components and thus overestimates the narrow sense heritability, which includes only additive variance. Despite that, only one study (Goodrick, 1975) reported as high values as 48 and 79% for the broad sense heritability, which were comparable to ours of 44 and 62% for the narrow sense heritability. Other studies reported much lower values between 19 and 37% (Storer, 1966; Festing & Blackmore, 1971; Gelman et al., 1988).

Experiments 1 and 2 in our study might have shown higher levels of heritability than prior studies because we used genetically highly diverse 4-way-

Table 2. Regression between each female's (longevity – life expectancy) and age at her last litter

Cut-off\cross (%)	Experiment 1	Experiment 1r	Experiment 2	Experiment 3
0	$0.70 \pm 0.27(37)^{b}$	$0.55 \pm 0.16(33)^{c}$	$1.27 \pm 0.24(38)^{c}$	$1.23 \pm 0.36(35)^{c}$
5	$0.83 \pm 0.30(34)^{b}$	$0.64 \pm 0.21(29)^{b}$	$0.85 \pm 0.15(35)^{a}$	$0.78 \pm 0.41(32)^{a}$
10	$0.32 \pm 0.31(32)$	$0.71 \pm 0.25(27)^{b}$	$0.61 \pm 0.46(34)$	$0.82 \pm 0.37(29)^{a}$
15	$0.01 \pm 0.31(30)$	$0.47 \pm 0.28(25)$	$0.18 \pm 0.50(30)$	$0.67 \pm 0.39(27)^{a}$
20	$0.15 \pm 0.32(29)$	$0.29 \pm 0.31(23)$	$0.35 \pm 0.50(26)$	$0.49 \pm 0.47(24)$
25	$0.08 \pm 0.36(25)$	$0.29 \pm 0.31(23)$	$0.15 \pm 0.54(23)$	$0.62 \pm 0.44(21)$

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$ statistically significant.
Slopes are from linear regressions of female's (longevity – life expectancy at the age at the last litter) to female's age at the last litter. Different cut-off levels show the fractions of the shortest-lived mice and mice with early cessation of reproduction that were excluded from the analysis.

Table 3. Regression between mother's age at the last litter and life spans of her offspring

Cut-off (%)	Slope	R^2
0	$0.17 \pm 0.09(140)^{b}$	0.018
5	$0.09 \pm 0.11(128)$	0.005
10	$0.22 \pm 0.11(119)^{a}$	0.031
15	$0.26 \pm 0.12(102)^{a}$	0.044
20	$0.21 \pm 0.13(88)^{b}$	0.030
25	$0.16 \pm 0.13(76)$	0.019

[a] $P < 0.05$ statistically significant.
[b] $0.05 < P < 0.06$ marginally significant.
Slopes are from linear regressions of the average offspring life span on their mother's age at the last litter for all data combined. Different cut-off levels show the fractions of shortest-lived mothers and offspring that were excluded from the analysis.

cross mouse populations. Such populations, first defined by Roderick (1963), have been used to identify genetic loci affecting late-life diseases and longevity (Chrisp et al., 1996; Miller et al., 1998). In contrast to previous reports (Chrisp et al., 1996; Miller et al., 1998), our crosses each included a wild derived mouse strain, MOLD/Rk and CAST/Ei, respectively. These strains are derived from *Mus m. molossinus* and *Mus m. castaneus*, subspecies that diverged about half a million years ago (Bonhomme & Guenet, 1996) from the *musculus* and *domesticus* subspecies that had been used to produce conventional inbred strains of mice used in research. Although MOLD/Rk or CAST/Ei inbred mice are not long lived themselves, they each appear to carry at least one allele that increases maximum life spans, while no such alleles are found in conventional strains (S. Klebanov et al., in press).

There are explanations why alleles for longevity may occur in wild derived strains. Besides their greater genetic divergence, wild strains have never been domesticated, while conventional strains were derived from domesticated mice (Bonhomme & Guenet, 1996). During domestication, the most rapidly reproducing mice contributed most to the domesticated populations ancestral to conventional strains. This strong selective pressure for rapid development and reproduction might have systematically removed alleles retarding aging (Williams, 1957; Miller et al., 1999). In contrast, wild-derived strains, such as MOLD and CAST, were inbred immediately after they were imported from wild populations. Selective pressure was intense for survival despite rapidly increasing homozygousity and, therefore, slower rates of development and reproduction were accepted. Thus, alleles that retarded these characteristics as well as aging (Williams, 1957) might have been fixed in these inbred strains. The apparent lack of heritability in Experiment 3 could have resulted from the absence of a wild-derived strain in this experiment and, as a result, greatly reduced allelic variability at loci affecting longevity.

The difference between the estimates of heritability in Experiments 1 and 1r was not statistically significant (single comparison $P = 0.04$) when corrected for six comparisons (as all other pairwise combinations of heritabilities, another five, were tested as well). However, while we observed a significant heritability in Experiment 1, the heritability in Experiment 1r was not statistically different from zero. This probably resulted from environmental differences between Experiments 1 and 1r. Only Experiment 1r was per-

formed in a room with an unusually noisy air handler, and only the mice in Experiment 1r were subjected to physiological tests. These tests included anesthesia followed by bone density measures two or three times and tail collagen and blood removal two times during the life span. The stress caused by the room and these tests, however, did not affect life spans of conventional strains in other studies (Harrison, D.E., unpublished observations). Wild-derived strains may have a very different response to stress than that of conventional mouse strains. For example, the wild-derived strains used in this study, MOLD/Rk (Experiments 1 and 1r) and CAST/Ei (Experiment 2), had normal hearing at one year of age, while almost half of the 80 tested inbred strains developed severe hearing defects before that age (Zheng, Johnson & Erway, 1999). In general, progenitor populations for conventional mouse strains were inadvertently selected for docility, which in part might arise from low sensitivity to external stimuli. Possibly, genes from wild-derived strains increased the vulnerabilities of populations in Experiments 1 and 1r to stress, and this disguised the relationship between life spans of S0 and S1 generations in Experiment 1r.

We estimated life span heritability as the slope of the regression of offspring on midparent life spans. While father-offspring regressions qualitatively recapitulated midparent-offspring data, mother-offspring regressions tended to be lower, probably because mothers' life spans were compromised by continuous breeding.

If selection is conducted based on parental life spans, our estimates of heritability are very useful because they can directly predict a response to selection. At the same time, our estimates of heritability may closely approximate narrow sense heritability because several conditions necessary to exclude nonadditive sources of variance have been met. First, S0 mice were bred randomly, before any information about their longevity was collected. Selection began at a later generation. Second, the effect of the common environment shared between parents and offspring was probably trivial because S1 mice were weaned at three weeks and thus shared a common cage with their S0 parents on average for less than 3% of their life span, and because cages, diet, lighting, etc., were identical for all the mice. Third, a considerable linkage disequilibrium present in our design fortunately does not directly affect the slope of the offspring to midparent regression (Lynch & Walsh, 1998; p. 149, Table 7.3). Fourth, gametic phase disequilibrium, present in our design as the result of linkage, might contribute to the offspring to midparent regression slope. However, this effect is unbiased. It could either inflate the estimate if loci were in the coupling disequilibrium or reduce it in the case of repulsion disequilibrium (Lynch & Walsh, 1998; pp. 100–101, 152). More importantly, since mice have 20 pairs of chromosomes, effects of linkage are small, as most pairs of loci will be on different chromosomes. The average recombination frequency for loci in mice is 0.483, which is very close to 0.5, representing no linkage. By comparison, in *Drosophila melanogaster*, it is only 0.365 (Lynch & Walsh, 1998; p. 211, Table 9.2).

Finally, additive epistatic effects could contribute to the slope of the offspring to midparent regression, inflating it. The coefficients of non-additive variance contributions to the midparent-offspring regression are, however, 1/2 or less (Lynch & Walsh, 1998; p. 149, Table 7.3). Hence, if two-gene epistatic interactions accounted for as much as 40% of the total variance (genetic plus environmental), it would only inflate the regression slope estimate of heritability by a maximum of 0.2. In any case, our measures were intended to evaluate different criteria for selective breeding to increase life spans. If a particular combination of alleles at two independent loci is needed for positive effects, offspring carrying it will tend to be selected. Future genetic analysis in populations selected for extended life span will be required to analyze possible epistatic effects.

Besides gametic phase repulsion disequilibrium, another factor could possibly reduce the estimate of heritability. The trait measured in the parents is longevity of breeders, while that measured in the offspring is longevity of virgin mice. Thus, our measures of heritability may be reduced to the extent that breeder life spans differ in genetic regulation from virgin life spans.

The regression of the difference between the mother's actual longevity and her life expectancy at the age at the last litter, on the age at the last litter, resulted mostly from the trivial fact that reproduction ceased with death in females living only 100–300 days. When the shortest lived 15% or more were excluded, this relationship became not significant (Table 2). Thus, when mice were not extremely short-lived, reproductive life span was not a good predictor of total life span in females. Of course, these studies were not specifically designed to measure female reproductive life spans. Once females stopped breeding, they were not remated with a young male.

Therefore, reproductive life spans could in theory be underestimated. However, this is unlikely because, in our mouse populations, when a breeding pair stops producing pups, males nearly always are still fertile.

The mother's age at her last litter was only weakly related to the life span of her offspring. This became significant in the combined data set only (Table 3), but even then significance levels were not impressive.

It has been shown that, for a trait x, the ratio of the response to the indirect selection for a trait y to the response to the direct selection for the trait x itself is

$$CR_x/R_x = i_y{}^* r_A{}^* h_y/(i_x{}^* h_x)$$

where CR_x is a response of the trait x to the indirect selection for the trait y, R_x is a response of the trait x to the direct selection for the trait x itself, i_y and i_x are the intensities of selection for the traits y and x, respectively, h_y and h_x are the heritabilities of the traits y and x, respectively, and r_A is the genetic correlation between the traits x and y (Falconer & Mackay, 1996). Assuming equal selection intensities, this expression can be reduced to the ratio of the heritabilities multiplied by the genetic correlation. Our experimental data are insufficient to calculate the genetic correlation between reproductive life span and longevity, but the phenotypic correlation often can be used as a reasonable approximation, instead (Lynch & Walsh, 1998, pp. 639–640). The square of the phenotypic correlation is about 0.025 (Table 3); that is, the phenotypic correlation is about 0.16. Life span heritabilities in Experiments 1 and 2 are about 0.4 (Table 1). Assuming the same degree of heritability for reproductive life span, the response to the indirect selection will only be $0.16^*0.4/0.4 = 16\%$ of the response to the direct selection. Although a round of selection for reproductive life span requires half the time needed for a round of direct selection for longevity, ultimately three times as much time and six times as many rounds of selection would be needed. Therefore, in mice, selective breeding for late life female reproduction will likely not lead to increased longevity as quickly as the direct selection. Besides that, the genes that determine a female's reproductive life span probably include only a subset of the genes that regulate longevity (Harrison & Roderick, 1997).

A previous study reported an increase in longevity in response to selection for increased female's reproductive life span (Nagai, Lin & Sabour, 1995). Unfortunately, it is difficult to evaluate whether maximal life span was affected in this study because only longevity of the shortest-lived 50% of the animals was reported. Besides that, unselected control reproductive life span was very short, about 140 days. Importantly, the response over 16 generations was not significant in one of the selected lines and was only about 25% in another line. Thus, even in the line that responded to selection for the reproductive life span, increase in longevity was about 1.5% per generation.

When wild-derived strains are included in the population, the high degree of life span heritability suggests that selection for increased life span may be successful within a few generations. To succeed in selective breeding to increase maximal life span, many different biological systems must be simultaneously affected and a wide variety of diseases postponed. Increases in maximal life span may point not only to genetic but also to physiological mechanisms retarding rates of aging.

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