

ONTOGENY AND INDIVIDUAL VARIATION: ANALYSIS OF PATTERNED COVARIANCE MATRICES WITH COMMON PRINCIPAL COMPONENTS

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Abstract.—Morphometric data from longitudinal growth studies with multiple measurements made in several growth stages on the same specimens confront researchers with difficult statistical problems because traits are correlated both within and across stages. Here, we introduce a statistical model especially designed to deal with this complexity. The common principal component (CPC) model for dependent random vectors is based on the assumption that the same pattern underlies both variation within stages and covariation across stages. Thus, a single transformation, when applied to all stages, renders the resulting CPCs uncorrelated not only within but also across stages. Because of these simplifying assumptions, the CPC model greatly reduces the number of parameters to be estimated; it is thus an efficient tool for data reduction. This model is demonstrated using growth of the water strider *Limnoporus canaliculatus*. The CPCs can be interpreted as patterns of “size” variation and contrasts between parts that are common to all stages, although there are minor deviations from the model. The “size” CPC accounts for most variation in all instars and is therefore an effective measure of overall growth. Moreover, the CPC model clarifies the link between static and ontogenetic variation by including both levels in a joint analysis and can be used to study morphological integration and constraints on the evolution of ontogenies. [Allometry; common principal components; Gerridae; growth; longitudinal data; multivariate morphometrics; size.]

In recent years, there has been renewed interest in the extent to which ontogeny acts as a mechanism influencing patterns of evolutionary change in morphological traits (e.g., Gould, 1977; McKinney and McNamara, 1991; Hall, 1992). Some studies have focused on the outcome of these processes by comparing multivariate patterns of ontogenetic and evolutionary variation (e.g., Shea, 1985; Voss et al., 1990; Klingenberg and Zimmermann, 1992; Voss and Marcus, 1992), whereas others have emphasized microevolutionary processes by studying individual variation in growth and its genetic basis (e.g., Cheverud et al., 1983; Kirkpatrick, 1988; Lynch, 1988; Atchley and Hall, 1991; Cowley and Atchley, 1992; Björklund, 1993).

Numerous studies of the evolution of ontogeny have focused on individual variation in growth curves (also called growth trajectories), i.e., trait measurements as a function of age or developmental stage.

Age-specific measurements can be treated as separate variables, or continuous growth functions can be accommodated by interpolating between the ages at which measurements were made (Kirkpatrick, 1988; Kirkpatrick and Lofsvold, 1989; Kirkpatrick et al., 1990; Björklund, 1993). Usually, the analyses focus on covariances or correlations among measurements made at several ontogenetic stages but consider only one trait at a time, thereby ignoring correlations among traits (e.g., Cheverud et al., 1983; Leamy and Cheverud, 1984; Lynch, 1988; Kirkpatrick et al., 1990; Björklund, 1993). Cheverud et al. (1983) characterized relationships among variables by first computing the eigenvectors of among-stage covariance matrices for each trait separately and then comparing them using vector correlations. Björklund (1993) used an analogous procedure within the framework for continuous growth. A more formal approach, however, would simulta-

neously include all measurements and stages into the analysis. Yet even for moderate numbers of stages and traits, the full statistical model, with no constraints imposed, would contain a very large number of parameters to be estimated and thus would render the application to real data sets difficult, which is probably why such a study has not been attempted. Nevertheless, a simultaneous analysis of the ontogenies of several traits is feasible if one makes some simplifying assumptions, as suggested by the similarity among patterns of ontogenetic variation found in different traits (Cheverud et al., 1983; Björklund, 1993).

Another approach to understanding the connections between ontogeny and evolution focuses on the static variation among individuals at a particular stage, which is the raw material upon which natural selection can act. This variation is the product of variation in the developmental processes that generated the structures under study and can therefore be used to investigate these processes (Cheverud, 1982; Zelditch, 1987; Cowley and Atchley, 1990; Atchley et al., 1992) and their regulation (Tanner, 1963; Atchley, 1984; Riska et al., 1984). Several studies comparing patterns of static variation across ontogenetic stages have found that a single "size" component dominated the variation within each stage (Cuzin-Roudy, 1975; Zelditch, 1988; Klingenberg and Zimmermann, 1992). None of these studies, however, considered the correlations of measurements among stages, either because they were based on cross-sectional data, with a different sample taken independently for each stage, or because the statistical methods were unable to deal with such correlations.

Here, we introduce a new statistical model (Neuenschwander, 1991; Flury and Neuenschwander, 1995a), which we use to analyze variation in multiple measurements at several ontogenetic stages. The model specifically uses the information contained in longitudinal data, with measurements at all stages for each individual, and it explicitly considers covariation among traits as well as across stages. As

an extension of the common principal component (CPC) model for independent groups (Airoldi and Flury, 1988; Flury, 1988), the model assumes that CPCs are uncorrelated within each group and between groups, e.g., the first CPC in one ontogenetic stage is correlated only with the first CPC of other stages. This assumption was derived from observations in earlier studies of the commonalities among traits or stages, and our example of growth in the water strider *Limnoporus canaliculatus* demonstrates that it can be realistic. Because CPCs are uncorrelated within and between stages, the model effectively divides a very complex analysis into several simpler ones. Furthermore, it sheds light on the connection between morphometric variability and growth variation and suggests a coherent framework to study them jointly.

STATISTICAL MODELS

Principal component (PC) analysis (PCA) and its recent extensions are frequently used in morphometric applications, especially in multivariate allometry (Jolicoeur, 1963; Pimentel, 1979; Airoldi and Flury, 1988; Marcus, 1990; Klingenberg, 1996). In most of these applications, the data have a relatively simple structure and consist of measurements made either on a single group of specimens or on specimens from several separate groups (e.g., species, sexes, ecomorphs, or geographical variants). Longitudinal growth data, however, have a more complex structure because the same individuals are measured for each growth stage, and the stages therefore cannot be treated as independent groups. This interdependence requires substantial adjustments of the statistical models used to analyze such data. Because multivariate studies of multiple groups and longitudinal studies are based on rather complex data, the need to summarize these data using simplified models is especially urgent. In this section, we briefly review one-group PCA and models of CPCs for independent groups before we introduce patterned covariance matrices for longitudinal data and a model of CPCs

for dependent random vectors. We emphasize the use of PC and CPC models as tools for data reduction.

One-Group Principal Components and Common Principal Components for Independent Groups

PCA is a tool used to analyze variation within a single group of specimens. In the space spanned by the variables (e.g., in two dimensions, the plane of a scatter plot), PCA can be used to assess the amount and direction of this variation. It transforms the original variables into PCs, a set of new variables that successively account for the largest possible part of total variation and yet are mutually uncorrelated (Fig. 1a). This transformation fundamentally changes the covariance matrix (Fig. 1b) and renders it into diagonal form (Fig. 1c); because PCs are uncorrelated, all off-diagonal elements of the covariance matrix (covariances between pairs of PCs) are zero, whereas the diagonal contains the variances of the PCs, or eigenvalues (see Appendix, PCA).

In many applications, the first few PCs account for the largest portion of total variance. In morphometrics, it is not uncommon for the first one or two PCs to take up $\geq 95\%$ of the variation in a much larger number of variables. The first few PCs therefore summarize most of the variation in fewer dimensions, perhaps only one. Models of this kind, including only one allometric "size" axis while regarding the remaining variation as random scatter around it, have been used traditionally in morphometrics (e.g., Hopkins, 1966; Bookstein et al., 1985; Klingenberg, 1996). This extreme data reduction using simplified models of within-group variation is helpful for comparisons between two or more groups of specimens.

PCA has been generalized for situations involving several groups. The CPC model assumes that the groups all share the same (common) PCs, but it allows the groups to differ in the amounts of variation associated with each one (Airoldi and Flury, 1988; Flury, 1988). The scatter ellipsoids for all groups therefore have parallel principal

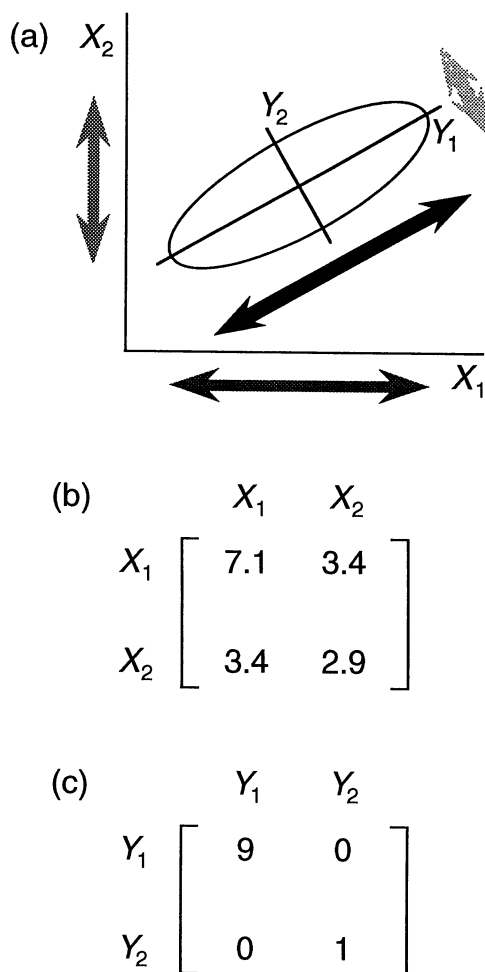


FIGURE 1. Principal component analysis of a single group. (a) Bivariate plot with a contour ellipse representing the distribution of data points. The original variables X_1 and X_2 are transformed into the principal components Y_1 and Y_2 , which account for maximal and minimal variation, respectively (arrows). This transformation corresponds to a rotation of the coordinate system, in which the new coordinates are aligned with the major and minor axes of the contour ellipse. (b) Covariance matrix of the original variables. Units are arbitrary. (c) Covariance matrix of the principal components. The off-diagonal elements of the matrix are zero. Units are arbitrary.

axes, but the lengths of corresponding axes may vary. Under the CPC model, a single transformation simultaneously converts the covariance matrices of all groups to diagonal form (Appendix, CPCs for Independent Groups). The CPC model has

been applied to biological data sets by Airoldi and Flury (1988), Klingenberg and Zimmermann (1992), and Klingenberg and Spence (1993).

Longitudinal Data and Patterned Covariance Matrices

The CPC model was designed for independent groups, e.g., samples drawn from different sexes, ecomorphs, or species. Numerous growth studies use separate samples of specimens in different ontogenetic stages; such cross-sectional data can be analyzed using the CPC model for independent groups (e.g., Klingenberg and Zimmermann, 1992). Longitudinal data, however, consist of measurements made on the same specimens in several growth stages (Fig. 2a), and these stages therefore are not independent groups. Following individuals through growth has obvious benefits; questions about individual variation in growth processes and their regulation can be addressed, e.g., whether there is compensatory growth (Tanner, 1963; Monteiro and Falconer, 1966; Riska et al., 1984; Lynch, 1988; Kirkpatrick et al., 1990; Cowley and Atchley, 1992). As a consequence of this additional information, however, the structure of longitudinal data is more complex than that of cross-sectional data.

For a typical longitudinal study, in each of k different growth stages p measurements are taken on the same n specimens (Fig. 2a). The data are most conveniently arranged in an $n \times kp$ matrix, i.e., the measurements for the different stages are treated as separate variables. The resulting covariance matrix has a distinctive pattern: it consists of an array of $k \times k$ submatrices, each of dimension $p \times p$ (Fig. 2b). The blocks along the main diagonal of the matrix (Fig. 2b, shaded boxes) are the within-stage covariance matrices, and they also are used in cross-sectional analyses. They characterize static variation between individuals within each stage. The off-diagonal blocks (Fig. 2b, cross-hatched boxes) contain covariances between measurements taken in different growth stages.

The number of variables in a longitu-

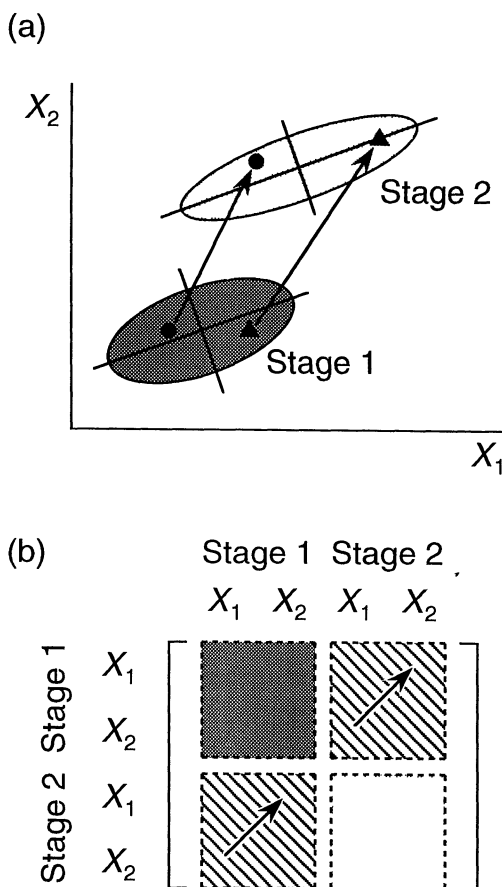


FIGURE 2. The structure of longitudinal data. (a) Bivariate scatter plot with contour ellipses for two growth stages (i.e., $p = 2$, $k = 2$). The measurements for two specimens (\bullet , \blacktriangle) are plotted in each stage and connected by arrows. (b) Patterned covariance matrix describing the data. The morphometric measurements X_1 and X_2 of each stage are entered separately as variables for the analysis. The blocks on the main diagonal (shaded) are the within-stage covariance matrices of stages 1 and 2. The off-diagonal blocks (cross-hatched, with arrows) contain covariances of measurements between stages, which can be used to study regulatory phenomena such as compensatory growth.

nal analysis can be very large, even with moderate numbers of measurements and stages. The example we use to demonstrate this approach contains four measurements and six growth stages and is thus smaller than the data sets used in many similar studies. Nevertheless, there are 24 variables in the analysis, and with-

out further constraints, the number of parameters required for a full statistical description of the covariance structure is $300 (24 \times [24 + 1]/2; \text{ see Appendix})$. This complexity of longitudinal data calls for techniques of data reduction; therefore, we introduce a simplified model for which fewer parameters need to be estimated but that can still represent the data with reasonable accuracy.

Common Principal Components for Dependent Random Vectors

The CPC model described above assumes that a single transformation simultaneously converts the covariance matrices of k groups to diagonal form. Because the groups are assumed to be independent of one another, these covariance matrices characterize the variation within groups sufficiently. If the groups are interdependent, however, there are k sets of measurements (groups, growth stages), each with the same p variables, for every observation (examples given by Flury and Neuenschwander, 1995a). The result is a patterned $kp \times kp$ covariance matrix composed of k^2 submatrices (each of format $p \times p$); the k within-group covariance matrices are arranged as blocks along the diagonal, and the off-diagonal blocks are matrices of covariances between groups. Figure 3a shows such a patterned covariance matrix for ontogenetic data, where the groups correspond to discrete growth stages.

Like the CPC model for independent groups, the model of CPCs for dependent random vectors assumes that all the groups share the same PCs. Therefore, the transformation to CPCs converts all the within-group covariance matrices to diagonal form (the blocks along the diagonal in Fig. 3b). In addition, however, the same transformation must also render diagonal all the remaining submatrices, which contain the covariances of measurements across groups (Appendix, CPCs for Dependent Random Vectors; Neuenschwander, 1991; Flury and Neuenschwander, 1995a). This means that only corresponding CPCs are correlated among groups; for example, only the pairs of first or pairs of

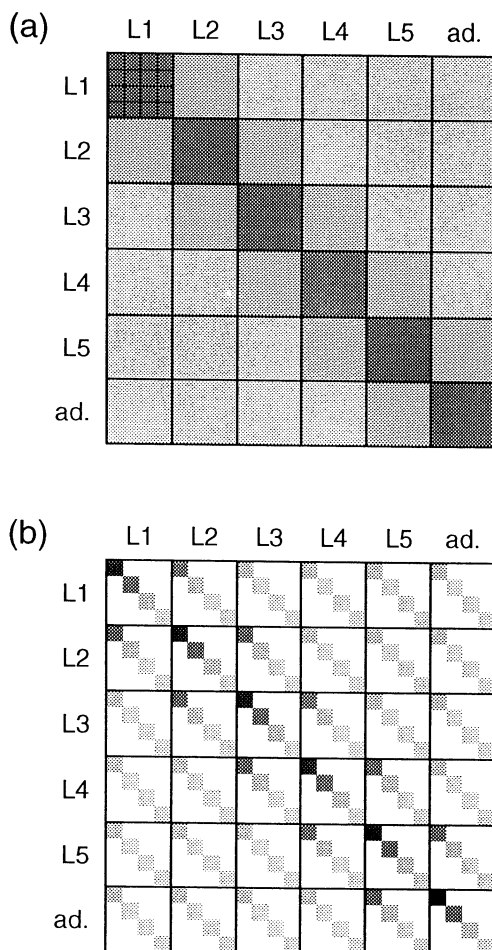


FIGURE 3. The model of common principal components for dependent random vectors. The structure of longitudinal data is shown as in the water strider example, with six discrete growth stages (five larval instars, denoted L1–L5, and the adult stage) and four morphometric variables. Shading represents the approximate magnitude of matrix elements (blank = 0). (a) Covariance matrix. The blocks along the diagonal are the covariance matrices within stages, and the off-diagonal blocks contain covariances between stages. Each block is a 4×4 matrix, as indicated by the grid in the covariance matrix for L1. (b) Covariance matrix of the CPCs. All the submatrices have diagonal form because only pairs of corresponding PCs are correlated between instars.

second CPCs are correlated among groups, not the first CPC in one group with the second CPC in another group.

This CPC model results in a substantial reduction in the number of parameters to

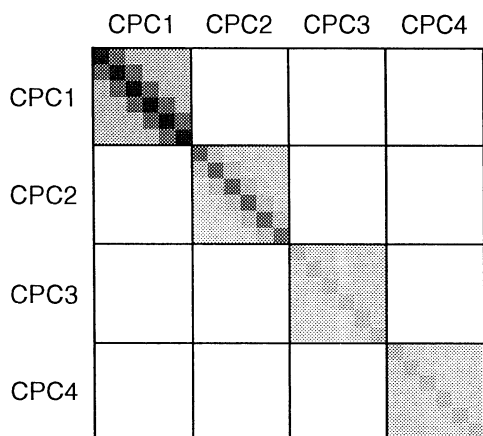


FIGURE 4. Rearrangement of the patterned covariance matrix after CPC transformation (cf. Fig. 3b). The transformation considerably simplified the structure of longitudinal growth data. Because the CPCs are uncorrelated both within and among stages, they can be examined separately. The multivariate problem is thus reduced to a study of the matrix of covariances between stages for each CPC. If some CPCs only account for a small amount of variation, they may be omitted from the analysis.

be estimated. In the example with $k = 6$ and $p = 4$, there are only 90 parameters instead of 300 in the unconstrained model (see Appendix, Number of Parameters). The advantage of the model becomes more apparent if the covariance matrix shown in Figure 3b is rearranged so that rows and columns are ordered by CPCs rather than by groups (Fig. 4). This rearranged matrix consists of p^2 blocks of format $k \times k$. All the elements of the off-diagonal blocks are zero values. The CPCs can now be studied separately because they are uncorrelated.

For longitudinal growth studies with multiple measurements, this model reduces a complex multivariate problem into a number of simpler analyses, each considering one CPC. Thus, p matrices of covariances of CPC scores among developmental stages must be examined, using the methods developed for analyses of a single measurement. Moreover, CPCs accounting for only minor amounts of variation may be ignored in the interpretation of results, as in one-group PCA, to simplify the analysis even further.

EXAMPLE: GROWTH IN WATER STRIDERS

Data

Water striders (Heteroptera: Gerridae) are especially suitable for studying the ontogeny of individual variation because their growth occurs in six discrete stages; five larval instars, denoted L1–L5, precede the adult stage. Because there is no variation in the number of larval instars, they are comparable developmental stages. Because of the rigid cuticle, the growth of numerous structures can be followed easily for the entire postembryonic development, as in other hemimetabolous insects. Moreover, water striders can be reared individually in the laboratory, and it is easy to obtain a complete record of each individual's growth by collecting the exuviae at every molt.

For this study, we used longitudinal growth data from the water strider *Limnoporus canaliculatus*, reared under controlled laboratory conditions (20°C, 16 hr light:8 hr dark). Water striders collected in the wild (Morris County, New Jersey; 1 May 1992) were set up as a mass culture, from which eggs were taken for individual rearings. Within about 12 hr of hatching, first instar larvae were put separately into plastic containers (diameter = 11.5 cm, height = 8 cm), each with about 1 cm of water and a small Styrofoam strip floating on the surface. Each larva was fed a frozen flesh fly (*Neobelliera bullata*) daily and checked for molts at intervals of about 12 hr, and all exuviae were collected. After the adults emerged, they were killed by freezing. Exuviae and adults were stored in 70% ethanol for several months before measuring.

The variables used in this study are the lengths of the femora and tibiae of the middle and hind legs, measured on both body sides. Shrinking and other artifacts of preservation are negligible, because the cuticle of the legs consists of rigidly sclerotized tubes even in the otherwise delicate first instar exuviae. All measurements were taken with a video system attached to a dissecting microscope.

If the measurements could be made on

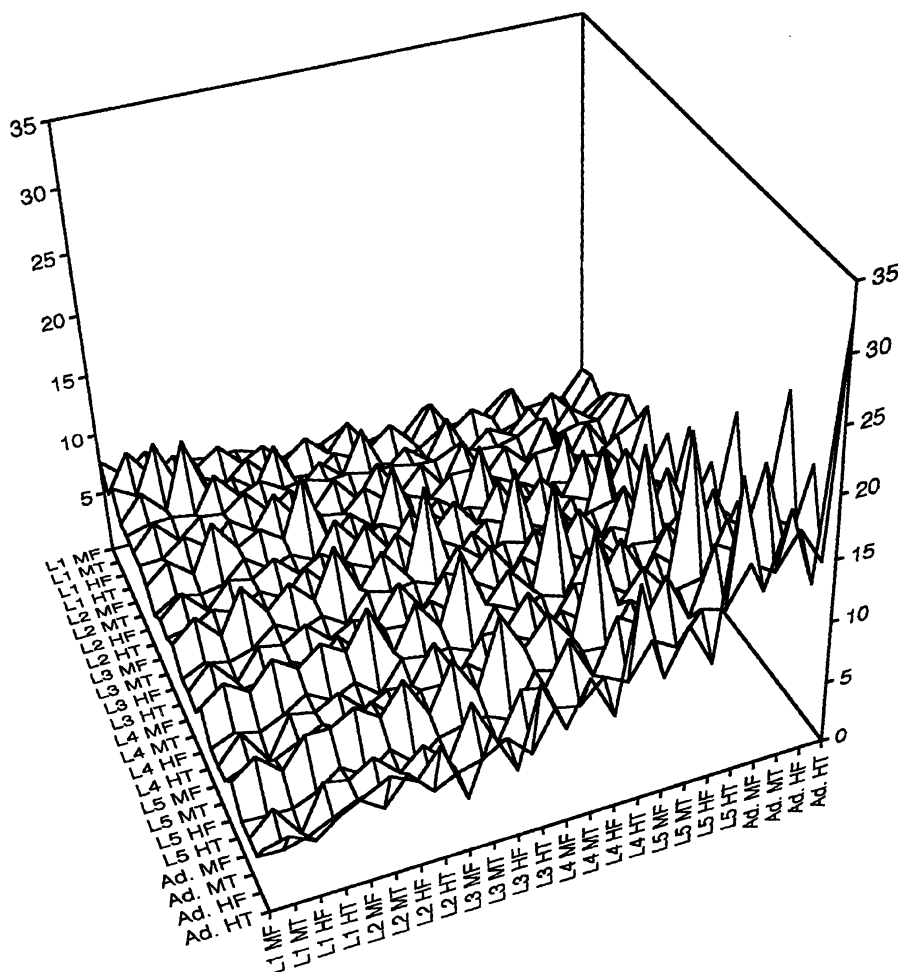


FIGURE 5. Covariance matrix for stage-specific measurements in females of the water strider *Limnoporus canaliculatus*. The lengths of middle and hind femora and tibiae (MF, MT, HF, HT) are the four measurements taken for all five larval instars (L1–L5) and adults (Ad.). Values on the vertical axis are variances and covariances for natural log-transformed measurements, multiplied by 10^4 .

both body sides, we used arithmetic means of left and right sides; otherwise we used measurements from one side. Data were checked for outliers for each variable separately in every instar. A few individuals were excluded from the data set because of deformities related to abnormal molting. In this study, we used the data for 89 females for which complete data were available in each instar (the data set is available from C.P.K. on request). All mea-

surements were transformed to natural logarithms before the analysis.

Statistical Analysis and Results

The covariance matrix of stage-specific measurements is the basis for the following analysis. The most conspicuous feature of this matrix is a general increase in variances from early to late instars, but especially in the L5 instar and adult (Fig. 5). The tibia of the hind leg is the most vari-

able trait in each instar. This pattern is not only repeated in every instar, but to a certain extent it also applies to the covariances among instars; the division of the covariance matrix into blocks is therefore visible mostly from the "peaks" representing the hind tibia in different instars (Fig. 5). All covariances within and between instars are positive, and covariances tend to be higher between consecutive instars than between those farther apart.

The patterns of variation within instars can be characterized separately with PCA. Despite the general increase in the amount of variation from instar to instar (Fig. 5), the proportion accounted for by each PC remains fairly constant. Within each instar, PC1 accounts for the largest proportion of the total variance, and PC1s have coeffi-

cients that are all positive (Table 1). Therefore, they can be interpreted as "size" vectors, reflecting static allometry. The allometric patterns are similar in all instars, as indicated by the high vector correlations among PC1s, which all exceed 0.99, and the corresponding angles, which range from 0.97° to 7.5°. PC2, which takes up a moderate amount of variation in all instars, is a contrast of the hind tibia with the middle and hind femora; the middle tibia has coefficients of smaller magnitude, which even vary in their sign. PC3 and PC4 only account for small proportions of the total variation.

CPCs for dependent random vectors.—Whereas one-group PCA always can transform the covariance matrix of a sample to exactly diagonal form, simultaneous anal-

TABLE 1. Patterns of individual variation in water striders, analyzed by separate PCAs in each life stage (instars L1–L5 and adult). Values presented are the PC coefficients for each variable and the percentages of total variance (% variance) for which each PC accounts.

Stage	Variable ^a	PC1	PC2	PC3	PC4
L1	MF	0.455	0.342	-0.144	0.809
	MT	0.500	-0.236	0.832	-0.034
	HF	0.516	0.612	-0.159	-0.578
	HT	0.526	-0.672	-0.511	-0.102
	% variance	76.9	14.8	5.1	3.2
L2	MF	0.504	0.434	0.217	-0.714
	MT	0.486	-0.128	0.716	0.484
	HF	0.482	0.489	-0.556	0.468
	HT	0.527	-0.745	-0.361	-0.191
	% variance	80.7	11.7	4.8	2.8
L3	MF	0.445	0.532	0.128	-0.709
	MT	0.496	-0.167	0.787	0.328
	HF	0.457	0.510	-0.426	0.591
	HT	0.589	-0.656	-0.428	-0.199
	% variance	77.4	14.9	4.1	3.7
L4	MF	0.444	0.497	0.059	-0.743
	MT	0.481	-0.057	0.816	0.315
	HF	0.448	0.514	-0.450	0.576
	HT	0.609	-0.697	-0.357	-0.131
	% variance	80.2	13.4	4.5	1.9
L5	MF	0.436	0.465	-0.095	0.765
	MT	0.474	0.058	0.856	-0.199
	HF	0.446	0.499	-0.423	-0.610
	HT	0.622	-0.729	-0.282	0.054
	% variance	80.3	13.0	4.8	1.8
Adult	MF	0.430	0.462	-0.138	0.763
	MT	0.513	0.051	0.840	-0.169
	HF	0.422	0.513	-0.414	-0.623
	HT	0.611	-0.722	-0.322	0.034
	% variance	79.4	14.3	4.8	1.5

^a MF = middle femur; MT = middle tibia; HF = hind femur; HT = hind tibia.

TABLE 2. Joint pattern of variation in all five larval instars and adult of the water strider data. Values presented are CPC coefficients (jackknife standard errors in parentheses).

Variable ^a	CPC1	CPC2	CPC3	CPC4
MF	0.471 (0.020)	0.436 (0.042)	-0.059 (0.092)	0.765 (0.023)
MT	0.414 (0.024)	0.073 (0.051)	0.878 (0.035)	-0.229 (0.107)
HF	0.491 (0.028)	0.466 (0.043)	-0.427 (0.087)	-0.600 (0.059)
HT	0.605 (0.033)	-0.767 (0.031)	-0.209 (0.051)	0.048 (0.051)

^a MF = middle femur; MT = middle tibia; HF = hind femur; HT = hind tibia.

yses of multiple groups generally pose more difficult problems. Even if the CPC model holds, sampling variation will generally make it impossible for any single transformation to render all blocks of the covariance matrix perfectly diagonal. Estimation of the CPC coefficients is therefore an optimization process, searching for a transformation that minimizes a measure of deviation from simultaneous diagonality in all blocks of the covariance matrix (Appendix, Estimation of CPCs). For this purpose, we used a version of the orthogonal FG^+ algorithm (Neuenschwander, 1991; Flury and Neuenschwander, 1995b) written in the SAS/IML language (a text file with this routine is available through the Internet: file://life.bio.sunysb.edu/morphmet/dpc.exe.ibmipc; a version written in GAUSS is available from B.E.N. or B.D.F.). Standard errors for the estimates of the CPC coefficients were computed using the jackknife method (leave-one-out procedure; e.g., Efron and Tibshirani, 1993: chapter 11). The ordering of CPCs is somewhat arbitrary because the amounts of variation they take up in each instar can vary. Here, we ranked them according to the average proportion of total variance or between-instar covariance for which they accounted in each block of the covariance matrix.

The CPC coefficients (Table 2) closely correspond to the patterns seen in the PCAs for single instars. CPC1 is an overall "size" axis, weighting the hind tibia some-

what more and the middle tibia slightly less than the two femur lengths, whereas the other CPCs are contrasts between measurements that are fairly similar to the corresponding within-instar PCs. We applied the CPC transformation to all larval and adult stages and thereby changed the covariance matrix of the original variables (Fig. 5) into that of the CPCs (Fig. 6; cf. Figs. 3a, 3b). The covariance matrix of transformed variables is an array of "spikes" and thus shows that CPC1 clearly dominates the variation in all developmental stages and accounts for almost all the covariance among stages (Fig. 6). The percentages of within-instar variance taken up by the CPC1 are only slightly lower than those for which the PC1s account (cf. Tables 1, 3). Therefore, the CPC1 is a fairly good summary of static allometry in all stages jointly.

Rearranging the covariance matrix by CPCs makes the dominance of CPC1 even more visible (Fig. 7). The variance in this "size" component is fairly constant in L1–L3 but later increases markedly from stage to stage. In CPC1 and CPC2, the covariances among instars are highest between successive instars and decline substantially as the number of intervening instars increases. The variance of CPC2 remains fairly constant in the younger instars and gradually increases in the L5 and adult. Because this increase parallels that in CPC1, the CPCs account for similar proportions of total variance in each instar (Table 3).

Most of the covariances between different CPCs (off-diagonal blocks in Fig. 7) are low, suggesting that the CPCs are almost uncorrelated. There are, however, weak to moderate positive correlations between CPC1 and CPC3, ranging from 0.12 to 0.41, and between CPC2 and CPC3 (0.07–0.32). Negative correlations are especially frequent between the CPC4 scores in the L1–L4 and the CPC2 and CPC3. The other correlations between CPCs do not display any apparent pattern, and most are substantially weaker (total range of -0.27 to 0.23). This indicates that the CPC model fits these data fairly well.

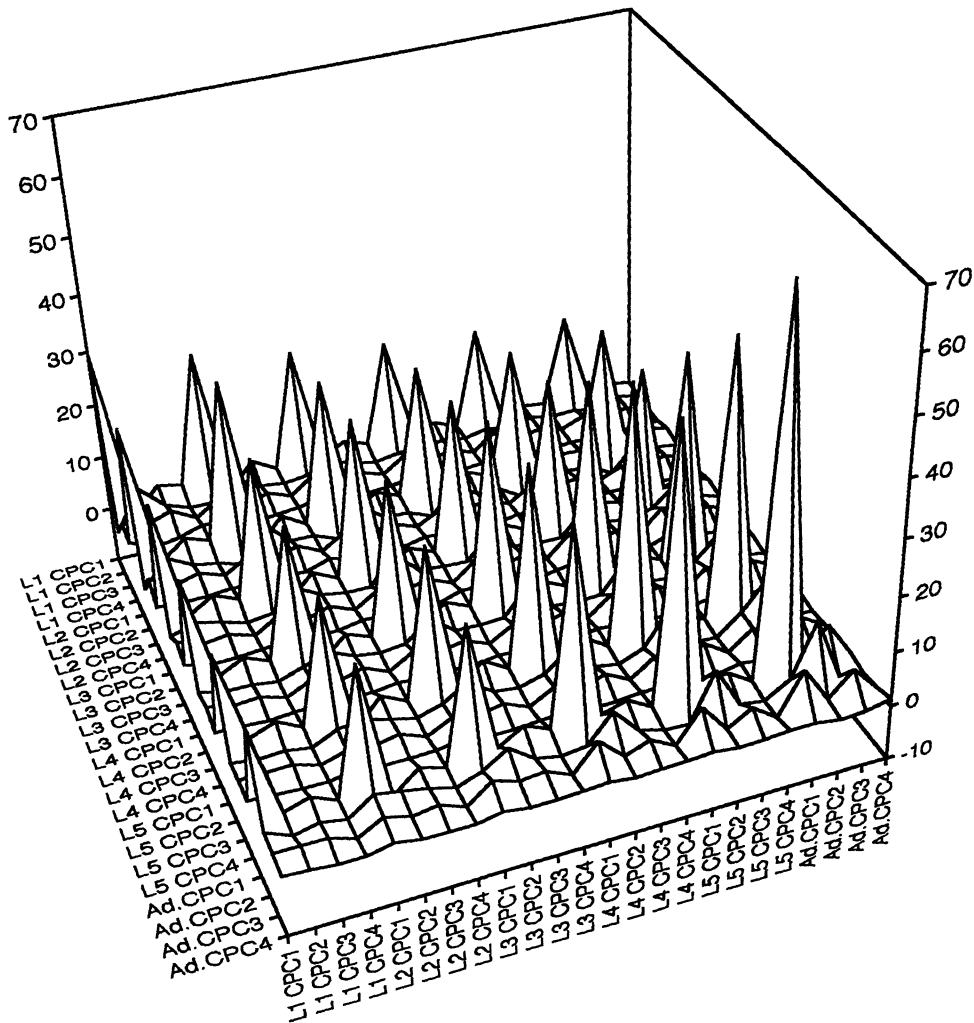


FIGURE 6. Covariance matrix after transformation to CPCs within each instar. "Spikes" are produced by the variances and between-instar covariances of the first CPC. Values on the vertical axis are variances and covariances of CPCs for natural log-transformed measurements, multiplied by 10^4 . L1–L5 = larval instars; Ad. = adult.

Test of the CPC model.—To evaluate whether these correlations seriously violate the assumptions of the CPC model, we used permutation tests, also known as randomization tests (Edgington, 1986, 1987; Manly, 1991; Efron and Tibshirani, 1993: chapter 15; Westfall and Young, 1993; Good, 1994). This class of tests uses repeated permutations of the original data to simulate the distribution of a test statistic under the null hypothesis stating that two

or more samples are drawn from the same population or that several variables are uncorrelated. Our test is based on the fact that, under the CPC model, different CPCs are uncorrelated within and between instars (Figs. 3b, 4). This test follows the procedure for testing bivariate correlations against the null hypothesis of independence by reshuffling the values of one variable repeatedly (Pitman, 1937; Edgington, 1987:198–201). We first computed the CPC

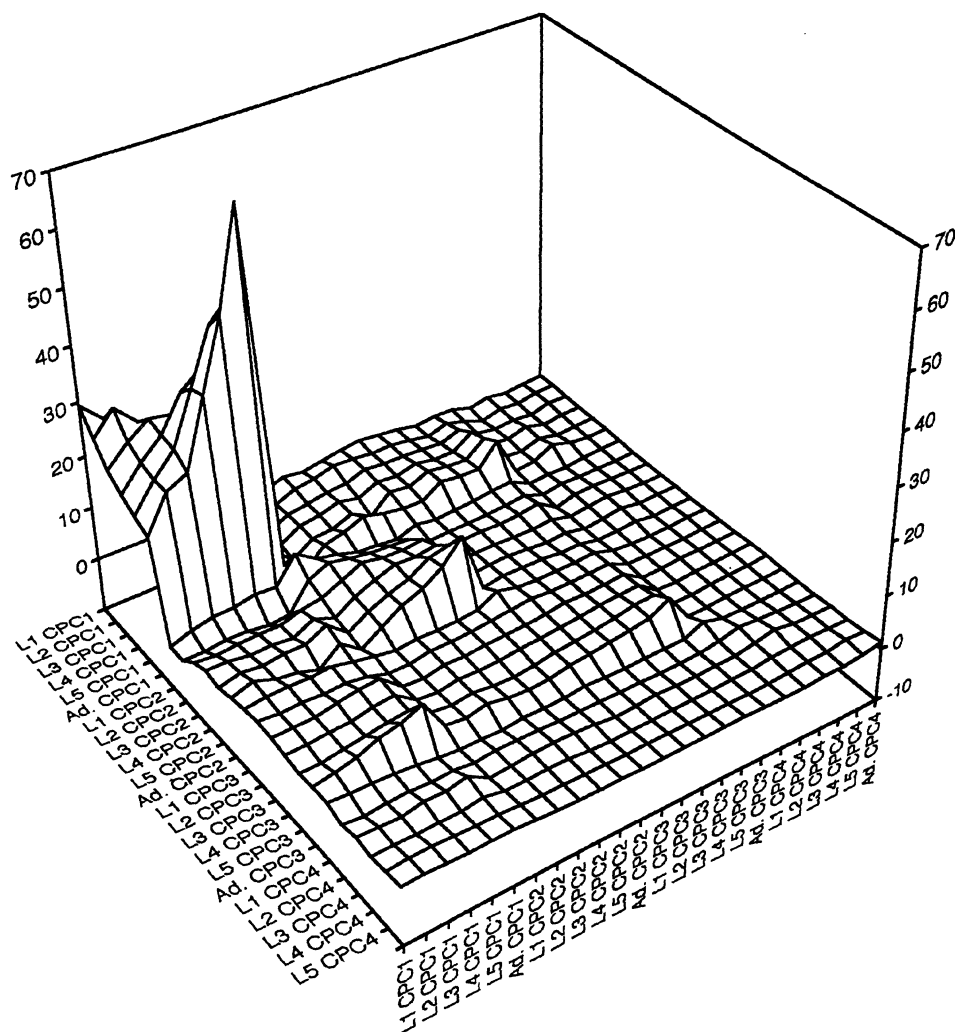


FIGURE 7. Covariance matrix arranged by CPCs. The covariation of the CPCs among instars is more apparent here than in Figure 6. Values on the vertical axis are variances and covariances of CPCs for natural log-transformed measurements, multiplied by 10^4 . L1–L5 = larval instars; Ad. = adult.

TABLE 3. Percentages of total within-instar variance taken up by each CPC of the water strider data.

Stage	CPC1	CPC2	CPC3	CPC4
L1	75.9	13.8	6.7	3.6
L2	79.7	11.9	5.3	3.1
L3	76.8	14.0	5.4	3.9
L4	79.7	13.2	5.1	1.9
L5	79.8	13.2	5.2	1.8
Adult	78.2	14.4	5.8	1.5

scores for each individual and then randomly reshuffled the observations separately for CPC2–CPC4, each time keeping all developmental stages together. This left the associations among stages unchanged for each CPC, because the permutation procedure affected only the correlations between different CPCs. This step was repeated 1,000 times. We calculated the CPCs for each of the randomized data sets and computed three different test statistics:

(1) the e statistic of deviation from simultaneous diagonality in all blocks of the covariance matrix (Appendix, Estimation of CPCs) as an overall test, (2) the maximum absolute covariance, and (3) correlation between different CPCs (i.e., excluding the diagonal entries in each block of the covariance matrix).

The e statistic did not reach the observed value in any of the 1,000 simulation runs of the CPC model and therefore provides strong evidence that the CPC model does not fit the data well overall. The maximal covariance and correlation between different CPCs of the original data, both between CPC1 and CPC3 in adults (see Fig. 7), were attained in only six or eight randomization runs and thus supported the result obtained with the e statistic. Because CPC3 accounts for a small fraction of the total variation, despite its relatively high correlations with other CPCs, we repeated the randomization test with two new statistics: the maximal absolute covariance and correlation not involving CPC3. These statistics matched or exceeded the observed values in 40.1% and 34.2% of the randomization runs, respectively. From this, we conclude that the statistically significant deviations from the CPC model are related to CPC3, but neither covariances nor correlations among the other CPCs are distinguishable from random effects.

DISCUSSION

The statistical model introduced here is designed to analyze the variation of multiple variables in several interdependent groups. Our example is an application of this method to the familiar problem of longitudinal data with a number of measurements taken in several ontogenetic stages (e.g., Cuzin-Roudy, 1975; Cheverud et al., 1983; Björklund, 1993). Because statistical methods to deal with such a complex data structure have not been available, previous authors had to treat different stages independently and thus ignore the longitudinal nature of the data or they were forced to perform the analyses separately for each measurement, thereby neglecting the cor-

relations among traits. A model that specifically addresses the complexity of the data structure offers several advantages in this situation. First, it allows inclusion of all traits in a single analysis rather than only an informal comparison of the results from separate analyses. Moreover, the simplifying assumptions made by the CPC model, when met, can provide further insight into the underlying patterns of variation and can lead to substantial data reduction if most variation can be approximated by just a few CPCs.

However, we must assess the fit of the model to the data set. The model assumes that the CPCs are independent of each other both within and across developmental stages and that nonzero covariances and correlations between different CPCs are due to sampling variation. Covariances between CPCs are generally low (Fig. 7), although there are relatively high correlations involving CPC3. Unfortunately, tests based on large-sample theory (Neuenschwander, 1991; Flury and Neuenschwander, 1995a) are not reliable in this case because the sample size (89) is fairly small compared with the 24 variables in the model (i.e., four measurements in each of six stages). For this reason, we used a permutation test (Pitman, 1937; Edgington, 1986, 1987; Manly, 1991; Westfall and Young, 1993; Good, 1994). Overall, there are significant deviations from the CPC model, but closer examination showed that they all concern CPC3, which accounts for only a minor proportion of the total variation. The other CPCs are nearly uncorrelated within and across stages, and therefore, with some caution, the CPC model can be applied here.

Use of the CPC model, although it may not fit the data perfectly, dramatically simplifies the problem by reducing the number of parameters to be estimated. In our example, the CPC model uses less than one third of the parameters it takes for a full statistical description with the unconstrained model. Only these simplifying assumptions make longitudinal growth data with multiple measurements statistically tractable. Unlike the original 24×24 co-

variance matrix (Fig. 5), the transformed and rearranged matrix (Fig. 7) shows some simple patterns, for which biological interpretations can be sought. This benefit far outweighs the relatively minor misfit of the model.

The CPC model can also be an effective tool for further data reduction. In our example, CPC3 and CPC4 account for small amounts of variation in all stages and probably can be ignored for most purposes (see Table 3). CPC2 has a moderate degree of variability in all stages (Fig. 7), and to give a fairly complete description of morphometric variability throughout ontogeny it should be considered along with CPC1.

CPC1, which is an "overall size" component, takes up the largest proportion of static variability within each stage and also accounts for most of the covariance between stages. The variances of CPC1 are fairly constant in the L1–L3 instars but then increase from stage to stage, suggesting that variability in "size" added at each molt is first compensated by some regulatory mechanism that is then switched off in L4, leading to divergent growth in the later stages (Riska et al., 1984). Such variability in growth regulation between ontogenetic stages has also been shown in other arthropods (e.g., Hartnoll and Dalley, 1981; Tanaka, 1981; West and Costlow, 1987). The hypothesis that growth is not strongly "targeted" is further supported by the covariances of CPC1 between instars, which are all positive and relatively high (correlations are 0.37–0.93), indicating that individuals tend to be either relatively small or relatively large in all instars (Klingenberg, unpubl.). CPC2 and CPC3 take up moderate or small amounts of variance throughout the entire life cycle.

The basis of the CPC model is an assumption about the covariation of morphometric traits: all ontogenetic stages share the same structure of static variation, which also forms the pattern of covariation among stages. As a consequence of this parallelism, the CPCs are uncorrelated within each stage and among stages. Therefore, variation of the CPCs during growth can be studied separately, using

the methods developed for single traits (e.g., Cheverud et al., 1983; Lynch, 1988; Kirkpatrick and Lofsvold, 1989). This procedure, although superficially similar to the approach using a separate analysis for each measurement, does not ignore the correlations among traits because the CPCs explicitly account for them. Therefore, the CPC model divides a very complex analysis into a few simpler ones. A quick comparison of the covariance matrices shown in Figures 5 and 7, which contain the same information because both CPC transformation and rearranging are reversible, demonstrates the effectiveness of this approach.

Morphometric variation within stages has long been the focus of numerous studies under the headings of static allometry (e.g., Cuzin-Roudy, 1975; Gibson et al., 1984; Klingenberg and Zimmermann, 1992) and morphological integration (e.g., Cheverud, 1982, 1995; Leamy and Atchley, 1984; Zelditch, 1987, 1988; Wagner, 1990). Although various authors have differed widely in their goals and methods used, they all examined the strength of associations among traits. In the CPC framework, the dominance of CPC1 reflects these associations. Because this pattern applies to covariances between as well as within developmental stages (Fig. 6), the CPC approach extends the study of integration from isolated examinations of within-stage variability to a unified analysis of growth. Such an analysis can also be used to investigate the variability and possible evolutionary constraints of growth curves by studying the covariances of each CPC between stages (Kirkpatrick and Lofsvold, 1992; Björklund, 1993).

Covariances among traits in different ontogenetic stages indicate variation and possible constraints for the evolution of ontogenetic trajectories. This unique information is only available from longitudinal studies. Nevertheless, because such studies are very labor intensive and only feasible for organisms that can be reared in the laboratory (but see Björklund, 1993), few such studies exist. Cross-sectional studies comparing the ontogenies of different species

(e.g., Klingenberg and Spence, 1993) or patterns of allometry (Klingenberg and Zimmermann, 1992) are alternative approaches to these problems. A combination of all these methods is most promising for an integrated understanding of individual variation, growth, and evolution.

The CPC model demonstrates the intimate link between the comparison of static variation in several stages and the study of variability in growth curves, which have been the two most important approaches to the study of the connection between ontogenetic processes and evolution. This model, applied to phenotypic or genetic covariance matrices, will be a useful tool for further exploration of this connection.

ACKNOWLEDGMENTS

We thank Rosalind Barrington Leigh for her patient assistance in rearing water striders and John Spence for valuable discussions and advice. We are grateful to Les Marcus, John Spence, Miriam Zelditch, and Manfred Zimmermann for constructive comments on earlier versions of the manuscript. Financial support was provided in part by a postdoctoral grant from the Swiss National Science Foundation to B.E.N., by a University of Alberta Ph.D. Scholarship and an Izaak Walton Killam Memorial Scholarship to C.P.K., and by operating and equipment grants from the Natural Sciences and Engineering Research Council (Canada) to John R. Spence.

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Received 25 May 1995; accepted 27 December 1995

Associate Editor: Paula Mabey

APPENDIX

PCA

Classical PCA deals with observations in a single group, i.e., with a p -variate random vector $\mathbf{X} = (X_1, X_2, \dots, X_p)$ with covariance matrix Σ . The PCs, $\mathbf{U} = (U_1, U_2, \dots, U_p)$, are linear combinations of the original variables, $\mathbf{U} = \mathbf{X}\beta$. This transformation is achieved by the matrix of eigenvectors, β , which is orthogonal and normalized so that $\beta'\beta = \mathbf{I}_p$.

The covariance matrix of the PCs,

$$\text{Cov}(\mathbf{U}) = \beta'\Sigma\beta = \Lambda,$$

is diagonal because the PCs are uncorrelated, i.e.,

$$\Lambda = \begin{bmatrix} \lambda_1 & 0 & \cdots & 0 \\ 0 & \lambda_2 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & \lambda_p \end{bmatrix}.$$

The eigenvalues $\lambda_1, \lambda_2, \dots, \lambda_p$ are the variances of the corresponding PCs (for further information, see Pi-

mentel, 1979; Jolliffe, 1986; Flury, 1988: chapter 2; Jackson, 1990; Jobson, 1992).

CPCs for Independent Groups

The CPC model for independent groups (Flury, 1988) assumes that all groups share the same eigenvectors. This means that the transformation given by the common matrix of eigenvectors, β , renders all covariance matrices to diagonal form simultaneously,

$$\beta' \Sigma_i \beta = \Lambda_i, \quad i = 1, \dots, k,$$

where the Λ_i are diagonal matrices (as above) and k is the number of groups.

CPCs for Dependent Random Vectors

The CPC model for dependent random vectors considers kp variables simultaneously, which have a $kp \times kp$ covariance matrix that shows a pattern of $k \times k$ blocks, each of size $p \times p$ (shown here for $k = 2$):

$$\begin{bmatrix} \Sigma_{11} & \Sigma_{12} \\ \Sigma_{21} & \Sigma_{22} \end{bmatrix}.$$

The diagonal blocks Σ_{11} and Σ_{22} are the within-group covariance matrices (as in the previous section), whereas the off-diagonal blocks contain covariances of measurements in different groups ($\Sigma_{12} = \Sigma'_{21}$).

The CPC model assumes that the same transformation (using the $p \times p$ orthogonal matrix β), when applied to all groups, simultaneously renders all blocks diagonal. Therefore, the covariance matrix after transformation to CPCs is

$$\begin{bmatrix} \beta' \Sigma_{11} \beta & \beta' \Sigma_{12} \beta \\ \beta' \Sigma_{21} \beta & \beta' \Sigma_{22} \beta \end{bmatrix} = \begin{bmatrix} \Lambda_{11} & \Lambda_{12} \\ \Lambda_{21} & \Lambda_{22} \end{bmatrix},$$

where all Λ_{ij} are diagonal. This model was discussed in detail by Neuenschwander (1991) and Flury and Neuenschwander (1995a); algorithms for estimating CPCs were presented by Flury and Neuenschwander (1995b).

Number of Parameters

The number of parameters in the unconstrained model is

$$pk(pk + 1)/2.$$

Under the CPC model for dependent random vectors, this number is

$$p(p - 1)/2 + pk(k + 1)/2,$$

where the first term accounts for the CPC coefficients and the second term for the within-group variances of the CPCs and their covariances across groups. As p and k increase, the reduction in parameters under the CPC model becomes very substantial.

Estimation of CPCs

In a sample, the $kp \times kp$ covariance matrix S is patterned as explained above for Σ (again illustrated for $k = 2$), i.e.,

$$S = \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix}.$$

Then we search for an orthogonal $p \times p$ matrix B (normalized so that $B'B = I_p$) that simultaneously renders the four blocks of F as closely to diagonal as possible, where

$$F = \begin{bmatrix} F_{11} & F_{12} \\ F_{21} & F_{22} \end{bmatrix} = \begin{bmatrix} B'S_{11}B & B'S_{12}B \\ B'S_{21}B & B'S_{22}B \end{bmatrix}$$

is the covariance matrix of the transformed variables.

The measure of deviation from simultaneous diagonality is

$$e = \frac{\det \begin{bmatrix} \text{diag } F_{11} & \text{diag } F_{12} \\ \text{diag } F_{21} & \text{diag } F_{22} \end{bmatrix}}{\det \begin{bmatrix} F_{11} & F_{12} \\ F_{21} & F_{22} \end{bmatrix}},$$

where "det" is the determinant of a matrix and the "diag" operator sets the off-diagonal elements of a matrix to zero. It can be shown that e is a minimum if all F_{ij} are diagonal. The FG^+ algorithm (Flury and Neuenschwander, 1995b) is designed to find an orthogonal matrix B that minimizes this measure (for further discussion, see Neuenschwander, 1991; Flury and Neuenschwander, 1995a, 1995b).