

## THE EVOLUTION OF AGING AND AGE-RELATED PHYSICAL DECLINE IN MICE SELECTIVELY BRED FOR HIGH VOLUNTARY EXERCISE

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**Abstract.**—We tested whether selective breeding for early-age high voluntary exercise behavior over 16 generations caused the evolution of lifelong exercise behavior, life expectancy, and age-specific mortality in house mice (*Mus domesticus*). Sixteenth-generation mice from four replicate selection lines and four replicate random-bred control lines were individually housed from weaning through death and divided between two activity treatments (either with or without running wheels). Thus, there were four treatment groups: selection versus control crossed with active versus sedentary. The effects of selective breeding on life expectancy and age-specific mortality differed between females and males. In females, sedentary selection mice had early and high initial adult mortality and thus the lowest increases in mortality with age. Active selection females had the lowest early adult mortality, had limited mortality during midlife, and exhibited rapid increases in mortality rates at the very end of life; thus, they had deferred senescence. Median life expectancy was greater for both groups of selection females than for the two complementary groups of control females. Like females, sedentary selection males had the highest early adult mortality, and slow but steadily increasing mortality over the entire lifetime. Unlike the active selection females, active control males had the lowest mortality across the lifespan (until the end of life). Interestingly, the males with the lowest median life expectancy were those in the active selection treatment group. In both sexes, running (km/week) decreased over the lifetime to very low and virtually equivalent levels at the end of life in control and selection mice. Overall, these results demonstrate an evolutionary cost of selective breeding for males, regardless of exercise level, but a benefit for females when they have an outlet for the up-selected behavior. We conclude that correlated evolution of senescence occurs in mice selectively bred for high voluntary wheel running; exercise per se is beneficial for control mice of both sexes, but the impact on the effect of selection depends on sex; and the behavioral effect of exercise selection at an early age declines throughout the life span, which demonstrates decreasing genetic correlations over age for the genes involved in increased exercise.

**Key words.**—Aging, artificial selection, experimental evolution, frailty, genetic correlation, Gompertz, senescence, trade-off.

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Evolutionary senescence theory argues that increases in intrinsic mortality with age result from the changing relationship between selection and mutation with age (Hamilton 1966; Charlesworth 2001). Specifically, the strength of selection against deleterious mutations decreases with age because cohort size decreases with age. Therefore, even in the absence of senescence, the net impact of a late-age deleterious mutation is less than that of an early-age deleterious mutation (Charlesworth 1994). It follows that deleterious mutations with late-age phenotypes may accumulate over generations with little selection against them (mutation accumulation hypothesis, Medawar 1952). Furthermore, as Williams (1957) pointed out, mutations that are deleterious at late ages but beneficial at early ages are more likely to be maintained in populations than are mutations with the opposite age-specific effects (antagonistic pleiotropy theory, Williams 1957). A quarter century of studies on *Drosophila melanogaster* has revealed that both genetic mechanisms contribute to the evolution of rate of senescence (e.g., Hughes and Charlesworth 1994; Sgrò and Partridge 2000; earlier work reviewed in Rose

1991). Many of these and other studies have performed either mortality or reproduction manipulations and documented the subsequent evolution of rate of increasing age-specific mortality to test between mutation accumulation and antagonistic pleiotropy. A second approach was taken by Stearns et al. (2000), who used environmental manipulations to alter the external mortality environment imposed; the experimental evolution of senescence followed. This latter study concluded that genetic or environmental manipulations that alter the relative value of older individuals (i.e., that alter their contributions to population viability) alter the age-specific profile of selection strengths, which makes possible the subsequent evolution of the rate of senescence. In this report, we introduce a third approach: we ask whether selection on a behavioral trait related to health results in the correlated evolution of senescence and life expectancy. Specifically, we test for correlated evolution of senescence traits in response to artificial selection for early-age voluntary wheel-running behavior.

The relationship between exercise and senescence is complex. On the one hand, exercise seems to slow the onset of age-related diseases (Astrand 1992; Wisløff et al. 2005) and lengthens the median life expectancy in laboratory rodents (Holloszy 1988, 1993; McCarter 2000). On the other hand, exercise has never been shown to increase maximum lifespan

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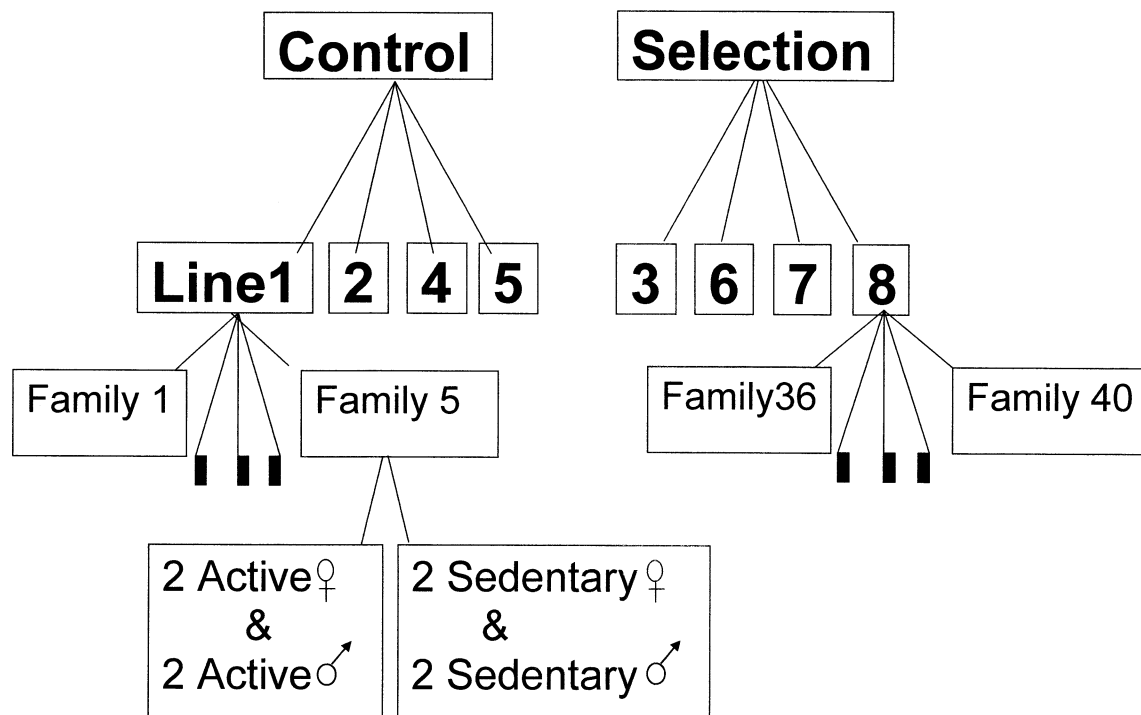


FIG. 1. Experimental design for the aging and ontogeny study. Line numbers refer to laboratory designations.

and indeed can result in increased levels of oxidative damage, which has been implicated as an important and perhaps universal proximate mechanism of aging (Ji 2000; Gredilla and Barja 2005; reviewed in Beckman and Ames 2000). Therefore, in this study we tested for correlated responses of senescence traits to selection on voluntary activity behavior in both high-activity and low-activity exercise environments.

To test these proximate and evolutionary mechanisms of aging related to exercise, we began an aging colony of house mice that were members of the 16th generation of selective breeding for increased voluntary wheel-running behavior at six to eight weeks of age. At generation 15, selection animals were running approximately 150% farther than control animals (selection males averaged 8.6 km/day; control males = 3.7 km/day; selection females = 11.3 km/day; control females = 4.3 km/day, Bronikowski et al. 2002a; Garland 2003). A total of 360 mice were used to establish the aging colony; half of the mice in the colony were from the four lines that were selectively bred for high running behavior and half were from the four control lines that were randomly bred with respect to running behavior (Fig. 1). Mice from the selection and control groups were individually housed from weaning through death; half of each selection group were housed with running wheels (active mice) and half were housed in cages without wheels (sedentary mice). From four to 83 weeks of age, selection mice ran significantly farther than did control mice but also had a steeper decline in weekly distance run with increasing age than did control mice (Morgan et al. 2003). We also assume, based on Koteja et al. (1999), that mice housed in cages without wheels are significantly less active than those housed with wheels and that there are no significant differences in activity behavior be-

tween selection and control mice housed in sedentary cages (but see Rhodes et al. 2005).

Using this colony of aging mice we addressed questions of whether aging evolves in a correlated fashion with early age selection for high wheel-running behavior and whether increased exercise affects senescence by comparing median life span, frailty, and rate of aging among the four experimental groups. Based on antagonistic pleiotropy models of aging we would predict an evolutionary cost of artificial selection for high voluntary wheel-running activity. In this scenario alleles selected for high voluntary wheel running would have detrimental effects at older ages, leading to decreased life span and increased senescence in selection mice. We would thus predict (H1) that selection mice have faster aging and shorter life spans than control mice, and that this effect would be accentuated in the active environment. Some weak support for this hypothesis from an earlier generation of this system of mice is presented by Thomson et al. (2002), who found lower Sod-2 activity in 15-week-old selection females regardless of activity environment; in males, a  $G \times E$  interaction was identified, with selection males having lower Sod-2 activity in the sedentary environment but higher Sod-2 activity in the active environment. Although activity of antioxidant enzymes is not directly correlated with longevity, the results of Thomson et al. (2002) suggest some potential evolutionary costs to selection for high wheel-running activity.

Alternatively, based on the ameliorating effects of exercise on senescence (see above), we would predict an evolutionary benefit of artificial selection for high voluntary wheel-running activity. In this scenario, alleles selected for high voluntary wheel running would have beneficial effects that lead to in-

creased life span and decreased senescence in selection mice. We would thus predict (H2) that mice selectively bred for high voluntary wheel-running activity have slower aging and longer life spans than control mice. If this beneficial effect is being directly caused by the increase in activity behavior itself (i.e., a training effect) then we would expect to see selection mice with increases in life span and decreases in senescence in the active environment only. If the beneficial effect is caused by some other mechanism that is unrelated to training effects (i.e., pleiotropic effects on traits not directly associated with exercise) we would expect to see increases in life span and decreases in senescence in both the active and the sedentary environments.

A second objective in this study was to measure late-life changes in the ontogenies of wheel running, body mass and food consumption. Morgan et al. (2003) identified significant differences between selection and control mice in both position and shape of these trajectories up through 83 weeks of age; herein we continue these measurements until the end of life with the goal of understanding the evolutionary impact of exercise selection on several organismal axes of aging.

## MATERIALS AND METHODS

### *Animals*

Experiments were performed on house mice (*Mus domesticus*) from the 16th generation of a selective-breeding experiment for increased voluntary wheel-running exercise (Swallow et al. 1998; Garland 2003). The selection experiment comprises four selection and four control lines; each line is propagated with 10 breeding pairs that contribute one male and one female to the next generation, with the condition that siblings are not mated. Breeders are chosen either randomly with respect to wheel running (control lines) or selectively, within family, as the mice that run the most revolutions on days 5 and 6 of six days of wheel exposure administered at eight weeks of age (selection lines).

The individuals in the aging colony of this report are the offspring of the generation 15 breeders for generation 16 of the selection experiment. Five breeding pairs from each of the eight lines were remated to produce second litters at Washington State University, Pullman, Washington (WSU; Fig. 1). Details of this design are presented in Morgan et al. (2003). Briefly, pups were weaned at 21 days of age and placed in treatment groups at  $28 \pm 3$  days of age in the animal care facility at Washington State University. Four males and four females from each family were used, with half designated for the active treatment and half designated for the sedentary treatment (Fig. 1). Each activity group thus contained two females and two males from each of the five families within each of the eight lines, for a total of 160 individuals per activity group; that is, 320 individuals in the aging colony. Mice in the active treatment group were placed individually in cages with a 11.75-cm radius running wheel and electronic wheel-revolution counter built into the cage-top. The mouse thus had the option of voluntarily getting into the wheel and running, or of remaining in the cage and not running. On the same day, sedentary mice were placed individually in standard rodent cages. Photoperiod was 12:12 h, and water was available ad libitum. Mice were provided

excess food weekly (Harland Teklad [Indianapolis, IA] rodent diet W8604), and apparent food consumption was determined by weighing food hoppers. This measure does not account for possible variation in food wasting, as when mice shred food pellets and allow fragments to drop in the litter. A study of food wasting at generation 10 found no significant differences between selection and control lines, but significant variation among the replicate lines within selection group (Koteja et al. 2003). Additionally, once a week each animal was weighed and its weekly wheel revolutions downloaded from the counter device. Cages were cleaned weekly and running wheels were cleaned monthly.

Extra sibs from all 40 families were placed in similar housing and were used as sentinels to monitor the colony monthly for the presence of specific pathogen exposure. At 24 months after the start date, a sentinel tested positive for MHV exposure, presumably from a barrier breakdown. No other sentinels tested positive. No treatment was initiated for the virus. Subsequent necropsied mice were monitored specifically for the presence of diseased liver tissue. None were observed to have contracted hepatitis (necropsies performed by Laboratory Animal Resources veterinarians at WSU).

About half of the individuals were euthanized for experimental reasons at 84 weeks of age; these individuals are right censored for the purpose of constructing survival curves. All other individuals died of natural causes. The data for running, food consumption, and body mass for the first 84 weeks were analyzed in a separate report on the full colony (Morgan et al. 2003). In addition to lifetime mortality curves, we focus here on the ontogeny of wheel running, and consider body mass and food-consumption ontogenies as they relate to wheel running, for the approximately 160 animals that were alive starting at experimental week 84. To provide tissues for studies of aged tissues, the experiment was ended when the remaining animals were 138 weeks old, at which time treatment groups had only one or two animals remaining.

### *Statistical Analysis*

Four traits were measured over the lifetime of this colony (about three years). The ages at death of all animals were analyzed using failure time analytical techniques (Smith 2002). The weekly measures of wheel revolutions, food consumption, and body mass were analyzed from weeks 84–138 with repeated-measures analysis of covariance.

Age at death was recorded for all animals. Deaths for intentional experimental purposes constitute right-censored data; the remaining colony members died from natural causes and are observed natural deaths. We constructed life tables in SAS (ver. 8.2 SAS Institute, Cary, NC) using the Lifetest procedure separately for males and females. The survival analysis procedures in SAS correctly interprets censored data in a nonparametric framework. Thus, we used these procedures rather than analysis of variance for age at death despite the fact that hierarchical nested analyses are not possible in SAS survival analysis. Specifically, the proportions surviving to each age  $x$  ( $l_x$ ) were calculated and the age-specific instantaneous mortality rates ( $u_x$ , also known as the age-specific hazards) were calculated and used to fit several models of increasing mortality with age. These  $l_x$  and  $u_x$  estimates were

calculated separately for each sex and for each treatment group: selection-active (SA), selection-sedentary (SS), control-active (CA), and control-sedentary (CS). We parsed the number of deaths into four-month age intervals to ensure a better likelihood fit and computed all vital rates over these four-month intervals to avoid  $u_x$  values of zero.

We tested several models of increasing mortality with age for goodness-of-fit to these data: the Gompertz model ( $u_x = Ae^{bx}$ ) where  $A$  is the initial mortality at age four months of age and  $b$  is the rate of exponential increase in adult mortality; the logistic model ( $u_x = Ae^{bx}[1 + (As/b)(e^{bx} - 1)]^{-1}$  where  $s$  is the deceleration of mortality increase at the end of life (if  $s = 0$ , logistic = Gompertz); both the Gompertz and logistic with a constant additive term for early adulthood mortality (Makeham term); and the Weibull model ( $u_x = \beta/\alpha((x - \tau)/\alpha)^{\beta-1}$  where  $\beta$  is the Weibull shape parameter,  $\alpha$  is the Weibull scale parameter, and  $\tau$  is the Weibull initial adult mortality rate (note that  $\tau$  is an additive early adult term). In practice, the Gompertz model implies that increasing mortality with age is a function of the causes of mortality of young adults, the logistic model implies the same but with a decoupling of this increase at late ages, and the Weibull model allows for causes of death of young adults to vary from causes of death of older adults (Ricklefs and Scheuerlein 2002). The Gompertz and logistic models with and without Makeham terms were fit to the age-at-death data with maximum likelihood estimation (implemented with program Winmodest; Pletcher 1999). In all comparisons, the Gompertz model was never rejected (all  $\chi^2 < 2.6$ ,  $P > 0.10$ ) when compared to the other three more parameterized models. The better fit of Gompertz rather than logistic suggests that increasing age-specific mortality did not decelerate or plateau at the end of life. However, our sample sizes at the end of the experiment (about five individuals per treatment group) do not provide sufficient statistical power to model late-life mortality patterns. The better fit of Gompertz alone without a constant early-adult Makeham term means that mortality rates began increasing in early adulthood. The two-parameter ( $\alpha$  and  $\beta$ ) Weibull model was fit to the data with failure time regression (Proc Lifereg in SAS). Weibull initial mortality ( $\tau$ ) was estimated using Proc Nlin in SAS (as in Ricklefs and Scheuerlein 2002). Within SAS, Weibull regression was never favored over exponential increases in mortality from the Gompertz model. We report the aging estimate developed by Ricklefs and Scheuerlein (2002), but do not consider the Weibull model further.

Once models were fit, a likelihood ratio test was used to test for significant variation in the Gompertz parameters among treatment groups within sex ( $A$  is the initial adult mortality rate, and  $b$  is the rate of exponential increase in mortality with age). For each pair of treatment groups, a fully parameterized case (i.e., independently estimated  $A$  and  $b$ ) was compared by likelihood ratio tests to models that assume common  $A$ , common  $b$ , or common  $A$  and  $b$ . This was done for all six pairwise comparisons of treatment groups.

Weekly wheel revolutions were converted to kilometers per week (km); km, body mass (g), and food consumption (g) were analyzed separately for each sex using the GLM procedure and the Mixed procedure in SAS. Analyses of fixed effects were in agreement using both procedures; we prefer

the use of GLM and present only the results from GLM because it allows straightforward testing of hypotheses for differences among replicate lines nested within selection group when there is an additional level below replicate line (mouse nested within replicate line nested within selection group). The following linear model was used in GLM:

$$y = \mu + G + L(G) + M[L(G)] + E + A + E \times G + E \times L(G) + A \times G + A \times L(G) + A \times E + A \times E \times G + A \times E \times L(G) + \text{covariates} + \varepsilon,$$

where  $G$ ,  $E$ , and  $A$  are the fixed effects of selection group, activity environment, and age, respectively. Line nested within selection group [ $L(G)$ ] and mouse nested within line nested within group [ $M(L(G))$ ] are random effects. Family nested within line nested within group was originally included but was subsequently dropped because it was not significant, and we substantially increased sample size by including unbalanced families. In these analyses, the effect of linetype was tested over the effect of line with 1 and 6 df, and the effect of activity group was tested over the activity  $\times$  line (linetype) interaction term, also with 1 and 6 df.

## RESULTS

### *Survival and Mortality*

The plots of proportion surviving to each age ( $l_x$ ) versus age  $x$  (Fig. 2A and 2B) start at 1 (100%) by definition, and approach the 0% survival time point with varying patterns. Each estimate of  $l_x$  depends on events in previous age classes. Unlike  $l_x$ ,  $u_x$ , the instantaneous age-specific mortalities, are independent of one another. Thus, our focus is on modeling the effects of selective breeding on ages at death. With respect to survival, median life expectancy is the age  $x$  at which  $l_x = 0.50$ . Median life expectancy differed significantly between selection and control mice within both females and males (standard errors varied between one and two days) based on the nonoverlap of the 95% confidence intervals. For females, selection active (SA) females had the highest median life span (872 days), followed by selection sedentary (SS), control active (CA), and control sedentary (CS). Overall, female median life span had the following relationship: SA > SS > CA > CS with values 872 > 837 > 801 > 760 days (Table 1). For males, selection sedentary animals had the greatest median life span (880 days) followed by control active, control sedentary, and selection active. Overall, male median life span had the following relationship: SS > CA > CS = SA with values 880 > 863 > 762 = 760 days. The primary difference between males and females was the reversal of selection animals that had access to running wheels: SA females had the greatest median life span, whereas SA males had the least. Maximum life span could not be analyzed due to small sample sizes at  $l_x = 0.10$  (for discussion see Bronikowski and Promislow 2005).

Gompertz model parameters are presented in Table 1 and scatter plots of observed and fit hazards are in Figure 2C,D. Gompertz initial adult mortality rate, when natural log-transformed, has been referred to as physiological frailty (Finch et al. 1990). Frailty was greatest for selection sedentary (SS) animals for both males and females. For females, frailty sort-

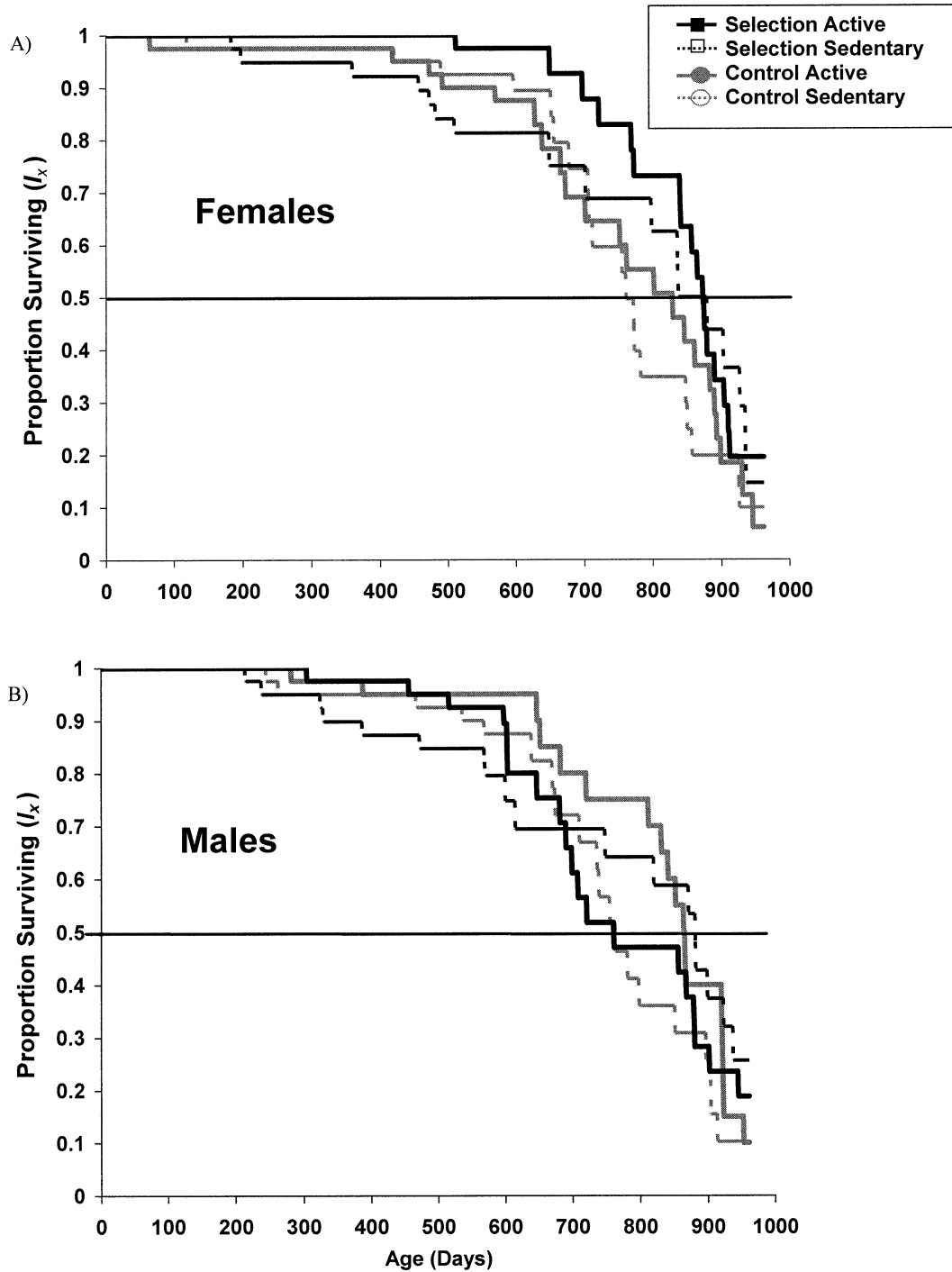
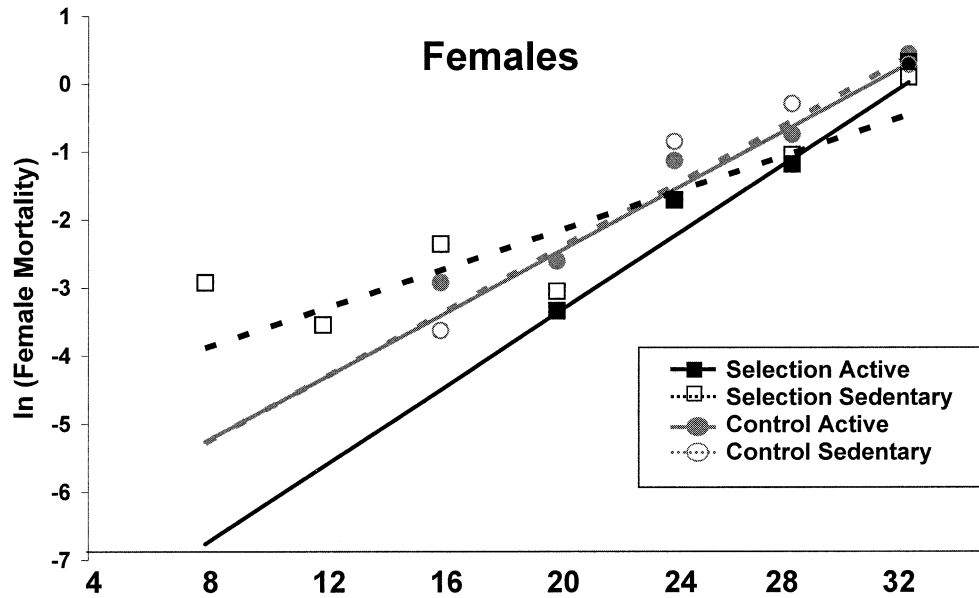


FIG. 2. Age-specific survival ( $l_x$ ) and mortality ( $u_x$ ) curves. Female survival (A) and male survival (B) all began with 40 individuals per treatment group; at 590 days of age, half of the colony was euthanized, reducing the working sample size to 20 individuals per treatment group. Female mortality (C) and male mortality (D) show the actual point estimates and the Gompertz model fits estimated with maximum likelihood techniques.

ed as:  $SS > CA = CS > SA$ ; and for males as:  $SS > SA > CS > CA$ . In practice, this can be interpreted as the SS group for both sexes exhibited early and persistent deaths. Rates of aging also differed among treatment groups. The significance of differences in the Gompertz aging slope ( $b$ ) sorted as  $SA > CA = CS > SS$  for females and  $CA > CS > SA > SS$

for males. Overall, frailty varied by an order of magnitude more than Gompertz slope, as shown by their coefficients of variation: frailty for females  $CV = 155\%$ , slope  $CV = 27\%$ ; frailty and slope for males:  $CV = 125\%$  and  $30\%$ , respectively. Note that because we could not estimate variation in maximum life span, we focus here on early adult mortality

C)



D)

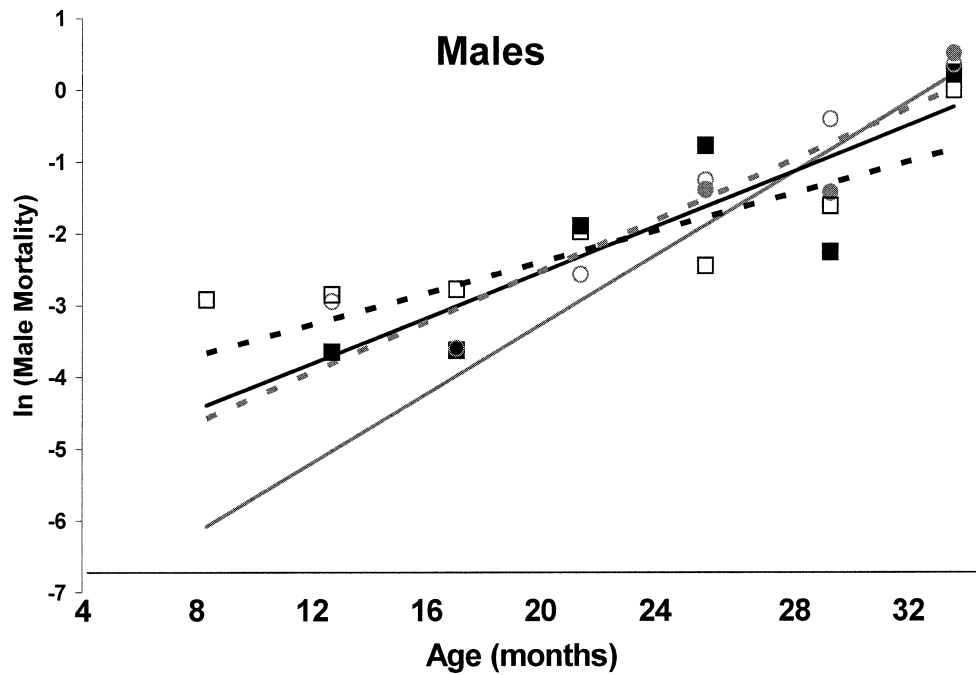


FIG. 2. Continued.

(frailty) rather than rate of aging across the entire life span, which varies partly in relation to maximum life span.

Ricklefs and Scheuerlein (2002) have proposed an index of rate of aging that incorporates both terms of the Gompertz model ( $\omega_G$ ):  $\omega_G = (Ab)^{1/2}$ . Alternatively, Finch et al. (1990) suggested that mortality rate doubling time (MRDT), which is equal to  $\ln(2)/b$ , be used as an index of rate of aging. We converted all model parameters to monthly values (from four-

monthly values) for ease of interpretability and then computed these force-of-mortality indices. These values are also presented in Table 1 for comparative purposes.

*Late-Life Ontogenies*

Kilometers run per week from 84 weeks of age to death were measured for each individual and analyzed using re-

TABLE 1. Gompertz and Weibull estimates of aging. Model parameters were estimated with a four-month interval. Significant groupings of Gompertz parameters within the same sex are indicated in bold. MRDT, mortality rate doubling time.

	Control active (CA)	Control sedentary (CS)	Selected active (SA)	Selected sedentary (SS)
Median life span (days $\pm$ SE)				
Females	801 (1.7)	760 (1.6)	872 (1.6)	837 (1.3)
Males	863 (1.6)	762 (1.5)	760 (1.7)	880 (1.5)
Gompertz initial mortality ( <i>A</i> )				
Females ( $\times 10^{-4}/4$ months)	<b>8.1</b>	<b>7.6</b>	<b>1.4</b>	<b>66</b>
Males ( $\times 10^{-4}/4$ months)	<b>2.9</b>	<b>16</b>	<b>26</b>	<b>95</b>
Gompertz slope ( <i>b</i> )				
Females	<b>0.95</b>	<b>0.96</b>	<b>1.12</b>	<b>0.58</b>
Males	<b>1.05</b>	<b>0.85</b>	<b>0.74</b>	<b>0.51</b>
Gompertz MRDT (months)				
Females	2.96	2.88	2.48	4.75
Males	2.64	3.26	3.75	5.44
Gompertz $\omega$				
Females ( $\times 10^{-3}$ )	6.9	6.8	3.1	15.5
Males	4.4	9.2	11.0	17.4

peated-measures ANCOVA with and without body mass as a covariate (Table 2; see Morgan et al. 2003 for early-age measures of wheel running). (Main effect results were independent of whether body mass was included, so we present our results with body mass as a covariate.) Running behavior declined significantly with age for both males and females, regardless of genetic background (Fig. 3). The effect of selection group was not significant for either males or females, which implies there was no difference in the position (height) of the ontogeny curve between selection and control mice. However, the interaction of age with selection group was significant for both males and females, which indicates a difference in the shape of the ontogeny curve between selection and control mice (see also Morgan et al. 2003). This result is likely driven by selection mice running more than control mice during middle age but not late age, which results in a steeper descent to the low level of running seen at the end of life (Fig. 3).

TABLE 2. Kilometers run per week: mixed-model repeated measures ANOVA. Bold indicates significance.

Source	df	MS	F	P
(A) Female km/week				
Selection group	1	143	0.49	0.51
Line(selection group)	6	537	1.29	0.29
Mouse(line(selection group))	33	903	34.03	< <b>0.0001</b>
Age	51	537	27.0	< <b>0.0001</b>
Age $\times$ selection group	51	34	1.74	<b>0.0021</b>
Age $\times$ line(selection group)	269	19	0.71	0.99
Body mass	1	1030	38.85	< <b>0.0001</b>
Error	770	26		
(B) Male km/week				
Selection group	1	0	0	0.99
Line(selection group)	6	439	0.95	0.48
Mouse (line(selection group))	26	827	28.68	< <b>0.0001</b>
Age	51	371	17.43	< <b>0.0001</b>
Age $\times$ selection group	51	29	1.39	<b>0.0478</b>
Age $\times$ line(selection group)	282	20	0.70	0.99
Body mass	1	22	0.75	0.39
Error	636	29		

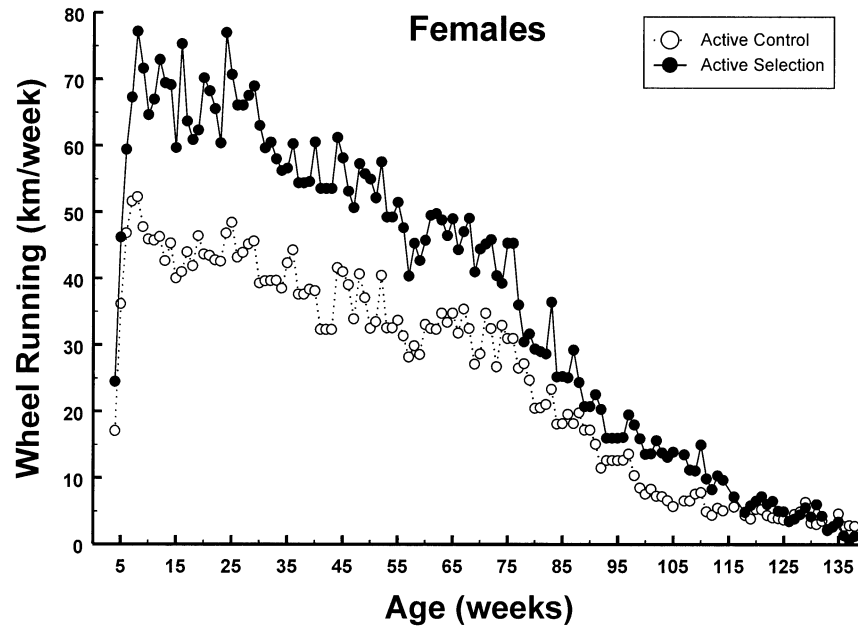
Body mass was also measured for each individual every week from age 84 weeks to death (Fig 4). For females, active mice were significantly smaller over all ages and for both genetic backgrounds (Table 3). For males, a significant age-by-activity group interaction was identified, meaning that the effect of activity on body mass depended on age, and that there was a difference in shape of the body mass ontogeny between active and sedentary mice. Active males were smaller than sedentary males during middle age, but as the end of life approached, this effect of activity on body mass disappeared. This result might be driven by the decline in activity levels at late ages or by small sample sizes within each treatment group at the end of the experiment. As can also be seen from Figure 4, selection mice are smaller than controls, consistent with many previous studies of these lines.

The analysis of weekly food consumption revealed an interaction effect of age and activity environment for females (Fig. 5; Table 4). Active mice ate less food in middle age, but not in late age (results from analysis with body mass as a covariate). For males, the significant three-way interaction among age, activity, and selection group indicates that the larger amounts of food eaten by active males of both genetic background during middle age decreased over time. These results are consistent with the observation that late in life, active selection mice were not running more than active control mice.

## DISCUSSION

We tested two competing hypotheses focused on whether 16 generations of selective breeding for increased voluntary exercise would result in either positive or negative correlated responses in median life span and physiological frailty. Our results show that, indeed, life span and frailty evolved in our selection lines of mice, but the directionality of evolution differed between the sexes (Table 1; Fig. 6). For females, median life expectancy showed a positive correlated response to selection: within each environment the selection females had higher median longevity than control females. Activity

A)



B)

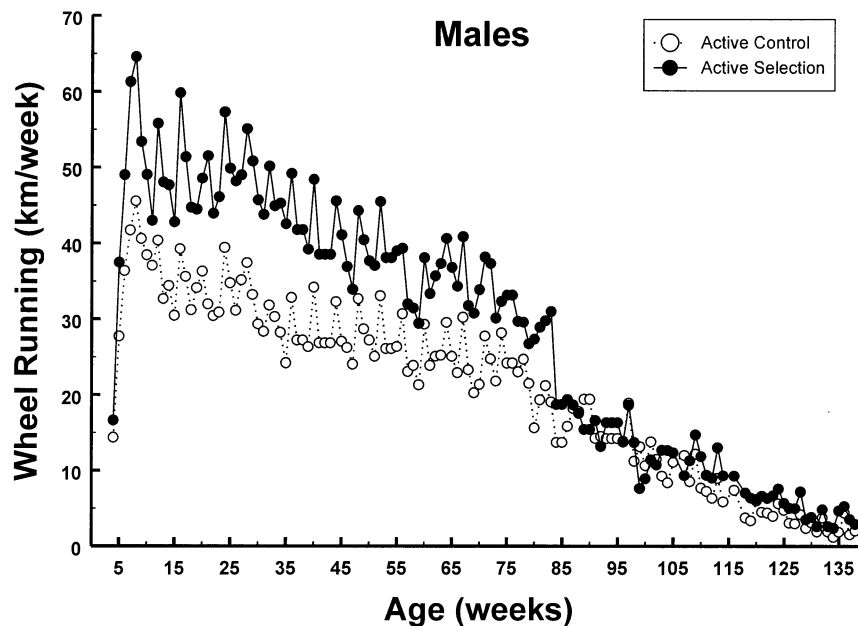


FIG. 3. The ontogeny of running (km/week) from four through 138 weeks of age. Both females (A) and males (B) had significant differences in the shape of the ontogeny between selection and control animals over age. Data from four through 83 weeks of age were published in slightly different form in Morgan et al. (2003).

environment likewise had a clear positive effect: active females had a greater median longevity than sedentary females regardless of selection history (Fig. 6A, Table 1). These results support hypothesis H2: selection for increased wheel-running behavior has caused a correlated response in median longevity by pleiotropic effects directly related to exercise (i.e., training effects, because active selection mice have a longer life span than sedentary selection mice) and by pleiotropic effects not directly related to exercise (because sed-

entary selection mice have a longer life span than sedentary control mice). In addition, these results support previous studies that demonstrated the positive impact on median life span of exercise throughout the lifetime (Holloszy 1988, 1993; McCarter 2000).

Cohort frailty, however, showed a  $G \times E$  interaction in females (Fig. 6C, Table 1). Frailty demonstrated a negative correlated response to selection, but only in the active environment: active selection females were less frail than sed-

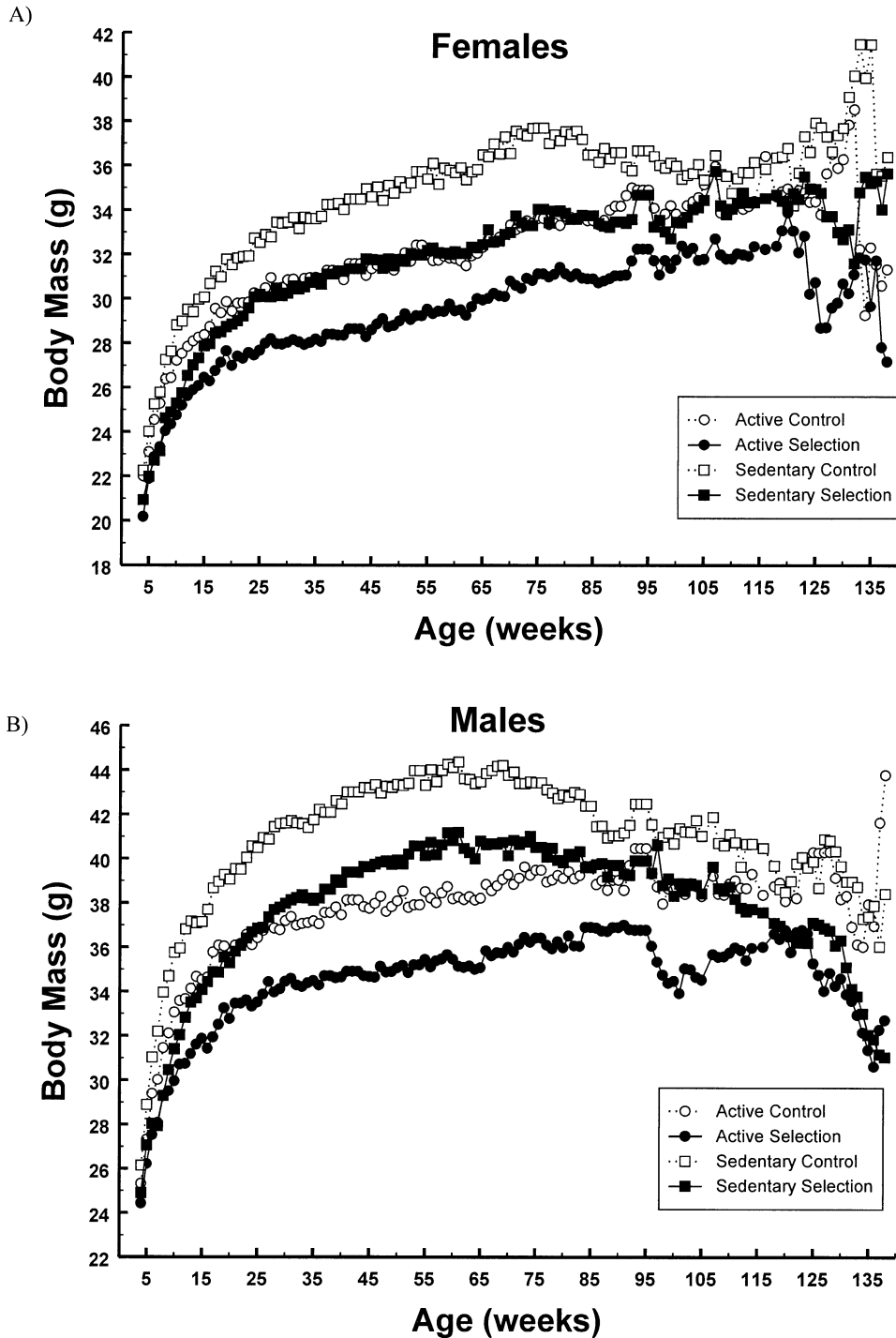


FIG. 4. The ontogeny of body mass (g) from four weeks through 138 weeks of age for females (A) and males (B). Data from four through 83 weeks of age were published in slightly different form in Morgan et al. (2003).

entary selection females and less frail than both control groups, but the sedentary selection females were the most frail of all four experimental groups. Hence, with regard to frailty, selection clearly imposed a cost in the sedentary environment, indicating some type of antagonistic pleiotropic effect, but resulted in a benefit in the active environment, indicating a pleiotropic effect directly related to exercise (i.e.,

some kind of training effect that occurred only in the selection females).

In males different results emerged. A  $G \times E$  interaction was identified for median longevity: sedentary selection males had the highest median longevity of any of the four experimental groups, but active selection males had a lower median longevity than either sedentary selection mice or ac-

TABLE 3. Body mass: mixed-model repeated measures ANOVA. Bold indicates significance.

Source	df	MS	F	P
<b>(A) Female body mass</b>				
Selection group	1	1469	2.44	0.17
Line(selection group)	6	884	7.84	<b>0.0125</b>
Mouse(line(selection group))	55	308	113.68	<b>&lt;0.0001</b>
Activity	1	503	7.30	<b>0.0319</b>
Age	51	20	4.80	<b>&lt;0.0001</b>
Activity × selection group	1	38	0.45	0.52
Activity × line(selection group)	6	133	0.78	0.59
Age × selection group	51	3.1	0.74	0.90
Age × line(selection group)	285	4.4	1.15	0.13
Age × activity	51	2.9	0.79	0.85
Age × activity × selection group	47	3.6	0.95	0.57
Age × activity × line(selection group)	194	3.9	1.45	<b>0.0001</b>
Error	1463	2.7		
<b>(B) Male body mass</b>				
Selection group	1	1663	2.03	0.20
Line(selection group)	6	1024	3.24	0.09
Mouse(line(selection group))	48	290	91.93	<b>&lt;0.0001</b>
Activity	1	108	0.44	0.53
Age	51	13	2.43	<b>&lt;0.0001</b>
Activity × selection group	1	59	0.22	0.66
Activity × line(selection group)	6	348	1.86	0.11
Age × selection group	51	2.7	0.51	0.99
Age × line(selection group)	292	5.6	2.33	<b>&lt;0.0001</b>
Age × activity	51	6.8	2.79	<b>&lt;0.0001</b>
Age × activity × selection group	43	2.2	0.90	0.65
Age × activity × line(selection group)	252	2.3	0.74	0.99
Error	1320	3.2		

tive control mice and did not differ from sedentary control males (Fig. 6B, Table 1). Hence, selection imposes a cost but only in the high-activity environment: in the sedentary environment, selective breeding confers a benefit. This indicates that some type of antagonistic pleiotropy is occurring but only when the genes can be expressed in an active environment; in the sedentary environment, selection for increased wheel running results in increased median life span.

Cohort frailty showed a clear negative response to selection in males: selection males were more frail than control males, regardless of environmental group. This result is consistent with hypothesis H1: selection for increased voluntary wheel-running activity imposes an evolutionary cost in terms of frailty, indicating antagonistic pleiotropy between these two traits in males. This is independent of the environmental effects of exercise because, within both the selection and control groups, mice in the active group have lower frailty; thus, regardless of genotype, exercise confers a benefit.

#### Correlated Evolution

When we focus on age-specific mortality we find several interesting insights into the correlated evolution of aging in these lines of mice. First, for both males and females, selection sedentary mice had the highest initial adult mortality rate (modeled at four months of age with maximum likelihood estimation; Table 1). In practice, this means that the evolution of increased exercise behavior resulted in the evolution of increased physiological frailty of these treatment groups when housed without an exercise outlet (i.e., a wheel). One possible cause of this could be changes in activity of certain brain regions, relative to control mice, that occur when se-

lection mice are deprived of wheel access (Rhodes et al. 2003, 2005). These changes could reflect a variety of behavioral or physiological states, and in turn could affect a variety of neuropsychological pathways, including the hypothalamic-pituitary-adrenal axis, a major component of both stress and exercise physiology (increased insulin-stimulated glucose uptake, Dumke et al. 2001; see also Girard and Garland 2002; Bunkers et al. 2003). Particularly relevant is the potential similarity between caloric restriction (CR) and exercise. For example, CR causes many of the same beneficial phenotypes at both the organismal and transcriptional levels (McCarter 2000; reviewed in Bronikowski et al. 2003). In addition, CR animals have low levels of glucocorticoid expression as well as other transcripts involved in stress response (Kayo et al. 2001).

Regardless of the underlying mechanism, our results reveal an apparent evolutionary cost of selection at the physiological level for animals housed without running wheels. Frailty is thought to represent the underlying physiological vulnerability of a treatment group and is both a consequence of genetics and environmental manipulation. Studies have reported estimates of heritabilities of frailty on the order of 0.30 (*Drosophila melanogaster*: Promislow et al. 1996; *Homo sapiens*: Yashin et al. 1999). In contrast, current limited estimates of the heritability of the Gompertz rate of aging,  $b$ , are orders of magnitude lower (Promislow et al. 1996). In our study, as in other mammalian studies (Promislow 1991; Bronikowski et al. 2002b), the rate-of-aging estimates were much less variable than those for frailty; coefficients of variation differed by an order of magnitude. We conclude that, although accelerating mortality with age is taken as *the sig-*

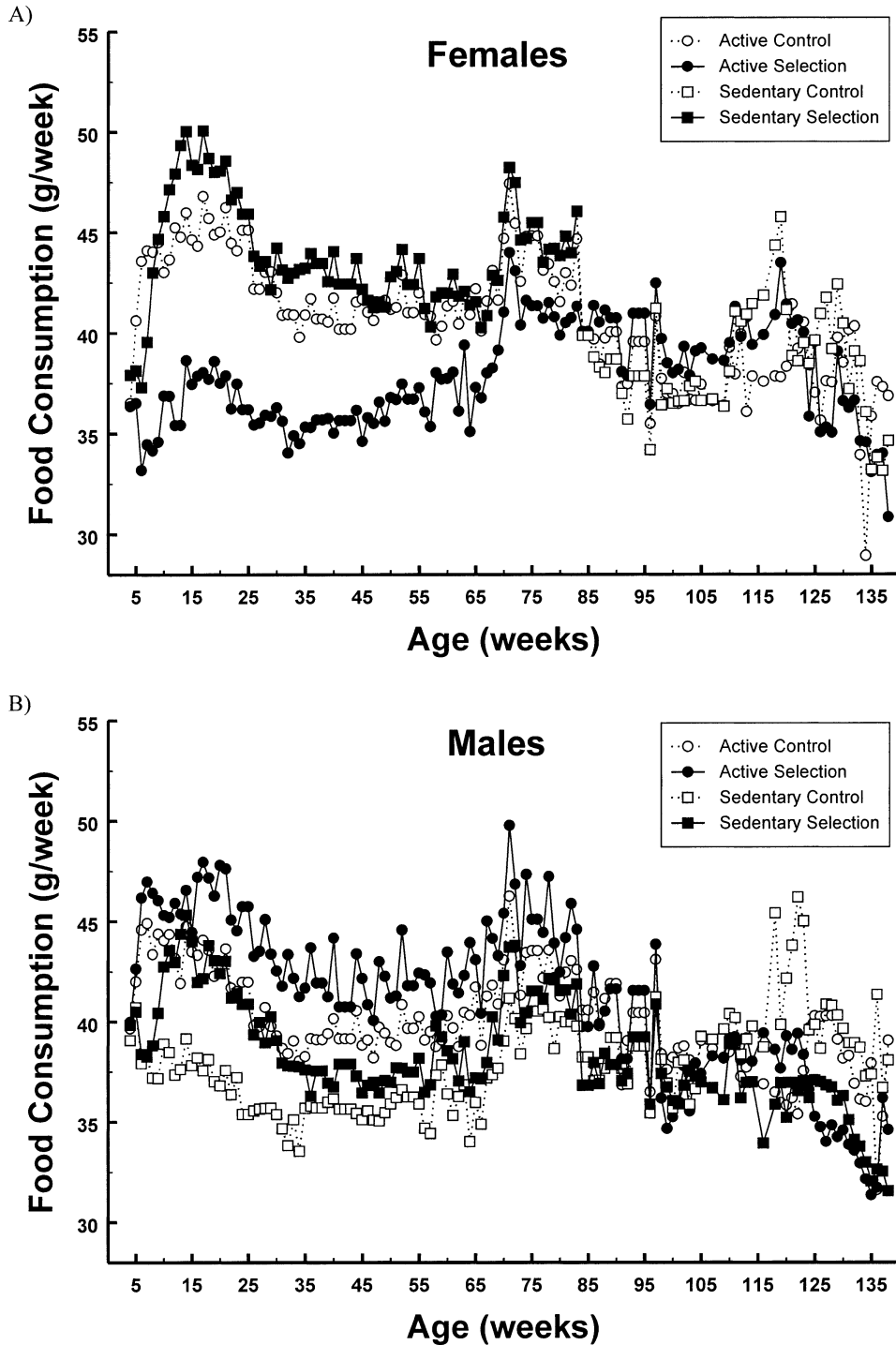


FIG. 5. The ontogeny of food consumption (g/week) from four weeks through 138 weeks of age for females (A) and males (B). Data from four through 83 weeks of age were published in slightly different form in Morgan et al. (2003).

nature of senescence, its relatively low heritability compared to frailty may mean that aging is controlled by physiological events early in the adult stage (Speakman 2005).

Ricklefs and Scheuerlein (2002) have proposed rate-of-aging metrics based on age-specific mortality increases but that combine initial adult mortality with the rate of increasing mortality. These combined values (see Table 1) are consistent

with the interpretation that, for both males and females, sedentary selection animals have the fastest rate of aging. For females, active selection animals have the slowest rate of aging; for males, active control mice have the slowest rate of aging. As has been observed in other studies on aging, the interpretation of how aging responds in correlated fashion to evolution of other traits is muddled by various definitions

TABLE 4. Food consumption: mixed-model repeated measures ANOVA. Bold indicates significance.

Source	df	MS	F	P
(A) Female food consumption				
Selection group	1	654	2.01	0.20
Line(selection group)	6	576	5.23	<b>0.0274</b>
Mouse(line(selection group))	55	278	25.2	<b>&lt;0.0001</b>
Activity	1	3.8	0.06	0.82
Age	50	82	6.21	<b>&lt;0.0001</b>
Activity × selection group	1	11	0.11	0.75
Activity × line(selection group)	6	137	0.88	0.51
Age × selection group	50	12	0.91	0.64
Age × line(selection group)	279	14	1.45	<b>0.0016</b>
Age × activity	50	15	1.57	<b>0.0117</b>
Age × activity × selection group	46	12	1.26	0.13
Age × activity × line(selection group)	189	9.2	0.83	0.95
Body mass	1	1600	145	<b>&lt;0.0001</b>
Error	1408	11		
(B) Male food consumption				
Selection group	1	255	0.59	0.47
Line(selection group)	6	649	4.41	<b>0.0548</b>
Mouse(line(selection group))	48	417	41.1	<b>&lt;0.0001</b>
Activity	1	0.53	0	0.99
Age	50	56	4.70	<b>&lt;0.0001</b>
Activity × selection group	1	94	0.63	0.46
Activity × line(selection group)	6	178	0.7	0.65
Age × selection group	50	16	1.33	0.07
Age × line(selection group)	286	12.1	1.08	0.27
Age × activity	50	53	4.76	<b>&lt;0.0001</b>
Age × activity × selection group	42	20	1.81	<b>0.0028</b>
Age × activity × line(selection group)	244	11	1.12	0.12
Body mass	1	3046	300.64	<b>&lt;0.0001</b>
Error	1275	10		

of aging (e.g., Reznick et al. 2004). Because of the mathematical dependency issue with survival estimates, we use mortality rates.

If exercise reduces the rate of aging, then this may be intimately linked to level of exercise. For both males and females, exercise declined with age; at middle age (about 20 months of age) selection animals were running about twice as much as control animals (Morgan et al. 2003). By the end of life, however, selection and control animals were running almost equally low amounts (Fig. 3). This result is independent of body mass differences between selection and control mice (Fig. 3 and 4) and suggests the following several interesting interpretations. First, our results provide further support to the interpretation made by Morgan et al. 2003 that the genetic covariances between young-age and late-age wheel running must be quite small. Second, the observation that physical activity declines with age is ubiquitous and has been reported in various vertebrate and invertebrate organisms with age (reviewed in Ingram 2000; Sallis 2000). An analysis of mechanism has shown it is not only declining ability that accounts for this age-specific drop-off in physical activity, but potentially declining interest as well (based on age-related alterations to the dopaminergic signaling system). Our work is consistent with this result. Early-age selection for increased voluntary exercise is accompanied by small but significant upregulation of a number of neurotransmitters and neuropeptides involved in neurogenesis and dopamine signaling in two-month-old females (Bronikowski et al. 2004). The decrease in running behavior across the lifetime suggests

weak genetic correlations across age in the genes responsible for this upregulated neuronal signaling. Therefore, a priority for future studies should be to examine patterns of gene expression in the brain in aged mice.

#### Mechanistic Basis of Correlated Evolution

The recent finding that rate of senescence can evolve by mutations in metabolic and stress-resistance pathways has intensified the academic interest in evolutionary studies of aging (reviewed in Bronikowski and Promislow 2005). Over the past 15 years, aging studies with such laboratory organisms as the nematode (*Caenorhabditis elegans*), the mouse (*Mus musculus*), and the fruit fly (*Drosophila melanogaster*) implicate a highly conserved pathway, triggered by the insulin-like growth factor signaling, as the regulator of rate of senescence by affecting downstream forkhead transcription factors (*foxo* in mice: Holzenberger et al. 2003; Migliaccio et al. 1999). This finding of highly conserved transcription factor activation across diverse taxa, potentially linked to diet and exercise, has led to innovative experiments on the part of evolutionary biologists to alter various components of the insulin pathway and alter the rate of aging (e.g., Tatar 2004). For our selection males, the expression levels of several categories of genes have been discovered to change with age in sedentary mice, but not active mice (Bronikowski et al. 2003). These included several receptors in the IGF signaling pathway, as well as several genes involved in innate immunity and anti-inflammation. Also, fewer genes changed with age

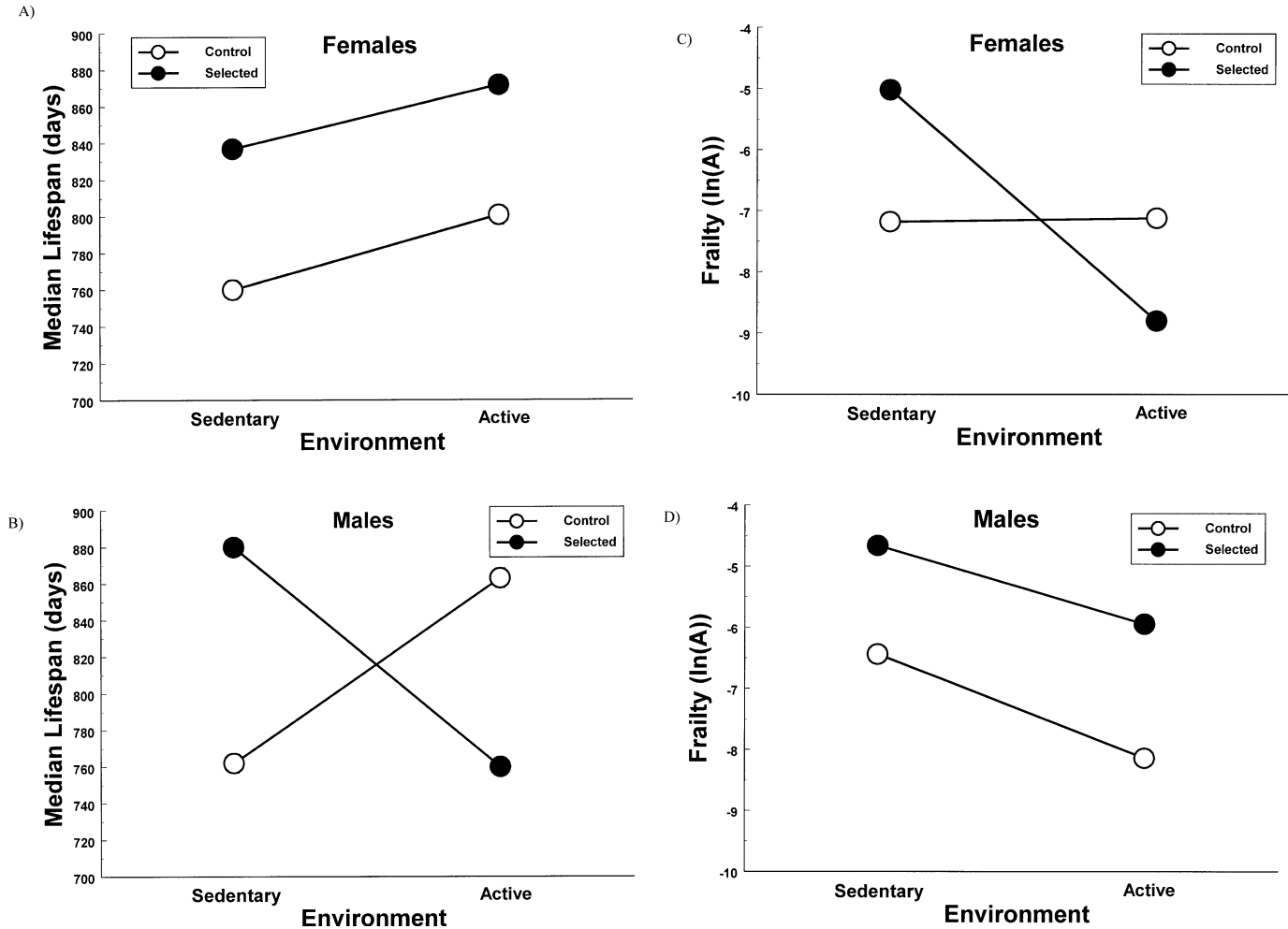


FIG. 6. Genotype-by-environment interactions. Mean median life span for females (A) and males (B), and Gompertz frailty ( $\pm$  SE) for females (C) and males (D).

in the selection active males. We concluded from these findings that activity, in the selection genetic background, attenuates aging at the transcription level. Other studies of heart aging in exercising mice have shown lower levels of reactive oxygen species (ROS) production and antioxidant activity (e.g., Judge et al. 2005). In addition, tissue in smaller individuals tends to expend more energy (daily energy expenditure) than tissue in larger individuals (reviewed in Speakman 2005). Selection mice in our study are smaller than control mice, as well as faster when running on the wheels (Swallow et al. 1998). Interestingly, Speakman et al. (2004) reported that mice can exhibit individual variation, with some showing high levels of mitochondrial uncoupling. This allowed some individual mice to respire at higher rates without concomitant production of ROS, which apparently caused an increased life span. Although not effective for adenosine triphosphate (ATP) production, uncoupling would allow for small amounts of ATP production (in glycolysis and the citric acid cycle) and heat generation with no damage-accumulation costs. Therefore, the available evidence suggests that, within generations, exercise attenuates aging through impacts on oxidative stress and stress resistance, perhaps through mi-

tochondrial uncoupling. Across generations, comparison studies between selection and control mice in terms of gene expression profiles of aging suggest that in males, control mice have fewer gene expression changes with age, and activity is more effective at attenuating the aging process (A. M. Bronikowski, T. Garland, Jr., and P. A. Carter, unpubl. data). This result agrees with the correlated evolution of aging in male selection and control mice (control active mice have slower aging than selection active mice), and suggests a mechanism to be investigated: the upregulation of uncoupling proteins.

#### Sex Differences

Why would selection for increased exercise cause opposite correlated responses to selection between the sexes? The sexes obviously differ in many ways, so it is impossible with the present information to identify causes with any certainty (cf. Garland and Adolph [1994] on the difficulties of making adaptive or mechanistic inferences when comparing only two species). Nevertheless, we can identify several sex differences that could impact the relationship between selective

breeding for exercise and correlated evolution in aging. First, except possibly during very late life (see Fig. 3), females always run more than males on an absolute basis in both selection and control lines (see Morgan et al. [2003] for the present mice from ages 4 to 83 weeks; examples of shorter-term wheel running are found in Swallow et al. 1998, 1999, 2001; Garland 2003). Second, the difference between selection and control mice tends to persist longer during ontogeny in females than in males (see Swallow et al. 1999, 2001; Morgan et al. 2003); the position effect in females (i.e., age by selection group) for the trajectory is much stronger than in males (see Fig. 3). Third, the increased running of selection females is almost entirely caused by greater average speed, whereas in males a significant increase in amount of time spent running per day has also evolved (see above references; Koteja and Garland 2001). Indeed, females sometimes run at speeds that approach their maximum aerobic speed (Girard et al. 2001; Rezende et al. 2005), whereas males probably do not, given that the sexes generally do not differ in maximum aerobic capacity (Rezende et al. 2006a; T. Garland, Jr., unpubl. data). Aside from differences in wheel running itself, the sexes obviously differ in various other behaviors as well as the hormonal milieu. For example, selection mice have higher basal corticosterone levels compared with control mice, and females have higher levels than males in both selection and control lines (Girard and Garland 2002; Bunkers et al. 2003).

We have argued elsewhere (Bronikowski et al. 2004; Rhodes et al. 2005) that the primary difference between selection and control mice that underlies the former's elevated running levels has more to do with neurobiological evolution than with the evolution of exercise physiology per se (but see Garland et al. 2002; Girard and Garland 2002; Swallow et al. 2005; Rezende et al. 2006a,b). Thus, the difference between male and female aging in correlated response to selection may relate to sex differences in brain chemistry or signaling molecules in general, rather than different anatomical or morphological adaptations for exercise between the two sexes. Little is known regarding sex differences in genes that control aging in mammals, although the sex difference between male and female survival is widely demonstrated (humans: Tan et al. 2005; Caselli et al. 1987; primates: Bronikowski et al. 2002b). Genetic dissection of life span in *Drosophila* has revealed that different genes control rate of aging and life span in males versus females (Nuzhdin et al. 1997). Whether this result will be generalized across species and classes is unknown at this time, but would have important implications for evolutionary studies of aging.

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