Copyright © 1997 by the Genetics Society of America

Developmental Quantitative Genetics, Conditional Epigenetic Variability and Growth in Mice

William R. Atchley* and Jun Zhu†

*Center for Quantitative Genetics, Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695-7614 and †Agronomy Department, Zhejiang Agricultural University, Hangzhou 310029, China

Manuscript received December 5, 1996
Accepted for publication June 26, 1997

ABSTRACT

Ontogenetic variation in the causal components of phenotypic variability and covariability is described for body weight and tail length in mice derived from a full 7 × 7 diallel cross. Age-related changes in additive, dominance, sex-linked and maternal variance and covariance between 14 and 70 days of age are described. Age-specific variance components at time t are conditioned on the causal genetic effects at time $(t - 1)$. This procedure demonstrates the generation of significant episodes of new genetic variation arising at specific intervals during ontogeny. These episodes of new genetic variation are placed in the context of epigenetic models in developmental quantitative genetics. These results are also concordant on recent findings on age-specific gene expression in mouse growth as shown by QTL analyses.

RECENTLY, a series of developmental quantitative genetic models have been proposed to deal with complex morphological structures (Atchley 1984, 1987; Atchley and Hall 1991; Cowley and Atchley 1991; Atchley *et al.* 1994). These models assume that development of complex morphological structures occurs through the actions and interactions of many genes that act differentially during ontogeny and whose expression is modified by interactions with other genes and by the cell or organism environment. An important aspect of these models is that the underlying genetic control of a complex trait may change significantly during ontogeny (Atchley 1984, 1987; Atchley *et al.* 1994). Indeed, there is considerable experimental evidence from quantitative genetic analyses in rodents that the causal components of phenotypic variability and covariability, including additive, non-additive and maternal genetic effects, exhibit a dynamic behavior during ontogeny (Atchley and Rutledge 1980; Cheverud *et al.* 1983; Atchley 1984; Riska and Atchley 1984; Riska *et al.* 1985; Atchley *et al.* 1991; Cowley and Atchley 1992; and others). These findings are consistent with a model of the genetic architecture of complex traits like body weight in mice involving expression of many genes with age-dependent patterns of gene expression. These ontogenetic dynamics add considerable complexity to discussions about quantitatively inherited traits particularly for such important problems as age-specific response to selection and trait definition (Atchley *et al.* 1994).

At the center of these quantitative genetic models is the concept of heritable epigenetic effects (Atchley and Hall 1991; Cowley and Atchley 1992; Atchley *et al.* 1994). Epigenetic effects occur because of the regulatory, interactive, sequential and hierarchical nature of development. Inductive interactions are common in sequential and hierarchical systems. One component (*e.g.*, a gene, protein, cell or tissue) may "induce" activities in other components and alter the eventual phenotype in a unidirectional or "cause and effect" fashion. Heritable epigenetic interactions often occur in developmental cascades and involve a time component. Thus, an event at time t can have a significant consequence on subsequent phenotypes later in ontogeny. Since these time-dependent cascade effects are heritable and unidirectional (inductive), they qualify as epigenetic phenomena. For example, a hormone or mitogen may induce a population of cells to undergo differentiation or proliferation (Vogl *et al.* 1993) or a muscle may induce a developing bone to alter its final form (Herring 1993). When these epigenetic interactions have a heritable basis, they are potentially important factors in evolution (Cowley and Atchley 1992; Atchley *et al.* 1994).

One of the complications of these models is that it is often difficult to elucidate age-specific gene effects in quantitative traits. In this paper we explore the dynamics during postnatal ontogeny of the causal components of phenotypic variability for two complex morphological traits in mice. Further, using recently developed statistical procedures we examine the impact of time-related epigenetic events on the dynamics of the causal components of phenotypic variation. We inquire whether these procedures can be used to find evidence for ontogenetic patterns of gene expression

Corresponding author: William R. Atchley, Center for Quantitative Genetics, Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614. E-mail: atchley@ncsu.edu

in quantitative traits. We examine the postnatal dynamics in variance components for body weight and tail length. These two traits have separate developmental pathways, genetic control and dynamics.

MATERIALS AND METHODS

Mouse strains and husbandry: The data described in this report arose from a diallel cross among seven different inbred mouse strains including A/J (A1), BALB/cByJ (A2), C3He/FeJ (A3), C57BL/6ByJ (A4), SEA/GnJ (A5), SEC/1ReJ (A6) and SWR/J (A7). All strains were obtained from the JACKSON Laboratory. In a full diallel cross design, reciprocal crosses were made among all seven inbred strains so there are no empty cells. Data are then recorded on the resultant F_1 and parent mice. In these analyses, a total of 2970 mice, including 1478 males and 1492 females, were used.

All litters were standardized (randomly) to six pups at birth. As much as possible, an equal sex ratio was maintained. Body weight (in g) and tail length (in mm) was taken at weekly intervals between 2 and 10 weeks of age. All litters were produced contemporaneously.

Statistical methodology: A genetic model with sex-linked and maternal effects (ZHU and WEIR 1996) was used for analyzing the mice data. The phenotypic mean measured at time t for male mice from the cross between maternal line i and paternal line j can be partitioned as

$$y_{ij1(t)} = \mu_{(t)} + A_{i(t)} + A_{j(t)} + D_{ij(t)} + L_{i1(t)} + M_{i(t)} + \epsilon_{ij1(t)}$$

and for female mice as

$$y_{ij2(t)} = \mu_{(t)} + A_{i(t)} + A_{j(t)} + D_{ij(t)} + \tfrac{1}{2} L_{i2(t)} + \tfrac{1}{2} L_{j2(t)} + M_{i(t)} + \epsilon_{ij2(t)},$$

where $\mu_{(t)}$ is population mean at time t, $A_{i(t)}$ (or $A_{j(t)}$) is additive effects at time t for autosomal genes, $D_{ij(t)}$ is dominance effects at time t for autosomal genes, $L_{i1(t)}$ (or $L_{i2(t)}$, $L_{j2(t)}$) is additive effects at time t for sex-linked genes, $M_{i(t)}$ is maternal effects at time t, and $\epsilon_{ij1(t)}$ or $\epsilon_{ij2(t)}$ is residual errors at time t.

Variance components (additive V_A, dominance V_D, sex-linked V_L, maternal V_M, and residual V_ϵ) and covariance components (additive C_A, dominance C_D, sex-linked C_L, maternal C_M, and residual C_ϵ) were estimated by minimum norm quadratic unbiased estimation (MINQUE) method (RAO 1970, 1971) by setting 1 for all prior values (ZHU and WEIR 1996). MINQUE is a statistical method for estimating variances and covariances of mixed linear models. When variance components were obtained, phenotypic variance was calculated by $V_P = V_A + V_D + V_L + V_M + V_\epsilon$ and then the proportion of genetic variance components as a function of phenotypic variance was estimated. Correlation coefficients between traits X and Y were calculated for additive effects $r_A = C_A/\sqrt{V_{A(X)}V_{A(Y)}}$, dominance effects $r_D = C_D/\sqrt{V_{D(X)}V_{D(Y)}}$, sex-linked effects $r_L = C_L/\sqrt{V_{L(X)}V_{L(Y)}}$,, and maternal effects $r_M = C_M/\sqrt{V_{M(X)}V_{M(Y)}}$.

Genetic variation ($V_{(t)}$) revealed by analyzing developmental behavior at time t can only provide inference for accumulated causal effects at time $(t-1)$ and extra effects within the period $(t-1)$ to t. These two kinds of gene effects ($G_{(t-1)}$ and $G_{(t)}$) are usually not independent ($\mathrm{Cov}(G_{(t-1)}, G_{(t)}) = C_{G(t-1,t)}$). The phenotypic mean at time t conditional on phenotypic mean measured at time $(t-1)$ for male mice from the cross between maternal line i and paternal line j can be partitioned as

$$y_{ij1(t|t-1)} = \mu_{(t|t-1)} + A_{i(t|t-1)} + A_{j(t|t-1)} + D_{ij(t|t-1)} + L_{i1(t|t-1)} + M_{i(t|t-1)} + \epsilon_{ij1(t|t-1)}$$

and for female mice as

$$y_{ij2(t|t-1)} = \mu_{(t|t-1)} + A_{i(t|t-1)} + A_{j(t|t-1)} + D_{ij(t|t-1)} + \tfrac{1}{2} L_{i2(t|t-1)} + \tfrac{1}{2} L_{j2(t|t-1)} + M_{i(t|t-1)} + \epsilon_{ij2(t|t-1)},$$

where $\mu_{(t|t-1)}$ is conditional population mean, $A_{i(t|t-1)}$ (or $A_{j(t|t-1)}$) is conditional additive effects for autosomal genes, $D_{ij(t|t-1)}$ is conditional dominance effects for autosomal genes, $L_{i1(t|t-1)}$ (or $L_{i2(t|t-1)}$, $L_{j2(t|t-1)}$) is conditional additive effects for sex-linked genes, $M_{i(t|t-1)}$ is conditional maternal effects, and $\epsilon_{ij1(t|t-1)}$ or $\epsilon_{ij2(t|t-1)}$ is conditional residual errors.

Conditional genetic effects and conditional variance components (additive $V_{A(t|t-1)}$, dominance $V_{D(t|t-1)}$, sex-linked $V_{L(t|t-1)}$, maternal $V_{M(t|t-1)}$, and residual $V\epsilon_{(t|t-1)}$) were analyzed by a mixed model approach (ZHU 1995). The genetic effects at time t conditional on the causal genetic effects at time $(t-1)$ will imply the new effects of genes that are independent to the causal genetic effects. The changes of conditional genetic variation can be used to measure the epigenetic effects of the causal components on the dynamic variability of developmental behaviors.

Genetic effects and conditional genetic effects in the genetic model used are all random effects, which are not estimable but predictable. An adjusted unbiased prediction (AUP) method (ZHU 1993; ZHU and WEIR 1996) was employed to predict these random genetic effects. Jackknifing over genetic entry with two sex means was applied for estimating standard error of estimates (variances, proportion of variances, correlation coefficients) or predictors (genetic effects) (MILLER 1974; ZHU and WEIR 1994). Then a t-test was conducted for testing null hypothesis of zero parameter for each estimate or predictor, respectively.

RESULTS

Variance components: Phenotypic means of body weight and tail length for seven parents (P) and 21 F_1s were presented for male (M) and female (F) mice in Figure 1. The growth curves indicated apparent difference between males and females for body weight or between parents and F_1s after 28 days of birth. The variances associated with the various causal components for body weight and tail length are showed in Figures 2 and 3.

Body weight: With a few minor exceptions there is a systematic increase in $V(A)$, $V(D)$, $V(L)$, and $V(M)$ that parallels the general increase in phenotypic variance during postnatal ontogeny (Figure 2). However, differential changes occur in causal components as seen by changes in the variance components as proportions of the phenotypic variance (Figure 3). Narrow sense heritability, defined as $V(A)/V(P)$, for body weight is initially 0.55 at 14 days of age, then decreases to 0.19 at 49 days of age and then increases slightly to 0.25 at 70 days of age. The proportion of the phenotypic variance due to dominance is reasonably constant during varying from 0.08 at 21 days to 0.03 at 56 days. The proportions of both additive and dominance variance are changing by a factor of approximately three. The proportion of variance due to sex-linked effects rises from zero at 14 days of age to 0.47 at 49 days of age

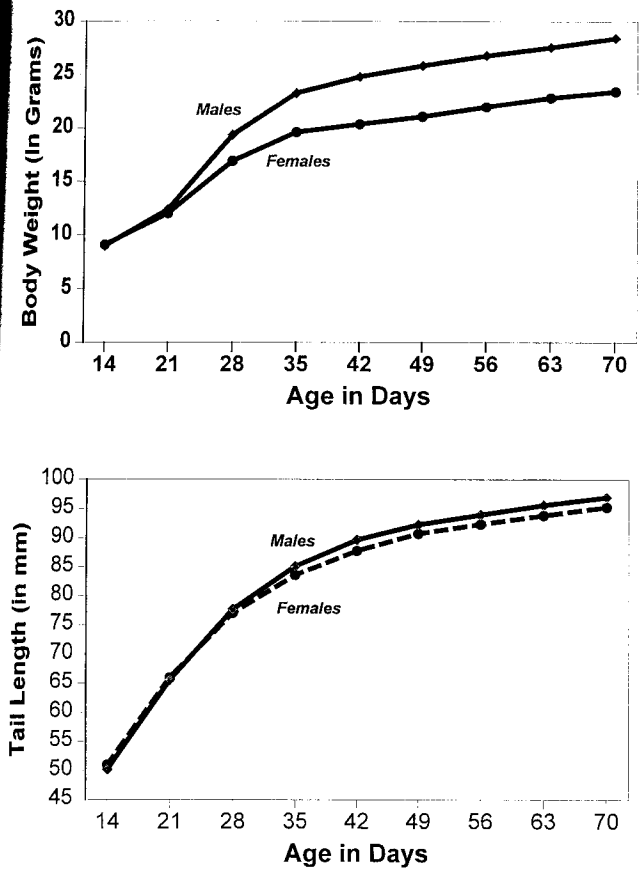

FIGURE 1.—Ontogenetic changes in body weight and tail length means. Female means are shown with dashed lines.

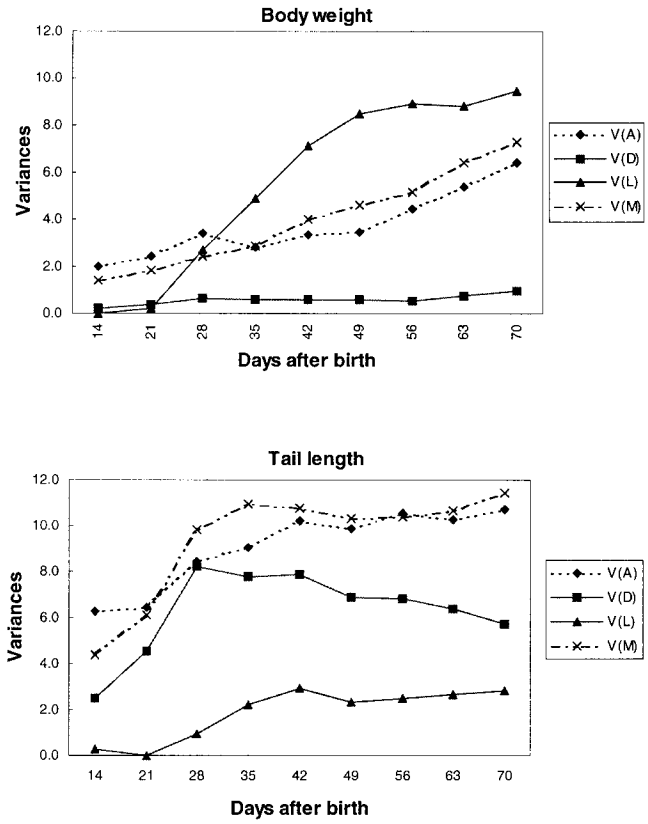

FIGURE 2.—Variance components for body weight and tail length of mice.

and then decreases to 0.38 at 70 days. The proportion of variance due to maternal effects decreases from 0.38 at 14 days to 0.29 at 70 days of age.

Tail length: The pattern of variance changes for tail length differs from that seen for body weight (Figure 2). The phenotypic variance increases through 42 days of age, asymptotes and remains relatively stable. Similarly, the additive, dominance, sex-linked and maternal genetic variances increase through 42 days of age and then stabilize. When expressed as proportions of the phenotypic variance, the components remain relatively constant during postnatal ontogeny (Figure 3). Narrow-sense heritability of tail length is initially 0.46 at 14 days of age and decreases to 0.31 at 70 days, which is a smaller proportional change than seen for body weight. The proportion of variance due to dominance rises from 0.18 at 14 days of age to 0.29 at 28 days and then falls back to 0.18 again at 70 days. The proportion due to sex-effects increases slightly to 0.09 at 70 days while maternal variance is relatively stable between 0.32 and 0.37. Again, the changes in broad-sense heritability reflect the dynamics of the individual components and varies from 0.64 at 14 days to 0.53 at 70 days of age.

Genetic correlations: It is useful to examine the genetic correlation of the mature trait with itself at different stages during ontogeny (*e.g.*, between 14 and 70 day body weight). This approach facilitates tests of several null hypotheses including (1) whether the same genes control the trait throughout ontogeny, (2) whether one can predict the final expression of a trait later in ontogeny based upon the early phenotype, and (3) if differential gene expression patterns during ontogeny will alter the patterns of genetic association among separate traits at different age intervals.

Body weight: The components of the genetic correlation of body weight with itself at various intervals are described in Tables 1—4. The additive genetic correlation decreases slightly over the experimental period. It is 0.86 between 14 and 21 day body weight and 0.78 between 14 and 70 day body weight. After 42 days of age, the additive genetic correlation of body weight with body weight the week before is >0.90 for each comparison. The dominance genetic correlation is 0.51 between 14 and 21 day weights and 0.34 between 14 and 70 day weights. Since the sex-effects variance is zero at 14 days, the first correlation is between 21 and 28 days (0.77) and 0.55 between 28 and 70 days of age. The genetic correlation due to maternal effects is 0.86 between 14 and 21 days and 0.70 between 14 and 70 days of age.

Tail length: The pattern of genetic correlations for tail length at different ages differs from that of body weight in several respects. The additive genetic correlation between 14- and 21-day tail length is 0.68 and this value decreases to 0.24 between 14- and 70-day values.

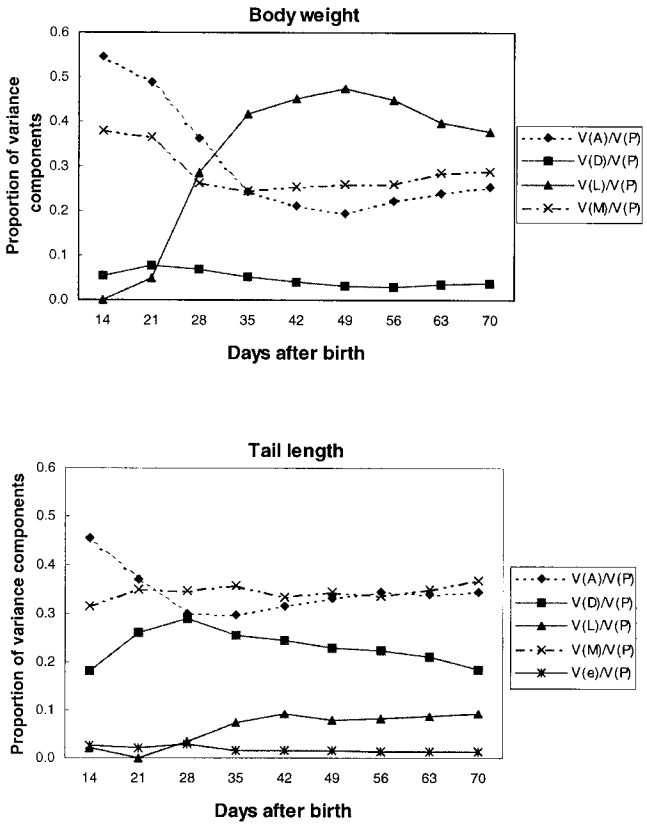

FIGURE 3.—Proportion of variance components for body weight and tail length of mice.

The values between successive ages never reach the values seen with body weight. For example, after 42 days of age, the additive genetic correlation of tail length with tail length the week before, ranging from 0.84 to 0.87 for each comparison. For dominance effects, the correlation is 0.77 between 14 and 21 days of age falling slightly to 0.68 between 14 and 70 days of age. Sex-effects correlations exhibit a more complex pattern. The variance due to sex-linked effects is initially very low for 14 and 21 days of age so that no significant correlation is detected for tail length before 21 days and the following days. The correlation of sex effects is highly significant for tail length among different ages after 28 days. Maternal effects gives a correlation of 0.62 between 14 and 21 days and decreases to 0.51 between 14 and 70 days of age.

Conditional genetic variance components: The causal components of variance described in the previous section are now analyzed where the values at time t are conditioned by events at time $t - 1$. These results provide a perspective of the ontogenetic component to quantitative genetic variability and insights into temporal patterns of gene expression.

Figures 3 and 4 give the conditional variance components for body weight and tail length where the genetic effects are conditioned on the gene expression of the traits 7 days before. Figure 5 describes these changes as proportions of the conditional phenotypic variance. Thus, 21-day body weight is conditioned by body weight at day 14, 28-day weight by 21-day weight and so on.

Body weight: Conditional phenotypic variance peaks at 35 days of age and decreases to the value seen at 70 days. Conditional additive genetic variance expressed as narrow-sense heritability peaks at 0.38 at 28 days, decreases to zero at 49 days and then shows a small increase (0.08) at 56 days and then returns to zero at 63 days. The proportion of conditional dominance variance is 0.16 at 21 days of age and decreases to zero at 42 days. The conditional sex-effects variance is initially 0.16 at 21 days, increases to 0.54 at 28 days but falls to zero at 42 days. Conditional maternal variance is 0.24 at 21 days, falls to zero at 28 days, rises to 0.12 at 35 days and then returns to zero for the duration.

The plot of the conditional additive genetic variance shows that the value becomes zero at 49 days, suggesting that there is no additive genetic variance for body weight present at 49 days that was not present at 42 days of age. There is some new additive genetic variance being observed at 56 days of age. This suggests new effects of gene expression occurring after 49 days of age that gives rise to new additive genetic variance.

Tail length: The ontogenetic pattern of the conditional variances for tail length are quite different from

TABLE 1

Additive correlation coefficients for seven-parent diallel cross of mice

R_A	14D	21D	28D	35D	42D	49D	56D	63D	70D
14D		0.859	0.753	0.749	0.770	0.769	0.803	0.777	0.785
21D	0.675		0.874	0.861	0.846	0.851	0.868	0.845	0.846
28D	0.513	0.531		0.874	0.849	0.863	0.874	0.858	0.854
35D	0.389	0.430	0.504		0.883	0.899	0.899	0.882	0.878
42D	0.273	0.294	0.434	0.545		0.918	0.926	0.909	0.912
49D	0.218	0.222	0.386	0.514	0.562		0.937	0.923	0.923
56D	0.204	0.196	0.377	0.513	0.568	0.583		0.945	0.952
63D	0.240	0.212	0.396	0.531	0.581	0.593	0.609		0.939
70D	0.245	0.212	0.413	0.552	0.602	0.613	0.631	0.665	

Upper triangular for body weight and lower triangular for tail length. All table values significant at $P < 0.01$, two-side alternative for correlation coefficients.

TABLE 2

Dominance correlation coefficients for seven-parent diallel cross of mice

R_D	14D	21D	28D	35D	42D	49D	56D	63D	70D
14D		0.505	0.396	0.491	0.509	0.478	0.441	0.398	0.342
21D	0.765		0.693	0.661	0.644	0.606	0.554	0.508	0.416
28D	0.699	0.818		0.650	0.590	0.530	0.439	0.394	0.285
35D	0.636	0.772	0.837		0.670	0.595	0.556	0.551	0.472
42D	0.619	0.771	0.821	0.863		0.610	0.563	0.574	0.475
49D	0.622	0.758	0.824	0.860	0.868		0.505	0.479	0.468
56D	0.634	0.769	0.814	0.846	0.856	0.864		0.557	0.511
63D	0.653	0.780	0.826	0.846	0.856	0.863	0.869		0.621
70D	0.675	0.788	0.804	0.825	0.839	0.838	0.852	0.870	

Upper triangular for body weight and lower triangular for tail length). All table values significant at $P < 0.01$, two-side alternative for correlation coefficients.

those for body weight (Figures 3 and 4). Phenotypic variance peaks at 28 days of age, decreasing to a value not different from zero at 56 days and reappearing at 63 and 70 days of age. Conditional additive genetic variance (narrow-sense heritability) is relatively large at 21 days of age (0.34), increases to 0.46 at 35 days of age, becomes zero at 56 days and is 0.22 at 70 days. Conditional dominance is 0.38 at 21 days, decreases to a low of 0.10 at 42 days and increases to 0.25 at 70 days. Conditional sex-linked variance is greatest (0.37) at 28 days, becomes zero at 49 days and rises to 0.06 by 70 days. Conditional maternal variance is 0.16 at birth, zero at 28 days, increases to 0.30 at 42 days, zero at 49 days and is 0.32 by 70 days of age.

Effect of time of conditioning: Clearly, the magnitude of the conditional variance component will change considerably depending upon the interval of time between when a trait is measured and when the conditioning trait is recorded. Figure 6 describes the impact on variance components of 70-day-old body weight and tail length (the observed traits) as a function of the interval between the observed trait and the conditioning trait is changed. Thus, the conditional genetic variance components for 70-day-old body weight are greatest when conditioned by 14-day-old body weight. The magnitude of the conditioned 70-day body weight variance component decreases as the interval between measured variable and conditioning date is decreased. For additive genetic variance and the maternal component, it reaches zero when 56-day-old body weight is the conditioning trait. For dominance and the sex-effects components, the variance of 70-day body weight becomes zero when 35-day weight is the conditioning trait. It was suggested that most genetic variation of 70-day-old body weight was due to gene expression at the age of 35 days and after.

Similar trends in decrease in conditional variance occur with tail length except the rates of change are different. With the additive genetic component, the conditional variance of 70-day-old tail length becomes zero when 63-day tail length is used as the conditioning trait. For the dominance and maternal components, the variance never become zero. With the sex-effects component, the conditional variance in 70-day tail length becomes zero when 28-day-old tail length is the conditioning trait. The variance remains zero except for a positive value when 49-day-old tail length is used. A peak is observed for conditional variance of sex effects when 70-day-old tail length is conditioned on 21-day tail length. It is indicated that sex effects of gene expression

TABLE 3

Correlation coefficients of sex-linked effects for seven-parent diallel cross of mice

R_L	14D	21D	28D	35D	42D	49D	56D	63D	70D
14D		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21D	0.000		0.766**	0.563**	0.529**	0.540**	0.530**	0.552**	0.553**
28D	0.205	0.000		0.897**	0.869**	0.876**	0.874**	0.878**	0.871**
35D	0.021	0.000	0.704**		0.994**	0.994**	0.989**	0.982**	0.979**
42D	−0.027	0.000	0.707**	0.939**		1.000	1.000**	0.994**	0.992**
49D	−0.145	0.000	0.678**	0.914**	0.949**		1.000**	0.997**	0.995**
56D	−0.019	0.000	0.766**	0.959**	0.975**	0.942**		0.999**	0.998**
63D	−0.068	0.000	0.764**	0.948**	0.971**	0.949**	0.968**		1.000**
70D	−0.001	0.000	0.794**	0.957**	0.977**	0.941**	0.977**	0.977**	

Upper triangular for body weight and lower triangular for tail length. ** $P < 0.01$, two-side alternative for correlation coefficients.

TABLE 4

Correlation coefficients of maternal effects for seven-parent diallel cross of mice

R_M	14D	21D	28D	35D	42D	49D	56D	63D	70D
14D		0.863	0.814	0.735	0.709	0.702	0.744	0.704	0.705
21D	0.621		0.853	0.819	0.791	0.779	0.824	0.798	0.795
28D	0.542	0.533		0.848	0.828	0.817	0.856	0.837	0.833
35D	0.539	0.530	0.491		0.887	0.883	0.911	0.903	0.902
42D	0.497	0.458	0.449	0.516		0.882	0.905	0.898	0.896
49D	0.488	0.448	0.445	0.515	0.538		0.899	0.894	0.892
56D	0.486	0.418	0.427	0.503	0.543	0.549		0.913	0.912
63D	0.483	0.400	0.413	0.494	0.052	0.552	0.575		0.909
70D	0.505	0.406	0.425	0.504	0.560	0.571	0.602	0.626	

Upper triangular for body weight and lower triangular for tail length. All the table values significant at $P < 0.01$, two-side alternative for correlation coefficients.

during 14–21 days of age might have a different contribution on the 70-day-old tail length.

Dynamics of individual effects by genetic strains: In a diallel experiment, the individual line (inbred strains) contributions to the variance components can be partitioned out. Examining these individual line effects may facilitate an understanding of the ontogenetic behavior of the causal components in the various genotypes (inbred lines) and clarify whether the expression of new genes as suggested above occurs in all inbred strains. The plotted values are given in Figures 7 and 8 as deviations of each inbred strain so that the individual line contributions sum to zero. For each variance component, we have shown the dynamics of the variance component during ontogeny and then the variance component conditioned by the value from the previous measurement interval $(t-1)$. To keep the figures and discussion simple, we have not labeled the particular inbred strains in these plots since we are primarily interested in the overall patterns of the results. We will identify the inbred strains at certain relevant points in the written account when it is important.

The dynamics of the unconditioned and conditional genetic effects are shown side by side for body weight

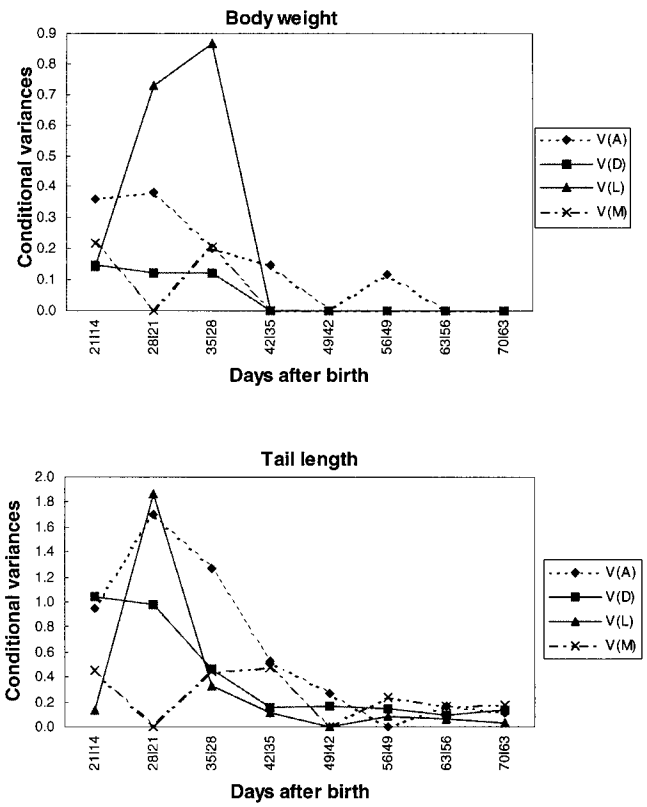

FIGURE 4.—Conditional variance components for body weight and tail length of mice.

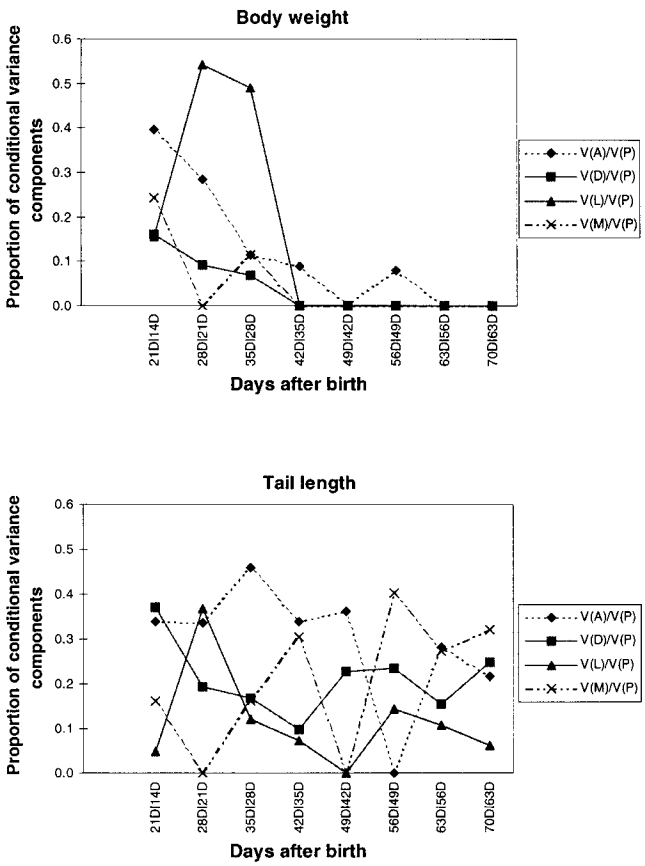

FIGURE 5.—Proportion of conditional variance components for body weight and tail length of mice.

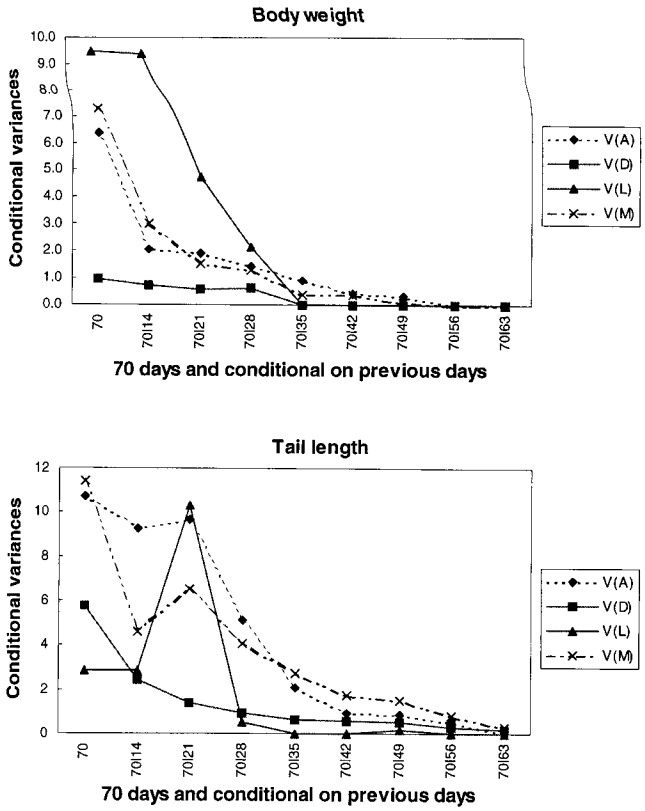

FIGURE 6.—Conditional variances at 70 days of age given the observation at different previous time for body weight and tail length of mice.

(Figure 7) and tail length (Figure 8) and clearly show the systematic patterns of ontogenetic change within the individual genotypes. However, these patterns are changed significantly when the observed values are conditioned by the epigenetic effects of the phenotype during the previous measurement interval. For example, the inbred strain BALB/cByJ is characterized by open circles with the long dashed line in these figures. It exhibits a large negative deviation of additive effects for body weight in the first two intervals and then the deviation is not different from zero from 35 days onward. These figures show the impact of different genotypes and different patterns of gene expression.

Nowhere is the impact of a time-dependent epigenetic effect more noticeable than in the dynamics of sex-linked and maternal effects for body weight. In both instances, the genetic variability is accumulated early in postnatal ontogeny and after about 35 days no new genetic variation associated with these causal components is being generated. With regard to maternal effects, there is significant maternal variability among lines at 21 days not accounted for by previous measurements. This is to be expected since the mice are weaned at 21 days. These maternal effects are gone when 28 day weight is conditioned by 21 day weight and the value is zero. However, at 35 days of age significant new maternal effects are present apparently arising as a delayed effect of nursing.

The conditional additive genetic variance for tail length also exhibits a point where the genetic variance is zero followed by the appearance of significant new additive genetic variability. As with body weight, the various different inbred strains exhibit different dynamics in conditional variance. Maternal effects in tail length show the same burst of new variability at 35 days that is not accounted for by previous phenotypes. This new variability persists until 49 days whereupon the variance goes to zero again. However, there is again new variability appearing at 56 days in some strains not accounted for by previous effects.

To conserve space, dominance effects are not discussed because dominance must be computed using pairs of lines that would involve 21 crosses and seven parents in this 7 × 7 diallel. These comparisons are beyond the available space.

Ontogenetic dynamics of genetic association between traits: Tables 5–8 provide the components of genetic correlation between body weight and tail length. At 70 days of age, body weight and tail length have phenotypic and additive genetic correlations of 0.13 and −0.20, respectively. However, simply reporting static values for adult traits ignores the dynamics of how two traits arrived at their mature phenotypes. Did these two traits exhibit the same phenotypic and genetic correlation at different stages during ontogeny? Alternatively, do pairs of traits exhibit dynamic changes in the magnitude and sign of their correlation components during ontogeny? There are several ways to describe some of the ontogenetic dynamics of the associations between traits.

First, let us consider the genetic variance for tail length in an allometric sense. A portion of variability in quantitative genetic traits can often be accounted for by genetic covariability with another trait. This can be measured by

$$1.0 - \frac{\text{conditional variance}}{\text{unconditional variance}}.$$

The changes in the proportion of variance accounted for by covariability in the other trait are presented in Figure 9 for body weight and tail length.

For the early developmental stages (14–28 days after birth), gene expression of mice have large values for additive, dominance and maternal effects for both body weight and tail length. The proportion of variability due to additive, dominance and maternal effects contributed by other trait is ~0.50 with a peak at 21 days for both traits. Therefore, these two traits share some common influence due to additive, dominance and maternal gene expression during the early developmental stages of mice. The proportions decrease until only ~20% at 70 days of age for additive and dominance effects. After 35 days of age, maternal variation of tail length is contributing <10% of body weight variation. For the later developmental stages (after 35 days), a large proportion (>50%) of sex variation for body

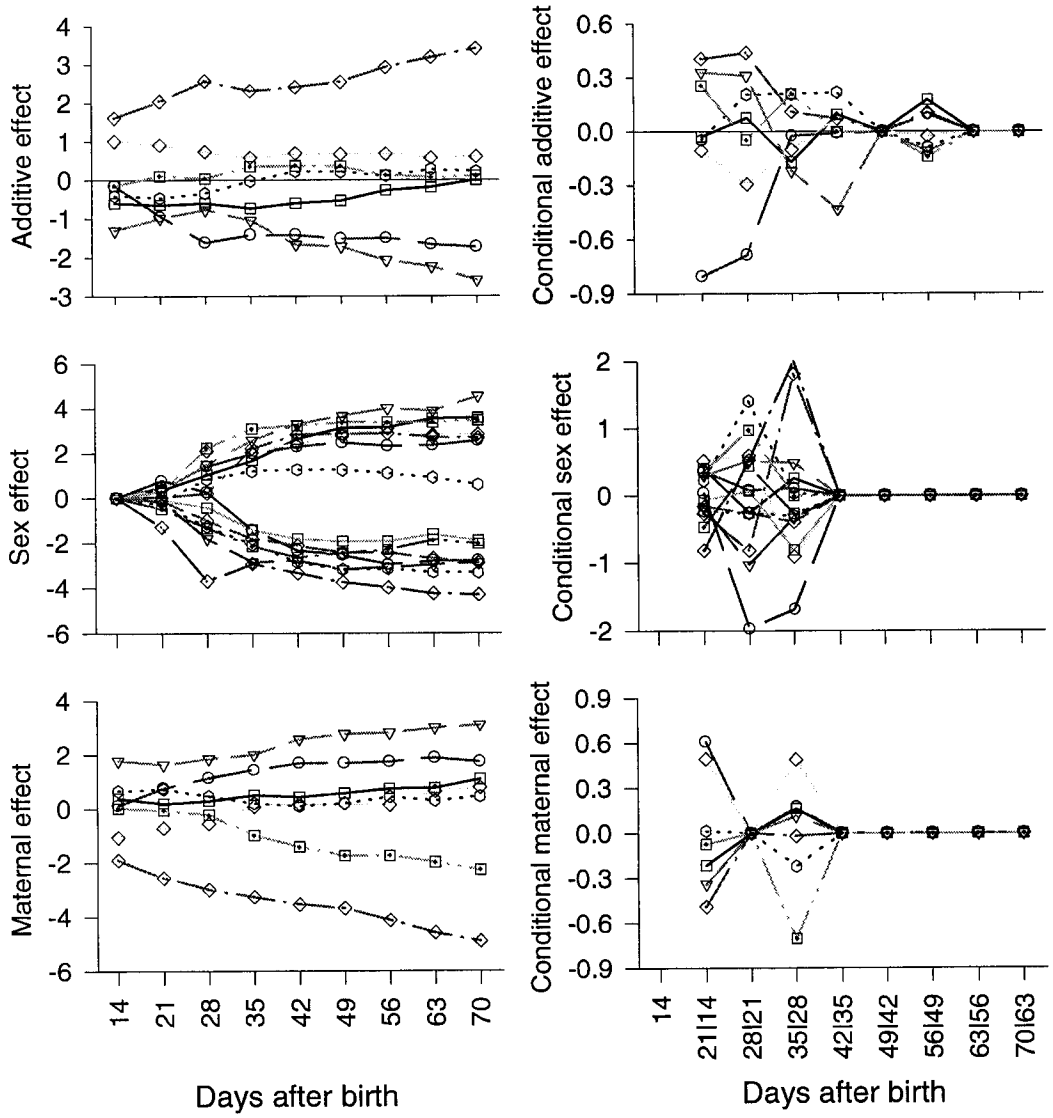

FIGURE 7.—Predicted genetic effects and conditional genetic effects for body weight of mice.

weight is due to variation in tail length, and also for tail length contributed by body weight.

One of the most striking aspects of the correlation components is the ontogenetic behavior of the additive genetic correlation that is the classical measure of pleiotropy. At 14 days of age, the additive genetic correlation between body weight and tail length is 0.56, it increases to 0.65 at 21 days of age, it falls precipitously to −0.08 at 42 days and is −0.20 at 70 days of age (Table 5). Thus, by considering only the genetic correlation between body weight and tail length in mature animals, one would miss these very significant dynamics of the genetic associations between these two traits and its ramifications that will be discussed later.

The component of phenotypic covariability due to dominance is initially 0.49, it increases to 0.63 and is 0.35 at 70 days (Table 6). The covariability due to sex effects is initially zero but climbs to 0.76 at 35 days of age and remains there (Table 7). Maternal effect variability is initially ~0.52 and decreases to ~0.08 at 70 days (Table 8).

DISCUSSION

Complex traits like body weight are composed of a number of embryologically distinct components whose patterns of growth and morphogenesis, and underlying controlling factors may differ considerably at different stages in ontogeny (ATCHLEY and HALL 1991). For example, in mammals very early postnatal organ growth is often predominated by changes in cell number while later in ontogeny changes in cell size dominate (RISKA and ATCHLEY 1985). Further, growth and morphogenesis of different organ systems may occur at predominantly different times. For example, growth of the nervous system begins much earlier than some other tissues. Finally, there are often differing influences of the mother on postnatal development. The impact of uter-

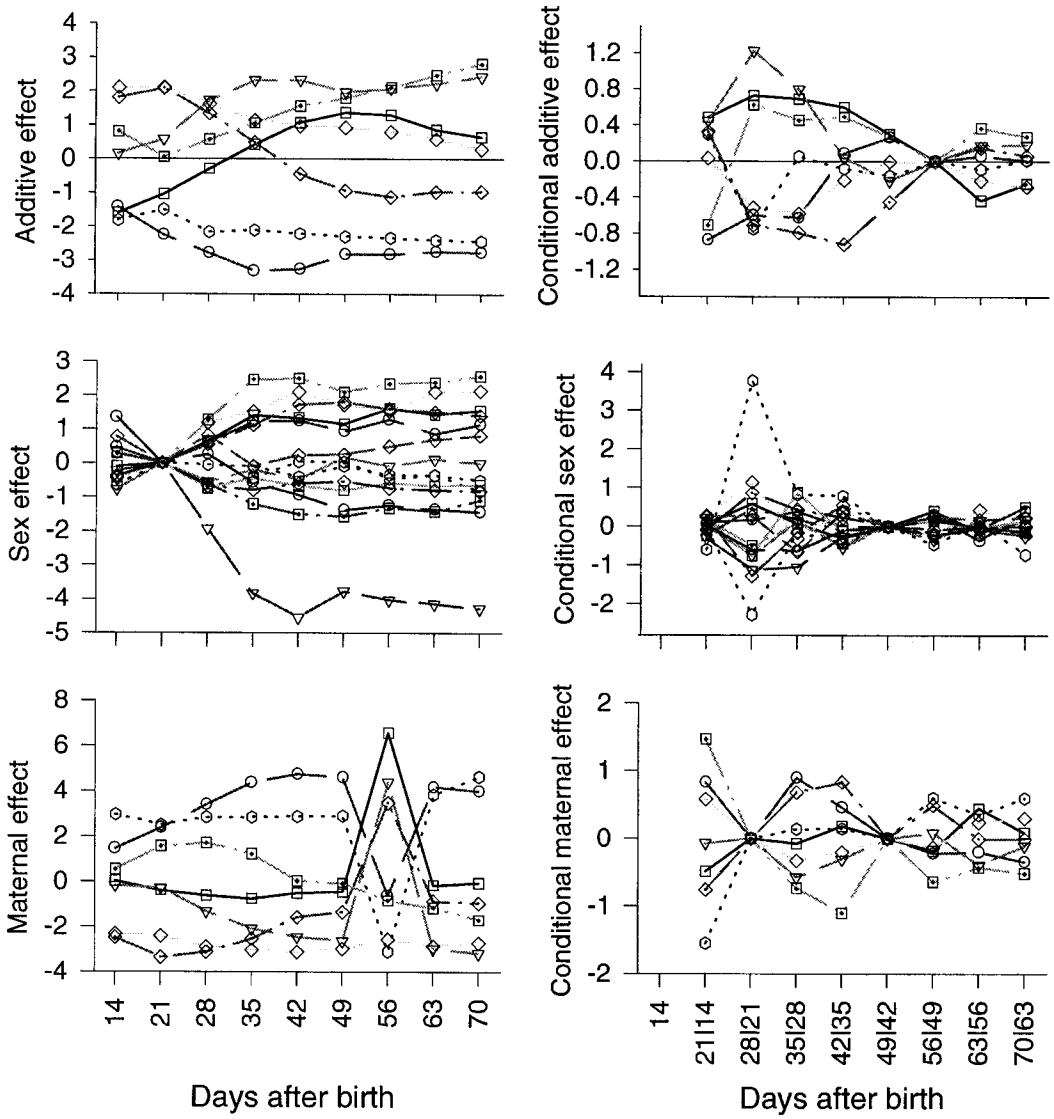

FIGURE 8.—Predicted genetic effects and conditional genetic effects for tail length of mice.

ine maternal and postnatal nursing effects may have significant yet very differential effects on progeny growth.

At the molecular level, ontogenetic changes in tissues and organs that comprise traits like body weight are manifested by different temporal and spatial patterns

TABLE 5

Correlation coefficients of additive effects between body weight and tail length for seven-parent diallel cross of mice

R_A	14TL	21TL	28TL	35TL	42TL	49TL	56TL	63TL	70TL
14BW	0.564**	0.521**	0.180*	−0.050	−0.190*	−0.236**	−0.266**	−0.268**	−0.291
21BW	0.651**	0.654**	0.368**	0.179*	0.020	−0.051	−0.083	−0.072	−0.084
28BW	0.590**	0.645**	0.398**	0.250**	0.090	0.003	−0.030	−0.018	−0.024
35BW	0.541**	0.555**	0.293**	0.145†	0.001	−0.074	−0.098	−0.079	−0.076
42BW	0.470**	0.476**	0.196*	0.050	−0.078	−0.138†	−0.163*	−0.156*	−0.161
49BW	0.475**	0.492**	0.217**	0.072	−0.058	−0.123	−0.149†	−0.142†	−0.146
56BW	0.452**	0.485**	0.208**	0.053	−0.077	−0.137†	−0.169*	−0.172*	−0.185
63BW	0.417**	0.455**	0.192*	0.046	−0.084	−0.142*	−0.176*	−0.181*	−0.196
70BW	0.408**	0.447**	0.181*	0.033	−0.089	−0.141†	−0.174*	−0.181*	−0.197

† $P < 0.10$, *$P < 0.05$, **$P < 0.01$, two-side alternative for correlation coefficients.

TABLE 6

Correlation coefficients of dominance effects between body weight and tail length for seven-parent diallel cross of mice

R_D	14TL	21TL	28TL	35TL	42TL	49TL	56TL	63TL	70TL
14BW	0.487	0.478	0.432	0.366	0.340	0.366	0.384	0.429	0.462
21BW	0.676	0.680	0.579	0.525	0.517	0.545	0.569	0.610	0.652
28BW	0.749	0.771	0.679	0.632	0.623	0.637	0.652	0.686	0.726
35BW	0.660	0.741	0.673	0.628	0.637	0.628	0.636	0.668	0.694
42BW	0.609	0.689	0.630	0.585	0.581	0.588	0.607	0.651	0.679
49BW	0.492	0.585	0.601	0.569	0.567	0.579	0.593	0.639	0.652
56BW	0.472	0.523	0.502	0.461	0.447	0.439	0.455	0.505	0.529
63BW	0.377	0.480	0.407	0.330	0.329	0.292	0.315	0.366	0.400
70BW	0.281	0.386	0.356	0.322	0.318	0.279	0.286	0.329	0.351

All table values significant at $P < 0.01$, two-side alternative for correlation coefficients.

of gene expression. Indeed, temporal and spatial patterns of gene expression will have differential effects on the genetic variances and covariances. Actions and interactions of genes and their products during ontogeny have primary effects on genetic variances, secondary effects on genetic covariances through pleiotropic and epigenetic interactions, as well as differential effects on the genetic background in which individual genes are expressed. The result is a dynamic pattern of change in the causal components of variability and covariability during the ontogeny of complex traits. Indeed, such phenomena are the underpinnings of the developmental quantitative genetic models proposed by ATCHLEY (1984, 1987), ATCHLEY and HALL (1991), COWLEY and ATCHLEY (1991), ATCHLEY *et al.* (1994) and others.

The models suggest that the ontogenetic variability in the genetic architecture of quantitative traits includes the number of loci involved, their relative magnitude of effects, and temporal variability in expression. Recently, CHEVERUD *et al.* (1996) provided information on age-specific patterns of gene expression in quantitative trait loci influencing growth in body weight in mice. They used quantitative trait locus mapping techniques to document gene expression at different points in postnatal ontogeny. For example, seven QTLs were found that were associated with 7-day body weight while 17 QTLs were associated with 70-day body weight. There were 11 QTLs found to be involved with early growth rate. Further, at 7 days postnatal age a particular locus is expressed that explains ~11% of the 7-day phenotypic variance and that has a strong dominance effect. Another locus acts much later in ontogeny and explains 5% of the phenotypic variance in late growth (*i.e.*, that occurring after 49 days of age).

The results described in the present article describe the age-related changes in the causal components of variance in body weight and tail length from a diallel experiment in seven inbred strains of mice. These results document the ontogenetic behavior of the additive, dominance, sex-linkage and maternal components of phenotypic variance as seen in previous studies.

The magnitude of sex-linked variance for body weight is quite high in these mice, particularly when one considers the size of the *X* chromosome in mice relative to the remainder of the genome. Recently, however, HASTINGS and VEERKAMP (1993) and VEERKAMP *et al.* (1993) described a large additive *X*-linked effect in reciprocal crosses between high and low body weight selection lines in mice. This *X*-linked effect accounted

TABLE 7

Correlation coefficients of sex-linkage effects between body weight and tail length for seven-parent diallel cross of mice

R_L	14TL	21TL	28TL	35TL	42TL	49TL	56TL	63TL	70TL
14BW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21BW	0.304**	0.000	0.310**	0.381**	0.299**	0.291**	0.354**	0.327**	0.328
28BW	−0.020	0.000	0.476**	0.683**	0.656**	0.657**	0.663**	0.673**	0.657
35BW	−0.235**	0.000	0.488**	0.757**	0.766**	0.803**	0.784**	0.781**	0.756
42BW	−0.298**	0.000	0.452**	0.721**	0.726**	0.778**	0.756**	0.753**	0.729
49BW	−0.297**	0.000	0.468**	0.728**	0.726**	0.768**	0.750**	0.750**	0.729
56BW	−0.316**	0.000	0.458**	0.719**	0.714**	0.759**	0.741**	0.745**	0.725
63BW	−0.303**	0.000	0.451**	0.711**	0.698**	0.740**	0.730**	0.728**	0.711
70BW	−0.293**	0.000	0.437**	0.698**	0.680**	0.727**	0.715**	0.715**	0.698

** $P < 0.01$, two-side alternative for correlation coefficients.

TABLE 8

Correlation coefficients of maternal effects between body weight and tail length for seven-parent diallel cross of mice

R_M	14TL	21TL	28TL	35TL	42TL	49TL	56TL	63TL	70TL
14BW	0.521**	0.500**	0.322**	0.224**	0.137*	0.105	0.085	0.059	0.060
21BW	0.531**	0.545**	0.383**	0.309**	0.221**	0.188*	0.155*	0.132	0.133
28BW	0.447**	0.478**	0.327**	0.267**	0.188**	0.157†	0.116	0.095	0.093
35BW	0.357**	0.380**	0.247**	0.202**	0.149*	0.124†	0.090	0.080	0.085
42BW	0.309**	0.330**	0.204**	0.164*	0.122†	0.094	0.065	0.052	0.056
49BW	0.308**	0.317**	0.187**	0.145*	0.110	0.083	0.060	0.052	0.062
56BW	0.367**	0.377**	0.238**	0.191**	0.150*	0.123	0.099	0.092	0.104
63BW	0.320**	0.335**	0.207**	0.163*	0.121†	0.097	0.069	0.064	0.075
70BW	0.326**	0.329**	0.194**	0.145*	0.106	0.083	0.060	0.061	0.077

† $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, two-side alternative for correlation coefficients.

for ~25% of the total divergence. Subsequent QTL mapping studies by RANCE *et al.* (1997) showed that the *X*-linked factor has ontogenetically varying effects and explains ~8% of the phenotypic variance in body weight at 3 weeks, 14.2% at 6 weeks and 28% at 10 weeks. Similarly, DRAGANI *et al.* (1995) reported *X*-linked QTL that affected body weight in mice and ZENG (1994) described two complementary QTLs on the *X* chromosome that accounted for 26% of the phenotypic variance in a test cross between two mouse lines selected

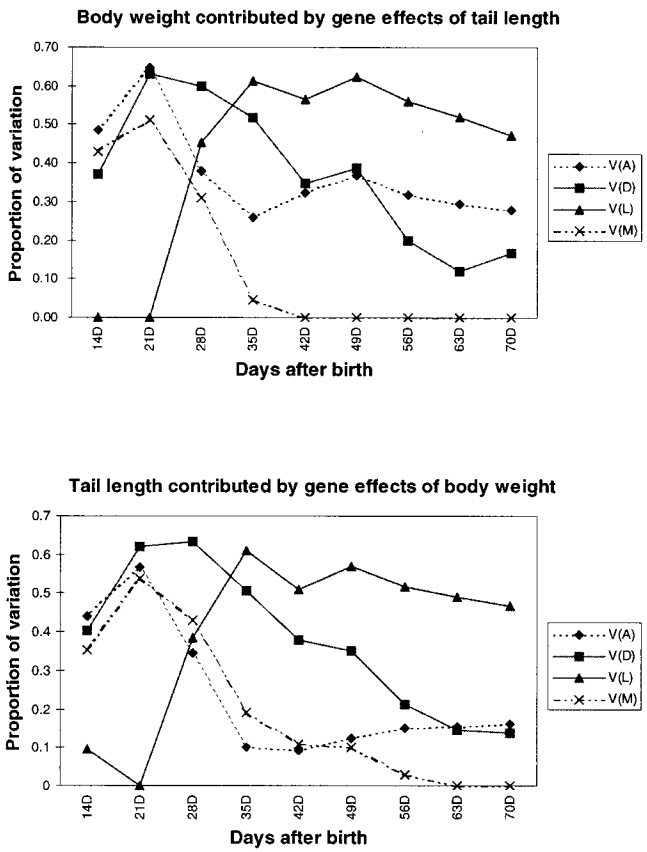

FIGURE 9.—Proportion of genetic variation contributed by the conditional gene effects for body weight and tail length of mice.

for body weight. Thus, our results on the magnitude and dynamics of the sex-linked variance in mice from this 7 × 7 diallel cross are in agreement with other recent experimental results.

The sex-linked variance for tail length, on the other hand, differs from body weight in that the magnitude of the effect is substantially less. It only reaches ~9% of the phenotypic variance at 70 days of age.

The proportion of phenotypic variance due to maternal effects might seem high since at 14 days of age, $V(M)/V(P)$ is almost 40% and drops to <30% at 70 days of age. However, large sample estimates of the contributions of maternal effects in random-bred ICR mice (RISKA *et al.* 1984) indicate that postnatal maternal effects account for ~65% of the phenotypic variance at 14 days of age. At 6 weeks of age maternal effects still account for 28% of the phenotypic variance. At 70 days of age, maternal effects account for 24% of the phenotypic variance in females and only 6% in males. Hence, the primary difference between the mice from the diallel and those from other studies seems to be that these mice show less ontogenetic change in the relative proportion of phenotypic variance due to maternal effects.

The results reported here on the dynamics of variance components are unique in that they involve variances conditioned on variability at earlier ages. Through this procedure the variance components can be "corrected" for variability at different ages so as to demonstrate when significant episodes in the generation of new variation might occur. Thus, we have described the additive genetic variance in body weight conditioned by the additive genetic variance 7 days previously. For body weight, there is a pattern of decreasing genetic variance from 14 days of age until it reaches a point not different from zero at 49 days of age. Thus, the genetic variance at 49 days of age is completely explained by the additive genetic variance at 42 days of age. However, between 49 and 56 days of age there is new additive genetic variance generated at a magnitude equal to ~10% of the phenotypic variance. This newly

generated additive genetic variance after 49 days of age is compatible with the observation by CHEVERUD *et al.* (1996) of the expression of genes influencing late growth in body weight.

The pattern of conditional additive genetic variance in tail length on the other hand suggests that when conditioned by the variation 7 days before, there is still considerable new variation being introduced. Indeed, there is an increase in additive genetic variance at a magnitude of over 10% between 28 and 35 days over and above the variance produced the week before. The additive genetic variance in tail length at 56 days of age can be completely accounted for by the variance at 49 days. However, between 56 and 70 days of age there is a significant introduction of up to 20% new additive genetic variability for tail length not accounted by the previous intervals.

As shown here and by previous authors (*e.g.*, ATCHLEY 1984), the additive genetic correlation between body weight and tail length in rodents is large and positive early in ontogeny indicating that early in postnatal ontogeny these traits share a significant genetic component. However, the genetic correlation then drops markedly to a value not different from zero after about 35 days of age indicating that the initial genetic commonality is significantly reduced later in ontogeny. Figure 9 describing the proportions of conditional variance in these two traits provides additional insight into why this marked drop in genetic association between these traits. These two traits have a common genetic control early, but as the genetic control of these traits changes during ontogeny, they become genetic more separate and distinct.

The use of conditioning causal components as shown here provides a useful tool for understanding the dynamics of the causal components controlling variability during ontogeny in complex traits. These data further clarify the role of time-dependent epigenetic effects and their importance in integrating developmental and quantitative genetic approaches to complex traits.

We greatly appreciate the critical comments by E. J. EISEN, RONG WEI and BRUCE WEIR on the manuscript. This work was supported by grants from the National Institutes of Health (GM-45344 and 32518).

LITERATURE CITED

ATCHLEY, W. R., 1984 Ontogeny, timing of development, and genetic variance-covariance structure. Am. Nat. **123:** 519–540.

ATCHLEY, W. R., 1987 Developmental quantitative genetics and the evolution of ontogenies. Evolution **41:** 316–330.

ATCHLEY, W. R., 1990 Heterochrony and morphological change: a quantitative genetic approach. Sem. Dev. Biol. **1:** 289–297.

ATCHLEY, W. R., and B. K. HALL, 1991 A model for development and evolution of complex morphological structures. Biol. Rev. **66:** 101–157.

ATCHLEY, W. R., and J. J. RUTLEDGE, 1980 Genetic components of size and shape variation. I. Dynamics of components of phenotypic variability and covariability during ontogeny in the laboratory rat. Evolution **34:** 1161–1174.

ATCHLEY, W. R., T. E. LOGSDON, D. E. COWLEY and E. J. EISEN, 1991 Uterine effects, epigenetics and postnatal skeletal development in the mouse. Evolution **45:** 891–909.

ATCHLEY, W. R., S. XU and C. VOGL, 1994 Developmental quantitative genetic models of evolutionary change. Dev. Genet. **15:** 92–103.

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

CHEVERUD, J. M., E. J. ROUTMAN, F. A. M. DUARTE, B. VAN SWINDEREN, K. COTHRAN *et al.*, 1996 Quantitative trait loci for murine growth. Genetics **142:** 1305–1319.

COWLEY, D. E., and W. R. ATCHLEY, 1992 Quantitative genetic models for development, epigenetic selection and phenotypic evolution. Evolution **46:** 495–518.

DRAGANI, T. A., Z-B. ZENG, F. CANZIAN, M. GARIBOLDI, M. T. GHILARDUCCI *et al.*, 1995 Mapping of body weight loci on mouse chromosome X. Mamm. Genome **6:** 778–781.

HASTINGS, I. M., and R. F. VEERKAMP, 1993 The genetic basis of response in mouse lines divergently selected for body weight or fat content. I. The relative contributions of autosomal and sex-linked genes. Genet. Res. **62:** 169–175.

HERRING, S. W., 1993 Epigenetic and functional influences on skull growth, pp. 153–206 in *The Vertebrate Skull*, Vol. 1, *Development*, edited by J. HANKEN and B. K. HALL. The University of Chicago Press, Chicago.

MILLER, R. G., 1974 The jackknife: a review. Biometrika **61:** 1–15.

RANCE, K. A., W. G. HILL and P. D. KEIGHTLEY, 1997 Mapping quantitative trait loci for body weight on the X chromosome in mice. I. Analysis of a reciprocal F2 population. Genet. Res. (in press).

RISKA, B., and W. R. ATCHLEY, 1985 Genetics of growth predicts patterns of brain-size evolution. Science **229:** 668–671.

RISKA, B., J. J. RUTLEDGE and W. R. ATCHLEY, 1985 Genetic analysis of crossfostering data with sire and dam records. J. Hered. **76:** 247–250.

RAO C. R., 1970 Estimation of heteroscedastic variances in linear models. J. Am. Stat. Assoc. 65: 161–172.

RAO C. R., 1971 Estimation of variance and covariance components MINQUE theory. J. Multivar. Anal. **1:** 257–275.

VEERKAMP, R. F., C. S. HALEY, S. A. KNOTT and I. M. HASTINGS, 1993 The genetic basis of response in mouse lines divergently selected for body weight or fat content. II. The contribution of genes with a large effect. Genet. Res. **62:** 177–182.

VOGL, C., W. R. ATCHLEY, D. E. COWLEY, P. CRENSHAW, J. D. MURRAY *et al.*, 1993 The epigenetic influence of growth hormone on skeletal development. Growth Dev. Aging **57:** 163–182.

ZENG, Z-B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457–1468.

ZHU, J., 1993 Mixed model approaches for estimating covariances between two traits with unequal design matrices. J. Biomathematics **8:** 24–30.

ZHU, J., 1995 Analysis of conditional genetic effects and variance components in developmental genetics. Genetics **141:** 1633–1639.

ZHU, J., and B. S. WEIR, 1994 Analysis of cytoplasmic and maternal effects: I. a genetic model for diploid plant seeds and animals. Theoret. Appl. Genet. **89:** 153–159.

ZHU, J., and B. S. WEIR, 1996 Diallel analysis for sex-linked and maternal effects. Theoret. Appl. Genet. **92:** 1–9.

Communicating editor: M. KIRKPATRICK