ONTOGENIES IN MICE SELECTED FOR HIGH VOLUNTARY WHEEL-RUNNING ACTIVITY. I. MEAN ONTOGENIES

THEODORE J. MORGAN,[1,2,3] THEODORE GARLAND, JR.,[4,5] AND PATRICK A. CARTER[1,6]

[1]*School of Biological Sciences, Washington State University, Pullman, Washington 99164*
[2]*E-mail: tjmorgan@unity.ncsu.edu*
[4]*Department of Biology, University of California, Riverside, California 92521*
[5]*E-mail: tgarland@citrus.ucr.edu*
[6]*E-mail: pacarter@wsu.edu*

Abstract.—The evolutionary importance of postnatal ontogenies has long been recognized, but most studies of ontogenetic trajectories have focused exclusively on morphological traits. For animals, this represents a major omission because behavioral traits and their ontogenies often have relatively direct relationships to fitness. Here four replicate lines of house mice artificially selected for high early-age wheel running and their four replicate control lines were used to evaluate the effects of early-age directional selection, genetic drift, and activity environment (presence or absence of a running wheel) on variation in the ontogenies of three traits known to be genetically correlated: voluntary wheel running, body mass, and food consumption. Early-age selection significantly changed both the shape and position of the wheel-running and food-consumption ontogenies while influencing the position, but not the shape, of the body mass ontogeny. Genetic drift (as indicated by variation among replicate lines) produced significant changes in both the position and shape of all three ontogenies; however, its effect differed between the selection and control groups. For wheel running and food consumption, genetic drift only influenced the control ontogenies, whereas for body mass, genetic drift had a significant effect in both selection groups. Both body-mass and food-consumption ontogenies were significantly altered by activity environment, with the environment causing significant changes in the shape and position of both ontogenies. Overall the results demonstrate strong effects of early-age selection, genetic drift, and environmental variation on the evolution and expression of behavioral and morphological ontogenies, with selection changing only the position of the morphological ontogeny but both the position and shape of the behavioral ontogenies.

Key words.—Artificial selection, body mass, correlated characters, food consumption, ontogeny, wheel running.

Correlated response to selection has received increasing attention as an important mechanism by which traits evolve and trait evolution may be constrained (e.g., Bennett and Ruben 1979; Lande and Arnold 1983; Arnold 1992; Lynch 1994; Schwenk 1995; Swallow et al. 1998b; Carter et al. 2000; Garland 2002). A correlated response to selection occurs when one trait responds to selection on a second trait and is dependent on the existence of a genetic correlation between the two traits (Falconer and Mackay 1996). Most studies of correlated characters have focused on phenotypicaly different traits (e.g., wheel running and body mass; Swallow et al. 1999), but correlated responses to selection can also occur between the same trait across ontogeny (e.g., body mass at 10 and 55 days of age; Atchley and Rutledge 1980). Such evolutionary responses in an ontogeny to selection at a specific age are dependent on genetic correlations of a trait with itself over ontogeny (McCarthy and Bakker 1979; Cheverud et al. 1983; Zelditch and Carmichael 1989); hence, the patterns of genetic covariation of a trait with itself across ontogeny will ultimately determine its possible rates and directions of evolutionary change (Atchley and Rutledge 1980; Cheverud et al. 1983; Arnold 1990; Kirkpatrick and Lofsvold 1992).

The correlated response of an ontogeny to selection is rarely studied, yet it is an important mechanism for the evolution of various forms and functions on both macro- and microevolutionary time scales (e.g., Gould 1977; Alberch et al. 1979; Creighton and Strauss 1986; Zelditch 1988; Brooks 1991; Jones 1992; Klingenberg and Zimmermann 1992; Björklund 1997; Hoying and Kunz 1998; Zelditch et al. 2000; Badyaev et al. 2001; Loy et al. 2001). The rate and direction of the short-term response to selection on an ontogeny will depend on the strength of selection, the age at which it occurs, as well as the underlying genetic architecture of the ontogeny (Hahn et al. 1990; Kirkpatrick et al. 1990; Kirkpatrick and Lofsvold 1992; Falconer and Mackay 1996). If the underlying genetic architecture is highly integrated across ages (i.e., the genetic correlations among ages are strong), then selection at a single age is expected to result in a similar correlated response across the entire ontogeny; that is, selection will cause the entire ontogeny to be shifted in position without changing its shape. In contrast, if the underlying genetic architecture is less integrated across all ages (i.e., the genetic correlations differ among ages or are weak), then selection at a single age will change only portions of the ontogeny, thus resulting in a change in ontogeny shape. Hence, the manner in which the same type of selection can result in different patterns of ontogenetic evolution is ultimately determined by the underlying genetic architecture of the trait ontogeny (Cheverud et al. 1983).

Previous studies have investigated the influence of selection on morphological ontogenies. For example, Atchley and Rutledge (1980) found that selection on six-week weight gain in mice resulted in a correlated change in body-mass, body-circumference, and tail-length ontogenies; for each trait, selection caused a change in position of the ontogenies but not their overall shape, suggesting strong genetic correlations across ages for each trait. Other studies in mice found similar changes in morphological ontogenies as a result of early-age

selection on body mass or development rate (Roberts 1961; Timon and Eisen 1969; McCarthy and Bakker 1979; Atchley et al. 1997). Similar effects of early-age selection have also been shown in other systems. For example, selection for increased or decreased body length at 70 days in guppies (*Poecilia reticulata*) resulted in significant changes in the position but not the shape of the body-length ontogenies (Rocchetta et al. 2000). Each of these studies applied early-age selection to morphological characters, focused on correlated responses in morphological ontogenies, and generally found evidence of strong genetic correlation across ontogenies.

Despite the evolutionary importance of behavior, studies of the effects of age-specific selection on behavioral ontogenies are virtually nonexistent. One behavior of great ecological importance in animals is locomotion (Miles et al. 2000; Irschick and Garland 2001; Kelt and Van Vuren 2001; Kramer and McLaughlin 2001). Locomotion underlies many different activities and is itself affected by morphological, physiological, biochemical, and neurobiological traits. As a highly complex phenotype, locomotor behavior is ideal for studies of correlated evolution (e.g., Garland and Carter 1994; Garland and Losos 1994; Boggs and Frappell 2000; Garland 2002).

Replicated artificial selection on increased early-age locomotor activity (specifically, voluntary wheel running) in house mice (*Mus domesticus*) has been used to examine the correlated evolution of behavioral, physiological, and biochemical traits at specific ages (Swallow et al. 1998a,b, 2001; Koteja et al. 1999b; Carter et al. 2000; Rhodes et al. 2000, 2001; Girard et al. 2001, 2002; Garland et al. 2002; Thomson et al. 2002). Here we use this model system to examine the effects of early-age selection, genetic drift, and activity environment (presence or absence of a running wheel) on the shape and position of wheel-running, food-consumption, and body-mass ontogenies. Because previous studies have demonstrated the effect of early-age selection on each of these traits at specific ages (Swallow et al. 1998a, 1999; Koteja 1999b), we expect that correlated responses will occur in the ontogenies as well, with the specific effect depending on the patterns of underlying genetic covariation in each ontogeny.

MATERIALS AND METHODS

Selection History

The lines of house mice (*M. domesticus*) used here are from replicate lines selected for 16 generations for increased voluntary wheel-running activity and from replicate control lines. Details of the production of these lines are described in Swallow et al. (1998a), so only a brief description will be provided here. Male and female (112 of each) laboratory house mice of the outbred Hsd:ICR strain were purchased from Harlan Sprague Dawley (Indianapolis, IN). These individuals were paired randomly to produce generation −1. From generation −1, one male and one female were chosen randomly from each litter, and these individuals were paired randomly with the provision of no sibling mating. Ten of these pairs were assigned randomly to each of eight closed lines. Four of these lines were randomly assigned to each selection group (selection or control). Offspring from these pairings were designated generation 0, and selection was begun at generation 1. Lines were maintained with 10 pairs per

generation through generation 16, which is the single generation used in the experiment described below.

Each generation, mice were weaned from the dams at 21 days of age, weighed, toe-clipped for individual identification, and housed in groups of four by sex. At approximately eight weeks of age, mice were placed in cages with activity wheels for six consecutive days. The selection trait was average number of wheel revolutions on days 5 and 6. To obtain a response to selection without producing highly inbred lines, within-family selection was used: in selected lines, the male and female who ran the greatest number of revolutions in each family were chosen as breeders, while in control lines one male and one female from each family were randomly chosen as breeders. Breeders were paired randomly within lines each generation with the provision of no sibling mating.

Mouse Colony and Study Animals

The experiment described here used a single generation of mice from the selection experiment described above. The breeder mice from generation 15 were shipped from University of Wisconsin to Washington State University (WSU) to serve as breeders of the new colony at WSU. Five breeding pairs from each of the four control lines and five breeding pairs from each of the four selection lines were used to produce the next generation. The resulting pups were weaned at 21 days of age and given ear tags (National Tag Company, Newport, KY) for individual identification. At 28 ± 3 days of age, from each of the five families in each line, two individuals of each sex were randomly assigned to the active environment (i.e., with access to a running wheel) and two individuals of each sex were randomly assigned to the sedentary environment (i.e., without access to a running wheel). Thus, four experimental groups were established: active selected, sedentary selected, active control, and sedentary control, with each activity environment containing two females and two males from each of the five families from each of the eight lines, for a total of 160 individuals per activity environment and 320 individuals used in the entire study. This design ensured that each activity environment contained equal numbers of individuals from all selection and control lines, and that each family contributed equal numbers of full-siblings of each sex to both the active and sedentary groups.

Mice in the active group were placed individually in 36 × 19 × 15-cm cages that contained a 10-cm radius running wheel (Nalgene Cages, Bend, OR) and electronic wheel-revolution counter built into the cage top. The active mice thus had the option of voluntarily getting into the wheel and running or remaining in the cage and not running. Sedentary mice were placed individually in standard rodent cages. Mice were checked daily; food and water were available ad libitum. Cage bottoms were cleaned once every two weeks and wheels were cleaned once every four weeks. Clean wheels were randomly assigned to active mice after each cleaning to insure no consistent effect on individual mice of any differences in mechanical resistance among wheels.

Data Collection and Statistical Analysis

Wheel-running activity, body mass, and apparent food consumption were measured weekly from ages 4 to 84 weeks.

Wheel-running activity in revolutions was converted to kilometers run per week for all analyses, and food consumption and body mass were measured in grams.

Univariate repeated measures analysis of variance was used to analyze the mean ontogenies of wheel running, body mass, and food consumption. Because sex differences in wheel-running activity existed in generation 0, before selection was imposed, traits have been analyzed separately for males and females in all studies on these mice (Swallow et al. 1998a). The general linear model used to analyze the ontogenies by univariate repeated measures is:

$$y = \mu + G + L(G) + F[L(G)] + M\{F[L(G)]\} + E + A$$
$$+ (E \times G) + [E \times L(G)] + \{E \times F[L(G)]\}$$
$$+ (A \times G) + [A \times L(G)] + \{A \times F[L(G)]\}$$
$$+ (A \times E) + (A \times E \times G) + [A \times E \times L(G)]$$
$$+ covariates + \epsilon \qquad (1)$$

where G, E, and A are the fixed effects of selection group, activity environment, and age, respectively. Line nested within selection group, $L(G)$; family nested within line nested within selection group, $F[L(G)]$; and mouse nested within family nested within line nested within selection group, $M\{F[L(G)]\}$ are random effects (Swallow et al. 1998a). The significance of the F-ratio for each effect was calculated by dividing the mean square of an effect by the appropriate error mean square. Hence, selection group, G, was tested over line nested within selection group, $L(G)$; line nested within selection group was tested over family nested within line nested within selection group, $F[L(G)]$; family nested within line nested within selection group was tested over mouse nested within family nested within line nested within selection group, $M\{F[L(G)]\}$; environment, E, and the interaction of environment by selection group, $E \times G$, were tested over the interaction of environment by line nested within selection group, $E \times L(G)$; age, A and the interaction of age by selection group, $A \times G$, were tested over the interaction of age by line nested within selection group, $A \times L(G)$; the interaction of activity by line nested within selection group, $E \times L(G)$, was tested over the interaction of activity by family nested within line nested within selection group, $E \times F[L(G)]$; the interaction of age by line nested within selection group, $A \times L(G)$, was tested over the interaction of age by family nested within line nested within selection group, $A \times F[L(G)]$; and the interaction of age by activity by selection group, $A \times E \times G$, was tested over the interaction of age by environment by line nested within selection group, $A \times E \times L(G)$. All other effects were tested over the mean square error.

The terms of primary interest in the model were the main effect by age interactions (e.g., selection group by age, $A \times G$; line within selection group by age, $A \times L(G)$; and activity group by age, $A \times E$), because they identify differences in both the position and shape of the ontogenies. If the interaction between the main effect and age is not significant, then significant differences in the main effect alone can be interpreted as differences in ontogeny position (i.e., the least square mean over all ages). All of the effects were evaluated by two-tailed F-tests except for selection group alone (i.e., a change only in position), which was evaluated by one-tailed

F-tests because of a priori knowledge about the direction of the correlated response to selection at specific ages and thus in the position of the entire ontogeny. Specifically, mice from selected lines were predicted to run more, eat more, and be smaller in body mass as compared with mice from the control lines (Swallow et al. 1998a, 1999; Koteja 1999b). When significant effects of replicate lines were detected, analyses were run on the selected and the control lines separately only for the purpose of determining if divergence was within one or both selection groups.

The general linear model in equation (1) was used to analyze the ontogenies for body mass and food consumption; however, the analysis of wheel running did not contain the activity environment fixed effect or any of the interactions with activity environment. This is because wheel running could only be measured in the active activity environment. The analyses of wheel running and food consumption were done with weekly body mass as a covariate in the model to account for the effects of changes in body mass over ontogeny.

All three traits were tested and determined to satisfy the assumptions of normality of residual error variance. The variance-covariance matrix for all analyses were found to be in violation of compound symmetry or the Huynh-Feldt (H-F) conditions (Johnson 1998); thus, significance of the age-by-main effect interactions were determined using H-F adjusted degrees of freedom (Milliken and Johnson 1984). The H-F adjustment to the F-ratio degrees of freedom has the effect of reducing the numerator and denominator degrees of freedom by the factor of the H-F ϵ. The H-F ϵ can vary between $1/(t - 1)$ and 1, where t is the number of repeated observations (Milliken and Johnson 1984). Thus, the H-F adjusted degrees of freedom provide a conservative test of differences in ontogenies among treatments (Milliken and Johnson 1984). The H-F F-ratio adjustment was used instead of multivariate analysis of variance tests or maximum-likelihood methods because our dataset has numerous incomplete longitudinal observations and is also quite large; standard statistical packages and computing resources are unable to accommodate such datasets. All analyses were performed in SAS version 8.1 using Type III sums of squares in Proc GLM (SAS Institute 1994).

RESULTS

Wheel Running

The ontogenies for wheel running, regardless of sex, selection group, or line within selection group, were characterized by a rapid increase in running early in ontogeny (i.e., between the ages of four and eight weeks), peaking at approximately week 8, which was also the average age of selection in the main selection experiment (Swallow et al. 1998a). This peak was followed by an essentially linear decline in wheel running until the end of the experiment at week 84 (Fig. 1). The effect of selection group on both position and shape of the ontogeny was estimated by the age by selection group interaction ($A \times G$) in the repeated measures analysis of variance model in equation (1); this interaction was significant in females and males ($F = 2.69$; df = 33, 199; $P < 0.0001$ and $F = 1.53$; df = 33, 198; $P = 0.0410$, respectively; Table 1). Thus, selection has resulted in the evolution of the shape and position of the ontogeny in both
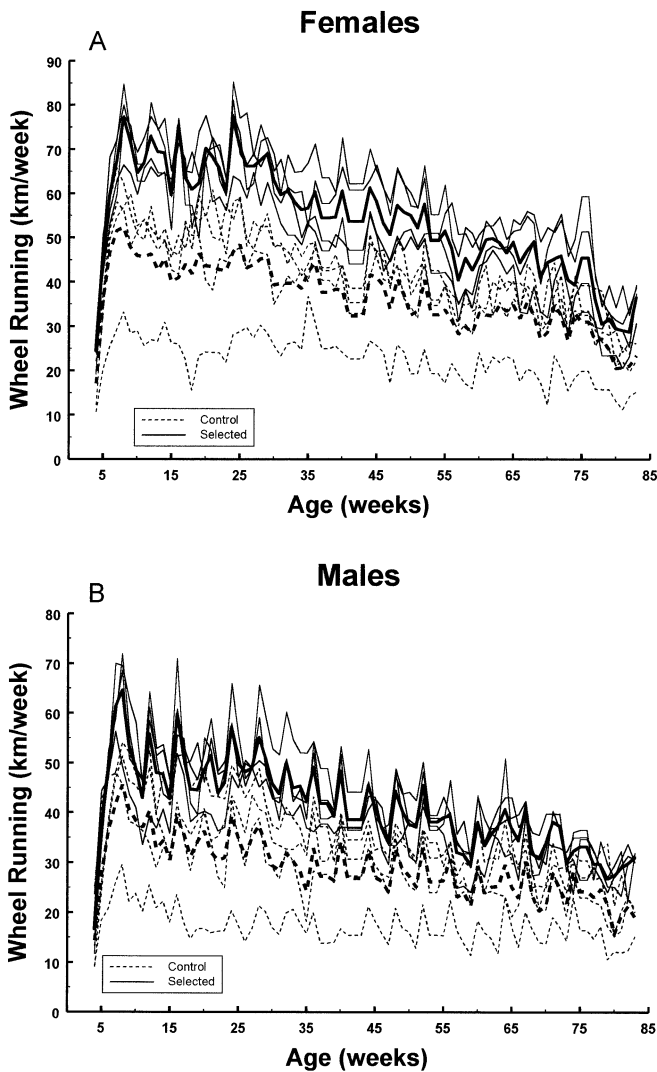
FIG. 1. Mean ontogenies of each genetically closed line of house mice through age 84 weeks for wheel-running activity (km/week) for females (A) and males (B). Selected lines are shown in solid lines, control lines are shown in dashed lines. Bold lines represent the mean ontogenies for the selected (bold solid line) or control (bold dashed line) selection groups.

females and males. The effect of selection is likely caused by the increased level of running in the selected mice early in ontogeny and a steeper rate of the decline in the selected mice as ontogeny progressed (Fig. 1).

The effect of genetic drift on the wheel-running ontogeny shape was tested by the significance of the age-by-line within selection group interaction $[A \times L(G)]$. This interaction term was significant for females and males ($F = 1.33$; df = 199, 1,118; $P = 0.0031$ and $F = 1.38$; df = 198, 1,081; $P = 0.0010$, respectively; Table 1), indicating significant effects of random genetic processes on the ontogenies in both selection groups. To determine if the effect of genetic drift was the same in each selection group, analyses were run on the selected and the control lines separately. The effect of genetic drift is the result of a highly significant divergence among lines within the control ($F = 1.56$; df = 99, 556; $P = 0.0010$ for females and $F = 1.88$; df = 100, 560; $P < 0.0001$ for

males) and a nonsignificant level of divergence within the selected mice($F = 1.16$; df = 99, 556; $P = 0.1554$ for females and $F = 1.04$; df = 100, 527; $P = 0.3862$ for males). Thus, it appears that genetic drift has had a greater influence on ontogeny shape and position in the control lines. The effects of genetic drift are likely the result of heterogeneity in the rate of decline in wheel-running activity among the lines within each of the selection groups over ontogeny (Fig. 1).

Body Mass

The mean ontogenies for body mass exhibit a characteristic pattern of change over ontogeny regardless of sex, selection group, line within selection group, or activity environment (Fig. 2). The body-mass ontogenies show rapid growth between weeks 4 and 20 followed by a period of slow growth over the remaining portion of ontogeny (i.e., weeks 20 to 84). The shape of the body mass ontogeny was not significantly influenced by selection as indicated by a nonsignificant age-by-selection group ($A \times G$) interaction term (eq. 1) in females or males ($F = 1.04$; df = 25, 148; $P = 0.4204$ and $F = 1.01$; df = 20, 117; $P = 0.4562$, respectively; Table 2). Because the interaction term was not significant, the selection group main effect (i.e., the difference in the selection group least square means across all ages) could be appropriately tested to determine if selection had shifted the position of the ontogeny without changing the shape. Selection had a significant effect on the position of the body-mass ontogenies resulting in smaller body mass at all ages in both females and males relative to the control lines (Fig. 2; one-tailed $F = 3.90$; df = 1, 6; $P = 0.0479$ and $F = 3.96$; df = 1, 6; $P = 0.0469$, respectively; Table 2).

A significant change in the shape and position of the ontogenies was caused by genetic drift in both females ($F = 1.92$; df = 148, 839; $P < 0.0001$) and males ($F = 1.88$; df = 117, 644; $P < 0.0001$; Table 2). This effect was the result of significant divergence within both the control ($F = 1.80$; df = 59, 332; $P = 0.0008$ for females and $F = 2.39$; df = 74, 419; $P < 0.0001$ for males) and the selected ($F = 1.43$; df = 74, 395; $P = 0.0002$ for females and $F = 1.43$; df = 74, 395; $P = 0.0177$ for males) selection groups.

The effect of activity environment on the position and shape of the body mass ontogeny was estimated by evaluating the age-by-activity environment interaction ($A \times E$). These were significant for both females ($F = 13.24$; df = 25, 148; $P < 0.0001$) and males ($F = 21.94$; df = 20, 117; $P < 0.0001$; Table 2), suggesting that access to running wheels significantly impacted the shape and position of the body mass ontogeny (Fig. 2). This difference appears to be caused by sedentary individuals continuing to grow at a faster rate over ontogeny compared to active individuals from the same selection group (Fig. 2).

Food Consumption

The mean ontogenetic trajectories for food consumption are more complex than those for wheel running or body mass and are characterized by peaks early and late in ontogeny (Fig. 3). In females, the three-way interaction between age, activity, and selection group was significant ($F = 1.83$; df = 28, 169; $P = 0.0105$; Table 3), suggesting the presence

TABLE 1. Wheel revolutions repeated-measures analyses of variance.

Source	df	MS	F	P
(A) Female wheel-evolutions				
Selection group[1]	1	62287.39	1.74	0.1174
Line (selection group)[2]	6	35719.34	3.54	0.0078
Family [line (selection group)][3]	34	10078.89	2.57	0.0026
Mouse {family [line (selection group)]}	38	3914.87	29.01	<0.0001
Age[4,5]	33	5898.07	30.32	<0.0001
Age × selection group[4,5]	33	524.05	2.69	<0.0001
Age × line (selection group)[5,6]	199	194.55	1.33	0.0031
Age × family [line (selection group)][5]	1118	146.81	1.09	0.0424
Body mass	1	8252.01	61.14	<0.0001
Error	2629	134.97		
(B) Male wheel revolutions				
Selection group[1]	1	19160.47	0.81	0.2012
Line (selection group)[2]	6	23597.42	3.52	0.0083
Family [line (selection group)][3]	33	6696.70	1.98	0.0207
Mouse {family [line (selection group)]}	39	3378.89	32.48	<0.0001
Age[4,7]	33	4281.80	24.49	<0.0001
Age × selection group[4,7]	33	266.97	1.53	0.0410
Age × line (selection group)[6,7]	198	174.85	1.38	0.0010
Age × family [line (selection group)][7]	1081	126.29	1.21	<0.0001
Body mass	1	10356.97	99.55	<0.0001
Error	2714	104.04		

[1] Significance determined with MS line (selection group) as MSE and one-tailed F-test.
[2] Significance determined with MS family [line (selection group)] as MSE.
[3] Significance determined with MS mouse {family [line (selection group)]} as MSE.
[4] Significance determined with MS age × line (selection group) as MSE.
[5] Female Huynh-Feldt corrected degrees of freedom (H-F ϵ = 0.4206).
[6] Significance determined with MS age × family [line (selection group)] as MSE.
[7] Male Huynh-Feldt corrected degrees of freedom (H-F ϵ = 0.4182).

of a selection group-by-environment interaction that produced complex changes in the shape and position of the trajectory (Fig. 3). The presence of a selection group-by-environment interaction in females makes interpretation of the separate effects of selection history and activity environment difficult because the shape and position of the trajectory depends on which selection group is present in which environment. Nevertheless, the pattern generally suggests that selected mice consume greater amounts of food than do control mice within each activity environment, although the ordering of the activity environment trajectories within each selection group is different (i.e., selected-sedentary mice have greater food consumption than selected-active mice, whereas the opposite pattern is found in the control lines), thus resulting in the significant selection group-by-environment interaction. In males, the three-way interaction of age-by-activity environment-by-selection group was not significant (F = 1.31; df = 36, 217; P = 0.1245; Table 3; Fig. 3). Selection had a significant effect on the position and the shape of the trajectory (F = 2.17; df = 36, 217; P = 0.0004; Table 3), which is the result of selected lines having a larger early-age peak than control lines in both activity environments (Fig. 3).

In females, genetic drift had a significant effect on the shape and position of the trajectories (F = 1.21; df = 169, 945; P = 0.0469; Table 3), although this effect was almost entirely caused by a highly significant divergence among control lines and no divergence among the selected lines. In males, the shape and position of the food-consumption ontogenies were not significantly changed as a result of genetic drift (F = 1.17; df = 217, 1,185; P = 0.0601; Table 3). Genetic drift did have a significant effect on the position of

the trajectories in males as evaluated by the line within selection group main effect (F = 2.65; df = 6, 33; P = 0.0330; Table 3). This suggests that random genetic changes have caused the mean trajectories of lines to diverge in shape and position in females and in position but not in shape in males (Fig. 3).

The age-by-activity environment interaction term was significant in both males (F = 3.61; df = 36, 217; P < 0.0001; Table 3) and females (F = 2.60; df = 28, 169; P = 0.0062; Table 3) although interpretation of the female effect is problematic because of the significant three-way interaction among selection group, activity environment, and age. The significant age-by-activity environment effect in males suggests that access to running wheels significantly changed the shape and position of the ontogenies (Fig. 3). The activity-environment effect appears to be the result of active individuals within both selection groups exhibiting increased levels of food consumption across ontogeny, followed by a pattern of ontogenetic change that is essentially parallel between the two activity environments (Fig. 3).

DISCUSSION

The evolution and expression of a trait ontogeny is determined by the interaction of the strength and timing of selection, the underlying genetic architecture of the ontogeny (Cheverud and Leamy 1985; Cowley and Atchley 1992), as well as various environmental influences. Previous studies of the evolution of ontogenies have focused almost exclusively on morphological characters, but behavioral ontogenies are also critical because many behaviors are complex phenotypes
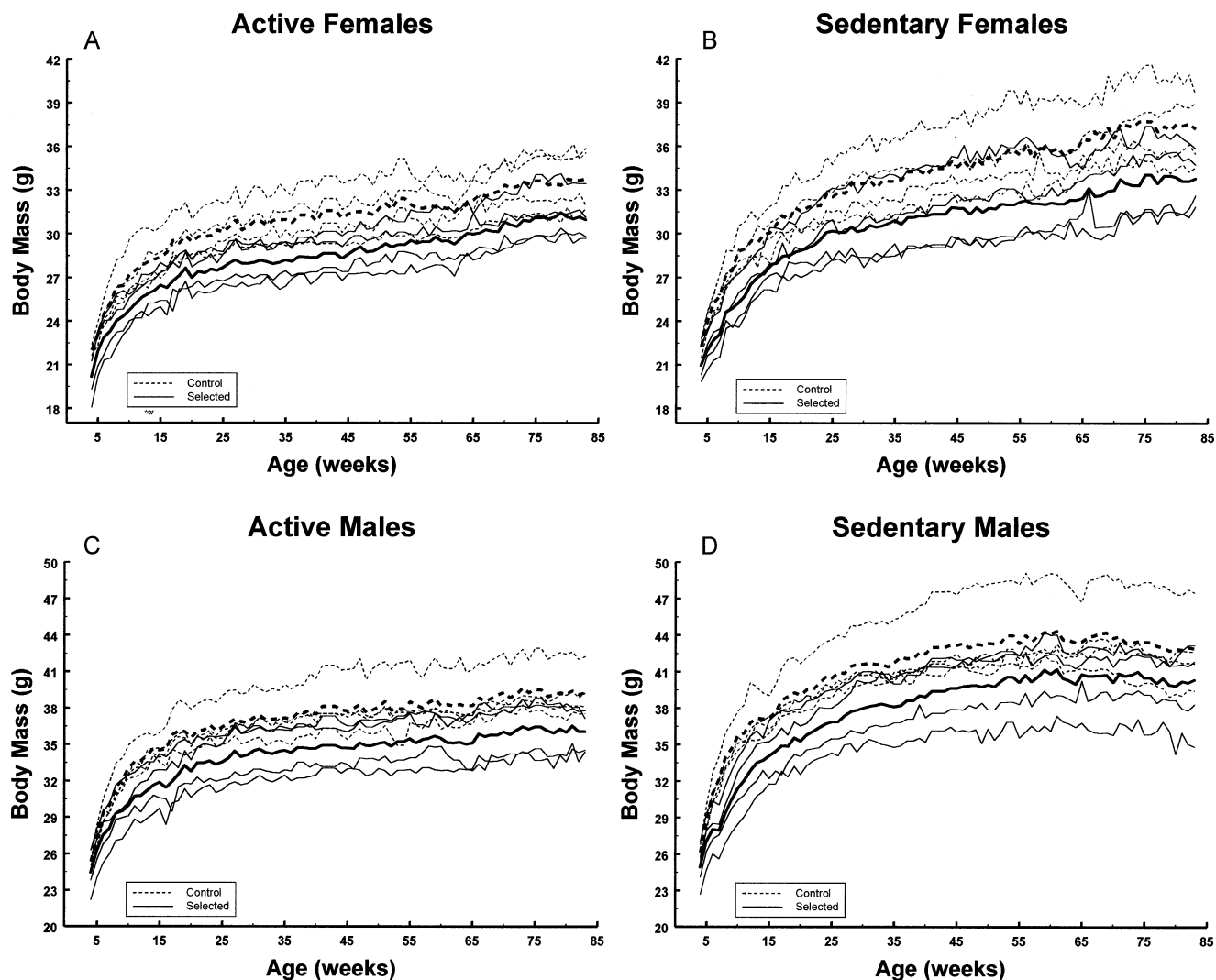
FIG. 2. Mean ontogenies of each genetically closed line of house mice within the two activity environments through age 84 weeks for body mass (g) for active females (A), sedentary females (B), active males (C), and sedentary males (D). Selected lines are shown in solid lines, control lines are shown in dashed lines. Bold lines represent the mean ontogenies for the selected (bold solid line) or control (bold dashed line) selection groups.

upon which natural and sexual selection may act relatively directly (Arnold 1990; Garland and Carter 1994). Here we used replicate lines of house mice artificially selected for high early-age wheel-running activity and their replicate control lines to evaluate the effects of early-age directional selection, genetic drift, and environmental variation on the ontogenies of three genetically correlated traits: voluntary wheel running, body mass, and food consumption (Swallow et al. 1998a, 1999; Koteja et al. 1999b). For each trait, significant changes in ontogeny shape and/or position caused by selection, genetic drift, and/or environment were identified. To our knowledge, this study is the first analysis of behavioral ontogenies in populations with known evolutionary histories.

Selection

Early-age selection for high wheel-running activity produced significant changes in the position and shape of the

wheel-running and food-consumption ontogenies (Figs. 1, 3) and produced significant changes in the position but not the shape of the body-mass ontogeny (Fig. 2). The differing effect of selection among the three traits suggests that, although selection has affected all of these traits at young ages (Swallow et al. 1998a, 1999; Koteja et al. 1999b), its effect on the entire ontogeny of the three traits is not the same.

Selection on wheel running resulted in a correlated response in the shape and position of the wheel running ontogeny in both females and males. This effect of selection is the result of the selected mice having a larger peak in wheel running early in ontogeny and having a steeper rate of decline in wheel-running activity across ontogeny than control mice (Fig. 1). Hence, selection has caused a pronounced response in the amount of wheel running early in ontogeny, when selection is imposed, but the correlated response in later portions of the ontogeny is less pronounced. Because the exact manner in which selection at a single age changes an ontog-

TABLE 2. Body mass repeated-measures analyses of variance.

Source	df	MS	F	P
(A) Female body mass				
Selection group[1]	1	18185.89	3.90	0.0479
Line (selection group)[2]	6	4661.35	3.97	0.0041
Family [line (selection group)][3]	34	1172.75	4.23	<0.0001
Mouse {family [line (selection group)]}	76	277.33	153.62	<0.0001
Activity[4]	1	15905.12	48.51	0.0004
Age[5,6]	25	1083.73	236.76	<0.0001
Activity × selection group[4]	1	230.46	0.70	0.4339
Activity × line (selection group)[7]	6	327.88	0.71	0.6422
Activity × family [line (selection group)]	34	460.37	181.62	<0.0001
Age × selection group[5,6]	25	4.76	1.04	0.4204
Age × line (selection group)[6,8]	148	4.58	1.92	<0.0001
Age × family [line (selection group)][6]	839	2.38	1.32	<0.0001
Age × activity[6,9]	25	28.60	13.24	<0.0001
Age × activity × selection group [6,9]	25	1.48	0.69	0.8607
Age × selection × line (selection group)[6]	148	2.16	—	—
Error	8115	1.81		
(B) Male body mass				
Selection group[1]	1	29463.81	3.96	0.0469
Line (selection group)[2]	6	7445.44	5.54	0.0005
Family [line (selection group)][3]	33	1344.94	2.47	0.0006
Mouse {family [line (selection group)]}	78	544.00	265.10	<0.0001
Activity[4]	1	38121.39	149.15	<0.0001
Age[5,10]	20	1360.11	242.53	<0.0001
Activity × selection group[4]	1	538.74	2.11	0.1967
Activity × line (selection group)[7]	6	255.59	0.28	0.9414
Activity × family [line (selection group)]	33	906.81	441.90	<0.0001
Age × selection group[5,10]	20	5.65	1.01	0.4562
Age × line (selection group)[8,10]	117	5.61	1.88	<0.0001
Age × family [line (selection group)][10]	644	2.99	1.46	<0.0001
Age × activity[9,10]	20	45.61	21.94	<0.0001
Age × activity × selection group[9,10]	20	1.63	0.78	0.7325
Age × activity × line (selection group)[10]	117	2.08	—	—
Error	8145	2.05		

[1] Signfiiance determined with MS line (selection group) as MSE and one-tailed F-test.
[2] Significance determined with MS family [line (selection group)] as MSE.
[3] Significance determined with MS mouse {family [line (selection group)]} as MSE.
[4] Significance determined with MS activity × line (selection group) as MSE.
[5] Significance determined with MS age × line (selection group) as MSE.
[6] Female Huynh-Feldt corrected degrees of freedom (H-F ϵ = 0.3122).
[7] Significance determined with MS activity × family [line (selection group)] as MSE.
[8] Significance determined with MS age × family [line (selection group)] as MSE.
[9] Significance determined with MS age × activity × line (selection group) as MSE.
[10] Male Huynh-Feldt corrected degrees of freedom (H-F ϵ = 0.2469).

eny is determined by the underlying pattern of genetic co-variation of the trait expressed at different ages (Kirkpatrick et al. 1990), our results suggest that early-age wheel running has a relatively large genetic covariance with wheel running at other young ages but a relatively small genetic covariance with wheel running at later ages. This differs from studies of selection on morphological traits in which selection generally shifted ontogeny position rather than ontogeny shape, suggesting large genetic covariances across young and old ages (Laird and Howard 1967; Eisen et al. 1969; Eisen 1976; Atchley and Rutledge 1980; Atchley et al. 1997).

Selection on wheel-running activity changed the body-mass ontogeny in the expected manner: selected mice had smaller body masses throughout the entire ontogeny in both sexes. However, selection did not significantly affect the shape of the body mass ontogeny. These results are similar to those from other studies that measured changes in the position of body-mass ontogenies after direct selection on body mass or early-age development rate (Timon and Eisen

1969; McCarthy and Bakker 1979; Atchley and Rutledge 1980; Rocchetta et al. 2000). The finding that the selected mice are smaller at all ages than the control mice for both sexes in both environments (Fig. 2) is also in agreement with previous studies on this system of mice, which have shown a significant negative correlated response in body mass at the ages near the age of selection (Swallow et al. 1999).

The significant change in the position of the body-mass ontogeny coupled with the differences between the correlated response of the body-mass and the wheel-running ontogenies suggests that the genetic covariance between wheel running and body mass is independent of the age at which body mass is measured. Furthermore, the different correlated responses of the body-mass and wheel-running ontogenies suggests that the body-mass ontogeny may be constrained to a particular shape by stronger genetic covariances of body mass with itself across ontogeny, in which case selection will more rapidly result in a correlated response in the position and less rapidly to change the shape of the ontogeny, implying that
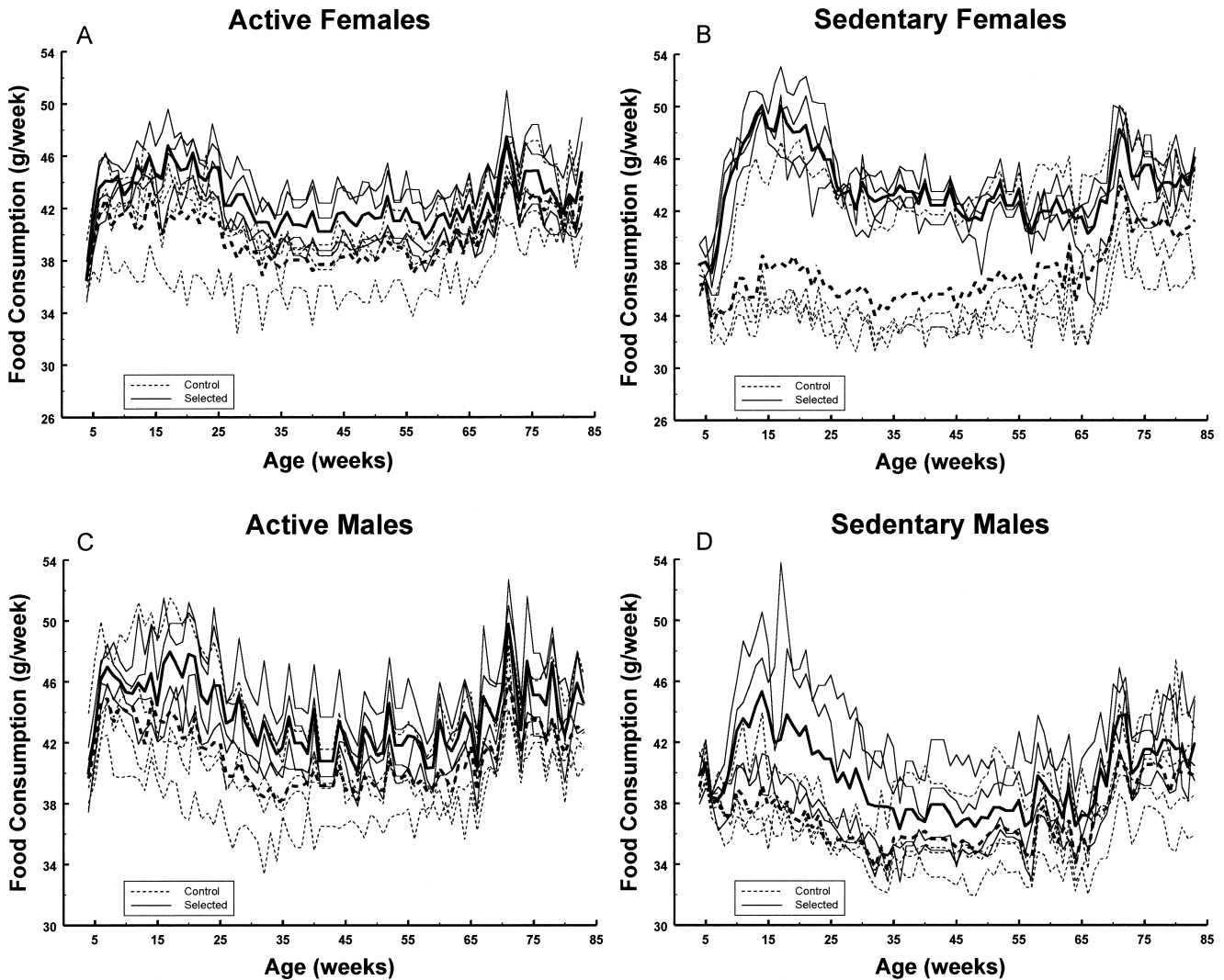
FIG. 3. Mean ontogenies of each genetically closed line of house mice within the two activity environments through age 84 weeks for food consumption (g/week) for active females (A), sedentary females (B), active males (C), and sedentary males (D). Selected lines are shown in solid lines, control lines are shown in dashed lines. Bold lines represent the mean ontogenies for the selected (bold solid line) or control (bold dashed line) selection groups.

body mass at later ages may not be able to evolve independently of early-age body mass (Kirkpatrick and Lofsvold 1992; Fig. 2). Thus, the differences between ontogenetic changes in wheel running and body mass may be caused partly by body mass being constrained by strong genetic covariances with itself across ontogeny, partly by weak or variable genetic covariances for wheel running with itself across ontogeny, and partly by relatively weak or variable genetic covariances between wheel running at older ages and body mass at all ages.

In addition, behavioral traits are in general more complex than morphological ones, and we can hypothesize various factors that might lead to a general difference in the pattern of evolutionary responses to age-specific selection. For example, wheel running is a composite trait that involves both motivation and physiological ability. Perhaps selection for early-age high voluntary wheel running occurs mainly by

increases in motivation. Motivational differences between selected and control lines might persist across ontogeny, but wheel running at later ages might be limited by a general decline in physiological abilities, such that both selected and control lines converge on similar activity levels later in life. Alternatively, activity at very young ages might be limited by ability rather than motivation. In any case, it is of interest to note that an age-related decline in activity levels appears to be a common phenomenon but one that presently lacks a good explanation (Ingram 2000; Sallis 2000).

The effect of selection on the food-consumption ontogenies was more complex. Selection on early-age wheel running resulted in food consumption increasing early in life, peaking between weeks 10 and 20, then declining during the middle portion of ontogeny, followed by an increase late in ontogeny (Fig. 3). In both females and males, selection had a significant effect on the shape and position of the food-consumption on-

TABLE 3. Food consumption repeated-measures analyses of variance.

Source	df	MS	F	P
(A) Female food consumption				
Selection group[1]	1	22680.29	6.85	0.0199
Line (selection group)[2]	6	4918.66	2.09	0.0802
Family [line (selection group)][3]	34	2353.08	2.66	0.0002
Mouse {family [line (selection group)]}	76	883.69	75.36	<0.0001
Activity[4]	1	3252.66	1.75	0.2338
Age[5,6]	28	484.51	25.66	<0.0001
Activity × selection group[4]	1	10510.33	5.66	0.0548
Activity × line (selection group)[7]	6	1856.37	1.14	0.3632
Activity × family [line (selection group)]	34	1634.98	139.44	<0.0001
Age × selection group[5,6]	28	124.27	6.58	<0.0001
Age × line (selection group)[6,8]	169	18.88	1.21	0.0469
Age × family [line (selection group)][6]	945	15.64	1.33	0.0001
Age × activity[6,9]	28	52.83	2.60	0.0062
Age × activity × selection group[6,9]	28	37.18	1.83	0.0105
Age × activity × line (selection group)[6]	169	20.35	—	—
Body mass	1	1981.54	168.99	<0.0001
Error	7889	11.73		
(B) Male food consumption				
Selection group[1]	1	7835.56	1.81	0.1134
Line (selection group)[2]	6	4322.13	2.65	0.0330
Family [line (selection group)][3]	33	1632.92	1.86	0.0130
Mouse {family [line (selection group)]}	78	875.95	69.42	<0.0001
Activity[4]	1	14801.18	3.76	0.1006
Age[5,10]	36	448.07	24.95	<0.0001
Activity × selection group[4]	1	2.73	0.00	0.9798
Activity × line (selection group)[7]	6	3937.18	2.85	0.0238
Activity × family [line (selection group)]	33	1380.25	109.41	<0.0001
Age × selection group[5,10]	36	39.05	2.17	0.0004
Age × line (selection group)[8,10]	217	17.96	1.17	0.0601
Age × family [line (selection group)][7,10]	1185	15.33	1.22	<0.0001
Age × activity[9,10]	36	66.89	3.61	<0.0001
Age × activity × selection group[9,10]	36	34.27	1.31	0.1245
Age × activity × line (selection group)[10]	217	18.55	—	—
Body mass	1	39.75	3.15	0.0759
Error	7913	12.61		

[1] Significance determined with MS line (selection group) as MSE and one-tailed F-test.
[2] Significance determined with MS family [line (selection group)] as MSE.
[3] Significance determined with MS mouse {family [line (selection group)]} as MSE.
[4] Significance determined with MS activity × line (selection group) as MSE.
[5] Significance determined with MS age × line (selection group) as MSE.
[6] Female Huynh-Feldt corrected degrees of freedom (H-F ϵ = 0.3555).
[7] Significance determined with MS activity × family [line (selection group)] as MSE.
[8] Significance determined with MS age × family [line (selection group)] as MSE.
[9] Significance determined with MS age × activity × line (selection group) as MSE.
[10] Male Huynh-Feldt corrected degrees of freedom (H-F ϵ = 0.4586).

togeny; the selected lines generally consumed more food than did the control lines across ontogeny, which is similar to other studies that found selection for activity resulted in a correlated response in food consumption at landmark ages (Dunnington et al. 1981; Swallow et al. 2001). This suggests that the selected mice are generally more active and require more energy, resulting in greater consumption of food (Swallow et al. 2001). In addition, the late ontogeny increase in food consumption coupled with the late ontogeny decline in wheel running suggests the possibility of changes in body composition at older ages (i.e., 45–84 weeks) caused primarily by an increase in fat deposition (Slentz and Holloszy 1993; Swallow et al. 2001). Although our data do not directly address this change, we hypothesize that an analysis of body composition in these mice would reveal an increase in body fat within both selection groups at later ages; such hypotheses can be addressed in future studies of this model system of mice.

Genetic Drift

Because each of the lines within a selection group should be experiencing approximately similar strengths of selection, divergence among lines within a selection group should largely be caused by random fluctuations in allele frequencies (Falconer and Mackay 1996). In this system significant genetic drift (i.e., among-line variation within each selection group) at specific ages has been measured (Swallow et al. 1998a; Garland et al. 2002), and divergence of quantitative genetic characters among lines within selection groups is in agreement with neutral expectations as determined by comparison of levels of divergence based on neutral molecular and quantitative traits (T. J. Morgan, M. A. Evans, T. Garland, Jr., J. G. Swallow, and P. A. Carter, unpubl. ms.).

Genetic drift influenced the shape and position of the ontogenies for wheel running and body mass in both males and

females and the position of the food-consumption ontogeny in males. For wheel running in both sexes and food consumption in males, genetic drift had a larger effect in the control lines than the selected lines, suggesting that for these traits selection is limiting the divergence among replicate line ontogenies. Body mass genetic drift had similar effects in both the selected and control groups, suggesting that genetic drift was not influenced by the early-age correlated response to selection (Swallow et al. 1999). Thus, for the two ontogenies that changed in shape and position as a result of selection (wheel running and food consumption), drift only influenced the control line; however, for the ontogeny that changed only in position as a result of selection (body mass), drift affected both the selected and control lines. Other studies have observed similar effects of genetic drift on the genetic architecture of trait ontogenies (Rhees and Atchley 2000) and of correlated traits (Arnold and Phillips 1999; Roff and Mousseau 1999; Roff 2000; Phillips et al. 2001), demonstrating that genetic drift can significantly influence the rate and direction of evolutionary change and that its effects in non-selected populations, at least for some traits may be stronger (but see Garland et al. 2002).

Activity Environment

The body-mass and food-consumption ontogenies were measured within two activity environments, presence or absence of an activity wheel. Access to a running wheel has significant effects on body mass (Swallow et al. 1999) and on food consumption (Slentz and Holloszy 1993; Swallow et al. 2001) in young mice and provides a variable that allows evaluation of the environmental plasticity of each ontogeny.

The body-mass ontogeny differed between active and sedentary mice because of an increased rate and duration of the early-age growth period in the sedentary mice, regardless of selection group, in both males and females (Fig. 2); hence, the activity environment caused the shape and position of the body mass ontogeny to change. Previous studies have shown a significant change as a result of activity environment in the body composition of middle-aged rats (25 months; Slentz and Holloszy 1993) and 49-day-old mice (Swallow et al. 2001). Both of these studies showed that physical inactivity resulted in an increase in body fat content and marginal changes in lean body mass. Thus, the change in the pattern of growth between the activity environments is likely the result of environment-induced changes in body composition over ontogeny (Slentz and Holloszy 1993; Swallow et al. 2001), which dovetails with the hypothesis proposed above that fat deposition increases in active mice late in ontogeny as they run less and eat more.

The activity environment also had significant effects on the food-consumption ontogeny, but the location of the change in the shape of the food-consumption ontogeny is more complex. In both the selected and control males and the control females, activity environment produced the expected effect of active mice generally consuming larger quantities of food across ontogeny relative to sedentary mice from the same selection group. However, selected-sedentary females have a larger peak in early-age food consumption than do active individuals (Fig. 3), which is opposite the pattern

seen in the control lines. The pattern of ontogenetic change in selected females is different from previous results on the relationship between activity and food consumption (Slentz and Holloszy 1993; Swallow et al. 2001), which generally suggests active animals expend more energy and therefore require greater quantities of food (Koteja et al. 1999b; Swallow et al. 2001). The unusual result in the sedentary-selected females may be the result of early-age activity even in the absence of a running wheel (see also Rhodes et al. 2001). If this were the case, then these sedentary mice could have energy needs in excess of their active counterparts, thereby producing the unexpected pattern. This result is also supported by the observation that the body-mass ontogenies of the active- and sedentary-selected females did not begin to diverge until about week 15 (Fig. 2), which suggests that selected individuals in both environments were growing at similar rates at early ages and thus potentially had similar energy requirements. In addition, Koteja et al. (1999a) found effects of activity environment on within-cage locomotion in a previous generation of these mice ($P = 0.066$ in females and $P = 0.0001$ in males; Koteja et al. 1999a), with sedentary mice exhibiting increased within-cage activity relative to active individuals. Thus, the pattern observed in the selected females at early ages may be the result of high within-cage activity, regardless of activity environment. A possible alternative explanation is that the amount of food wasted (e.g., see Hastings et al. 1997) shows a selection group-by-environment interaction such that selected-sedentary females waste more food. However, studies on other mice from the selected and control lines have not found differences in food wasting in relation to selection history (P. Koteja, P. A. Carter, J. G. Swallow, and T. Garland, Jr., unpubl. data).

Because the environment in which the mice were raised had a significant effect on the shape and position of both the body-mass and food-consumption ontogenies, a large degree of plasticity exists in these ontogenies. Other studies on growth in different environments have found similar effects in both endotherms and ectotherms. Significant effects of dietary protein content on early growth were measured in mice (Nielsen and Anderson 1982). Cowley et al. (1989) found significant effects of maternal environment on mouse postnatal growth and adult body size. Thompson (1999) found significant diet-induced phenotypic plasticity in the pattern of development of morphological characters in a grasshopper (*Melanoplus femurrubrum*), and Bronikowski (2000) found significant effects of temperature on first-year growth rate in the garter snake (*Thamnophis elegans*). Thus, the effect of environment-induced plasticity in ontogenies appears to be common in many species.

Future Studies

Our results demonstrate the dynamic nature of the evolution and expression of trait ontogenies and demonstrate that single-age selection, genetic drift, and environmental variation can each have an effect on patterns of ontogenetic evolution. The evolution of these three ontogenies ultimately depends on patterns of genetic covariance between the traits and within traits across ages. Although the present study has not directly addressed the ontogenetic patterns of genetic

covariation, future analyses of these data using function-valued methods (Kingsolver et al. 2001) will directly examine the underlying patterns of genetic variances and covariances among traits and within traits across ontogeny (T. J. Morgan and P. A. Carter, unpubl. ms.).

Literature Cited

Alberch, P., S. J. Gould, G. Oster, and D. Wake. 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5:297–317.

Arnold, S. J. 1990. Inheritance and the evolution of behavioral ontogenies. Pp. 167–189 in M. E. Hahn, J. K. Hewitt, N. D. Henderson, and R. H. Benno, eds. Developmental behavior genetics: neural, biometrical, and evolutionary approaches. Oxford Univ. Press, New York.

———. 1992. Constraints on phenotypic evolution. Am Nat 140: S85–S107.

Arnold, S. J., and P. C. Phillips. 1999. Hierarchical comparison of the genetic variance-covariance matrices. II. Coastal-inland divergence in the garter snake, *Thamnophis elegans*. Evolution 53: 1516–1527.

Atchley, W. R., and J. J. Rutledge. 1980. Genetic components of size and shape. I. Dynamics of components of phenotypic variability and covariability during ontogeny in the laboratory rat. Evolution 34:1161–1173.

Atchley, W. R., S. Xu, and D. E. Cowley. 1997. Altering developmental trajectories in mice by restricted index selection. Genetics 146:629–640.

Badyaev, A. V., L. A. Whittingham, and G. E. Hill. 2001. The evolution of sexual size dimorphism in the house finch. III. Developmental basis. Evolution 55:176–189.

Bennett, A. F., and J. A. Ruben. 1979. Endothermy and activity in vertebrates. Science 206:649–654.

Björklund, M. 1997. Variation in growth in the blue tit (*Parus caeruleus*). J Evol Biol 10:139–155.

Boggs, D. F., and P. B. Frappell. 2000. Unifying principles of locomotion: foreword. Physiol Biochem Zool 73:647–650.

Bronikowski, A. M. 2000. Experimental evidence for the adaptive evolution of growth rate in the garter snake *Thamnophis elegans*. Evolution 54:1760–1767.

Brooks, M. J. 1991. The ontogeny of sexual dimorphism: quantitative models and a case study in labrisomid blennies (Teleostei: *Paraclinus*). Syst Zool 40:271–283.

Carter, P. A., J. G. Swallow, S. J. Davis, and T. Garland Jr. 2000. Nesting behavior of house mice (*Mus domesticus*) selected for increased wheel-running activity. Behav Genet 30:85–94.

Cheverud, J. M., and L. J. Leamy. 1985. Quantitative genetics and the evolution of ontogeny. III. Ontogenetic changes in correlation structure among live-body traits in randombred mice. Gen Res 46:325–335.

Cheverud, J. M., J. J. Rutledge, and W. R. Atchley. 1983. Quantitative genetics and development: genetic correlations among age-specific trait values and the evolution of ontogeny. Evolution 37:895–905.

Cowley, D. E., and W. R. Atchley. 1992. Quantitative genetic model for development, epigenetic selection, and phenotypic evolution. Evolution 46:495–518.

Cowley, D. E., D. Pomp, W. R. Atchley, E. J. Eisen, and D. Hawkins-Brown. 1989. The impact of maternal uterine genotype on postnatal growth and adult body size in mice. Genetics 122: 193–203.

Creighton, K. G., and R. E. Strauss. 1986. Comparative patterns of growth and development in cricetine rodents and the evolution of ontogeny. Evolution 40:94–106.

Dunnington, E. A., J. M. White, and W. E. Vinson. 1981. Selection for serum cholesterol levels, voluntary physical activity, 56-day body weight and feed intake in randombred mice. II. Correlated responses. Can J Genet Cytol 23:545–555.

Eisen, E. J. 1976. Results of growth curve analyses in mice and rats. J Anim Sci 42:1008–1023.

Eisen, E. J., B. J. Lang, and J. E. Legates. 1969. Comparison of growth functions within and between lines of mice selected for large and small body weight. Theor Appl Gent 39:251–260.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Addison Wesley Longman, Essex, U.K.

Garland, T., Jr. 2003. Selection experiments: an underutilized tool in biomechanics and organismal biology. Pp. 23–56 in V. L. Bels, J.-P. Gasc, and A. Casinos, eds. Vertebrate biomechanics and evolution. BIOS Scientific Publishers, Oxford, U.K.

Garland, T., Jr.,and P. A. Carter. 1994. Evolutionary physiology. Ann Rev Phys 56:579–621.

Garland, T., Jr.,and J. B. Losos. 1994. Ecological morphology of locomotor performance in squamate reptiles. Pp. 240–302 in P. C. Wainwright and S. M. Reilly, eds. Ecological morphology: integrative organismal biology. Univ. of Chicago Press, Chicago.

Garland, T., Jr., M. T. Morgan, J. G. Swallow, J. S. Rhodes, I. Girard, J. G. Belter, and P. A. Carter. 2002. Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. Evolution 56:1267–1275.

Girard, I., M. W. McAleer, J. S. Rhodes, and T. Garland Jr. 2001. Selection for high voluntary wheel running increases intermittency in house mice (*Mus domesticus*). J Exp Biol 204: 4311–4320.

Girard, I., J. G. Swallow, P. A. Carter, P. Koteja, J. S. Rhodes, and T. Garland Jr. 2002. Maternal-care behavior and life-history traits in house mice (*Mus domesticus*) artificially selected for high voluntary wheel-running activity. Behavioral Processes. 57: 37–50.

Gould, S. J. 1977. Ontogeny and phylogeny. Harvard Univ. Press, Cambridge, MA.

Hahn, M. E., J. K. Hewitt, N. D. Henderson, and R. H. Benno. 1990. Developmental behavior genetics: neural, biometrical, and evolutionary approaches. Oxford Univ. Press, New York.

Hastings, I. M., S. M. Moruppa, L. Bünger, and W. G. Hill. 1997. Effects of selection on food intake in the adult mouse. J Anim Breed Genet 114:419–434.

Hoying, K. M., and T. H. Kunz. 1998. Variation in size at birth and post-natal growth in the insectivorous bat *Pipistrellus subflavus* (Chiroptera: Vespertilionidae). J Zool 245:15–27.

Ingram, D. K. 2000. Age-related decline in physical activity: generalization to non-humans. Medicine & Science in Sports & Exercise 32:1623–1629.

Irschick, D. J., and T. Garland Jr. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. Ann Rev Ecol and Syst 32:367–396.

Johnson, D. E. 1998. Applied multivariate methods for data analysis. Duxbury Press, Pacific Grove, CA.

Jones, C. S. 1992. Comparative ontogeny of a wild cucurbit and its derived cultivar. Evolution 46:1827–1847.

Kelt, D. A., and D. H. Van Vuren. 2001. The ecology and macroecology of mammalian home range area. Am Nat 157: 637–645.

Kingsolver, J. G., R. Gomulkiewicz, and P. A. Carter. 2001. Var-

iation, selection, and the evolution of function-valued traits. Genetica 112–113:87–104.

Kirkpatrick, M., and D. Lofsvold. 1992. Measuring selection and constraint in the evolution of growth. Evolution 46:954–971.

Kirkpatrick, M., D. Lofsvold, and M. Bulmer. 1990. Analysis of the inheritance, selection and evolution of growth trajectories. Genetics 124:979–993.

Klingenberg, C. P., and M. Zimmermann. 1992. Static, ontogenetic, and evolutionary allometry: a multivariate comparison in nine species of water striders. Am Nat 140:601–620.

Koteja, P., T. Garland Jr., J. K. Sax, J. G. Swallow, and P. A. Carter. 1999a. Behavior of house mice artificially selected for high levels of voluntary wheel running. Animal Behavior 58:1307–1318.

Koteja, P., J. G. Swallow, P. A. Carter, and T. Garland Jr. 1999b. Energy cost of wheel running in house mice: implications for coadaptation of locomotion and energy budgets. Physiol Biochem Zool 72:238–249.

Kramer, D. L., and R. L. McLaughlin. 2001. The behavioral ecology of intermittent locomotion. Am Zool 41:137–153.

Laird, A. K., and A. Howard. 1967. Growth curves in inbred mice. Nature 213:786–788.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Loy, A., M. Bertelletti, C. Costa, L. Ferlin, and S. Cataudella. 2001. Shape changes and growth trajectories in the early stages of three species of the genus *Diplodus* (Perciformes, Sparidae). J Morphol 250:24–33.

Lynch, C. B. 1994. Evolutionary inferences from genetic analyses of cold adaptation in laboratory and wild populations of the house mouse. Pp. 278–301 *in* C. B. Boake, ed. Quantitative genetic studies of behavioral evolution. Univ. of Chicago Press, Chicago.

McCarthy, J. C., and H. Bakker. 1979. The effect of selection for different combinations of weights at two ages on the growth curve of mice. Theor Appl Genet 55:57–64.

Miles, D. B., B. Sinervo, and W. Anthony Frankino. 2000. Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. Evolution 54:1386–1395.

Milliken, G. A., and D. E. Johnson. 1984. Analysis of messy data. Vol. I. Designed experiments. Lifetime Learning Publications, Belmont, CA.

Nielsen, B. V., and S. Andersen. 1982. The effect of dietary protein content on growth in a genetically heterogeneous population of mice. Growth 46:343–354.

Phillips, P. C., M. C. Whitlock, and K. Fowler. 2001. Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. Genetics 158:1137–1145.

Rhees, B. K., and W. R. Atchley. 2000. Body weight and tail length divergence in mice selected for rate of development. J Exp Zool 288:151–164.

Rhodes, J. S., P. Koteja, J. G. Swallow, P. A. Carter, and T. Garland Jr. 2000. Body temperatures of house mice artificially selected for high voluntary wheel-running behavior: repeatability and effect of genetic selection. J Thermal Biol 25:391–400.

Rhodes, J. S., G. R. Hosack, I. Girard, A. E. Kelley, G. S. Mitchell, and T. Garland Jr. 2001. Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. Psychopharmacology 158:120–131.

Roberts, R. C. 1961. The lifetime growth and reproduction of selected strains of mice. Heredity 16:369–381.

Rocchetta, G., M. L. Vanelli, and C. Pancaldi. 2000. Analysis of inheritance of growth trajectories in laboratory populations of guppy-fish. Growth Dev Aging 64:83–90.

Roff, D. 2000. The evolution of the **G** matrix: selection or drift? Heredity 84:135–142.

Roff, D., and T. Mousseau. 1999. Does natural selection alter genetic architecture? An evaluation of quantitative genetic variation among populations of *Allonemobius socius* and *A. fasciatus*. J Evol Biol 12:361–369.

Sallis, J. F. 2000. Age-related decline in physical activity: a synthesis of human and animal studies. Medicine & Science in Sports & Exercise 32:1598–1600.

SAS Institute. 1994. SAS/STAT user's guide. Ver. 6.0. SAS Institute, Inc., Cary, NC.

Schwenk, K. 1995. A utilitarian approach to evolutionary constraint. Zoology 98:252–261.

Slentz, C. A., and J. O. Holloszy. 1993. Body composition of physically inactive and active 25-month-old female rats. Mech Ageing Devel 69:161–166.

Swallow, J. G., P. A. Carter, and T. Garland Jr. 1998a. Artificial selection for increased wheel-running behavior in house mice. Behav Genet 28:227–237.

Swallow, J. G., T. Garland Jr., P. A. Carter, W. Z. Zhan, and G. C. Sieck. 1998b. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). J Appl Physiol 84:69–76.

Swallow, J. G., P. Koteja, P. A. Carter, and T. Garland Jr. 1999. Artificial selection for increased wheel-running activity in house mice results in decreased body mass at maturity. J Exp Biol 202:2513–2520.

Swallow, J. G., P. Koteja, P. A. Carter, and T. Garland Jr. 2001. Food consumption and body composition in mice selected for high wheel-running activity. J Comp Physiol B 171:651–659.

Thompson, D. B. 1999. Genotype-environment interaction and the ontogeny of diet-induced phenotypic plasticity in size and shape of *Melanoplus femurrubrum* (Orthopter: Acrididae). J Evol Biol 12:38–48.

Thomson, S. L., T. Garland Jr., J. G. Swallow, and P. A. Carter. 2002. Response of Sod-2 enzyme activity to selection for high voluntary wheel running. Heredity. 88:52–61.

Timon, V. M., and E. J. Eisen. 1969. Comparison of growth curves of mice selected and unselected for postweaning gain. Theor Appl Gen 39:345–351.

Zelditch, M. L. 1988. Ontogenetic variation in patterns of phenotypic integration in the laboratory rat. Evolution 42:28–41.

Zelditch, M. L., and A. Carmichael. 1989. Ontogenetic variation in patterns of developmental and functional integration in skulls of *Sigmodon fulviventer*. Evolution 43:814–824.

Zelditch, M. L., H. D. Sheets, and W. L. Fink. 2000. Spatiotemporal reorganization of growth rates in the evolution of ontogeny. Evolution 54:1363–1371.