# Longer Life Spans and Delayed Maturation in Wild-Derived Mice

RICHARD A. MILLER,[1,*,†] JAMES M. HARPER,* ROBERT C. DYSKO, STEPHEN J. DURKEE,* AND STEVEN N. AUSTAD§

*Department of Pathology and Geriatrics Center and Unit for Laboratory Animal Medicine, University of Michigan School of Medicine, University of Michigan Institute of Gerontology, Ann Arbor, Michigan 48109; †Ann Arbor VA Medical Center, Ann Arbor, Michigan 48109; and §Department of Biological Sciences, University of Idaho, Moscow, Idaho 83844

**Nearly all the experimental mice used in aging research are derived from lineages that have been selected for many generations for adaptation to laboratory breeding conditions and are subsequently inbred. To see if inbreeding and laboratory adaptation might have altered the frequencies of genes that influence life span, we have developed three lines of mice (Idaho [Id], Pohnpei [Po], and Majuro [Ma]) from wild-trapped progenitors, and have compared them with a genetically heterogeneous mouse stock (DC) representative of the laboratory-adapted gene pool. Mean life span of the Id stock exceeded that of the DC stock by 24% ($P < 0.00002$), and maximal life span, estimated as mean longevity of the longest-lived 10% of the mice, was also increased by 16% ($P < 0.003$). Mice of the Ma stock also had a significantly longer maximal longevity than DC mice (9%, $P = 0.04$). The longest-lived Id mouse died at the age of 1450 days, which appears to exceed the previous longevity record for fully fed, non-mutant mice. The life table of the Po mice resembled that of the DC controls. Ma and Id mice differ from DC mice in several respects: both are shorter and lighter, and females of both stocks, particularly Id, are much slower to reach sexual maturity. As young adults, Id mice have lower levels of insulin-like growth factor 1 (IGF-I), leptin, and glycosylated hemoglobin compared with DC controls, implicating several biochemical pathways as potential longevity mediators. The results support the idea that inadvertent selection for rapid maturation and large body size during the adaptation of the common stocks of laboratory mice may have forced the loss of natural alleles that retard the aging process. Genes present in the Id and Ma stocks may be valuable tools for the analysis of the physiology and biochemistry of aging in mice.**

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[1] To whom requests for reprints should be addressed at Room 5316 CCGCB, Box 0940, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, MI 48109–0940. E-mail: millerr@umich.edu

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The traits that favor reproductive success in the laboratory differ in many respects from those that promote survival and fecundity in the natural environments that produced and continue to support wild populations of the house mouse *Mus musculus*. Modern research is conducted using laboratory mouse populations that have been raised under artificial conditions for many hundreds of generations (1), conditions that have led, without any deliberate efforts on the part of their caretakers, to alterations in physical, developmental, and physiological characteristics. Compared with wild-caught mice, laboratory-adapted mouse stocks are typically much easier to handle, are less inclined to bite, and tend to seek rather than to avoid cage-like enclosures. These adaptations are easy to understand: mice genetically predisposed to exhibit these characteristics are less likely to escape and thus more likely to produce progeny in the first few generations of confinement to laboratory housing. For similar reasons, genes that promote early reproductive maturity and large litters are particularly likely to increase in frequency in breeding programs in which each successive generation is derived, for the most part, from those mated pairs that produce large early litters soon after pairing, and in which each succeeding litter follows in quick succession. Selective pressures of this kind would be expected to lead both to rapid growth rate and to large body size, because rapid growth leads to relatively early reproductive maturity (2) and because large body size is a prerequisite to large litter size in females. Indeed, many previous reports have pointed out systematic increases in body size and in litter size in comparisons of laboratory-adapted rodent stocks with their closest wild-caught ancestors (2, 3).

Virtually nothing is known, in molecular terms, about the genetic changes that can bring about differences in longevity, developmental rates, and rates of aging among related species. As a rule, ecological niches that pose a relatively high risk of death around the age of reproductive maturation ("high-hazard" niches) select for genotypes in which early reproduction is favored. In contrast, low-hazard niches, in which survival to produce more than a single litter

is more common, can select for life history strategies that emphasize production of multiple litters, perhaps smaller ones, over a longer time span. A species whose reproductive strategy requires continued production of offspring over a relatively extended period of time will, of course, need to avoid neoplastic and degenerative illnesses for longer than would members of short-lived species whose reproductive efforts were more likely to be terminated by predation or early starvation; thus, low-hazard niches tend to promote extended longevity (4, 5). Inter-species comparisons make this point clearly: among rodents, for example, those species whose resistance to predation pressures is based upon recondite habitat (naked mole rat, [6]) or intimidating defensive armament (porcupine, [7]) are longer lived, both in the wild and in captivity, than rodents, like wild rats and mice, that are adapted to higher-risk niches. Field experiments in which predation rate was manipulated in guppies (*Poecilia reticulata*) have revealed that a mere 30–60 generations of increased predation was sufficient to evolve earlier sexual maturity, more offspring per litter, and more frequent litters (8). Studies of closely related populations of wild opossums have shown that even a relatively brief period of adaptation to a low-risk environment, such as the ~4000 generations of isolation that separate the Sapelo Island opossums from their mainland cousins, can lead to dramatic alterations in reproductive scheduling, progression of biochemical signs of aging, and survival curves (9).

Have the selective pressures that have molded the modern research rodent—pressures selecting for genotypes that produce rapid maturation and large litter sizes—led to the loss of naturally occurring alleles that diminish the maturation rate and (as a side effect) slow the aging process? To test this idea, we have performed a life span study of four groups of mice, three of which are only two generations removed from wild-trapped progenitors, and the fourth of which is derived from mouse stocks long adapted to laboratory breeding conditions. To avoid the confounding factor of inbreeding depression, i.e., the well-known depression of mean and maximal longevity seen in inbred populations when compared with their equally isogenic but comparatively heterozygous $F_1$ hybrid offspring (10–12), we have as our laboratory control used a stock of deliberately outbred mice derived from a four-way cross of common laboratory inbred stocks. We find that two of the three wild-derived lines are longer lived than the laboratory control stocks, and indeed that one of these lines (Idaho, "Id") seems to be longer lived than any previously studied captive mouse stock. Id mice also are slow to mature and have unusually low levels of insulin-like growth factor 1 (IGF-I) as young adults.

## Materials and Methods

**Mouse Stocks and Husbandry.** Lines Id, Majuro (Ma), and Pohnpei (Po) were derived from wild-caught progenitors as described in Miller *et al.* (13). Wild-caught animals were mated in the laboratory to produce the $G_0$ generation. $G_0$ mice were then mated, and newborn litters ($G_1$ generation) were transferred to foster mothers of the CD-1 stock (Charles River Laboratories, Wilmington, MA) to create a specific pathogen-free population. Serum samples from some of the $G_1$ mice, and from CD-1 sentinel mice caged from birth to weaning with $G_1$ pups, were tested for antibodies to each of 13 mouse pathogens and were found to be uniformly negative. Tests for pinworms and pinworm eggs in cecal samples and for fur mites were also negative. The $G_1$ mice were used for the longevity experiments reported in this paper. $G_2$ animals were produced from $G_1 \times G_1$ matings, $G_3$ mice from $G_2$ pairs, and so forth. In each generation, care was taken to minimize loss of genetic diversity by avoidance of sibling and (where possible) cousin mating, and by retaining, to the extent possible, equal numbers of mice from each matrilineal and patrilineal line. Nonetheless, some genetic drift is likely to have occurred because practical constraints limited the number of mated pairs in each generation (typically 6–11 pairs for Ma and Id stocks, and 25–31 pairs for Po mice). Mice of the $G_4$ generation were used for the tests of body size, tail length, hormone levels, hemoglobin glycation, and reproductive maturation.

The DC stock was produced in order to provide a genetically heterogeneous stock with a representative sample of genetic alleles from the laboratory-adapted inbred stocks most often used in aging and genetic research. Females of the (BALB/c × C57BL/6)F1 stock were crossed to males of the (DBA/2 × C3H/He)F1 stock to produce the $G_0$ generation. $G_0$ mice were then mated with one another to produce the $G_1$ generation, and so forth through successive generations. Mice of the $G_6$ generation were used as controls for the longevity experiment, and mice of the $G_{12}$ generation were used as controls for all other experiments.

**Longevity Experiments.** Mice of the $G_1$ generation for the Id, Ma, and Po stocks were housed in the Kresge Building at the University of Michigan (Ann Arbor, MI) until approximately 12–14 months of age, and they were then transferred to the Cancer Center and Geriatrics Center Building (CCGCB) vivarium for the remainder of their life span. The Id, Ma, and Po mice were chosen for the longevity study in such a way as to maximize the number of different parental and grandparental lineages represented. These mice were treated with the anti-helminth agent Fenbendazole (150 ppm in Harlan-Teklad Lab Diet 5001) for two 5-day periods just prior to their transfer to CCGCB because pinworm had been detected in sentinel mice within the Kresge Building. The Id, Ma, and Po mice were housed one per cage and were inspected at least daily to record dates of death. The DC controls for the longevity experiment lived in CCGCB from birth until death at an initial density of three to four per cage. Mice observed to be so moribund that an experienced technician thought it unlikely that they would survive more than a few additional days were euthanized for humane reasons. The mice were housed in polycarbonate cages with wire bar lids and microisolation

filter tops and corn-cob bedding ("Bed-O'Cobs,"; The Andersons, Maumee, OH). The mice were fed commercial rodent chow (Lab Diet 5001; PMI Nutrition International, Brentwood, MO) and were provided municipal tap water in glass bottles. The room was maintained at 74° ± 4°F with 10–15 fresh air changes per hour and a 14:10-hr light:dark cycle. The animal care program at the University of Michigan is fully AAALAC accredited.

A separate longevity analysis was conducted at the University of Idaho (Moscow, ID). The "Idaho-2" mice used in this study were all males, and were the second and third generation offspring of wild-trapped ancestors. The original founder stock, like the progenitors of the Id line, were live-trapped in barns and on farms near Moscow, Idaho. Wild mice were initially screened (Charles River Laboratory) for a standard panel of pathogens. All mice testing positive for pathogens were euthanized without being allowed to breed. The mice were weaned at 21 days of age, group-housed until 3 months of age, and then individually housed with a 12:12-hr light:dark cycle thereafter. All mice were *ad libitum*-fed Harlan Teklad 92051 diet throughout their life span. Although the mice were not kept under barrier conditions, there were no common pathologic lesions upon death that would suggest that infectious diseases were seriously affecting the health of the colony.

**Hormone Levels and Glycated Hemoglobin.** To control for circadian rhythmicity, all blood samples were collected between 0900 and 1200 hr via the puncture of the retro-orbital sinus with a heparinized capillary tube without anesthesia. After collection, all samples were transferred to 1.5-ml microfuge tubes and were allowed to clot for 2–4 hr at 4°C prior to the collection of blood sera for the hormone assays. Whole blood was used for the determination of total glycated hemoglobin (GHb). For the three hormone assays, all samples were tested in a single batch; intraassay coefficients of variation among replicate aliquots of pooled serum controls were <12%.

Serum thyroxine ($T_4$) levels were determined using a monoclonal solid phase radioimmunoassay (RIA) kit (ICN Pharmaceuticals, Costa Mesa, CA) according to the manufacturer's instructions except that all volumes were reduced by a factor of 4. Each sample was tested in duplicate using dilutions up to 1:2.3 with phosphate-buffered saline if necessary to achieve adequate sample volume.

Serum IGF-I levels were quantified via a double-antibody RIA kit (Diagnostic Systems Laboratories, Webster, TX) according to the manufacturer's instructions except that all volumes were reduced by a factor of 4. Prior to assay, 10 μl of serum from each individual was subjected to an acid-ethanol extraction procedure using the materials provided in the kit. Each sample was tested in duplicate.

Serum leptin levels were quantified with a double-antibody RIA kit (Linco Research, Inc., St. Charles, MO) according to the manufacturer's instructions except that all volumes were reduced by a factor of 4. Each sample was assayed in duplicate using dilutions up to 1:5.2 with phosphate-buffered saline if necessary to achieve adequate sample volume.

Total GHb was determined in 50 μl of whole blood via affinity chromatography (Procedure No. 422; Sigma Diagnostics, St. Louis, MO). All samples were processed according to the manufacturer's instructions using the materials provided.

**Date of Vaginal Opening.** Mice were examined daily from 15 days of age until vaginal patency was observed; ethanol-sterilized forceps were employed to provide optimal visualization of the relevant area.

**Statistics.** Survival curves were constructed using the Kaplan-Meier method, and intergroup differences were evaluated using both log-rank and Cox's $F$ test. The effect of gender on interstock differences in longevity was evaluated by a two-factor analysis of variance (ANOVA). Differences in rates of female sexual maturation were evaluated using the log-rank test. Differences in hormone levels, body size, and GHb were evaluated by one-factor ANOVA, followed by the Student Newman-Keuls test. The mean life span of the longest-lived 10% of mice in each group was taken as an estimate of maximal longevity. All statistical analyses used the Statistica software package (Statsoft, Inc., Tulsa, OK).

## Results

**Longevity Experiments.** Survival was measured in groups of 48–51 mice, approximately one-half males and one-half females, of four stocks of mice: Ma, Po, Id, and DC. Ma, Po, and Id animals were from the second laboratory-born generation derived from wild-caught grandparents. The DC mouse stock was developed to provide a laboratory-derived control stock that avoids both the inbreeding depression characteristic of fully homozygous stocks and the genetic uniformity of $F_1$ hybrid mice. Each DC mouse derives, on average, about 25% of its alleles from each of four common laboratory inbred stocks: BALB/c, C57BL/6, DBA/2, and C3H/He. Figure 1 shows the survival curves for these four stocks, and the first four lines of Table I show summary statistics for the longevity experiment. The survival curve for the Po stock closely resembles that of the laboratory-derived control stock DC. Survival of Ma mice is similar to that of DC until the age of about 900 days (60% mortality), but then diverges: mortality risks in the Ma stock seem to be somewhat lower than in DC mice after 900 days of age. Id mice were remarkably long-lived.

Differences in these life tables were evaluated using the log-rank test and using the Cox's $F$ statistic, which is particularly sensitive for experimental designs with small numbers ($n < 50$/group) and no censored observations (14). Both statistical tests showed that Id mice are longer lived than DC mice, with a 24% increase in mean life span ($P < 0.00002$ by the log-rank test and $P < 0.00001$ by Cox's test). The difference between Id and DC mice was more dramatic in female mice than in male animals (290 vs 90 days; the 'sex × group' interaction is significant by ANOVA at $P = 0.02$),

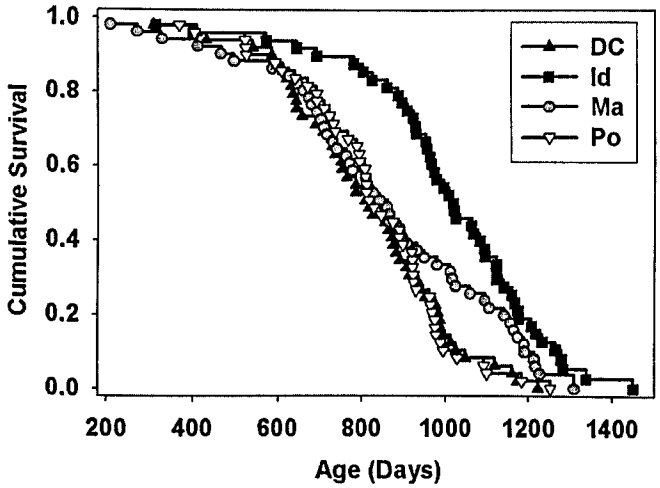


**Figure 1.** Survival curves for the four stocks of mice used in this project. Each symbol represents an individual mouse of the indicated stock. Two mice are not shown: an Ma mouse alive at age 1302 days, and an Id mouse alive at age 1313 days (as of January 8, 2002).

although the two longest-lived Id mice were both males (age at death 1338 and 1450 days). Statistical significance of the difference between DC and Ma mice is marginal, with $P = 0.06$ using the log-rank method and $P = 0.03$ by the Cox's test.

Differences among mouse stocks in aging rate is often evaluated by comparison of the maximum observed life span, although this number has been criticized because like any other extreme value statistic, it is highly unstable across replicate populations and is highly dependent on sample size (15). For the record, we note that the maximum life span in our Id population, 1450 days, was 19% higher than that of the similarly sized DC group. Calculating the mean life span of mice at the tail end of the survival curve provides a more robust measure of the potential effects of differential aging on overall survival, and Table I includes a calculation of average life span of the longest-lived 10% in each of the tested stocks. By this measure, both Id and Ma mice showed significantly higher extreme longevity than the DC mice; $P = 0.003$ for Id and $P = 0.04$ for Ma by the Student Newman-Keuls *post hoc* test.

Table I also shows summary statistics for a separate life table study of an independent population of mice, all males, derived from progenitors wild-caught in Idaho and raised from birth in a conventional (i.e., not specific pathogen-free) vivarium at the University of Idaho. Mice in this second Idaho-derived group were also long lived, with higher mean, median, and maximal longevity than the Michigan-raised DC group, and they rivaled Michigan-raised Id mice in the survival for the longest-lived 10% of the population. Although this population differed from the Michigan-based populations in several respects, it provides supportive evidence that the superior longevity of wild-derived mice may prove to be reproducible in different vivaria and with different initial sets of wild-trapped progenitor animals.

**Rate of Reproductive Maturation in Female Mice.** The age at which vaginal patency is observed can provide a useful and easily measured surrogate for maturation rate in female mice (16). Figure 2 shows the cumulative incidence of vaginal patency among groups of Id, Ma, and DC mice from generations $G_4$ (Id and Ma) or $G_{12}$ (DC); Po mice were not tested. Table II presents the summary statistics for this experiment. All three of the tested stocks differed from one another at $P < 0.0001$ using the log-rank test. Differences between Id and DC mice were dramatic with more than 90% of the DC females having achieved this milestone at an age at which fewer than 10% of Id females showed evidence of maturation. Ma mice were also slower to mature than DC females.

**Physical Characteristics.** In our previous publication (13), we have shown that Ma and Id mice were much lighter, at all ages from 1 to at least 8 months, than DC mice, and these differences were also observed in the current set of experiments (see Table III). To see whether the differences in body weight reflected corresponding differences in stature, i.e., in length, we measured both body length (from nose to base of the tail) and tail length at 6 months of age. These results are also shown in Table III. Both Id and Ma mice have shorter bodies and shorter tails than DC mice ($P < 0.0005$ by Student Newman-Keuls test); in addition, tail length is significantly shorter ($P < 0.0005$) in Id than in Ma mice.

**Endocrine and Glycation Assessments.** In search of insights into the biochemical basis for exceptional longevity in the Id and Ma mice, we measured the levels of thyroxine T4, leptin, and IGF-I in the serum of young adult mice (6 months of age), as well as the level of GHb as an indirect index of integrated glucose exposure. T4 and IGF-I were chosen because these hormones are particularly low in Snell dwarf and Ames dwarf mutant mice (17), and because

**Table I.** Summary Statistics from Longevity Experiments

| Group | Life span (days; mean ± SEM) (*n*) | Mean for males | Mean for females | Median | Mean life span for longest lived 10% | Maximum |
|---|---|---|---|---|---|---|
| DC | 811 ± 28.5 (49) | 909 | 723 | 816 | 1144 | 1223 |
| Id | 1006 ± 32.7 (48) | 1000 | 1013 | 1019 | 1323 | 1450 |
| Ma | 855 ± 37.4 (51) | 925 | 776 | 859 | 1245 | 1309 |
| Po | 827 ± 26.6 (49) | 829 | 824 | 827 | 1131 | 1252 |
| Idaho-2[a] | 888 ± 49 (39) | | | 985 | 1321 | 1403 |

[a] These mice were housed at the University of Idaho colony; all others were housed at the University of Michigan.

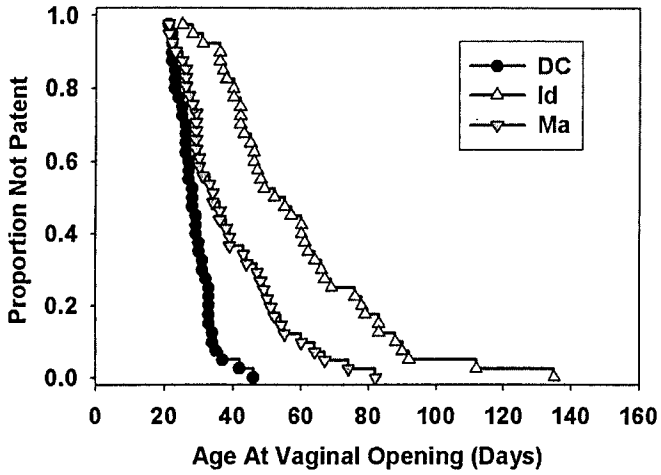


**Figure 2.** Proportion of female mice not showing vaginal patency at varying ages in groups of DC, Id, and Ma mice. Each symbol indicates an individual animal.

**Table II.** Age at Vaginal Patency for Female Mice

| Stock | Age (days; mean ± SD) (*n*) | Minimum (days) | Maximum (days) |
|---|---|---|---|
| DC | 28.9 ± 5.5 (40) | 21 | 46 |
| Id | 58.8 ± 23.5 (40) | 25 | 135 |
| Ma | 39.3 ± 15.1 (41) | 21 | 82 |

IGF-I and the thyroid hormones levels are low early in life in rodents subjected to an age-retarding regimen of caloric restriction (18–21). The results are presented in Table IV.

Serum IGF-I levels in Id mice were only about one-half of those seen in DC controls ($P < 0.0002$), consistent with the small body size of the wild-derived mice. The IGF-I level in the Ma mice, however, was reduced only 21% below that of DC, although the difference is still statistically significant ($P < 0.02$). The difference between Id and Ma mice is also significant at $P < 0.002$. In a separate study (not shown), IGF-I levels were also depressed in Ma and in Id mice compared with DC controls in serum samples obtained at 3, 4, 6, 8, and 12 weeks of age ($n$ = 5–6 mice/group), but these data did not achieve statistical significance. Mean IGF-I levels in C57BL/6, BALB/c, and B6D2F1 hybrid stocks were, respectively, 961 ± 98, 999 ± 87, and 640 ± 167 ng/ml (mean ± SD for $n$ = 6), suggesting that laboratory-derived stocks may in general have levels similar to that of DC mice and thus higher than the levels of Id animals. The small body size of Ma mice (see Table III, as well as weight data reported previously in [13]) may reflect a combination of factors beyond mere diminution of IGF-I levels in serum.

Table IV also shows that Ma mice have significantly lower levels of T4 than either DC or Id mice ($P < 0.0002$ by Student Newman-Keuls test in each case). The difference between DC and Id mice is much smaller and does not quite achieve statistical significance ($P = 0.06$). The levels in DC and Id are similar to those we have measured in young adult mice of the C57BL/6, BALB/c, and B6D2F1 hybrid stocks, for which means were respectively 4.9 ± 0.28, 3.8 ± 0.6, and 4.7 ± 1.3 (mean ± SD). In a separate study involving five to six mice/group, Ma mice were found to have similarly low T4 levels compared with DC and Id mice when tested at 3, 4, 6, 8, and 12 weeks of age (data not shown). Thus, Ma mice seem to have unusually low levels of thyroid hormone through the first 6 months of life.

Serum leptin levels, also shown in Table IV, were significantly lower at 6 months of age in Id mice than in DC controls (33% of the DC values, $P < 0.03$) or in Ma mice (24% of Ma; $P < 0.002$). However, the mean level of leptin in Id mice (3.1 ± 1.5 ng/ml) was not dissimilar from that seen in a separate analysis of serum from 6-month-old C57BL/6, BALB/c, and B6D2F1 hybrid mice, which were, respectively, 5.4 ± 1.3, 3.9 ± 1.0, and 2.8 ± 1.5. The variation in leptin levels among individual mice is quite high within each group, and a separate study of another group of mice at 3–6 months of age found no difference between Ma and Id animals (5.4 ± 1.0, $n$ = 18 for Ma and 4.8 ± 3.3, $n$ = 8 for Id mice). Clarification of these inconsistent results will require a more thorough study of the interactions of age, food intake, leptin, and adiposity in these stocks of mice.

Lastly, Table IV presents values for GHb levels measured at 6 months of age. Levels in Id mice are about 37% of those in the DC mice, but levels in Ma mice are almost twice that of the DC controls. Both of these contrasts are significant at $P < 0.0003$. Because the values in the Ma mice were surprisingly high, we repeated these measures on a separate set of blood samples taken from the same set of mice, and we found similar results (not shown). The values in C57BL/6, BALB/c, and B6D2F1 hybrid mice were, respectively, 3.6 ± 0.3, 21 ± 5.4, and 8.6 ± 1.6. Thus, the data show that levels of hemoglobin glycation vary widely among stocks of mice and that there is no clear cut correlation between glycation levels and stock life span, at least among these examples.

## Discussion

Selective pressures have great power to mold life history patterns both in natural and in artificial environments. These forces can lead to differences among species, and between local populations within a given species (8, 22, 23), in the age of reproductive competence, the size and spacing of litters, and the age at which fecundity begins to decline. The genes that control the timing of these key life history parameters must act through effects on hormonal and other developmental pathways whose nature is still poorly understood, and whose secondary effects on late-life illnesses and functional decline make them a topic of gerontological interest.

Nearly all of the mice and rats used for research are the products of a century-long process through which wild-trapped animals were converted, gradually, to a set of domesticated, highly inbred stocks whose characteristics made them of interest first to collectors and hobbyists and, later,

**Table III.** Body and Tail Length at 6 Months

| Stock | Body length (cm; mean ± SD) (*n*) | Tail length (cm; mean ± SD) (*n*) | 2-month body weight (g, mean ± SD) (*n*) | 4-Month body weight (g; mean ± SD) (*n*) |
|---|---|---|---|---|
| DC | 10.2 ± 0.51 (39) | 8.5 ± 0.40 (39) | 22.7 ± 3.1 (40) | 27.8 ± 3.9 (40) |
| Id | 8.7 ± 0.33 (16) | 7.3 ± 0.34 (16) | 13.3 ± 2.0 (40) | 16.3 ± 2.0 (26) |
| Ma | 8.6 ± 0.57 (33) | 7.7 ± 0.57 (33) | 13.5 ± 1.8 (41) | 16.2 ± 2.2 (41) |

**Table IV.** Hormone Levels and Glycated Hemoglobin

| Stock | IGF-I (ng/ml; mean ± SD) (*n*) | Thyroxine T4 (μg/dl; mean ± SD) (*n*) | Leptin (ng/ml; mean ± SD) (*n*) | Glycated hemoglobin (%; mean ± SD) (*n*) |
|---|---|---|---|---|
| DC | 591 ± 106 (18) | 5.00 ± 0.76 (18) | 9.4 ± 6.1 (15) | 10.3 ± 7.2 (38) |
| Id | 302 ± 111 (17) | 4.44 ± 1.20 (17) | 3.1 ± 1.5 (14) | 3.8 ± 0.6 (16) |
| Ma | 464 ± 186 (15) | 2.31 ± 0.51 (18) | 13.1 ± 10.8 (15) | 20.2 ± 5.8 (33) |

to research scientists (1). Some of the selective pressures were intentional, including retention of animals with unusual coat colors, and later, retention of animals unusually prone to spontaneous neoplasia. The domestication process also favored genetic alleles that led to docility, reduced behaviors that favored escape or diminished recapture, or favored reproductive success in unnatural environments with limited spatial scope, unrestricted access to food, infrequent opportunities for male/male conflict, and unvarying light/dark cycles. The effects of husbandry practices on life history traits are of particular relevance because the relative poor success rate of mated pairs early in the domestication process will have favored retention of alleles that produce early, large litters and short inter-litter intervals.

After domestication, laboratory rodents were inbred to produce a consistent and genetically uniform source of rodents, useful for replication of experimental findings and for examining the genetic bases for inter-strain differences in physiological and pathological traits. However, the inbreeding process is itself highly selective because alleles that might produce advantages when heterozygous can be lethal or can impair fertility when homozygous. The inbred stocks (and their $F_1$ hybrid offspring) used for most aging research thus represent a still farther departure from the genotypes present in natural populations of mice.

The longevity experiment presented in Figure 1 and Table I was designed to test the idea that domestication and inbreeding might have eliminated from the genome of the laboratory mouse alleles that retard the rate of aging and thus diminish mean and maximal longevity. Three wild-derived stocks were used, two of which (Ma and Po) were derived from islands in which low predation pressure and mild climate might in principle have relaxed the pressure for early reproduction in natural mouse populations in less temperate circumstances. Use of an inbred laboratory line as the control group would have been inappropriate because of the well-documented "hybrid vigor" effect, i.e., the observation that $F_1$ hybrid stocks nearly always live longer than inbred lines from which they were produced (10–12). For this reason, rather than selecting any single $F_1$ hybrid as a putatively representative laboratory control, we have constructed a genetically heterogeneous line, DC, in which each individual animal receives 25% of its alleles from the C57BL/6, BALB/c, C3H/He, and DBA/2 inbred strains. It is possible that the three wild-derived stocks might differ from one another, and from the DC stock, in the proportion of heterozygosity and in associated hybrid vigor; small founding populations and loss of heterogeneity by genetic drift may have contributed to the relatively short life span of Po and Ma mice compared with the Id stock.

The results show that the wild-derived line Id has higher mean, median, and maximal life span than the DC laboratory-derived stock, and that a second wild-derived line, Ma, also contains a high proportion of unusually long-lived individuals with a significantly higher maximum life span (estimated as mean survival of the longest-lived 10%). The unusual longevity of wild-derived mice was also documented in a separate stock of Idaho-derived mice housed in a conventional vivarium at the University of Idaho.

We believe that these results reflect unusually long life spans in the Id and Ma mice, rather than an unusually short life span in the DC control stock. Although historical controls are far less convincing than contemporaneous controls for longevity experiments, an examination of other longevity studies can help to put our own findings in perspective. Among the 59 inbred and $F_1$ hybrid stocks for which longevity records have been compiled by the Jackson Laboratories (24), no stock exceeded a mean longevity of 967 days for either males or females as compared with 1006 days for the Id stock in this report. Among the 20 recombinant inbred stocks studied by Gelman *et al.* (25), the longest lived (BXD19) had a mean life span of 904 days. A group of 15 genetically heterogeneous stocks studied at the University of Michigan CCGCB colony included none with a mean life span above 941 days, even though six of these stocks had been selectively bred for a trait, small body size, associated with longer life spans (26). Among the 21 studies compiled in an analysis of the effects of caloric restriction on life span (18), no group of fully-fed control mice had a mean lon-

gevity that exceeded 906 days (see Table 2.5, of Ref. 18), except in one group of B10C3F1 females whose food intake was slightly restricted (to 105 kcal/week) below *ad libitum* intake. Except for one mouse in a study of the long-lived Snell dwarf mutant, (27), we are also unaware of any reports of a fully fed mouse surviving for longer than the 1450-day life span of mouse IdG1-030, the longest-lived animal in the Id group. Among the fully fed control lines compiled in (18), for example, the longest recorded life span for an individual mouse was 40 months (1208 days), a value exceeded by 8 of the Id mice and 5 of the Ma mice. Among the many stocks studied by David Harrison and his colleagues (D.E. Harrison, personal communication), the longest-lived individual was the product of a four-way cross whose progenitors included mice from an inbred stock of the subspecies *Mus musculus molossinus*. The longest-lived animal in this four-way cross died at the age of 1386 days (28), a value surpassed in each of the two studies of Idaho-derived mice reported in Table I. Thus, we are confident that our results in Table I document unusually long survival of mice of the Id stock, rather than an atypically short-lived control group.

We have reported previously (13) that Id and Ma mice are dramatically (i.e. about 2-fold) lighter in weight than DC mice from 1 month of age through at least the 8th month of life, and here we report that these differences reflect body length and tail length rather than merely effects of adiposity. These findings are consistent with the idea that factors regulating early life growth trajectory may have secondary effects on late life processes, including disease risks, that limit life span. Studies of genetically heterogenous, laboratory-adapted mouse stocks (26) have shown that small stocks tend to be longer-lived than larger ones, and small size is associated with longer life span in dwarf mice (27, 29, 30), in calorically restricted rodents (18), in methionine-restricted rats (31), and among breeds of dogs (32, 33). It will be of interest to see whether small size is associated with longer life spans among individual members of segregating crosses between Id and laboratory-derived stocks.

We have not measured food intake in our wild-derived stocks, but we are reasonably confident that these mice will consume less food than mice of the much larger DC stock (and other laboratory-derived stocks) because larger mice require more fuel for maintenance of body temperature. An association between low food intake and small body size is seen in calorically restricted mice and is expected in wild-derived mice, but the direction of causality is opposite in the two situations. In the former case, experimental deprivation of food causes small size; in the latter, genetic alleles that produce small size are expected to diminish caloric requirements and hence food intake. The extent to which small body size per se might contribute to the deceleration of aging rate in these two experimental models is an open question.

Our initial analyses of hormonal and biochemical parameters among these stocks provide some new insight into a number of proposed mechanistic hypotheses about the control of life span in rodents. The low levels of thyroxine and IGF-I in the long-lived Snell and Ames dwarf models (17), as well as in young adult mice on calorically restricted diets, have hinted that early life levels of these two hormones may influence aging rate in rodents. Our longest-lived stock, Id, shows a clear and significant decline in IGF-I levels, but little if any decline in T4, lending support to the idea that diminished IGF-I may be of particular importance in life span regulation. Such a conclusion would be consistent with the observations of increased longevity in two mouse mutants in which GH/IGF-I abnormalities are seen without specific defects in the TSH/T4 axis (27, 30). It is also noteworthy that small dogs, which are longer lived than dogs of larger breeds (33), also tend to have relatively lower levels of serum IGF-I (34, 35). T4 levels are abnormally low in mice of the Ma stock, but show little if any difference between Id and DC control mice.

Alterations in metabolism of sugars and lipids may contribute to the life span extension seen in calorically restricted and dwarf mice. GHb, which provides an indirect measure of integrated glucose control, is lower in calorically restricted rodents (36–38) than in normally fed control mice. GHb levels were indeed somewhat lower in Id mice than in DC, but were similar to levels seen in the standard laboratory inbred strain C57BL/6. In contrast, Ma mice were found to have unusually high GHb values. Further analysis of glucose and insulin levels, as well as estimation of erythrocyte turnover, would be needed to determine if the high level of hemoglobin glycation seen in the Ma animals is indeed due to relatively high mean serum glucose levels, but at face value, the data suggest that diminution of blood glucose may not be a requirement for longevity extension in mice. Similarly, more detailed analysis of leptin and other regulators of lipid metabolism and adipose tissue mass will be required to determine what role, if any, these circuits may play in life span determination. We have shown previously (13) that Id and Ma mice have elevated glucocorticoid levels when compared with DC mice, and in this respect mimic the high glucocorticoid levels noted in calorically restricted rodents (39), but high glucocorticoid levels were also seen in Po mice, which are not particularly long lived (see Fig. 1). Analyses of gene expression profiles may help to provide a catalog of alterations that distinguish long-lived stocks of mice from short-lived ones.

The outcome of a longevity experiment will be influenced not only by genetic and environmental factors, but also potentially by interactions between genotype and environmental variables. It is possible, for example, that the stresses associated with transfer of a wild-captured animal to laboratory housing might either impair or even improve its expected life span. Our design, which used mice whose grandparents were wild-caught but whose parents were born and raised in the laboratory, was intended to mitigate such factors, but cannot exclude the possible effects of genetic factors that might render a specific stock more or less

healthy under vivarium conditions. It is possible, for example, that prolonged selection for rapid growth and maturation under laboratory conditions may have provided laboratory-derived stocks, like DC, with traits, such as an ability to detoxify or to absorb specific components of lab chow diets, that increase overall health and thus improve longevity of these control mice in the laboratory setting. Testing hypotheses of this sort would be difficult, expensive, and unrewarding, but in this context, it is all the more remarkable that Id and Ma mice should survive so well in an environment to which the ancestors of DC mice have been strongly selected for hundreds of generations.

At the outset of this study, we proposed (13) that mouse stocks derived from animals trapped in low-hazard environments (in this case the Po and Ma stocks) might be longer lived than stocks derived from wild-caught mainland mice such as the Id stocks. This idea was based in part on the idea that niches with low predation pressure and year round food abundance might relieve some of the selective pressure for rapid sexual maturation, and in part on the analogy to field studies of aging in island opossums (9). In the event, though, the longest lived of the three wild-derived stocks was that from the Idaho-trapped mice, and one of the two island stocks, Po, was indistinguishable from laboratory mice in its survival pattern. It is possible that the wild mouse populations on Pohnpei and on Majuro may have been derived from only a small number of founder animals, with low intrinsic genetic variability, followed by subsequent further random loss of polymorphic alleles. If so, the longevity of these mice may be limited by the same inbreeding depression noted in studies of laboratory inbred strains. It is also possible, as noted just above, that wild-derived stocks may in general be at a disadvantage compared with laboratory adapted stocks in physiological functions required for optimal health in captivity. In any case, a study using only two island-derived and one mainland-derived stock is too small to permit strong inferences, and additional longevity studies involving mice trapped on two other islands and at additional mainland locations is now under way.

Our data support the pessimistic interpretation that laboratory-adapted stocks of rodents may be particularly inappropriate for the analysis of the genetic and physiological factors that regulate aging in mammals. If evolution of mouse stocks for maximal fitness under laboratory breeding programs does indeed involve the loss of natural alleles that decelerate the maturation process and, perhaps as a pleiotropic effect, postpone the late life diseases that determine the mortality curve, then analyses of aging in laboratory-derived mouse and rat stocks may be unable to provide much information about the mechanisms that time aging in mammals except under the highly artificial conditions of the laboratory. It is noteworthy that heritability of longevity appears to be substantially larger in segregating crosses in which at least one of the grandparental stocks was inbred without a long period of laboratory domestication (28) compared with similar crosses among long-domesticated stocks.

Female mice of the Id stock are unusually slow to achieve reproductive maturity (Fig. 2 and Table II), and it is tempting to ask whether the rates of maturation and aging are under coordinated genetic control in this instance. We are currently measuring both maturation rate and longevity in segregating crosses between Id mice and laboratory-derived stocks to test the hypothesis that individual mice that mature slowly will prove to be relatively long lived. Age at sexual maturity is directly related to longevity among species of field populations of mammals (40). Longer life has previously been shown experimentally to correlate genetically with delayed age at sexual maturity and smaller body size in *Caenorhabditis elegans* (41) and fruit flies (42). Slower aging is also associated with delayed maturity rate in calorically restricted rodents (43).

Current ideas about the evolution of differential aging rates among species emphasize the importance of selective pressures, imposed on fitness characteristics in the juvenile period and among young adults, whose influence on aging represents secondary or pleiotropic effects. Genetic loci that influence the rate of growth and rate at which sexual competence is achieved are likely to be especially important mediators of adaptation to niches in which changes in intrinsic mortality rates permit decelerated life history schedules. Some models of natural selection (44) suggest that slower growth rates may allow commitment of metabolic resources to improved maintenance and repair pathways that in turn slow aging and prolong the period of disease resistance, but these models do not provide clear insights into the specific molecular mechanisms involved. If analysis of segregating stocks with Id and laboratory-derived progenitors shows a strong correlation between maturation rate and longevity in individual mice, these crosses will provide powerful tools for dissection of the relevant biochemical and hormonal control points that link early life history traits to disease resistance and hence survival in later life.

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