Acta Genet Med Gemellol 28:93–105 (1979)
The Mendel Institute/Alan R. Liss, Inc.

Received 27 October 1978
Final 22 December 1978

Analysis of Longitudinal Twin Data
Basic Model and Applications to Physical Growth Measures

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A formal model is presented for the analysis of longitudinal twin data, based on the underlying analysis-of-variance model for repeated measures. The model is developed in terms of the expected values for the variance components representing twin concordance, and the derivation is provided for computing within-pair (intraclass) correlations, and for estimating the percent of variance explained by each component. The procedures are illustrated with physical growth data extending from birth to six years, and concordance estimates are obtained for average size and for the pattern of spurts and lags in growth. A test of significance is also described for comparing monozygotic twins with dizygotic twins. The procedures are particularly useful for assessing chronogenetic influences on development, especially whether the episodes of acceleration and lag occur in parallel for genetically matched twins. The model may be employed with psychological data also.

Key words: Analysis of variance, Longitudinal studies, Twin models, Growth in twins, Chronogenetics

INTRODUCTION

The physical growth of twins during early childhood provides a valuable source of data for exploring chronogenetic influences on growth. Twins are typically premature in terms of birth weight and gestational age, but they recover rapidly in the first year and appear to become aligned on their intrinsic growth curves [14]. For monozygotic twins, this produces increasing similarity in size as the two growth curves progressively converge.

The course of growth, however, is not entirely uniform for a particular child, but rather moves in episodes of acceleration and lag [1, 8]. These episodes appear to depend upon the activity of age-linked gene action systems which switch on and switch off the phases of rapid growth. The timing of the growth spurts follows a distinctive pattern for each child, and as a consequence a child who may be smaller than average at one age may then enter a phase of rapid growth and catch up with, or surpass, his peers at a later age.

This research has been supported in part by PHS research grants OCD 90-C-922, HD 07200, and NSF research grant BNS 17315.

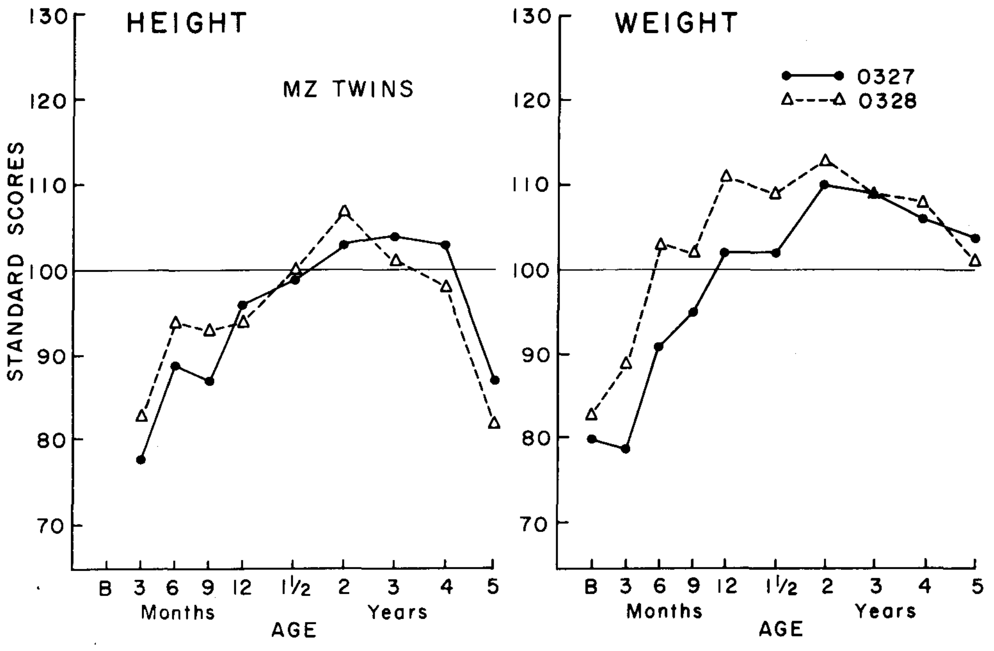

Fig. 1. Growth curves for a pair of monozygotic twins, birth to five years.

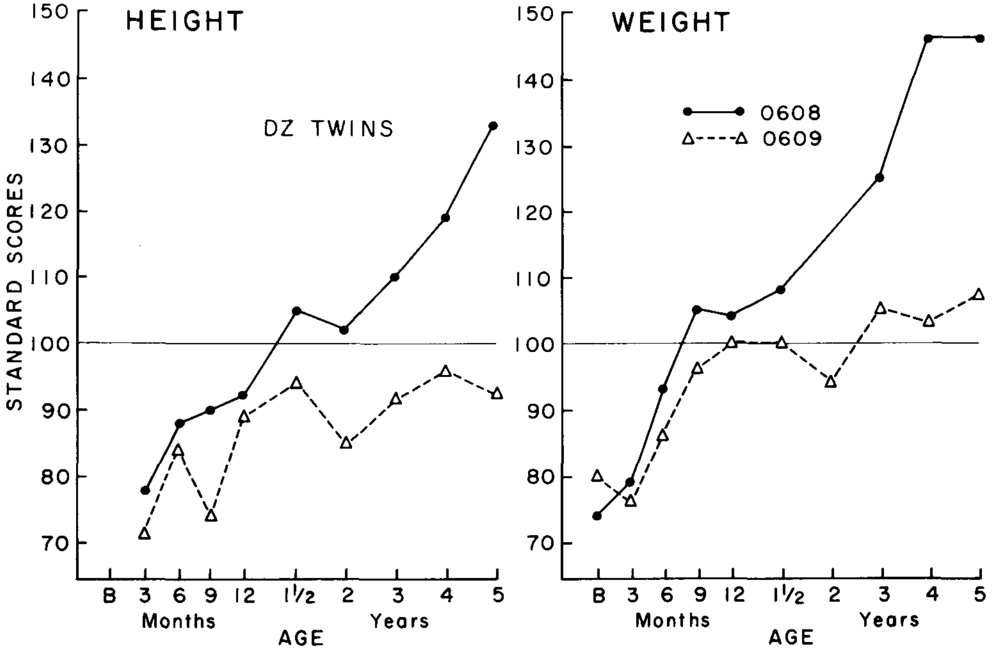

Fig. 2. Growth curves for a pair of dizygotic same-sex twins, birth to five years.

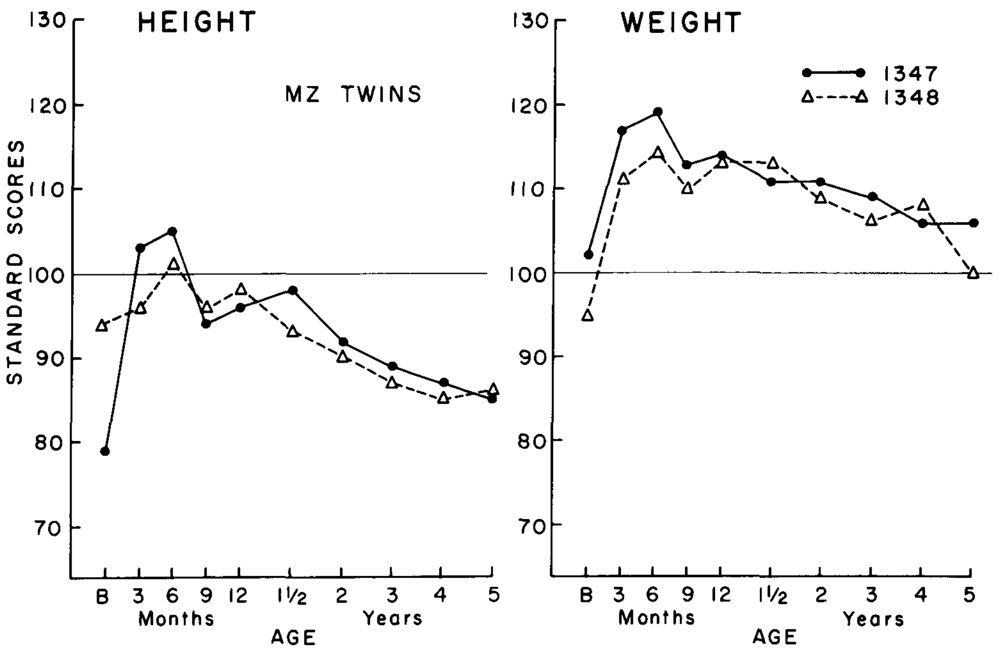

Fig. 3. Growth curves for a pair of monozygotic twins, birth to five years.

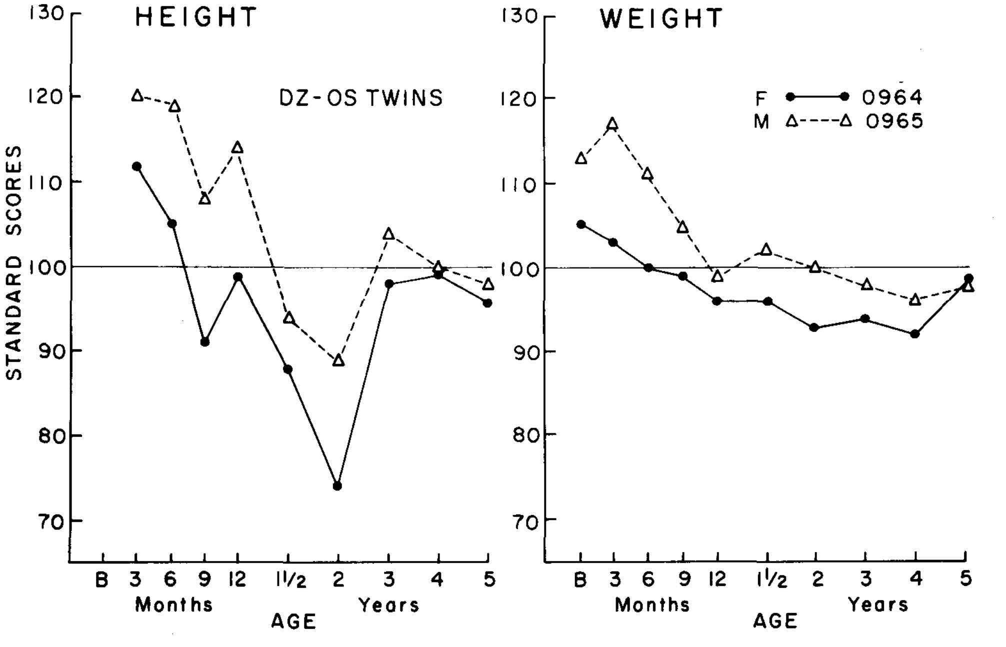

Fig. 4. Growth curves for a pair of dizygotic opposite-sex twins, birth to five years.

https://doi.org/10.1017/S0001566000009132 Published online by Cambridge University Press

The analysis of such individualized growth patterns requires a series of measurements made during infancy and childhood which can be evaluated for a large sample of children. Further, if the phasing of growth spurts is regulated by the genetic program, then monozygotic (MZ) twins who share the same genotype should exhibit these phases in common, while dizygotic (DZ) twins should be less concordant.

These implications may be illustrated by the growth curves for four pairs of twins (Figs. 1–4). The physical measures have been standardized at each age on a sample of more than 700 twins, with a mean of 100 and standard deviation of 16, so that a twin of average size at each age would be represented by a consistent score of 100. Fluctuations in the scores from age to age reflect phases of acceleration or delay in growth, relative to the trend for the full sample of twins.

The MZ twins in Figure 1 were initially much smaller than average, but by two years had moved above average; then, at age five years they dropped below average for height, but not for weight. The DZ twins in Figure 2 were also small initially, but moved upward together during the first year; then, one twin went on a prolonged growth spurt that eventually placed that twin far above average at five years. The twins in Figure 3 are notable for being below average in height, but above average in weight, while the twins in Figure 4 display dramatic changes in relative height over age, but much smaller changes in weight.

ANALYSIS OF GROWTH DATA

The statistical analysis of such growth curves for twins would focus on the twins' concordance for the pattern of age-to-age changes, or spurts and lags, and for concordance in average size during the age period. These concordance estimates may be obtained from a repeated-measures analysis of variance which has been specifically adapted for use with twins. The basic model may be found in Winer [15: pp 302 ff], where it is classified as a two-factor mixed design with repeated measures on one factor. The derivation of concordance estimates for the pattern of scores over ages is discussed in Haggard [5] and is illustrated in some detail for twins in Wilson [9, 10, 13].

The use of analysis of variance to determine the components of variance associated with genetic and environmental factors is well established [7], and has been the focus of much recent activity [eg, 2, 4, 6]. The methods to be presented herein differ from the above methods principally by adapting the basic statistical model to the twin situation and concentrating on the concordance for MZ and DZ pairs, rather than partitioning the variance among genetic and environmental components. If the results of the twin analysis are strong enough to confirm a systematic effect in the pattern of scores over age, then it may be appropriate to proceed with model fitting for genetic and environmental components.

Before illustrating the application of analysis of variance to these weight data, some preliminary results for the twin sample may be described. The sample includes over 700 twins, most of whom have been measured on a regular basis from birth to six years. The complete results for height and weight at each age may be found in Wilson [14], along with a description of the sample. For brevity, the present article will be confined to the weight data as a vehicle for demonstrating how repeated-measures analysis of variance (ANOVA) may be used to reveal concordance in growth patterns for twins.

By way of background, the within-pair correlations for weight at separate ages are shown in Table 1.

TABLE 1. Within-Pair Correlations for Weight From Birth to Six Years

	MZ pairs		DZ same-sex pairs		DZ opposite-sex pairs	
Age	n	R	n	R	n	R
Birth	152	0.62	91	0.66	70	0.68
6 months	137	0.78	80	0.61	60	0.46
1 year	137	0.87	82	0.57	63	0.45
2 years	142	0.87	85	0.58	58	0.56
4 years	116	0.85	63	0.54	47	0.66
6 years	107	0.88	62	0.56	39	0.67

The results showed that MZ twins rapidly increased in concordance during the first year and remained at a high level thereafter, effectively offsetting most of the within-pair differences in birth weight. By contrast, DZ same-sex twins declined in concordance following birth until the within-pair correlations stabilized around $R = 0.55$. The opposite-sex pairs showed a much more abrupt drop in concordance during the early months after birth, a reflection of sex differences in weight gain during infancy, but gradually reconverged and became more concordant than the same-sex DZ pairs.

MODEL FOR REPEATED-MEASURES ANALYSIS OF TWIN DATA

With this perspective from the single-age analysis, we turn to the repeated-measures ANOVA for assessment of concordance in growth trends over age. The analysis requires that each twin have scores at every age so that the pattern will be fully defined by a complete set of data points. Other considerations and constraints for using a repeated-measures analysis of variance are discussed in Wilson [11, 12] and in Winer [15].

The general linear model for the analysis of repeated-measures data for twins is given by:

$$X_{npq} = M + A_p + E_{n(p)} + B_q + AB_{pq} + BE_{qn(p)} + e_{n(pq)}$$

Each score (X_{npq}) represents the composite outcome of the various effects in the above model, which are identified as follows:

- M → population constant for the attribute being measured.
- A_p → differences between twin pairs in the sum (or average) of the repeated measures; in this case, the sum of the weight scores over successive ages.
- $E_{n(p)}$ → differences within pairs in the sum (or average) of the repeated measures.
- B_q → differences between ages for weight of the entire sample.
- AB_{pq} → differences between pairs for pattern of weight scores over age; in this case, different patterns of spurt and lag in weight gain.
- $BE_{qn(p)}$ → differences within pairs in the pattern of weight scores over age.
- $e_{n(pq)}$ → variability in each twin's score due to random error.

The variance of the total set of scores may then be expressed as a linear combination of the sources of variance identified above:

$$\text{Var}(X_{npq}) = \sigma^2_A + \sigma^2_E + \sigma^2_B + \sigma^2_{AB} + \sigma^2_{BE} + \sigma^2_e$$

where each variance component represents a population value that contributes to the total score variance.

TABLE 2. Summary Table for Analysis of Variance of Repeated-Measures Data for Twins

Source of variation	Degrees of freedom	Variance represented by mean squares
Between subjects		
Between pairs	p – 1	Deviation of pair means (averaged over ages) from the grand mean
Twins within pairs	p(n – 1)	Deviation of each twin's mean from the pair mean
Within subjects		
Ages	q – 1	Deviation of age means from the grand mean
Pairs × ages	(p – 1) (q – 1)	Distinctive pattern of age-to-age changes for each pair
Twins within pairs × ages	p(n – 1) (q – 1)	Deviation within pairs in pattern of age-to-age changes

TABLE 3. Expected Values of Mean Squares for Twin Analysis

Entry from ANOVA table	Expected value of mean squares
MS Pairs	$nq\sigma^2_A + q\sigma^2_E + \sigma^2_e$
MS Twins within pairs	$q\sigma^2_E + \sigma^2_e$
MS Ages	$np\sigma^2_B + n\sigma^2_{AB} + \sigma^2_{BE} + \sigma^2_e$
MS Pairs × ages	$n\sigma^2_{AB} + \sigma^2_{BE} + \sigma^2_e$
MS Twins within pairs × ages	$\sigma^2_{BE} + \sigma^2_e$

The thrust of most analyses is to partition the total score variance in such a way that the significant sources of variance can be identified and the magnitude of their effects estimated. To accomplish this, there are two steps at this point – a conceptual step, in which the variance components of the underlying model are combined to yield expected values for the measures of twin concordance; and a computational step, in which the actual data are processed by the standard calculations of analysis of variance to yield variance estimates for each main effect and interaction in the design. These variance estimates (typically referred to as mean squares, or MS) furnish the basic measures of systematic variance and error variance in the data, and they in turn relate to the contribution of each specific component in the underlying model.

Most investigators are familiar with the calculations of a mixed-design ANOVA with repeated measures on one factor. As employed with twins, the separate treatment groups become the p pairs of twins in the analysis, with each group (pair) having n = 2. All twins are measured on q different occasions, which provide the repeated measures for each subject. In this format, the measures of between-group variance and within-group variance now become the measures of between-pair and within-pair variance. The summary table for this analysis is illustrated in Table 2.

The mean squares for each main effect and interaction in the summary table represent the joint contributions of several variance components from the underlying model. Consequently each entry in the summary table has an expected value of mean squares based on the particular combination of variance components that enter into that source. The conceptual step mentioned earlier is now needed, and the expected values have been derived for the general case where the twin pairs in the sample represent a random effect, and the ages of measurement a fixed effect [cf 15: p 318]. The expected values are presented in Table 3. It will be seen, for example, that "MS pairs" represents the weighted contribution of true variance between pairs, plus true variance within pairs, plus error variance.

TEST OF TWIN CONCORDANCE

In the twin model, the basic test of twin concordance is whether the twins in each pair match one another more closely than they match the twins from other pairs. This translates into a comparison of the variance between pairs to the variance within pairs, since the latter becomes smaller as the twins become more concordant. The standard test of significance is an F ratio, which is initially constructed from the table of expected values, and then actually computed by substituting the appropriate variance sources from the summary table. The F ratios are shown in Table 4 for the two measures of twin concordance and for the test of ages as a main effect.

The resulting F ratios are then referred to the standard F tables with the appropriate degrees of freedom to determine the significance of each effect.

These results lead directly to intraclass correlations that express the concordance within pairs in the form of correlation coefficients. Intraclass correlations are also defined in terms of expected values, which are combined in such a way as to isolate the between-pairs variance components (σ^2_A or σ^2_{AB}), and then express each component as a proportion of the total expected variance for the effect in question. The appropriate combinations are shown in Table 5, along with the corresponding mean squares by which they are estimated.

TABLE 4. Tests of Twin Concordance in Repeated-Measures Analysis of Variance

Test	F ratio based on expected values of mean squares	F ratio from ANOVA summary table	Degrees of freedom
Concordance within pairs for sum of repeated measures (test of σ^2_A)	$\dfrac{nq\sigma^2_A + q\sigma^2_E + \sigma^2_e}{q\sigma^2_E + \sigma^2_e}$	MS pairs / MS twins w/i pairs	p−1 / p (n−1)
Concordance within pairs for pattern of age-to-age change (test of σ^2_{AB})	$\dfrac{n\sigma^2_{AB} + \sigma^2_{BE} + \sigma^2_e}{\sigma^2_{BE} + \sigma^2_e}$	MS pairs × ages / MS twins w/i pairs × ages	(p−1) (q−1) / p(n−1) (q−1)
Differences between ages (test of σ^2_B)	$\dfrac{np\sigma^2_B + n\sigma^2_{AB} + \sigma^2_{BE} + \sigma^2_e}{n\sigma^2_{AB} + \sigma^2_{BE} + \sigma^2_e}$	MS ages / MS pairs × ages	q−1 / (p−1) (q−1)

https://doi.org/10.1017/S0001566000009132 Published online by Cambridge University Press

TABLE 5. Within-Pair Correlations for Twins Derived From Repeated-Measures Analysis of Variance

Within-pair correlation	Based on expected values*	Derived from ANOVA table	Equivalent
Sum of repeated measures	$\frac{\sigma_A^2}{\sigma_A^2 + \sigma_E^2 + \sigma_{\bar{e}}^2}$	$\frac{\text{(MS pairs)} - \text{(MS twins w/in pairs)}}{\text{(MS pairs)} + (n-1)\text{ (MS twins w/i pairs)}}$	$\frac{F_p - 1}{F_p + 1}$
Pattern of age-to-age changes	$\frac{\sigma_{AB}^2}{\sigma_{AB}^2 + \sigma_{BE}^2 + \sigma_e^2}$	$\frac{\text{(MS pairs} \times \text{ages)} - \text{(MS twins w/i pairs} \times \text{ages)}}{\text{(MS pairs} \times \text{ages)} + (n-1)\text{ (MS twins w/i pairs} \times \text{ages)}}$	$\frac{F_{pxa} - 1}{F_{pxa} + 1}$

$^*\sigma_{\bar{e}}^2 = \sigma_e^2/q.$

TABLE 6. Summary Table for Analysis of Weight Measures Obtained at Birth, Three Months, and Six Months for MZ Twins

Source	Degrees of freedom	Mean squares	F ratio	Within-pair correlation	P
Between subjects					
Pairs	(p−1) = 112	929.8	4.51	0.64	< 0.001
Twins within pairs	p(n−1) = 113	206.3			
Within subjects					
Ages	(q−1) = 2	245.0	1.40[a]		ns
Pairs × ages	(p−1)(q−1) = 224	175.0	14.46	0.87	< 0.001
Twins within pairs × ages	p(n−1)(q−1) = 226	12.1			

[a]Since the weight measures were standardized at each age, there was no significant effect for ages.

The entries in the final column of Table 5 show the direct relationship between the intraclass correlation and the F ratio, since both depend upon combinations of the same variance components. In fact, the significance level of the correlation is exactly the same as the corresponding F test.

In a practical sense, the derivations above show that the within-pair correlations for for total score and for age-to-age changes are immediately available from the ANOVA summary table. For illustration, the results are shown in Table 6 for the weight scores obtained at birth, three and six months for 113 pairs of MZ twins.

The results indicated that MZ twins were moderately concordant for average weight over the three occasions of measurement (R = 0.64), and even more concordant for the pattern of age-to-age changes in relative weight (R = 0.87). The latter indicated that both twins were going through parallel episodes of spurt or lag in weight gain during the first six months.

TWIN CONCORDANCE AND GROWTH

With these derivations completed, the analyses were then performed on the weight scores for the entire twin sample. Four different age periods were selected for analysis – the

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TABLE 7. Twin Concordance for Average Weight and for Spurts and Lags in Weight Gain During Four Age Periods

	Average weight: Within-pair R's			Spurts and lags: Within-pair R's		
Ages	MZ	DZ-SS	DZ-OS	MZ	DZ-SS	DZ-OS
Birth, 3, 6 mos	0.64	0.73	0.55	0.87	0.56	0.50
6, 9, 12 mos	0.82	0.56	0.39	0.80	0.62	0.65
1½, 2, 3, 4 yrs	0.86	0.54	0.69	0.84	0.63	0.37
4, 5, 6 yrs	0.87	0.52	. . .	0.64	0.36	. . .

SS = same sex, OS = opposite sex.
Ellipses indicate that the number of pairs was insufficient for a reliable estimate.

first two periods to display short-term changes in concordance during the first year, when the effects of prematurity were progressively offset and weight gain was very rapid; then two longer subsequent periods to reveal more enduring episodes of growth acceleration or lag, as previously illustrated in Figure 2. The analyses were performed separately for MZ twins, DZ same-sex twins, and opposite-sex twins, and the results are presented in Table 7.

For MZ twins, the concordance for average weight remained consistently high after the first year. The concordance for spurts and lags in weight gain also remained high until the final age period, when it dropped somewhat. By this time, the episodes of spurt and lag had smoothed out considerably and each child was maintaining a consistent ranking in the weight distribution, as shown by an age-to-age correlation of $r = 0.96$. Therefore, there was very little systematic variance attributable to spurts and lags in growth during this final period for which MZ pairs could be concordant.

Turning to the DZ twins, the correlations for average weight in same-sex pairs stabilized in the low 0.50s after the first year, a reflection of the downward trend previously seen in Table 1. The spurt/lag correlations were also significantly below the corresponding MZ correlations, although still positive and sizable enough to indicate moderate concordance for the pattern of weight gain. Among opposite-sex twins, sex differences in weight gain had a marked age-linked influence on concordance, with the correlations for average weight dropping throughout the first year as the males went through an accentuated growth spurt, then reversing in subsequent years as the females caught up [14].

When comparing the correlations among different groups of twins, it is desirable to establish the significance of the differences by an appropriate test. Following Haggard [5], the appropriate procedure is to transform the correlation coefficients into z scores, and then test for the difference in z scores relative to the standard error of the differences. For concordance in average weight, this would take the following form:

$$\text{Critical ratio} = \frac{z_{MZ} - z_{DZ}}{\sqrt{\dfrac{1}{p_{MZ} - 2} + \dfrac{1}{p_{DZ} - 2}}}$$

where p is the number of pairs. The critical ratio is then referred to the table of the normal curve to establish the probability value.

For the spurt/lag correlations, the formula is modified slightly to take account of the increased degrees of freedom for each correlation:

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$$\text{Critical ratio} = \frac{z_{MZ} - z_{DZ}}{\sqrt{\dfrac{1}{p_{MZ} - 2} + \dfrac{1}{p_{DZ} - 2}}}$$

where q is the number of repeated measures.

ESTIMATION OF VARIANCE ACCOUNTED FOR

While the correlations reveal the extent of twin concordance, they do not directly reveal the magnitude of a given effect in relation to the total variance in the data. Where there are rapid developmental changes, a particular effect such as spurts and lags in growth may be a prominent source of variance during one age period but much reduced at the next. Estimating the magnitude of each effect for a given age period requires an estimation of the percent of variance accounted for by that effect.

The general procedures for estimating the percent of variance explained have been presented by Dwyer [3], and they are also based on the expected values for each main effect and interaction. The expected values are combined in such a way that each variance component is isolated individually, then a numerical figure is obtained by inserting the appropriate mean squares from the ANOVA summary table into the equation. The procedures are illustrated in Table 8, making use of the data previously presented in Table 6.

The concordance within MZ pairs for average weight during the first six months accounted for 47% of the variance, and the concordance for spurts and lags accounted for 21%. Differences in average weight accounted for 27% of the variance – again, a reflection of some initial within-pair differences in birth weight that were not fully offset during the first six months – while the differences for spurts and lags in weight gain

*TABLE 8. Calculation of Percent of Variance Explained for Each Component in Twin Model**

Variance component	Estimate	Variance accounted for	Percent of variance explained
σ^2_A	(1/nq) [(MS pairs) – (MS twins w/i pairs)]	120.6	47.1
$\sigma^2_E + \sigma^2_{\bar{e}}$	(1/q) (MS twins w/i pairs)	68.8	26.8
σ^2_B	(1/np) [(MS ages) – (MS pairs × ages)] (1 – 1/q)	0.2	0.1
σ^2_{AB}	(1/n) [(MS pairs × ages) – (MS twins w/i pairs × ages)] × (1 – 1/q)	54.6	21.3
$\sigma^2_{BE} + \sigma^2_e$	(MS twins w/i pairs × ages)	12.1	4.7
		256.3	100.0

*Values for mean squares drawn from Table 6.

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TABLE 9. Percent of Variance Explained by Different Components in Analysis of Weight Scores for Twins

Variance component	Age periods: Birth, 3, and 6 months			6, 9, and 12 months			1½, 2, 3, and 4 years			4, 5, and 6 years		
	MZ	DZ-SS	DZ-OS	MZ	DZ-SS	DZ-OS	MZ	DZ-SS	DZ-OS	MZ	DZ-SS	DZ-OS
σ^2_A (Pairs)	47.1%	59.4%	40.0%	76.0%	52.1%	35.6%	78.9%	49.0%	64.6%	83.1%	50.3%	. . .
σ^2_E (Twins w/i pairs)	26.8	22.0	33.4	16.9	41.3	56.3	12.6	41.8	28.9	12.7	46.8	. . .
σ^2_{AB} (Pairs × ages)	21.3	8.6	10.5	5.2	3.5	4.6	6.6	5.1	2.0	2.3	0.8	. . .
σ^2_{BE} (Twins w/i pairs × ages)	4.7	10.0	15.9	1.9	3.2	3.6	1.7	3.9	4.5	1.9	2.1	. . .

SS = same sex, OS = opposite sex.
Ellipses indicate that the number of pairs was insufficient for a reliable estimate.

accounted for 5%. Age as a main effect made no significant contribution to the variance since the measures were standardized on the full sample at each age.

It will be noted that the two variance components for within-pair differences, σ^2_E and σ^2_{BE}, also include a component of random error variance, σ^2_e. The latter represents all uncontrolled sources of variation affecting each twin's score, and typically it includes measurement error as the largest uncontrolled source. With physical growth data, however, the errors of measurement are negligible, and the resulting variance estimates for σ^2_E and σ^2_{BE} may be regarded as minimally affected by the random error component. In fact, the within-pair variance for physical measures may furnish a useful benchmark for identifying the amount of added variance due to measurement error in psychological data.

Extending the analysis to all age periods and zygosity groups, the percent of variance explained by each component has been computed and is presented in Table 9.

For MZ twins, the variance explained by concordance in average weight rose to 83% in the final period, while for spurts and lags it declined to less than 3%. The latter reflected the previously-mentioned smoothing out of growth spurts after four years, so there was relatively little fluctuation for which the twins could be concordant.

The percentage figures help clarify the significance of the spurt/lag correlations in each period (Table 7). For example, the MZ correlation for the spurt/lag component in the first period was $R = 0.87$, and this component accounted for 21.3% of the variance. In the third period, however, while the correlation remained at nearly the same level ($R = 0.84$), the concordance for spurt and lag accounted for only 6.6% of the variance. Intraclass correlations reveal whether there is concordance for a given source of variance, not whether the source has a powerful effect upon the data, and it is always desirable to interpret a correlation in the context of the percent of variance explained.

Turning to DZ same-sex twins, their similarities in average weight during the first six months were more pronounced than for either MZ twins or opposite-sex pairs, but in subsequent periods the variance explained by this component receded to 50%. Notably, by the final period the within-pair differences in average weight (σ^2_E) accounted for almost as much variance as the similarities. This coordinates with the same-sex correlation for average weight in the final period ($R = 0.52$), and reflects the intermediate degree of dispersion in average weight reached by the same-sex twins. By contrast, the opposite-sex twins were much less similar in average weight during the first year, but subsequently moved closer together and yielded a higher percent of variance explained for weight similarity in the following years.

These data illustrate the powerful chronogenetic influences on growth, and how a repeated-measures analysis of variance may be employed with twins to determine the magnitude of these influences during different age periods. The next step is to extend the analysis to measures of height and mental development, so that the patterns of concordance may be established for these variables. By putting the height and weight measures in the same standardized format as the mental development scores, the joint patterns of development may be simultaneously assessed for all three variables – for example, do the spurt/lag episodes occur in common, or does each variable follow an independent path? And is mental development as powerfully influenced by chronogenetic factors as physical growth? The statistical methods outlined herein offer a method for analyzing these questions; and for investigators who may be interested, a computer program for the basic twin ANOVA may be obtained from the author.

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