Journal of Gerontology: BIOLOGICAL SCIENCES
2000, Vol. 55A, No. 9, B455–B461

Differential Longevity in Mouse Stocks Selected for Early Life Growth Trajectory

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Small body size is associated with superior longevity in several intraspecies comparisons, including dogs bred for specific forms of work, mice and rats fed diets low in calories, rats fed diets low in methionine, and mutant mice whose levels of growth hormone and thyroid hormone are atypically low. To further investigate the interactions among body size, genetic endowment, and longevity, we measured the life span of female mice selectively bred from Institute for Cancer Research stock for differences in rate of body weight gain. These mice were selected for differential rates of growth either early (0–10 days) or later (26–56 days) in the first 2 months of life. The data show a good correlation between the average weight of the stock and its mean longevity, with low body size associated, as predicted, with longer life span. Weight at 3, 6, and 12 months, and weight at peak body weight, are all significant predictors of longevity (among stocks) in univariate regressions; weight at 6 months has the strongest association in stepwise multiple regression. There is no significant correlation between the life span for the stock and the proportion of deaths attributable to neoplasia in this group of mice. The data provide support for the hypothesis that genetic factors that influence early life growth trajectories can have a strong influence on life span. These size-selected mice provide useful tools for analysis of the genetic factors that influence life history parameters, including maturation and aging rates.

SOME authors have suggested that genetic factors influencing longevity and disease risk in later life represent pleiotropic effects of polymorphic alleles selected to improve reproductive fitness earlier in life (1–3). Despite considerable useful speculation, there is little direct evidence about the number and nature of loci whose effects on early development and fitness actually influence longevity and late life disease risk.

Several instances have been reported where differences among individuals in body size are associated with differences in longevity within species. In each case, superior longevity is associated with smaller body size. In some cases the differences in body size are a result of dietary manipulation, as in studies of caloric restriction in mice and rats (4) or lifelong methionine deprivation in rats (5). In other circumstances the differences in body size reflect differences in single genetic loci, as in the df/df dwarf mice (6) and the urokinase knockout mice (7). Differences in body size, associated with altered longevity, can also be produced by natural selection: among dogs, for example, differences among breeds in body weight are very strongly correlated to interbreed differences in mean life span (8), and similar correlations have been noted in populations of flies (9), whose size differences represent selected genetic adaptations to specific ecological niches.

To seek further insights into the relationship between body size and life span, we have examined longevity in a series of 15 mouse stocks that have been selected over 22 generations for differential rate of body weight by restricted index selection (10). Three of the stocks (EB = "early big") were selected for rapid gain in body weight from birth to 10 days of life, combined with average weight gain from 28–56 days. Three others (LB = "late big") were selected for high weight gain from 28–56 days, combined with average gain from day of birth to day 10. Three stocks (ES = "early small") were selected for exceptionally slow weight gain from day of birth to day 10, with average gain at 28–56 days. Three stocks (LS) were selected for slow gain from day 28–56, and average gain at 0–10 days. Lastly, three control stocks (C) were bred in parallel, from the same initial pool of heterogeneous progenitors, without deliberate selection for weight gain trajectories.

This report presents life span and pathology data for mice in these 15 size-selected mouse populations.

Materials and Methods

Mouse Selection

Foundation of these selection lines derives from 180 litters of ICR (Institute for Cancer Research, London, England) mice obtained in 1986 from Sprague-Dawley (Indianapolis, IN). These litters, produced from 261 female and 256 male founders, were randomly allocated into three replicates for each selection and control strain, producing a total of 15 lines. Each replicate contained 12 litters. At each generation mice were selected according to a restricted selection index procedure that ensures that growth at later intervals is held constant when selection is focused on early rate of development in body weight, and vice versa (10). Within-family selection was practiced to minimize maternal effects. Litters were standardized at birth to eight pups and an equal sex ratio. Litters with fewer than eight pups were augmented with excess pups from other litters. These excess pups were tail-clipped to distinguish them from the original members

of the litter. The substitute mice did not enter into any calculations for experiments. All mice were forcibly weaned at 21 days of age.

Two selection treatments (E) focused on increased or decreased rate of development in body weight from birth to 10 days of age. During this interval, postnatal growth is most influenced by changes in cell number (hyperplasia) in relevant organs and tissues (11–13) (and Atchley et al., unpublished data). The other two selection treatments (L) focused on increased or decreased rate of development in body weight between 28 and 56 days of age. During this interval, growth is more influenced by changes in cell size (Atchley et al., unpublished data).

The three replicated control lines were randomly selected using a random number generator to produce a pseudo selection index score. Cellular analyses of these mouse stocks indicate that for several organ systems, selection for rate of body weight gain between birth and 10 days of age changed the number of cells (hyperplasia), whereas selection for rate of gain between 28 and 56 days of age altered cell size (hypertrophy) (Wei et al., unpublished data).

The three replicated stocks designated here as EB (early big) were those selected for rapid weight gain between birth and 10 days of age while holding late gain constant. The three replicated LB (late big) strains were selected for high weight gain from 28–56 days holding gain between birth and 10 days of age constant. Three replicated ES stocks (early small) were selected for decreased rate of weight gain from day 0–10 holding 28–56 day gain constant and three replicated LS (late small) stocks were selected for decreased gain from day 28–56 holding 0–10 day gain constant.

Previous data (10) have shown that various EB and LB replicates had statistically indistinguishable mean body weights, tail lengths, and organ weights at 56 and 91 days of age. These authors described this phenomenon as developmental homoplasy (structural resemblance due to convergent evolution rather than common ancestry) to reflect that the same complex morphological phenotype can be produced by quite different genetic and developmental processes. The impact of selection in these mice on maternal effects, organ size, and other attributes is described separately (14) and in a series of submitted manuscripts (Ernst et al., 1999a, 1999b; Crenshaw et al., 1999; Atchley et al., 1999).

Method

For the present longevity experiment, weaned female mice were produced from the selection colony at North Carolina State University and shipped at about 25 days of age to the University of Michigan. At Michigan the mice were housed in a specific pathogen-free colony and observed at least daily until their natural death. Mice were housed initially at 3–4 mice/cage and given free access to Purina Mouse Chow and tap water. All mice were housed in the same room throughout the study period. Mice were weighed once a month beginning at 6 months of age. Each cage was protected by a filter-paper bonnet to minimize the risks of airborne infection. The pathogen-free status of the colony was documented every 3 months using a procedure in which spent bedding from the experimental mice was provided to sentinel mice of the outbred CD1 stock, and the sentinels later tested for antiviral antibodies and for parasites; all such tests were negative throughout the course of this study.

Mice found dead were subjected to a careful gross necropsy, and to a detailed histopathological examination using methods that have been described in detail elsewhere (15).

Results

Relation of life span to interstock differences in body weight.—Table 1 provides the mean (±SD), median, and maximal observed longevity, as well as mean weight at 6 months and the peak weight for each of the 15 stocks. The sample sizes used to calculate the mean weights are slightly lower than those used in the longevity calculations because three mice received as weanlings died before the first weight determination at 6 months of age. The 15 stocks are listed in descending order ranked by mean life span.

There has been significant direct and correlated response to selection for differential early and late rates of development in these mice. For example, there has been a 2.7-fold range (from 344 to 941 days) in mean stock life span, and a 2.0-fold range in peak body weight (33 to 65 grams). Thus, while these stocks were derived from a common random-bred ICR stock of progenitor mice, the various selection lines have undergone significant genetic divergence in a number of characteristics.

A protected least significant difference test (16) was carried out to partition the mouse strains into statistically homogeneous subsets for each trait. For 6 months body weight, there are five homogeneous subsets. The homogeneous subset containing the five heaviest mouse strains includes strains LB3, LB1, LB2, EB2, and EB3. Thus, those mice selected for gain in body weight from 28–56 days are the heaviest at 6 months of age, followed by two of the strains selected for gain from birth to 10 days of age. For average longevity, there are five statistically homogeneous groups including (from longest to shortest lived): (i) LS2, EB1, and C2; (ii) EB1, C2, LS1, C1, LS3, EB3, and LB2; (iii) LS1, C1, LS3, EB3, LB2, and ES3; (iv) LS3, EB3, LB2, ES3, ES2, LB1, ES1, and D3; and (v) C3, EB2 and LB3.

Figure 1 shows a scatterplot relating mean life span for each stock to mean body weight at 6 months of age. In this plot, the area of the circle is proportional to the number of individual mice tested from each indicated stock. Using the means of each stock, univariate linear regression analysis shows that weight at age 6 months is a good predictor of mean life span of the stock ($r = .69$, $p = .004$). This plot suggests that low body weight is associated with increased life span. Maximum observed life span per stock is also strongly correlated with weight at age 6 months ($r = .74$, $p = .002$; scatterplot not shown), although the value of this result is seriously compromised by the small numbers of mice studied in each stock. Weight at age 12 months ($r = .57$, $p = .03$) and lifetime peak weight ($r = .61$, $p = .02$) are associated with stock mean life span almost as strongly as the weight at 6 months, and indeed among the 15 stocks these three estimates of stock size are strongly correlated, with Pearson r correlations between .91 and .97.

Table 1. Life Span and Weight Statistics for 15 Mouse Stocks

Stock	Mean LS	*n*	Life-Span Cluster	Median	Min	Max	Weight (6 mon)	*n*	Weight-6 Cluster	Rank (a)	Weight (Peak)	*n*	Peak Wt Cluster
LS2	941 ± 117	15	a	905	804	1146	29 ± 2.4	15	ef	14	36 ± 1.5	15	g
EB1	832 ± 212	9	ab	899	304	1035	40 ± 5.7	9	bc	7	52 ± 7	9	de
C2	827 ± 150	17	ab	879	568	1043	32 ± 2.6	17	de	12	37 ± 2.5	17	g
LS1	785 ± 221	15	bc	825	150	1029	35 ± 3.2	14	d	10	47 ± 3.8	14	f
C1	777 ± 139	19	bc	783	561	1136	34 ± 3.0	19	d	11	43 ± 2.9	19	
LS3	719 ± 250	12	bcd	802	362	1149	29 ± 3.5	12	f	15	38 ± 4.4	12	g
EB3	704 ± 157	15	bcd	731	327	976	46 ± 6.3	15	a	5	65 ± 9.1	15	a
LB2	693 ± 147	6	bcd	700	521	854	49 ± 2.8	6	a	2	53 ± 2.3	6	ce
ES3	669 ± 177	12	cd	687	209	888	32 ± 2.9	11	def	13	33 ± 10.5	12	g
ES2	664 ± 187	21	d	627	321	1074	39 ± 3.3	21	c	9	49 ± 5.2	21	ef
LB1	615 ± 211	14	d	663	244	889	49 ± 4.9	14	a	3	58 ± 5	14	bc
ES1	592 ± 213	15	d	513	237	954	39 ± 4.5	15	bc	8	51 ± 5.4	15	ef
C3	572 ± 168	5	de	593	299	759	43 ± 4.8	5	b	6	57 ± 6.2	5	bd
EB2	473 ± 162	23	e	395	289	791	48 ± 5.1	23	a	4	55 ± 6.6	23	cd
LB3	344 ± 114	4	e	363	188	462	51 ± 12.7	3	a	1	61 ± 4	3	ab

Note: Letters in the cluster columns indicate groups that are not statistically different from one another.

We also examined the association between stock life span (measured in this study) and measures of weight at 91 days based on mice at Generation 19 housed at North Carolina State University (Crenshaw and Atchley et al., unpublished data). This weight measure, too, was strongly associated with mean stock life span ($r = 0.59$, $p = 0.02$), consistent with the interpretation that the associations noted above were not dependent on any artifacts of shipping stress or idiosyncrasies of the husbandry procedures used at the University of Michigan vivarium.

Body weights at various ages are not statistically independent. Consequently, we performed a stepwise multiple regression analysis, with mean stock life span as dependent variable and weight at 6, 12, and peak as potential independent variables. The stepwise regression procedure (16) adds body weight variables in terms of their ability to predict the dependent variable (life span in this case). At each step, a new predictor variable is added based upon its partial correlation with the dependent variable. The goal is to produce a predictive equation that gives the best prediction of the dependent variation using the smallest number of predictor variables.

Table 2 gives the stepwise regression results and shows that body weight at 6 months is the best predictor of stock life span ($t = -2.40$, $p = .035$). Body weight at 12 months is positively correlated with stock life span, though at only marginal significance ($p = .099$), after the correlation with body weight at 6 months is removed. Thus, although low body weight is associated with increased longevity, the ability to maintain or increase body weight between 6 and 12 months may also be associated with increased resistance to late-life illness and early death.

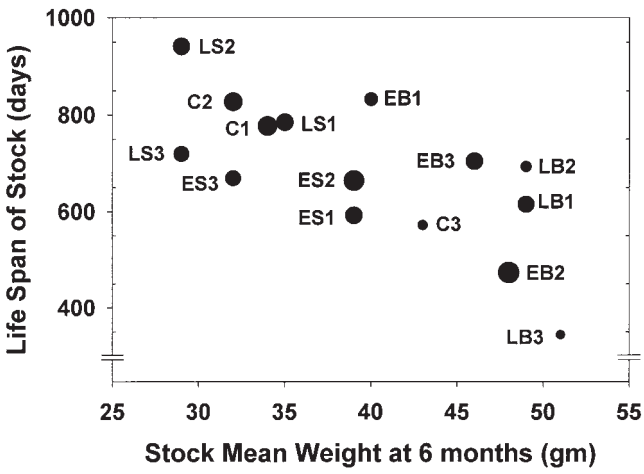

Figure 1. Association of longevity with weight across mouse stocks. Each symbol represents the mean weight at 6 months for mice of the indicated stock and the mean longevity of mice of that stock. The area of each symbol is proportional to the number of mice tested, which varies from 4 mice (stock LB3) to 23 mice (stock EB2). There is a significant correlation between these measures ($r = .69$, $p = .004$).

Within selection treatments.—Some interesting trends are evident within selection treatments (i.e., among replicates for a given selection protocol). For example, among the stocks selected for rapid gain between birth and 10 days, EB2 and EB3 do not differ significantly in 6-month weight, but EB1 had a significantly smaller body weight. In terms of life span, EB1 was longer lived than EB2 and EB3. This dif-

Table 2. Stepwise Multiple Regression Analysis

	Beta*	Standard Error of Beta	*b*†	Standard Error of *b*	*t* (11)	*p* Level‡
Intercept			1149	159.4	7.21	.000
Weight, 6 months	–1.29	.54	–25.6	10.7	–2.40	.035
Weight, 12 months	1.61	.89	31.5	17.5	1.80	.099
Peak weight	–.99	.79	–15.6	12.4	–1.26	.235

*Standardized regression coefficient.

†Nonstandardized regression coefficient for equation in which stock life span is the dependent variable and independent variables are weight (at 6 months), weight (at 12 months), and peak weight.

‡Significance level for the regression coefficient for each independent variable; only weight (at 6 months) is significant at $p < .05$.

ference in longevity is not statistically significant, but this lack of significance could be due to the small sample size in EB1 ($n = 9$).

In the three control replicates (C1, C2 and C3), random mating occurred within each replicate stock. The small population size (12 pairs per generation) might be expected to lead to stochastic variation in trait means despite the absence of directional selection. Indeed, there are significant differences in 6-month weight in these three lines, with C3 being significantly heavier than C1 and C2 (these latter two strains being not significantly different for the trait). With regard to longevity, C1 and C2 do not differ significantly (C1 = 777 ± 139 days and C2 = 827 ± 150 days). C3, on the other hand, had a mean longevity of 572 ± 168 days), which was significantly less than seen in C1 and C2. Thus, within the randomly selected control replicates, as in the set of EB stocks, there seems to be an inverse relationship between body weight at 6 months of age and longevity (i.e., the significantly heavier C3 replicate has significantly shorter average life span).

Within genetic strain analyses.—Our current data set provides only limited statistical power for testing the hypothesis that body weight is associated with longevity among individual mice within strains. This lack of power stems from small sample sizes, as our test group contained only 3–23 evaluable mice in any one stock. Among the nine stocks where $N \geq 14$, there were no significant correlations between mouse longevity and mouse weight at 6 months, nor any stocks in which $r > .50$; among these nine stocks, the slope of regression line was negative in six cases.

We also sought evidence of a correlation between individual mouse life span and body weight. As expected, a regression between mouse weight at 6 months and mouse life span was highly significant ($r = -.42$, $p < .0001$, $N = 199$), reflecting the strong interstock differences in both life span and weight. When individual mouse weights were normalized, however (i.e., expressed as the number of standard deviations above or below the mean weight for the given stock), the relationship between normalized weight and longevity was small and not statistically significant ($r = .05$, $p = .45$). Thus, within the limits imposed by our population sizes, the factors that influence the strong correlation among stocks between body weight and life span do not seem to have appreciable effect among mice within these stocks.

Impact of inbreeding on longevity.—Even with optimal breeding methods, any long-term selection experiment inevitably increases the level of inbreeding among the selected and control stocks. The level of inbreeding may influence longevity because of increased levels of homozygosity, the accumulation of alleles of reduced fitness, and related effects. We have computed estimates of the levels of inbreeding in females in these selection lines using the INBREED Procedure in the SAS Statistical Package (SAS Technical Report P-229, SAS/STAT Software: Changes and Enhancements, Release 6.07, Cary, NC). These values range from 0.232 in LS2 to 0.363 in LB1. A univariate regression of life span on inbreeding coefficient yielded $r = .33$ ($p = .23$). A scatterplot of inbreeding coefficient versus longevity for the 13 stocks with $N > 5$ mice showed a strong association between inbreeding coefficient and longevity among 11 of the stocks, with two outliers (EB2 and ES1). This association between high longevity and low inbreeding coefficient, which did not reach statistical significance in this group of stocks, is consistent with many previous analyses of the impact of inbreeding on longevity.

To see if the association between stock weight and longevity was influenced by interstock differences in inbreeding, we calculated a multiple regression using weight (at age 6 months) and inbreeding coefficient as independent variables and stock life span as the dependent variable. The effect of weight in this regression equation was highly significant ($r = .70$, $p = .01$), but there was no significant effect of inbreeding coefficient ($p = .62$). We conclude that the weight/longevity association does not reflect an unsuspected influence of inbreeding on these two factors.

Necropsy results.—Of the 202 mice originally entered into this study as weanlings, 181 were discovered soon enough after death to make a necropsy potentially informative. Of these 181 cases, 17 (9%) were uninformative, in that no conclusion could be drawn as to likely cause of death, usually due to advanced autolysis. In eight of the remaining cases, death was attributed to two serious diseases, one neoplastic and one not neoplastic, under such circumstances that it was not possible to assign either disease, by itself, as the likely cause of death.

A synopsis of the remaining 156 diagnoses is presented in Table 3. Among the 156 diagnosable cases, 5 different diagnoses each accounted for at least 5% of the deaths: lymphoma (29 cases), pituitary adenoma (19 cases, of which 16 were found in EB2 mice), congestive heart failure (15 cases), fibrosarcoma (11 cases), and pulmonary adenocarcinoma (10 cases). Thirty-six mice (23%) died of non-neoplastic lesions, in addition to the 15 that died of congestive heart failure; these lesions included cases of glomerulonephritis ($n = 6$), ovarian hematoma ($n = 4$), and dermatitis ($n = 4$), in addition to a variety of less common inflammatory conditions (pancreatitis, osteomyelitis, peritonitis, pneumonia with sepsis, etc.), and five cases in which combinations of non-neoplastic diseases contributed to death.

Among the 156 diagnoses, neoplasia was responsible for 105 deaths (67%), but the proportion of deaths due to neoplasia varied among stocks from 20% (1 of 5 cases in LB2) to 86% (6 of 7 deaths in EB1). Averaged across the 15 stocks, the mean proportion of deaths attributable to neoplasia was 63%. We used the proportions test of S-Plus (MathSoft, Inc., Seattle, WA) to test the null hypothesis that the proportion of deaths due to neoplastic lesions did not differ among the 15 stocks, and obtained a marginal $p = .17$. We therefore cannot conclude that these stocks differed among one another in this trait. A plot of mean longevity versus the proportion of deaths due to neoplasia revealed no evidence of an association between these two measures.

The number of deaths due to any single cause in any one stock was in most cases too low to provide much statistical power for testing hypotheses of stock-specific mortality patterns. The two exceptions are shown in Table 3. Among the 19 cases for stock EB2 mice, 16 deaths were attributable to

Table 3. Necropsy Results

Stock	Cases*	Diagnoses†	% Neoplastic‡	% Pituitary Adenoma	% Lymphoma
C1	19	17	82%	0%	18%
C2	14	13	54	0	8
C3	5	4	50	0	0
EB1	9	7	86	0	14
EB2	21	19	84	84	0
EB3	14	11	82	0	27
ES1	13	13	62	0	54
ES2	15	11	64	0	36
ES3	12	10	50	0	20
LB1	13	9	78	0	33
LB2	5	5	20	0	20
LB3	3	3	67	0	0
LS1	14	14	79	14	7
LS2	15	13	54	8	15
LS3	9	7	43	0	14
Sums:	181	156			
Mean (for stocks)			64	7	18
Proportion test§			$p = .17$	$p < .001$	$p = .056$

*The number of animals submitted for necropsy.

†The number of necropsies for which an unambiguous cause of death could be assigned.

‡The proportion of cases for which death was attributed to neoplasia (including cases of pituitary adenoma), as a percentage of all cases to which a cause of death was assigned.

§The probability that the indicated cause of death is equally likely among all 15 stocks.

pituitary adenoma. No other lethal cases of this lesion were seen among the other LB and EB lines, although pituitary adenoma was lethal in 2 of 14 LS1 mice and in 1 of 13 LS2 mice. The proportions test confirmed ($p < .001$) the impression that this lethal lesion was distributed differentially among the 15 stocks. The incidence of fatal lymphoma varied among the stocks from <10% in five stocks to >30% in three stocks; the proportions test provided marginal support ($p = .056$) for the hypothesis that the stocks differed among themselves in the proportion of fatal cases of lymphoma. No attempt was made in these calculations to adjust for the potential confounds of competing hazards, although it is possible that high early mortality attributable to one disease (e.g., pituitary adenoma in EB2) could lead to a corresponding decline in the frequency of other possible causes of death. Stock longevity was not significantly related either to the frequency of neoplasia (product moment $r = -.13$) or to the frequency of lymphoma ($r = .12$).

Discussion

These analyses suggest a significant correlated response of longevity to direct selection for differential rates of early and late growth during the first 56 days of life in a set of 15 genetic strains of mice. This result is consistent with the idea that polymorphic alleles with an impact on life span may have effects much earlier in the life history, and that their frequency in a population can be influenced by selective pressures acting on developmental, rather than adult or senescent, phenotypes. Our data also add further support to the hypothesis that longevity and body size are influenced at least partly by common influences within a species, by providing another example where small size is associated with superior longevity.

Many reports have associated longer life span with smaller body size. The best known and most often replicated association comes from an environmental intervention, in which caloric deprivation leads to dramatic life span extension in mice and rats (4). Although the effect varies somewhat with strain and dietary protocol, mice or rats allowed to eat only 60% of what they would eat given free access to food are found to live about 25%–40% longer than ad libitum-fed controls. The improvement in longevity is accompanied by a deceleration of age-dependent changes in many cell types and organ systems, and retards the appearance of a wide range of lethal and nonlethal diseases; this is consistent with the idea that the intervention has retarded or decelerated a fundamental aging process that helps to time the course of a wide range of changes in middle age and later life. Limitation of dietary methionine to the minimal levels needed for survival is also reported to give a 30%–45% increase in longevity in F344 rats (5,17); rats exposed to this diet are 43% lighter than controls, and the extent to which this intervention mimics the metabolic and pathophysiological effects of caloric restriction is still an open question.

Natural and induced mutations that result in smaller body size can also lead to dramatic increases in mouse longevity. Mice homozygous for the Ames dwarf mutation df/df, at the locus now known as Prop-1, live 50% to 70% longer than normal sized, non-mutant controls (6), as do mice of the Snell dwarf mutant, dw/dw, at the Pit-1 locus (18). Each of these mutations induces the same essential change, i.e., a loss, during embryogenesis, of signals needed to induce the cells of the anterior pituitary responsible for secretion of growth hormone, thyroid-stimulating hormone, and prolactin. The decline in growth hormone and thyroid hormone secretion prevents attainment of normal body size, and the mice are only about 35% of normal body weight at 8–12 weeks of age; both weight and linear dimensions are affected in parallel. Unless treated with growth hormone and/or thyroid hormone, the mice remain small all of their long lives, although they become quite obese in middle age.

Transgenic mice that overproduce urokinase-type plasminogen activator in brain consume 20% less food than littermates, and exhibit a 20% decline in body size and a 20% increase in longevity (7). It seems likely in this case that the genetic alteration is acting through an alteration in appetite rather than a change in endocrine control of growth and development (as in the dw/dw and df/df mutants).

Artificial selection for suitability to a variety of tasks has produced a very wide variation in size among dog breeds, up to 36-fold when measured as body weight (8). Breeds also vary in mean longevity, from 6.9 to 10.8 years, and the correlation between breed longevity and mean body weight is a remarkably high $R^2 = 56\%$. In some cases this variation in size has been shown to be due to alterations in production of IGF-1, the principal mediator of growth hormone effect (19,20). It is not known whether interbreed differences in size are in all cases due to changes in the GH/IGF-1 axis, nor how many loci contribute polymorphic alleles that affect breed size and longevity, but the available data are con-

sistent with the hypothesis that polymorphic loci affecting size have pleiotropic effects on dog life span. The evidence on size/longevity correlations in humans is complicated by a large number of potential confounding factors, including intergroup differences in health status, socioeconomic status, childhood nutrition, and ancestry; in general, however, they are consistent with the associations noted in the animal models. The effects are of substantial magnitude: in a study of 373 male veterans who died between 1984 and 1988, for example, men whose height did not exceed 1.75 meters were found (21) to live 4.95 years longer than taller members of the study population, and those shorter than 1.7 meters were on average 7.5 years longer-lived than those taller than 1.83 meters.

In the current study we did not make any measurement of body composition, such as fat/lean ratio, but we consider it unlikely that the effects seen are due simply to interstock differences in obesity. For one thing, the correlation between stock body weight and stock longevity was as strong ($r = .59$, $p = .02$) when the weight measure was derived from 3-month-old mice as when the weight data were obtained at peak body weight ($r = .61$, $p = .02$), or at 12 months of age, i.e., at ages at which mice have become substantially more obese than at 3 months ($r = .56$, $p = .03$). It is noteworthy in this context that the effect of caloric restriction on longevity is equally apparent in mice of the ob/ob stock, whose lack of leptin expression makes them obese, compared to ad lib fed controls, even when on a calorically restricted diet (22). Similarly, the striking growth extension of the df/df and dw/dw dwarf mouse lines is accompanied by progressive obesity in midlife and at late ages. Furthermore, the strong association between body weight and longevity among dog breeds is not attributable to parallel interbreed differences in obesity. In 12 of our set of 15 mouse stocks the interstock differences in adult and midlife body weight are very likely to reflect selection-driven alterations in allele frequencies that influence either the rate of weight gain from day 0–10 (ES and EB stocks) or weight gain from day 28–56 (LS and LB stocks), although it is hard to rule out effects of genetic drift that have led to substantial (and significant) differences in weight and life span in one of the three unselected control lines (i.e., C3). It is unlikely that these differences reflect selection for obesity in the first 10 or 56 days of life, although formal studies of body composition would be needed to address this possibility.

Each of the four selection protocols (EB, LB, LS, ES) was applied to generate three independent replicate stocks that share no common ancestor subsequent to the application of the selection pressure. The selection procedures were carried out on closed populations (i.e., there was no interbreeding between replicate lines within each selection protocol). It is interesting to note that the replicate mouse stocks thus produced can in some cases vary considerably from one another in pathology, peak weight, and longevity. The most dramatic example comes from the incidence rates of pituitary adenoma, which led to the death of 84% of the mice in stock EB2 but which were not noted in any of the necropsies of EB1, EB3, or any of the LB stocks. It is plausible, though unproven, that the rapid growth rate of EB2 mice from day 0–10 represents an abnormality of pituitary gland function that leads, later in life, to fatal adenoma formation at a relatively early age (life span = 473 ± 162 days).

It has been shown elsewhere that selection for early rates of increased or decreased rate of growth in body weight resulted in significant changes in cell number (i.e., hyperplasia, in the brain of these mice) (Atchley, Wei, and Crenshaw, unpublished data). It thus seems likely that the selection process has acted on genes that participate in cell proliferation, some of which (such as N-myc) are known to affect neural tissue preferentially. The high incidence of pituitary adenoma in EB2 may be the result of the fixation during selection of an aberrant form of this or some other oncogene.

The absence of pituitary adenomas in the other five stocks of EB and LB mice suggest that there are likely to be a number of other genetic combinations that can lead to rapid weight gain either early or later in the first 56 days of life but that act via pathways different from those that affect EB2 mice and thus do not lead to pituitary adenoma. The data also include examples where stocks selected according to the same protocol differ significantly in life span (e.g., LS2 > LS1, LS3; and EB1, EB3 > EB2; and LB2, LB1 > LB3) or in weight (e.g., for peak weight: EB3 > EB1, EB2; ES1, ES2 > ES3; LB3 > LB2, and LS1 > LS2, LS3).

These mouse stocks may provide useful starting points for further investigations of the genetic control of growth trajectory, body size, life span, and disease risk. Crosses among the stocks would be expected to segregate alleles with effects on longevity, body size, and other life history traits (such as litter size and maturation rate), and provide insights into the number, location, and effect size of alleles that influence one or more of these traits. Mice of the LS2 stock, in particular, may be a source of allele combinations that convey exceptional longevity compared to those common among typical laboratory inbreds. We have in previous studies used mice of the UM-HET3 stock, bred as the progeny of (BALB/c × C57BL/6)F1 dams and (C3H × DBA/2)F1 sires, to map quantitative trait loci associated with differential longevity (23). Females of the UM-HET3 stock, housed in the same vivarium over the same time interval, were significantly shorter lived than the LS2 females presented in this report (UM-HET3 life span was 806 ± 167 days, $N = 148$, $p = .003$ compared to LS2 females by two-tailed t test). Further work with the LS2 mice will be needed to see if their superior longevity can be replicated in other environments, affects males as well as females, and associates in backcross generations with small body size and/or altered patterns of fertility.

Acknowledgments

This research was supported by the Nathan Shock Center for the Biology of Aging, National Institutes of Health Grants AG13283 and GM-45344 to William Atchley. We thank Luann Linsalata and Gretchen Buehner for technical assistance, and Dr. Maria Moalli for veterinary supervision.

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References

1. Rose MR. *Evolutionary Biology of Aging*. New York: Oxford University Press; 1991.

2. Westendorp RG, Kirkwood TB. Human longevity at the cost of reproductive success. *Nature*. 1998;396:743–746.
3. Austad SN. *Why We Age: What Science is Discovering About the Body's Journey Through Life*. New York: John Wiley & Sons; 1997.
4. Weindruch, R, Walford RL. *The Retardation of Aging and Disease by Dietary Restriction*. Springfield, IL: Charles C Thomas; 1988.
5. Orentreich N, Matias JR, DeFelice A, Zimmerman JA. Low methionine ingestion by rats extends life span. *J Nutr*. 1993;123:269–274.
6. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. *Nature.* 1996;384:33.
7. Miskin R., Masos T. Transgenic mice overexpressing urokinase-type plasminogen activator in the brain exhibit reduced food consumption, body weight and size, and increased longevity. *J Gerontol Biol Sci*. 1997;52A:B118–B124.
8. Li Y, Deeb B, Pendergrass W, Wolf N. Cellular proliferative capacity and life span in small and large dogs. *J Gerontol Biol Sci.* 1996;51A: B403–B408.
9. Hillesheim E, Stearns SC. Correlated responses in life-history traits to artificial selection for body weight in *Drosophila melanogaster*. *Evolution*. 1992;46:745–752.
10. Atchley WR, Xu S, Cowley DE. Altering developmental trajectories in mice by restricted index selection. *Genetics.* 1997;146:629–640.
11. Enesco M, Leblond EP. Increase in cell number as a factor in the growth of the organs and tissues of the young male rat. *J Embryol Exp Morph.* 1962;10:530–562.
12. Goss RJ. Hypertrophy and hyperplasia. *Science*. 1966;153:1615–1620.
13. Falconer DS, Gauld IK, Roberts RC. Cell numbers and cell sizes in organs of mice selected for large and small body size. *Genet Res.* 1978; 31:287–301.
14. Ernst CA, Crenshaw PD, Atchley WR. Effect of selection for development rate on reproductive onset in female mice. *Genet Res.* 1999;74:55–64.
15. Chrisp CE, Turke P, Luciano A, Swalwell S, Peterson J, Miller RA. Lifespan and pathology in genetically heterogeneous (four-way cross) mice: a new model for aging research. *Vet Pathol.* 1996;33:735–743.
16. Sokal RR, Rohlf FJ. *Biometry: The Principles and Practice of Statistics in Biological Research*. 3rd ed. New York: Freeman; 1995.
17. Richie JP Jr, Leutzinger Y, Parthasarathy S, Malloy V, Orentreich N, Zimmerman JA. Methionine restriction increases blood glutathione and longevity in F344 rats. *FASEB J.* 1994;8:1302–1307.
18. Miller RA. Genes for ageing? *Trends Genet.* 1999;15:175–176.
19. Eigenmann JE, Patterson DF, Froesch ER. Body size parallels insulin-like growth factor I levels but not growth hormone secretory capacity. *Acta Endocrinol.* 1984;106:448–453.
20. Eigenmann JE, Amador A, Patterson DF. Insulin-like growth factor I levels in proportionate dogs, chondrodystrophic dogs and in giant dogs. *Acta Endocrinol.* 1988;118:105–108.
21. Samaras TT, Storms LH. Impact of height and weight on life span. *Bull World Health Organization.* 1992;70:259–267.
22. Harrison DE, Archer JR, Astle CM. Effects of food restriction on aging: separation of food intake and adiposity. *Proc Natl Acad Sci USA.* 1984;81:1835–1838.
23. Miller RA, Chrisp C, Jackson AU, Burke DT. Marker loci associated with lifespan in genetically heterogeneous mice. *J Gerontol Med Sci.* 1998;53A:M257–M263.

Received August 6, 1999
Accepted March 10, 2000
Decision Editor: Jay Roberts, PhD