Evolution, 54(4), 2000, pp. 1273–1285

MORPHOLOGICAL INTEGRATION BETWEEN DEVELOPMENTAL COMPARTMENTS IN THE *DROSOPHILA* WING

CHRISTIAN PETER KLINGENBERG[1,2,3] AND STEFANIE D. ZAKLAN[4]
[1]*Department of Zoology, Duke University, Durham, North Carolina 27708-0325*
[2]*Laboratory for Development and Evolution, University Museum of Zoology, Department of Zoology, Downing Street, Cambridge CB2 3EJ, United Kingdom*
[4]*Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada; E-mail: szaklan@ualberta.ca*

Abstract.—Developmental integration is the covariation among morphological structures due to connections between the developmental processes that built them. Here we use the methods of geometric morphometrics to study integration in the wing of *Drosophila melanogaster*. In particular, we focus on the hypothesis that the anterior and posterior wing compartments are separate developmental units that vary independently. We measured both variation among genetically diverse individuals and random differences between body sides of single individuals (fluctuating asymmetry, FA). For both of these sources of variation, the patterns of variation identified by principal component analyses all involved landmarks in both the anterior and posterior compartments simultaneously. Analyses focusing exclusively on the covariation between the anterior and posterior compartments, by the partial least-squares method, revealed pervasive integration of the two compartments, for both individual variation and FA. These analyses clearly indicate that the anterior and posterior compartments are not separate units of variation, but that the covariation between compartments is sufficient to account for nearly all the variation throughout the entire wing. We conclude that variation among individuals as well as the developmental perturbations responsible for FA generate shape variation primarily through developmental processes that are integrated across both compartments. In contrast, much less of the shape variation in our sample can be attributed to the localized processes that establish the identity of particular wing veins.

Key words.—Compartments, *Drosophila melanogaster,* fluctuating asymmetry, geometric morphometrics, modularity, morphological integration, wing veins.

Received September 28, 1999. Accepted February 3, 2000.

Morphological integration refers to the coordinated variation and mutual interdependence ensuring that the parts of organisms are organized into functioning wholes. It has been discussed extensively in developmental, functional, and evolutionary contexts (e.g., Olson and Miller 1958; Cheverud 1982a, 1996; Zelditch 1987; Roth 1996; Smith 1996; Leamy et al. 1999). Developmental integration focuses specifically on those relationships that exist because the characters have been built by interdependent developmental processes. In recent years, however, the focus of attention has shifted from developmental integration toward its counterpart, modularity, which denotes the organization of parts as distinct and partially independent units in development and evolution (Raff 1996; Wagner 1996; Wagner and Altenberg 1996; Kirschner and Gerhart 1998; von Dassow and Munro 1999). Nevertheless, regardless of whether the emphasis is on the integration of parts into a coherent structure or on their ability to vary as separate modules, the question is to which degree the variation of parts is coordinated.

Fly wings are an excellent system for studying these issues because the wing veins provide many morphological landmarks and *Drosophila* wing development is well understood (e.g., Waddington 1940; García-Bellido and de Celis 1992; Sturtevant and Bier 1995; Biehs et al. 1998; Stark et al. 1999). The subdivision of fly wings into anterior and posterior compartments (Fig. 1) has attracted special attention: Because they correspond to distinct cell lineages and domains of gene expression (García-Bellido et al. 1973; Lawrence and Morata 1976; Lawrence 1992), they have been considered promising candidates for being separate developmental modules (but see also Blair 1995; Lawrence and Struhl 1996; Milán and Cohen 2000). Accordingly, a number of studies have used morphometric approaches to examine whether anterior and posterior wing compartments are distinct as modules that are reflected in phenotypic and genetic variation (Cavicchi et al. 1981, 1985, 1991; Thompson and Woodruff 1982; Cowley and Atchley 1990; Guerra et al. 1997; Pezzoli et al. 1997; Baylac and Penin 1998). Based on the results of their selection experiments, Cavicchi et al. (1981, p. 194) hypothesized that ''two groups of genes control two compartments of the wing during development,'' and Thompson and Woodruff (1982, p. 75) suggested that whereas some polygenic modifiers affect the entire wing or only a specific structure, others affect development of a single compartment. In more general terms, Cavicchi et al. (1991, p. 157) proposed that ''the two compartments represent individual units of selection,'' and Pezzoli et al. (1997, p. 575) stated that they ''are distinct units of selection subjected to different genetic control.''

According to this hypothesis, the anterior and posterior compartments are distinct units, and one would expect that developmental integration in the wing occurs primarily within compartments, but not between them. Therefore, significant components of morphological variation should be confined to one compartment and independent of variation in the other compartment.

This hierarchical structure of developmental integration should not only affect variation among individuals, but the developmental compartmentalization should also be reflected in patterns of integration for fluctuating asymmetry (FA) between body sides (Palmer and Strobeck 1986; Palmer 1994; Markow 1995; Møller and Swaddle 1997). Because the left

[3] Address for correspondence: University Museum of Zoology, Downing Street, Cambridge CB2 3EJ, United Kingdom; E-mail: cpk24@cam.ac.uk.

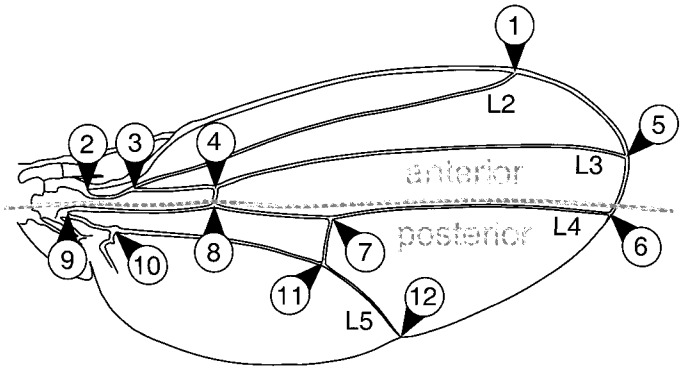

FIG. 1. Wing of *Drosophila melanogaster* with the morphological landmarks used in this study. The bold gray line indicates the approximate location of the boundary between the anterior and posterior compartments. The major longitudinal veins are labeled in the way customary in genetics and developmental biology as L2 ($radius_{2+3}$), L3 ($radius_{4+5}$), L4 ($media_{1+2}$), and L5 ($media_{3+4}$; comparative terminology after Colless and McAlpine 1991).

and right body sides of an individual share the same genome and nearly the same environment, FA results primarily from small random perturbations of developmental processes. Therefore, FA provides an intrinsic ''control'' for genetic and environmental effects. Correlated left-right asymmetries in two different traits thus indicate that the same perturbation has been transmitted to both traits. Therefore, integration of signed FA should be restricted to structures between which there are developmental interactions (e.g., Klingenberg and Nijhout 1998; Van Dongen et al. 1999). Although integration of FA has been analyzed before (Jolicoeur 1963; Leamy 1984, 1993; Leamy et al. 1997; Hallgrímsson 1998; Klingenberg and McIntyre 1998), it has not been used specifically to infer developmental relationships (but for an early attempt, see Sakai and Shimamoto 1965).

Here, we evaluate the hypothesis of independent variation in the anterior and posterior wing compartments by analyzing variation of wing shape using the framework of geometric morphometrics (Bookstein 1991, 1996; Dryden and Mardia 1998). We have previously used these methods to quantify FA and directional asymmetry and to compare patterns of integration of FA and individual variation in fly wings (Klingenberg and McIntyre 1998; Klingenberg et al. 1998). In this paper, we extend these methods by introducing the partial least-squares method (e.g., Bookstein et al. 1990) to analyze patterns of covariation between compartments of *Drosophila* wings. We discuss the results in light of the available information on *Drosophila* wing development, which suggests an alternative interpretation of the role of the compartment boundary for morphological integration in the wing.

MATERIALS AND METHODS

Data

We analyzed separate samples of 117 male and 117 female *Drosophila melanogaster*. The flies were from the F_2 generation of a cross between the Canton-S and Oregon-R stocks, which therefore contained ample genetic variation. The wings of these flies were mounted in Euparal on microscope slides. The *x*- and *y*-coordinates of 12 landmarks were measured with a dissecting microscope fitted with a camera lucida and a digitizing tablet. Each wing was digitized twice by the same person (SDZ). The landmarks (Fig. 1) are at the intersections of wing veins or at points where veins reach the wing margin, and all of them can therefore be considered type 1 landmarks (Bookstein 1991, p. 63 f.).

Procrustes Analysis

Our analysis of variation among individuals and of within-individual variation between left and right body sides uses the methods developed by Klingenberg and McIntyre (1998). These methods are based on the Procrustes approach for the analysis of shape (Bookstein 1996; Dryden and Mardia 1998) and extend prior work by Bookstein (1991, pp. 267–270), Auffray et al. (1996), and Smith et al. (1997), who used similar methods to study FA.

In short, the procedure can be described as follows (for a more detailed description, see Klingenberg and McIntyre 1998). First, the landmark configurations of all wings of one body side (e.g., all left wings) are reflected to their mirror images. Second, all the configurations are scaled to the same size, that is, to unit centroid size. Centroid size is the square root of the sum of squared distances from each landmark to the centroid (center of gravity) of a configuration (Dryden and Mardia 1998). Third, the centroids of the configurations are superimposed, for example, on the coordinates of the origin (0, 0). This step eliminates differences in position. And finally, to eliminate variation in the orientation of specimens, the configurations are rotated around their common centroid to achieve an optimal fit. This best fit is defined as the rotation that minimizes the sum of squared deviations of the landmarks of all configurations from the corresponding landmarks of the overall consensus (mean) configuration. The final step was done without additional scaling (the analysis is therefore a partial Procrustes fit according to the terminology of Dryden and Mardia 1998).

Procrustes analysis of variance

Because the criterion for obtaining the optimal fit is the sum of squared deviations between corresponding landmarks, Procrustes analysis is akin to the algebra of sums of squares used in conventional analysis of variance (ANOVA). Therefore, it is possible to adapt the conventional ANOVA models designed to study individual variation and FA (Leamy 1984; Palmer and Strobeck 1986; Palmer 1994) for use with geometric shape data (Klingenberg and McIntyre 1998). The main difference is that variation and asymmetry are examined not for length measurements, but for features of shape—for instance, the left wing of a fly may be more elongated or rounded than the right wing or it may have a more pointed or obtuse tip. The ANOVA design follows the usual two-factor model, in which the individuals and body sides enter as main effects that represent individual shape variation and directional asymmetry, respectively, and the individual $\times$ side interaction represents FA (Leamy 1984; Palmer and Strobeck 1986; Palmer 1994). The replicate measurements provide an estimate of measurement error, which can be of substantial importance in FA studies (Palmer 1994).

This Procrustes ANOVA provides an overall test of significance for FA and directional asymmetry, for which we used the permutation tests described in detail by Klingenberg and McIntyre (1998). In addition to significance tests, and more importantly, Procrustes ANOVA provides matrices of mean squares and cross-products (MSCP) for landmark coordinates, which are analogous to the mean squares obtained from conventional ANOVA. To obtain matrices of variance and covariance components for the various ANOVA effects, we used an approach corresponding to the decomposition of expected mean squares in univariate ANOVA (see Palmer and Strobeck 1986; Palmer 1994). Therefore, we calculated the among-individual matrix of variance and covariance components as $(\mathbf{MS}_I - \mathbf{MS}_{IS})/4$ and the matrix of variance and covariance components for FA as $(\mathbf{MS}_{IS} - \mathbf{MS}_M)/2$, where $\mathbf{MS}_I$ is the among-individual MSCP matrix, $\mathbf{MS}_{IS}$ is the individual $\times$ side interaction MSCP matrix, and $\mathbf{MS}_M$ is the measurement error MSCP matrix.

Morphological Integration and Geometric Morphometrics

This study characterizes integration of shape through multivariate analyses of the matrices of variance and covariance components for the various effects included in the Procrustes ANOVA. The standard tools of multivariate statistics can be used to analyze this variation, just as in traditional morphometric studies. However, the Procrustes procedure confers some special properties to the superimposed configurations, which are important for multivariate analyses. As the Procrustes superimposition removes size, position (in two dimensions), and orientation from the original coordinate data, four degrees of freedom are ''lost'' from the data. Accordingly, the resulting shape space has four dimensions fewer than the original data (i.e., for k landmarks, it has $2k - 4$ dimensions). Moreover, permutation procedures normally should not exchange the x- and y-coordinates of the landmarks, because otherwise the null hypothesis of those tests would imply the complete absence of all geometric structure.

Correlation versus covariance matrices

For the study of morphological integration, a most important difference between geometric and conventional morphometric methods is that the analyses must use covariance matrices instead of correlation matrices. Morphological integration has traditionally been studied through patterns of correlations among linear distance measurements (e.g., Olson and Miller 1958; Cheverud 1982a; Wagner 1984; Cheverud et al. 1989). In geometric morphometrics, however, the use of correlations could introduce a spurious dependence on the orientation of configurations relative to the coordinate axes. Rotating a set of superimposed configurations relative to the coordinate axes, which does not alter any aspect of shape, can fundamentally change the correlations between the x- and y-coordinates of a landmark depending on how well the major and minor axes of scatter around the mean position align with the coordinate axes. This would also change the results of subsequent analyses, especially those of integration. Correlation matrices are therefore inappropriate for the analysis of Procrustes coordinates. In contrast, statistical analyses based on covariance matrices are invariant with regard to such rotations of the coordinate system, and are therefore not affected by this problem.

Influence of size on shape variation

To examine whether there was an association between size and shape, reflecting allometry of the wing (Baylac and Penin 1998), we carried out multivariate regressions of Procrustes coordinates on centroid size (e.g., Mardia et al. 1979; Monteiro 1999). These regressions were done for the left-right means of size and shape, for shape asymmetry versus the left-right mean size, and for shape asymmetry against size asymmetry. In all these regressions, size accounted only for a small fraction of shape variation. Multivariate analyses using the residuals of a regression on centroid size produced results that were nearly identical to those of the complete variation. We therefore present only the results of analyses of the total shape variation.

Patterns of Variation across the Entire Wing

To analyze and display the patterns of covariation in the positions of landmarks throughout the wing, we used principal component analysis (PCA; e.g., Jolliffe 1986), which has been used regularly in the context of shape analysis (e.g., Dryden and Mardia 1998; Klingenberg and McIntyre 1998). This analysis extracts features of shape variation as a set of new shape variables (the principal components, PCs) that are uncorrelated to one another and successively account for maximal amounts of variation. It is possible to interpret them as independent features of variation that can be added together to make up the observed variation (note, however, that this is a description of the observed variation and not a hypothesis about underlying causes). In geometric shape analysis, the PCs can be visualized directly as patterns of simultaneous displacements of landmarks in relation to one another. Moreover, because a small subset of PCs may be sufficient to take up most of the total variation, PCA is an effective method for data reduction, which is especially important for shape analysis because of the large number of variables (coordinates) entering the analysis.

Covariation between Anterior and Posterior Compartments

Permutation test

To test statistically whether there was any significant covariation between the landmarks of the anterior and posterior compartments, we used a permutation test (e.g., Edgington 1995). The test simulated the null hypothesis of independent variation in the two compartments by randomly reshuffling the landmark coordinates of one compartment among observations (e.g., leaving the data unchanged for the landmarks in the anterior compartment, whereas the configurations of landmarks in the posterior compartment are randomly exchanged among individuals). As the test statistic, we computed the sum of squared cross-covariances between the two sets of landmark coordinates, which is a measure of the overall magnitude of covariances between compartments. For each random permutation of the dataset, this test statistic was calculated and compared to the value for the original data. The permutation step was repeated 10,000 times for each

test. In each sex, this test was run separately for individual variation (using the average landmark coordinates of superimposed left and right wings as observations) and for FA (using signed left-right asymmetries).

Partial least squares

The next step is to identify the features of shape that covary between the anterior and posterior compartments, that is, to extract patterns of covariation between compartments just as PCA extracts patterns of variation across the entire wing. For this purpose, we used the method of partial least squares (PLS). This method was originally introduced in psychometry as interbattery factor analysis (Tucker 1958), but it has recently been used in many different contexts, including dose-response studies in medical research (Bookstein et al. 1990; Streissguth et al. 1993), analyses of environmental variables and species abundance in ecology (Chessel and Mercier 1993), in ecomorphology (Corti et al. 1996; Klingenberg and Ekau 1996), or to relate different morphometric analyses carried out on the same specimens (Tabachnick and Bookstein 1990). In a context similar to the present one, Baylac and Penin (1998) used PLS to study covariation between the compartments of fly wings, but limited their analysis exclusively to the uniform part of shape variation. Here we use PLS to study all components of shape covariation between the landmarks of the anterior and posterior compartments and in separate analyses for both individual variation and FA.

The PLS method restricts itself to the analysis of *covariation between two sets* of variables such as the coordinates of landmarks in the two compartments, and provides a best fit to the matrix of covariances between sets (see also Bookstein 1991, p. 40 ff.). For this purpose, it finds pairs of shape variables (the PLS axes), each pair with one PLS axis for the anterior and one for the posterior compartment, which represent the features of shape variation that have maximal covariance between the two sets of landmarks. Each PLS axis only covaries with its counterpart in the other set of landmarks, but is uncorrelated with all other PLS axes in that set (within their own set, however, PLS axes can be correlated). The PLS axes and their covariances are obtained from a singular value decomposition of the matrix of cross-covariances between the two sets of coordinates (see Appendix; Bookstein et al. 1990; Bookstein 1991, p. 41 ff.). As a measure of the amount of covariation for which one or more PLS axes account, their squared covariances can be expressed as a proportion of the sum of all squared cross-covariances between the two sets of coordinates.

The PLS method has a number of properties that correspond to the more familiar PCA (for details, see the Appendix). Whereas PCA extracts features of variation within a single set of variables and represents them as mutually orthogonal axes (PCs) successively accounting for maximal variance, PLS can similarly display features that covary between two separate sets of variables. For any given number of PCs, PCA provides a best fit of the covariance matrix, according to a least-squares criterion, whereas the PLS method provides an analogous approximation for the matrix of covariances between two sets of variables. Many of the tools for presentation and interpretation of results of PCA can be applied to PLS, as long as users keep in mind the different structure and purposes of the analyses (e.g., Bookstein et al. 1990; Klingenberg and Ekau 1996).

Comparison of Partial Least Squares and Principal Component Analysis

To assess how much the covariation between anterior and posterior compartments contributes to the total variation across the whole wing, and to examine whether the same features of shape variation are involved at both these scales of analysis, we compared the PLS and PCA results. Because these two analyses are formally similar, many of the methods for comparing results between different PCAs (Jolliffe 1986; Klingenberg 1996) can be applied to the comparison of results from PLS and PCA. For instance, a PC and the vector of the combined pair of corresponding PLS axes, ''stacked'' after rescaling so that all variables have equal weight (see Appendix, eq. A4), can be compared in terms of the angle between them. For normalized vectors (i.e., $\mathbf{b}'\mathbf{b} = \mathbf{c}'\mathbf{c} = 1$), this angle is calculated as $\alpha = \arccos(\mathbf{b}'\mathbf{c})$, where $\mathbf{b}$ and $\mathbf{c}$ are the vectors of PC and rescaled PLS coefficients (Klingenberg 1996).

To test the correspondence of PCs and PLS axes statistically, we devised two tests based on opposite null hypotheses. The first assessed the observed angles against the conventional null hypothesis that the PCs and PLS axes are no more similar than pairs of random vectors. This test compared the angles between PC and PLS axes to a Monte Carlo simulation of angles between pairs of random vectors in 20-dimensional space (Cheverud 1982b; Klingenberg and Zimmermann 1992; Klingenberg and McIntyre 1998).

The second test, with the opposite null hypothesis, was based on a model of pervasive anterior-posterior integration. This model of integration assumed that the PLS vectors were also the PCs within each of the compartments. Although this model is not a model of complete integration, where rescaled PLS axes and overall PCs would be identical and all correlations of anterior-posterior pairs of PLS axes would be 1.0 (see Appendix), it does assume that all shape variation is characterized completely by those shape components that vary jointly between compartments. Therefore, under this model, there are no features of shape that vary exclusively within one compartment but are independent of the other compartment—there is always variation in the other compartment that is at least correlated to some degree. We tested this model with a parametric bootstrap test (Efron and Tibshirani 1993), that is, by using a computer simulation of the theoretical model based on the estimated parameter values. A more detailed justification of this model and description of the parametric bootstrap simulation can be found in the Appendix.

RESULTS

Amount and Dimensionality of Individual Variation and Asymmetry

In the samples from both sexes, the two-factor ANOVAs for centroid size consistently revealed significant variation in size among individuals as well as fluctuating asymmetry,

TABLE 1. Two-factor ANOVA of centroid size and shape. The analysis of centroid size is the standard ANOVA for fluctuating asymmetry (Palmer and Strobeck 1986; Palmer 1994), and the analysis of shape is the corresponding Procrustes ANOVA (Klingenberg and McIntyre 1998). Note that different units are used for the mean squares for size (mm^2; values have been multiplied by 10^4) and for shape (dimensionless units of the squared Procrustes metric; values have been multiplied by 10^6). Significance levels are from permutation tests, and have been adjusted with the sequential Bonferroni procedure to indicate overall error rates within each part of the table.

	Females	Males
Centroid size:		
Individual	945.4***	361.2***
Side	9.5	24.9*
Individual × side	4.7***	3.9***
Measurement	0.7	0.6
Shape:		
Individual	50.3***	54.2***
Side	213.3***	258.3***
Individual × side	12.9***	13.6***
Measurement	2.5	2.9

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

and Procrustes ANOVAs of shape yielded similar results (Table 1). For size, directional asymmetry was statistically significant only for males, whereas for shape, directional asymmetry was highly significant for both sexes (for further details and discussion, see Klingenberg et al. 1998). Moreover, in both sexes the Procrustes ANOVA of shape showed that the main effect of individuals and the individual × side interaction were highly significant, indicating that further analyses of individual variation and FA are warranted.

PCA of the matrices of variance and covariance components for the different ANOVA effects showed that most of the variance among individuals and most individual × side interaction (FA) variance was concentrated in the first few dimensions, slightly more so for individual variation than for FA (Fig. 2). The degree to which variation is concentrated in the first few PCs is an indicator of morphological integration (Cheverud et al. 1983; Wagner 1984). Consequently, individual variation appears to be integrated somewhat more tightly than FA, although this difference is small, and does not rule out the possibility that individual variation and FA share a common developmental basis.

In contrast, the distributions of eigenvalues for measurement error were markedly different (Fig. 2), as variation was spread out much more evenly over all the 20 dimensions available in shape space. These profiles resemble those found for models of random correlation (Wagner 1984; Jackson 1993), just as one might expect for small, random imprecisions of the digitizing process. We will not present further analyses for measurement error, but will focus entirely on individual variation and FA.

Patterns of Shape Variation among Individuals

PCA of overall shape variation yielded results that were fairly consistent between sexes. The patterns of variation corresponding to the first three PCs (Fig. 3A) featured combinations of several types of landmark shifts. They showed clear relations to the local arrangement of the veins, as the landmarks tended to move in the direction of one of the intersecting veins. For instance, the landmarks at either end of a crossvein tended to move in parallel and mostly along the adjoining longitudinal veins. The PC1 primarily affected the shape of the distal part of the wing, as the landmarks 1 and 12 moved distally along the proximo-distal axis, whereas landmarks 5 and 6 moved proximally, altogether producing a blunter tip of the wing. In addition, especially in the males, the PC1 was also associated with widening of the wing tip between landmarks 1 and 12. Because the direction of PCs is arbitrary, however, all these movements can be reversed simultaneously by 180°—the PC1 is therefore better described as variation between a broader and blunter or narrower and more pointed tip of the wing. The PC2 primarily consisted of a contraction (or expansion) of the distal part of the wing blade by movements of landmarks 1, 7, 11, and 12 (in males also landmark 5) toward or away from each other. The PC3 was mainly a rotation of the distal part of the wing blade relative to the proximal part, resulting primarily

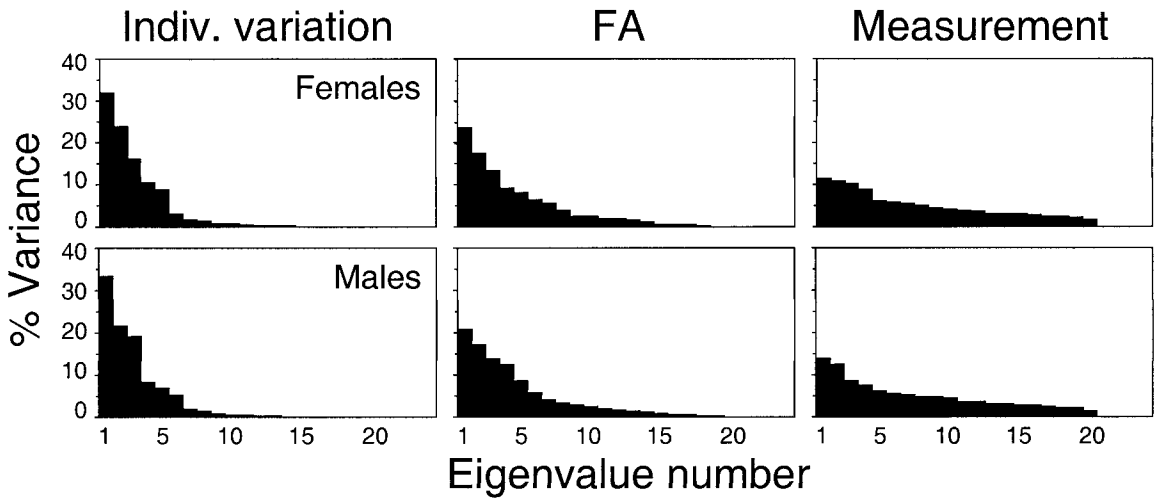

FIG. 2. Percentages of total shape variation taken up by the principal components of the matrices of variance and covariance components for individual variation fluctuating asymmetry (FA) and measurement error.

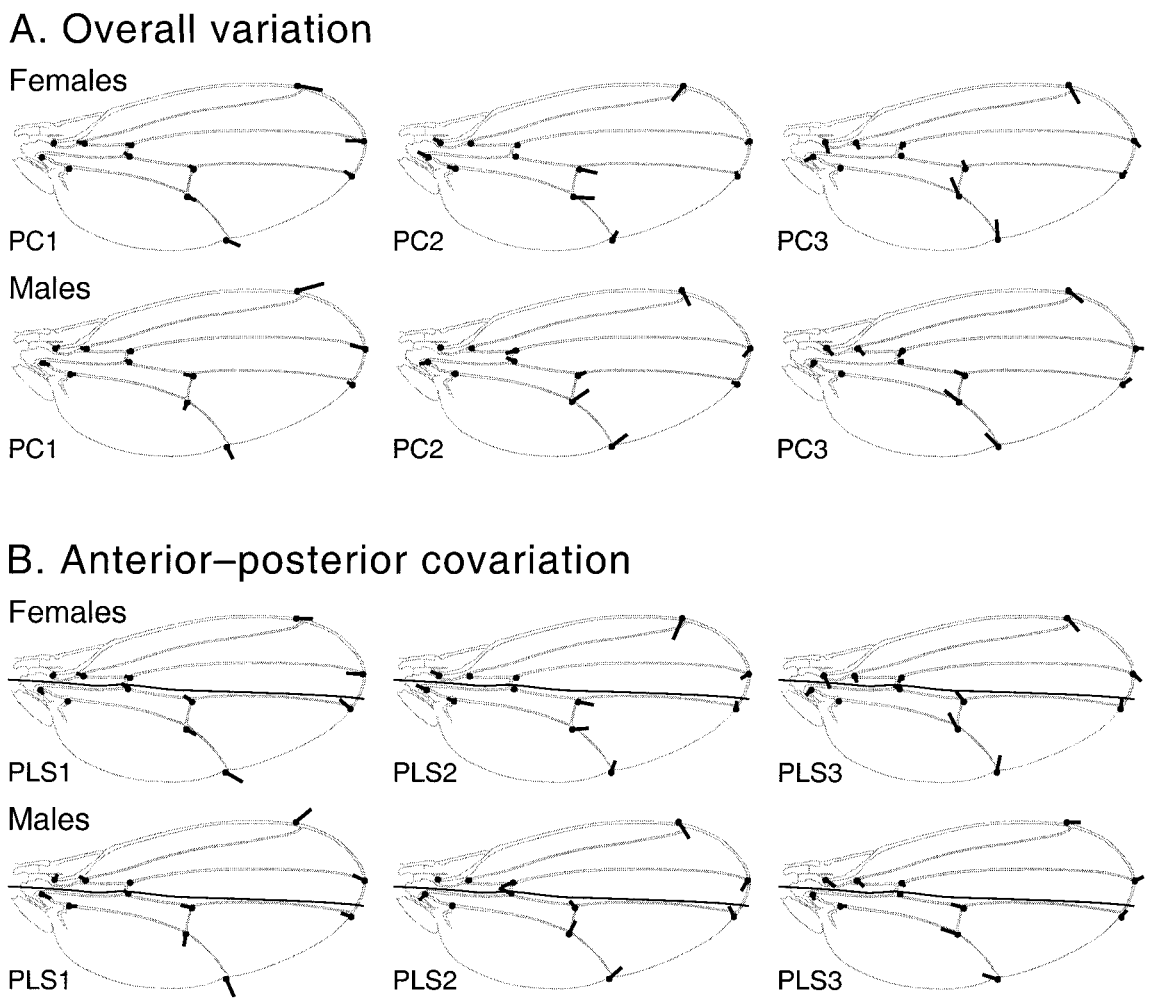

FIG. 3. Analysis of individual variation in shape. The diagrams visualize the coefficients for the first three principal components (PCs; A) or pairs of partial least squares (PLS) axes of anterior-posterior covariation (B) for the among-individual matrix of variance and covariance components in each sex. The dots denote the average landmark positions and lines point to the position of the landmark in a hypothetical configuration with an arbitrary score of +0.1 Procrustes units for the respective PC or pair of PLS axes (this is a very large shape change, far beyond the variation present in the samples). Because the signs of the axes are arbitrary, readers may also imagine the vectors of shape change with opposite signs, which means that the directions of *all* landmark movements are reversed simultaneously by 180°. The PLS axes in (B) have been rescaled to give each landmark the same weight (see Appendix, eq. A4).

from anterior and proximal movements of landmarks 7, 11, and 12 and a posterior and distal movement of landmark 1.

All these PCs simultaneously affected landmarks of both the anterior and posterior compartments, suggesting that they are well coordinated and do not vary independently. To test this suggestion statistically, not only for the first three PCs but for all the individual variation, we performed permutation tests with the null hypothesis of independence between the anterior and posterior compartments. For both sexes, this null hypothesis was clearly rejected ($P < 0.0001$), indicating there was highly significant covariance in landmark positions between the compartments.

We examined this covariation between compartments in more detail by means of PLS analyses. The profiles of covariances between pairs of PLS axes in either compartment (not shown) were similar to the corresponding profiles of variances in PCA (Fig. 2), and in both sexes, the first three PLS axes accounted for more than 75% of the covariances between compartments (proportion of the total sum of singular values from the PLS analysis). This indicates that, in terms of its concentration in a few dimensions, the covariation between compartments is of a dimensionality similar to that of the entire variation across the wing. The correlations between pairs of corresponding PLS axes for the anterior and posterior compartments ranged from 0.79 to 0.93. The fact that there were such high correlations, even though the PLS procedure maximizes covariances and not correlations, underscores the strength of the associations between sets of landmarks in the two compartments.

The most striking result of these analyses is how closely the PLS axes (Fig. 3B) resembled the corresponding PCs (Fig. 3A). Except for minor differences in magnitude and direction of landmark movements, there was a clear one-to-one correspondence between corresponding PCs and PLS axes. Likewise, the angles between the PCs and the combined PLS vectors were generally fairly small (upper part of Table 2).

TABLE 2. Angular comparisons between principal component analysis (PCA) of overall shape variation and partial least squares (PLS) analysis of covariation between anterior and posterior compartments. Parametric bootstrap tests suggested that none of these angles was significantly greater than expected under a model assuming that the PLS vectors are also the PCs within compartments, that is, the patterns of variation within and covariation between compartments are identical.

	Females	Males
Individual variation:		
PC1–PLS1	20.9°	24.1°
PC2–PLS2	14.7°	31.6°
PC3–PLS3	19.8°	30.1°
Fluctuating asymmetry		
PC1–PLS1	10.5°	20.6°
PC2–PLS2	42.2°	28.7°
PC3–PLS3	51.9°	15.0°

All of these angles were significantly smaller than expected for pairs of random vectors (Monte Carlo tests for all angles were highly significant), and the parametric bootstrap test indicated that none of the angles deviates significantly from the model in which the pattern of variation across the entire wing is generated by the patterns of anterior-posterior covariance. In sum, this indicates that the patterns of between-compartment covariation are nearly sufficient to characterize all shape variation across the entire wing. Furthermore, these results provide no evidence for any significant contribution by patterns of variation, and thus developmental processes, that are restricted to one of the compartments and independent of the other.

Patterns of Fluctuating Asymmetry of Shape

PCA indicates that the patterns of variation for FA (Fig. 4A) correspond in part to those for individual variation (Fig. 3A). For both sexes, the PC1s for FA corresponded best to the PC3s for individual variation (females: $\alpha = 46.4°$, $P < 0.001$ for the Monte Carlo test; males: $\alpha = 44.3°$, $P < 0.001$), and there was a fairly good match between the PC2s for both these sources of variation (females $\alpha = 55.4°$, $P < 0.05$; males $\alpha = 41.9°$, $P < 0.001$). In contrast, the PC3 for FA showed a new pattern that consisted primarily shifting of the anterior crossvein (landmarks 4 and 8) along the proximal-

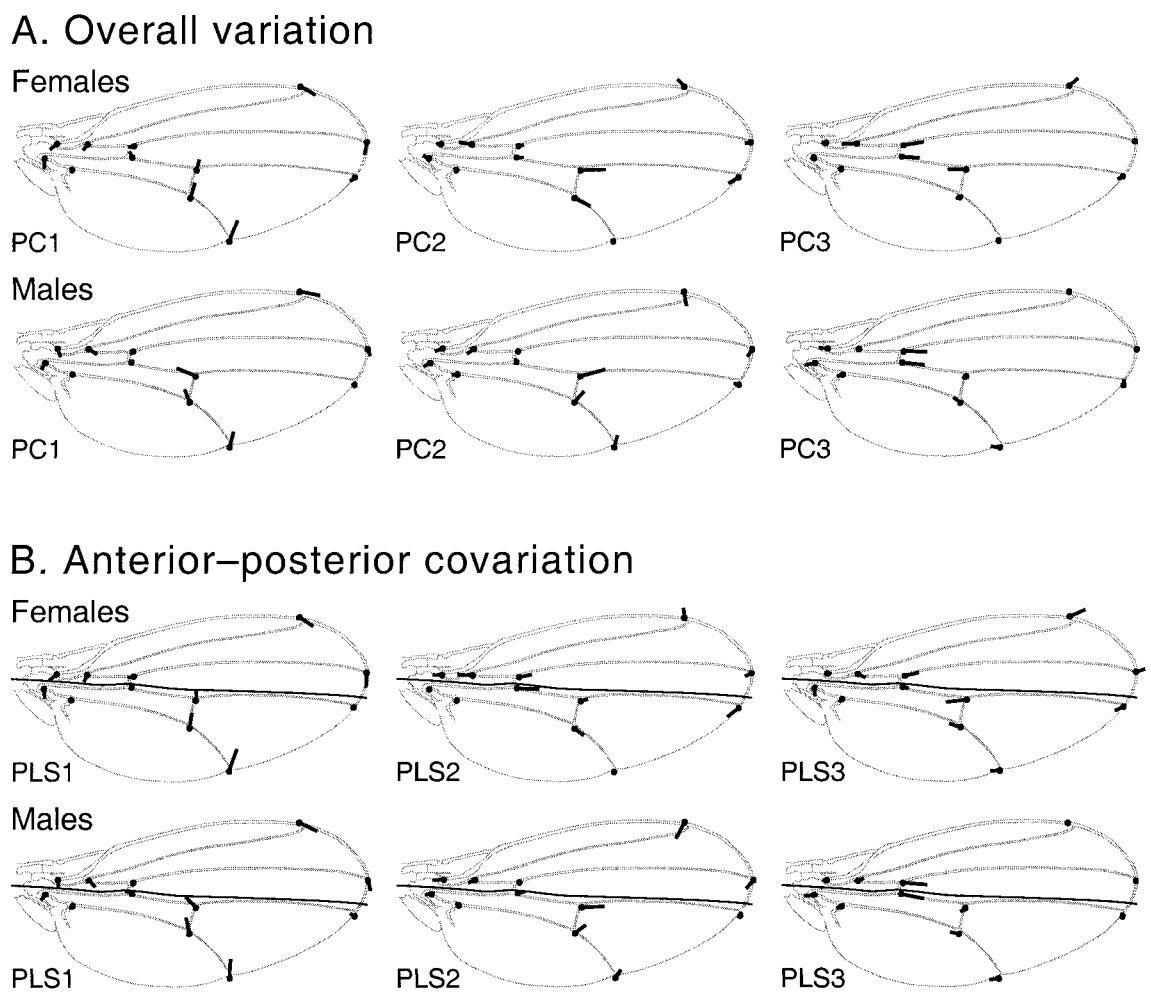

FIG. 4. Analysis of fluctuating asymmetry of shape. The diagrams show the first three principal components (A) or partial least squares axes (B) of covariance matrix for the individual × side effect both sexes. For further explanation, see Figure 3.

distal axis of the wing. Like the other PCs, this feature of shape variation affects both compartments simultaneously, because landmarks 4 and 8 are on opposite sides of the compartment boundary. Although this pattern did not have a clear equivalent among the first three PCs of individual variation, it was present as part of PC4–PC6 in females and of PC5 and PC6 in males (not shown).

The patterns of covariation between compartments for FA correspond to patterns of FA variation across the entire wing. This is apparent from the close match between the corresponding PCs (Fig. 4A) and PLS axes (Fig. 4B). Moreover, the angles between PCs and PLS axes (lower part of Table 2) were smaller than expected for pairs of random vectors (the Monte Carlo test was highly significant), and none of them deviated significantly from our model of pervasive anterior-posterior covariance (parametric bootstrap test). It appears, therefore, that the results from PCA and PLS analyses were just as similar, and integration between the two developmental compartments is just as strong for FA as for individual variation.

Discussion

Our analyses of morphometric variation in *Drosophila* wings have identified a number of features of shape variation and indicate that there is pervasive covariation of landmark positions across the entire wing, both for individual variation and for FA. This finding of overall integration is in contrast to the conclusions of earlier studies supporting the hypothesis that anterior and posterior compartments are separate developmental modules. Here we discuss our results, the methodological basis for the apparent conflict with the results of earlier studies, and implications in relation to existing work on morphological integration and information about wing development in *Drosophila.*

Patterns of Shape Variation in the Wing

The dominant pattern of variation among individuals (PC1 in Fig. 3A) is a contrast between a narrower and more pointed or broader and more rounded wing tip. This variation of the wing tip shape simultaneously affects the distal regions in both the anterior and posterior compartments and thus contributes to integration between them. This pattern may relate to shape change during the pupal period, when the wing tip narrows and becomes more pointed (Waddington 1940, p. 89). However, no direct equivalent to this pattern was found in the analyses of FA, in a study of size-related shape variation in *D. simulans* (Baylac and Penin 1998), or in an analysis of shape variation in tsetse flies (Klingenberg and McIntyre 1998).

Several of the PCs showed shifts of crossveins along the longitudinal veins to which they abut. The shifts of the anterior (PC3 of FA; Fig. 4A) and posterior crossvein (PC2 for individual variation and FA; Figs. 3A, 4A) appear to be independent of each other. Similar displacements of one or both crossveins have been found in intra- and interspecific studies, for instance, for intrapopulation variation in *D. simulans* (Baylac and Penin 1998, fig. 3), geographic variation in *D. melanogaster* (Imasheva et al. 1995), among species in a tribe of tachinid flies (O'Hara 1988), and among the families of flies (Colless and McAlpine 1991). Moreover, such shifts also occur in a number of mutants of *D. melanogaster* (Waddington 1940, pp. 119, 132; Villano and Katz 1995, fig. 3). That these shifts of crossveins are directed primarily along the longitudinal veins is consistent with evidence that the development of crossveins depends on the adjoining longitudinal veins. The crossveins are determined after the longitudinal veins (e.g., Waddington 1940; Yu et al. 1996, fig. 4), and mutations that eliminate crossveins do not affect the longitudinal veins, but some genes affecting the longitudinal veins also have effects on the crossveins (Waddington 1940; Diaz-Benjumea and García-Bellido 1990). However, this dependence is not a complete one: There appears to be mutual adjustment of the precise positions of longitudinal veins and crossveins according to the local geometry of the veins. Waddington (1940, p. 133) compared this to elastic threads under tension, which are able to slide along each other to some degree. Presumably this explains why, for species such as tsetse flies, in which the geometry of crossveins is more complex than in *Drosophila,* the patterns of variation also are more complicated and do not simply follow the longitudinal veins (Klingenberg and McIntyre 1998).

However, these shifts of crossveins did not occur in isolation, but included other landmarks as well, and thus were part of shape changes of larger sections of the wing that involved both compartments. The shifts of the posterior crossvein were coordinated not only with movements of the nearby landmark 12 (at the end of vein L5) but also with landmark 1 (end of vein L2) in the anterior compartment. Because the anterior crossvein intersects the anterior posterior boundary, simultaneous shifts of the landmarks at either end of the crossvein will automatically be shared between compartments. The shifts of crossveins appear to be coordinated with movements of most other landmarks through larger-scale patterning across the entire wing (e.g., Waddington 1940, p. 132 ff.; Villano and Katz 1995).

The pattern of the PC2s both of individual variation (Fig. 3A) and of FA (Fig. 4A) was a contraction or expansion of the distal part of the wing blade (landmarks 1, 7, 11, 12, and to a lesser extent 5, 6). A similar pattern was found as a part of size-related shape variation in *D. simulans* (Baylac and Penin 1998, fig. 3), and it may be a feature of geographic variation in *D. melanogaster* (Imasheva et al. 1995; however, this similarity should be viewed as tentative because of the methodological differences between studies). This pattern may relate to the relative contraction of the base of the wing and expansion of the wing blade that occur during the pupal stage, when the shape of the wing disc changes from roughly rectangular to the final wing shape (Waddington 1940, pp. 87–89, 95–106; González-Gaitán et al. 1994). It is also conceivable, however, that this pattern is due to localized variation in proliferation, growth, and death of cells (García-Bellido et al. 1994; Milán et al. 1997; Neufeld et al. 1998). Regardless of the mechanism involved, this pattern is clearly coordinated between the anterior and posterior compartments. In contrast to the results in *Drosophila,* there was no similar pattern for either individual variation or FA in tsetse flies (Klingenberg and McIntyre 1998). This discrepancy may be due to differences in developmental processes or simply to the different spatial arrangement of wing veins, especially

near the posterior crossvein, in these two quite different species of flies.

The rotation in the distal part of the wing blade seen in the PC3s for individual variation (Fig. 3A) and the PC1s for FA (Fig. 4A) corresponded to fairly similar patterns in the wing of tsetse flies (Klingenberg and McIntyre 1998, figs. 4, 5). Such a relative rotation could be generated by differences between anterior and posterior compartments in the amount of growth along the proximal-distal axis. A faster-growing compartment would expand around the slower-growing one, which may also lead to a bending of the proximal-distal axis of the wing toward the slower-growing compartment. Distortions of this kind have been produced by experimental reduction of wing growth in either the anterior or posterior compartment (using double mutant clones of *vein* and *rhomboid*; García-Bellido et al. 1994, figs. 1B, E, F). Whether this interpretation supports the hypothesis of compartment autonomy depends on whether the observed differences in absolute growth are independent or coordinated between compartments.

Developmental Integration of Fluctuating Asymmetry

Our analyses suggest a similar overall degree of shape integration for FA and individual variation, as the profiles of eigenvalues indicate that FA is concentrated within the first few PCs only slightly less than individual variation (Fig. 2). Moreover, the analyses revealed clear correspondences between spatial patterning of individual variation and FA. There were mostly minor differences in these patterns, and it was primarily the order of PCs that differed between individual variation and FA, indicating that the same patterns account for different amounts of variation at the two levels of morphological variation.

That integration for FA is nearly as strong as for individual variation contrasts with earlier findings that correlated FA among traits has been difficult to demonstrate (Leamy 1993; Palmer 1994, p. 359 ff.; Møller and Swaddle 1997, p. 53 ff.; Clarke 1998). However, a number of studies have found correlated asymmetries among traits that are morphologically adjacent, and have ascribed these correlations to ''neighborhood effects'' (Jolicoeur 1963; Leamy 1984; Hallgrímsson 1998; Van Dongen et al. 1999). However, the only examples of FA integration comparable to the integration of individual variation are from the wings of tsetse flies (Klingenberg and McIntyre 1998) and mouse mandibles (Leamy 1993).

Because FA is generated by random perturbations of developmental processes (for an explicit model, see Klingenberg and Nijhout 1999) that arise locally and independently, integrated FA in two parts of a structure can only occur if the parts share the effects of the same perturbations. This means there must be developmental interactions that transmit the effects of the perturbations to both parts. Such interactions can be due to the embryonic origin of parts (e.g., Riska 1986), localized competition among organ primordia for some limiting resource (Klingenberg and Nijhout 1998), or differential use of structures on left and right body sides (Trinkaus et al. 1994; Hallgrímsson 1998). Integration of FA is usually confined to a small ''neighborhood'' because these interactions will only transmit the perturbations effectively over limited distances.

It is no coincidence, therefore, that fly wings and mouse jaws are the only examples in which strong FA integration has been shown, because both are coherent structures within which a multitude of developmental interactions take place. In fly wings, an obvious basis for these developmental interactions is their origin from imaginal discs, within which all parts of a wing share a long ontogenetic history (Waddington 1940; Lawrence 1992; Sturtevant and Bier 1995). The fact that all the patterns of integration for FA, just as those for individual variation, extend across the entire wing indicates that even subtle random perturbations are transmitted throughout the developing wing, and most notably, across the anterior-posterior compartment boundary. With regard to vein patterning, the entire wing therefore appears to be fairly homogeneous, rather than composed of distinct subunits, and can be considered as a single developmental module (e.g., Raff 1996; Wagner 1996; Kirschner and Gerhart 1998).

We would not expect such homogeneity and high degree of integration if structures derived from multiple imaginal discs and different body regions were included (Cowley and Atchley 1990). In that situation, we would expect substantial and consistent differences between PCs for individual variation and FA. The agreement between patterns of integration for individual variation and FA suggests that the same connections among developmental pathways that transmit the perturbations responsible for FA may also be the dominant conduit for developmental integration of variation among individuals.

Covariation of Anterior and Posterior Compartments

The high degree of integration of the wing is underscored by the PLS analyses of covariation between the anterior and posterior wing compartments. For both individual variation and FA, the PLS axes were highly correlated between the compartments and remarkably similar to the PCs (Table 2; Figs. 3, 4). The PLS axes and PCs would exactly coincide under a model of complete integration between the two compartments, in which all variation is shared between compartments (and all correlations between PLS axes would be 1.0; see Appendix). Although this extreme condition was not met completely for any of the analyses, none of the comparisons of PCs to PLS axes showed a statistically significant deviation from a null model of pervasive integration in which the patterns of covariation between compartments also account for all morphometric variation within compartments. The anterior and posterior compartments are far from being separate units of morphological variation: the component of variation shared between compartments is nearly sufficient to account for *all* the variation in the entire wing.

At first sight, this result appears to contradict the conclusions of a number of morphometric and quantitative genetic studies in *Drosophila* that have emphasized the role of the anterior and posterior compartments as distinct units of morphometric variation in the wing (see quotations in the introduction). Most of these studies were based on measurements of several distances between landmarks, areas of intervein

regions, or measures of cell size and number in different regions of the wing. They concluded that the compartments are distinct units because of differences in amount or direction of the response to selection (Cavicchi et al. 1981, 1985, 1991; Guerra et al. 1997) or different degrees of geographic variation (Imasheva et al. 1995; Pezzoli et al. 1997). This reasoning, which equates morphological integration with positive correlations, does not entirely take into account the multivariate nature of the data. As Figures 3 and 4 show, for any given PC or PLS axis, the landmarks can move in the same or in opposite directions; accordingly, distances between landmarks may show positive, negative, or no correlations.

Indeed, examination of these earlier studies reveals that most of them also found some evidence for integration across the entire wing. Studies of geographic variation reported correlations throughout the wing besides differences between wing regions (Imasheva et al. 1995; Pezzoli et al. 1997). Artificial-selection experiments produced correlated responses throughout the whole wing (Cavicchi et al. 1981; Thompson and Woodruff 1982; Weber 1990, 1992), which may vary depending on the vein targeted by selection (Guerra et al. 1997). Finally, Cowley and Atchley (1990) showed that phenotypic and genetic correlations among measurements in different compartments were just as high as correlations within a compartment, but that the extent of predicted selection responses would vary depending on which trait is under selection. The results of these studies, as far as can be judged from the published reports, are consistent in that they simultaneously show both a high degree of integration across the wing and local specificity of morphometric variation. This is in agreement with our study, where relative landmark movements covary throughout the wing, although these movements often differ in extent and direction even between neighboring landmarks. Just as the pervasive integration revealed by our analyses does not rule out localized variation, none of the studies reviewed above contains evidence that is inconsistent with integration across compartments.

The apparent conflict between the finding of complete integration and the role of compartments in the development of fly wings can be resolved by considering the interactions between compartments. The compartments long have been viewed as important units for growth control in the imaginal discs (e.g., Lawrence and Morata 1976), and recent experiments have shown that cell number and cell size are regulated jointly within compartments to achieve a given size (Weigmann et al. 1997; Neufeld et al. 1998). Reduced growth caused by mutant clones in one compartment can also elicit a reduction in the growth of the other compartment, which attenuates the effect on the ratio between the two wing regions (García-Bellido et al. 1994), and experimentally induced cell death in the posterior compartment can lead to compensatory cell death in the anterior compartment (Milán et al. 1997). Thus, growth control appears to be both locally active within compartments and integrated throughout the wing.

Integration between anterior and posterior compartments is emphasized even more by recent research indicating that compartment boundaries themselves play a preeminent role in organizing the patterning of legs and wings (Blair 1995; Lawrence and Struhl 1996; Lecuit and Cohen 1997). For the wings, this role is underscored by experiments showing that clones producing ectopic boundaries of *engrailed/invected* expression, which mimic the anterior-posterior compartment boundary, can cause duplication of large parts of the wing including veins that have clear identities (Tabata et al. 1995). Rather than being a passive borderline delimiting two independent domains of patterning, the compartment boundary acts as an organizing center from which patterning signals emanate (Lawrence and Struhl 1996; Strigini and Cohen 1999; Milán and Cohen 2000). The boundary itself is thus an active contributor to anterior-posterior integration.

Signals originating from the anterior-posterior compartment boundary initiate regulatory interactions that subdivide the wing into a series of sectors with discrete boundaries (Sturtevant and Bier 1995; Sturtevant et al. 1997; Biehs et al. 1998; de Celis 1998; Lunde et al. 1998; Campbell and Tomlinson 1999; Jazwinska et al. 1999; Milán and Cohen 2000). Different sectors are distinguished by the expression of a different combination of genes, and specific genes are activated at their boundaries to initiate vein formation (Sturtevant et al. 1997; Biehs et al. 1998; Lunde et al. 1998). The specificity of this process gives each vein its identity. Moreover, because the veins coincide with clonal limits in the wing (González-Gaitán et al. 1994), these sectors are also linked to patterns of cell proliferation in the growing wing discs. Vein formation itself involves further genes that are active in all veins, for example, to determine the final width of veins and to differentiate vein from intervein tissue (Sturtevant and Bier 1995; Biehs et al. 1998; de Celis 1998; Roch et al. 1998). Finally, the morphogenetic movements that bring about evagination of the wing and the regional expansion and contraction that give the wing its final shape (Waddington 1940) can also contribute to the observed morphometric variation. All the steps in this process have different specificities, they may vary in their ability to transmit developmental perturbations, and the genes involved can have phenotypic effects that are localized to various degrees. Thus, the simultaneous occurrence of overall integration and local specificity appears as a natural outcome of the developmental system.

The combined analysis of individual variation and FA has emphasized the strength of integration throughout the wing. The role of the wing compartments, it appears, is not to delimit two autonomous developmental units, but the boundary itself is now recognized as an active center for patterning. The integration of FA shows that even subtle perturbations of developmental processes can thus have effects extending throughout the entire wing. In contrast, the processes that are specific to certain veins or small regions of the wing appear to contribute only a small proportion of the observed morphometric variation. It is not clear why so much less variation in these localized processes is manifest in the phenotype. There appears to be no reason to believe a priori that vein-specific processes should be inherently less variable or better canalized than those that are integrated across the entire wing. It is conceivable, however, that the effects of late-acting processes affecting the whole wing, like wing expansion, may mask localized variation from earlier processes. Of course, this scenario also would imply that the integrating effects of earlier processes, like the signaling from the anterior-posterior compartment boundary, would be masked as well.

Clearly, any such masking of variation will affect on how the genes involved in the successive processes of wing development would respond to selection on wing shape.

To distinguish between these possibilities, the effects of the various developmental processes on morphological variation in the fly wing need to be separated. This will require that the sources of variation in the developmental system are controlled experimentally. Combining the morphometric methods presented here with the tools of developmental genetics should make this feasible. Because of its explicit mechanistic basis, this approach will be complementary to the studies of morphological integration from the more phenomenological perspective of quantitative genetics. In combination, the two approaches will provide a better understanding of how developmental mechanisms contribute to morphological variation and its integration, and thus mold the evolutionary process.

Acknowledgments

We thank M. Akam, M. Baylac, F. L. Bookstein, L. Castilho, H. F. Nijhout, A. R. Palmer, F. Roch, F. J. Rohlf, M. L. Zelditch, and three anonymous reviewers for discussions during the analyses and comments on several versions of the manuscript. Financial support was provided by postdoctoral fellowships from the Swiss National Science Foundation and the Human Frontier Science Program to CPK, a University of Alberta Ph.D. Scholarship to SDZ, and by grants from the Natural Sciences and Engineering Research Council (Canada) to A. R. Palmer and from the U.S. National Science Foundation to H. F. Nijhout.

Literature Cited

Auffray, J.-C., P. Alibert, S. Renaud, A. Orth, and F. Bonhomme. 1996. Fluctuating asymmetry in *Mus musculus* subspecific hybridization: traditional and Procrustes comparative approach. Pp. 275–283 *in* L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor, and D. E. Slice, eds. Advances in morphometrics. Plenum Press, New York.

Baylac, M., and X. Penin. 1998. Wing static allometry in *Drosophila simulans* males (Diptera, Drosophilidae) and its relationships with developmental compartments. Acta Zool. Acad. Sci. Hung. 44:97–112.

Biehs, B., M. A. Sturtevant, and E. Bier. 1998. Boundaries in the *Drosophila* wing imaginal disc organize vein-specific genetic programs. Development 125:4245–4257.

Blair, S. S. 1995. Compartments and appendage development in *Drosophila*. BioEssays 17:299–309.

Bookstein, F. L. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge Univ. Press, Cambridge, U.K.

———. 1996. Biometrics, biomathematics and the morphometric synthesis. Bull. Math. Biol. 58:313–365.

Bookstein, F. L., P. D. Sampson, A. P. Streissguth, and H. M. Barr. 1990. Measuring ''dose'' and ''response'' with multivariate data using partial least squares techniques. Commun. Statist.—Theory Meth. 19:765–804.

Campbell, G., and A. Tomlinson. 1999. Transducing the Dpp morphogen gradient in the wing of *Drosophila*: regulation of Dpp targets by *brinker*. Cell 96:553–562.

Cavicchi, S., C. Pezzoli, and G. Giorgi. 1981. Correlation between characters as related to developmental pattern in *Drosophila*. Genetica 56:189–195.

Cavicchi, S., D. Guerra, G. Giorgi, and C. Pezzoli. 1985. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape variation. Genetics 109:665–689.

Cavicchi, S., G. Giorgi, V. Natali, and D. Guerra. 1991. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. III. Fourier and centroid analysis of wing shape and relationship between shape variation and fitness. J. Evol. Biol. 4:141–159.

Chessel, D., and P. Mercier. 1993. Couplage de triplets statistiques et liaisons espèces-environnement. Pp. 15–43 *in* J.-D. Lebreton and B. Asselain, eds. Biométrie et environnmenent. Masson, Paris.

Cheverud, J. M. 1982a. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36: 499–516.

———. 1982b. Relationships among ontogenetic, static, and evolutionary allometry. Am. J. Phys. Anthropol. 59:139–149.

———. 1996. Developmental integration and the evolution of pleiotropy. Am. Zool. 36:44–50.

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., G. P. Wagner, and M. M. Dow. 1989. Methods for the comparative analysis of variation patterns. Syst. Zool. 38:201–213.

Clarke, G. M. 1998. The genetic basis of developmental stability. IV. Individual and population asymmetry parameters. Heredity 80:553–561.

Colless, D. H., and D. K. McAlpine. 1991. Diptera (flies). Pp. 717–786 *in* I. D. Naumann, P. B. Carne, J. F. Lawrence, E. S. Nielsen, J. P. Spradberry, R. W. Taylor, M. J. Whitten and M. J. Littlejohn, eds. The insects of Australia. Cornell Univ. Press, Ithaca, NY.

Corti, M., C. Fadda, S. Simson, and E. Nevo. 1996. Size and shape variation in the mandible of the fossorial rodent *Spalax ehrenbergi*. Pp. 303–320 *in* L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor and D. E. Slice, eds. Advances in morphometrics. Plenum Press, New York.

Cowley, D. E., and W. R. Atchley. 1990. Development and quantitative genetics of correlation structure among body parts of *Drosophila melanogaster*. Am. Nat. 135:242–268.

de Celis, J. F. 1998. Positioning and differentiation of veins in the *Drosophila* wing. Int. J. Dev. Biol. 42:335–343.

Diaz-Benjumea, F. J., and A. García-Bellido. 1990. Genetic analysis of the wing vein pattern of *Drosophila*. Roux's Arch. Dev. Biol. 198:336–354.

Dryden, I. L., and K. V. Mardia. 1998. Statistical analysis of shape. Wiley, Chichester, U.K.

Edgington, E. S. 1995. Randomization tests. Marcel Dekker, New York.

Efron, B., and R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman and Hall, New York.

García-Bellido, A., and J. F. de Celis. 1992. Developmental genetics of the venation pattern of *Drosophila*. Annu. Rev. Genet. 26: 277–304.

García-Bellido, A., P. Ripoll, and G. Morata. 1973. Developmental compartmentalisation of the wing disk of *Drosophila*. Nature (London), New Biol. 245:251–253.

García-Bellido, A., F. Cortés, and M. Milán. 1994. Cell interactions in the control of size in *Drosophila* wings. Proc. Natl. Acad. Sci. USA 91:10222–10226.

González-Gaitán, M., M. P. Capdevila, and A. García-Bellido. 1994. Cell proliferation patterns in the wing imaginal disc of *Drosophila*. Mech. Dev. 40:183–200.

Guerra, D., M. C. Pezzoli, G. Giorgi, F. Garoia, and S. Cavicchi. 1997. Developmental constraints in the *Drosophila* wing. Heredity 79:564–571.

Hallgrímsson, B. 1998. Fluctuating asymmetry in the mammalian skeleton: evolutionary and developmental implications. Evol. Biol. 30:187–251.

Imasheva, A. G., O. A. Bubli, O. E. Lazebny, and L. A. Zhivotovsky. 1995. Geographic differentiation in wing shape in *Drosophila melanogaster*. Genetica 96:303–306.

Jackson, D. A. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. Ecology 74:2204–2214.

Jazwinska, A., N. Kirov, E. Wieschaus, S. Roth, and C. Rushlow. 1999. The *Drosophila* gene *brinker* reveals a novel mechanism of Dpp target gene regulation. Cell 96:563–573.

Jolicoeur, P. 1963. Bilateral symmetry and asymmetry in limb bones of *Martes americana* and man. Rev. Can. Biol. 22:409–432.

Jolliffe, I. T. 1986. Principal component analysis. Springer-Verlag, New York.
Kirschner, M., and J. Gerhart. 1998. Evolvability. Proc. Natl. Acad. Sci. USA 95:8420–8427.
Klingenberg, C. P. 1996. Multivariate allometry. Pp. 23–49 *in* L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor, and D. E.Slice, eds. Advances in morphometrics. Plenum Press, New York.
Klingenberg, C. P., and W. Ekau. 1996. A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). Biol. J. Linn. Soc. 59:143–177.
Klingenberg, C. P., and G. S. McIntyre. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52: 1363–1375.
Klingenberg, C. P., and H. F. Nijhout. 1998. Competition among growing organs and developmental control of morphological asymmetry. Proc. Roy. Soc. Lond. B Biol. Sci. 265:1135–1139.
———. 1999. Genetics of fluctuating asymmetry: a developmental model of developmental instability. Evolution 53:358–375.
Klingenberg, C. P., and M. Zimmermann. 1992. Static, ontogenic, and evolutionary allometry: a multivariate comparison in nine species of water striders. Am. Nat. 140:601–620.
Klingenberg, C. P., G. S. McIntyre, and S. D. Zaklan. 1998. Left-right asymmetry of fly wings and the evolution of body axes. Proc. Roy. Soc. Lond. B Biol. Sci. 265:1255–1259.
Lawrence, P. A. 1992. The making of a fly: the genetics of animal design. Blackwell Scientific, Oxford.
Lawrence, P. A., and G. Morata. 1976. The compartment hypothesis. Symp. R. Ent. Soc. Lond. 8:132–149.
Lawrence, P. A., and G. Struhl. 1996. Morphogens, compartments, and patterns: lessons from *Drosophila*? Cell 85:951–961.
Leamy, L. 1984. Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. Am. Nat. 123: 579–593.
———. 1993. Morphological integration of fluctuating asymmetry in the mouse mandible. Genetica 89:139–153.
Leamy, L. J., E. J. Routman, and J. M. Cheverud. 1997. A search for quantitative trait loci affecting asymmetry of mandibular characters in mice. Evolution 51:957–969.
———. 1999. Quantitative trait loci for early- and late-developing skull characters in mice: a test of the genetic independence model of morphological integration. Am. Nat. 153:201–214.
Lecuit, T., and S. M. Cohen. 1997. Proximal-distal axis formation in the *Drosophila* leg. Nature 388:139–145.
Lunde, K., B. Biehs, U. Nauber, and E. Bier. 1998. The *knirps* and *knirps-related* genes organize development of the second wing vein in *Drosophila.* Development 125:4145–4154.
Marcus, L. F. 1993. Some aspects of multivariate statistics for morphometrics. Pp. 95–130 *in* L. F. Marcus, E. Bello and A. García-Valdecasas, eds. Contributions to morphometrics. Museo Nacional de Ciencias Naturales, Madrid.
Mardia, K. V., J. T. Kent, and J. M. Bibby. 1979. Multivariate analysis. Academic Press, London.
Markow, T. A. 1995. Evolutionary ecology and developmental instability. Annu. Rev. Entomol. 40:105–120.
Milán, M., and S. M. Cohen. 2000. Subdividing cell populations in the developing limbs of *Drosophila*: do wing veins and leg segments define units of growth control? Dev. Biol. 217:1–9.
Milán, M., S. Campuzano, and A. García-Bellido. 1997. Developmental parameters of cell death in the wing disc of *Drosophila.* Proc. Natl. Acad. Sci. USA 94:5691–5696.
Møller, A. P., and J. P. Swaddle. 1997. Asymmetry, developmental stability, and evolution. Oxford Univ. Press, Oxford, U.K.
Monteiro, L. R. 1999. Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. Syst. Biol. 48:192–199.
Neufeld, T. P., A. F. A. de la Cruz, L. A. Johnston, and B. A. Edgar. 1998. Coordination of growth and cell division in the *Drosophila* wing. Cell 93:1183–1193.
O'Hara, J. E. 1988. Correlation between wing size and position of a hind crossvein in the Siphonini (Diptera: Tachinidae). J. Nat. Hist. 22:1141–1146.
Olson, E. C., and R. L. Miller. 1958. Morphological integration. Univ. of Chicago Press, Chicago, IL.
Palmer, A. R. 1994. Fluctuating asymmetry analyses: a primer. Pp. 335–364 *in* T. A. Markow, ed. Developmental instability: its origins and implications. Kluwer, Dordrecht, The Netherlands.
Palmer, A. R., and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Ann. Rev. Ecol. Syst. 17:391–421.
Pezzoli, M. C., D. Guerra, G. Giorgi, F. Garoia, and S. Cavicchi. 1997. Developmental constraints and wing shape variation in natural populations of *Drosophila melanogaster*. Heredity 79: 572–577.
Raff, R. A. 1996. The shape of life: genes, development and the evolution of animal form. Univ. of Chicago Press, Chicago, IL.
Riska, B. 1986. Some models for development, growth, and morphometric correlation. Evolution 40:1303–1311.
Roch, F., A. Baonza, E. Martín-Blanco, and A. García-Bellido. 1998. Genetic interactions and cell behaviour in *blistered* mutants during proliferation and differentiation of the *Drosophila* wing. Development 125:1823–1832.
Roth, V. L. 1996. Cranial integration in the Sciuridae. Am. Zool. 36:14–23.
Sakai, K.-I., and Y. Shimamoto. 1965. A developmental-genetic study on panicle charaters in rice, *Oryza sativa* L. Genet. Res. 6:93–103.
Smith, D. R., B. J. Crespi, and F. L. Bookstein. 1997. Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. J. Evol. Biol. 10:551–574.
Smith, K. K. 1996. Integration of craniofacial structures during development in mammals. Am. Zool. 36:70–79.
Stark, J., J. Bonacum, J. Remsen, and R. DeSalle. 1999. The evolution and development of dipteran wing veins: a systematic approach. Annu. Rev. Entomol. 44:97–129.
Streissguth, A. P., F. L. Bookstein, P. D. Sampson, and H. M. Barr. 1993. The enduring effects of prenatal alcohol exposure on child development. Univ. of Michigan Press, Ann Arbor, MI.
Strigini, M., and S. M. Cohen. 1999. Formation of morphogen gradients in the *Drosophila* wing. Semin. Cell Dev. Biol. 10: 335–344.
Sturtevant, M. A., and E. Bier. 1995. Analysis of the genetic hierarchy guiding wing vein development in *Drosophila.* Development 121:785–801.
Sturtevant, M. A., B. Biehs, E. Marin, and E. Bier. 1997. The *spalt* gene links the A/P compartment boundary to a linear adult structure in the *Drosophila* wing. Development 124:21–32.
Tabachnick, R. E., and F. L. Bookstein. 1990. The structure of individual variation in Miocene *Globorotalia.* Evolution 44: 416–434.
Tabata, T., C. Schwartz, E. Gustavson, Z. Ali, and T. B. Kornberg. 1995. Creating a *Drosophila* wing de novo, the role of *engrailed,* and the compartment border hypothesis. Development 121: 3359–3369.
Thompson, J. N., Jr., and R. C. Woodruff. 1982. Polygenic analysis of pattern formation: interdependence among veins in the same compartment of the *Drosophila* wing. Genetica 60:71–76.
Trinkaus, E., S. E. Churchill, and C. B. Ruff. 1994. Postcranial robusticity in *Homo.* II. Humeral bilateral asymmetry and bone plasticity. Am. J. Phys. Anthropol. 93:1–34.
Tucker, L. R. 1958. An inter-battery method of factor analysis. Psychometrika 23:111–136.
Van Dongen, S., E. Sprengers, and C. Löfstedt. 1999. Correlated development, organism-wide asymmetry and patterns of asymmetry in two moth species. Genetica 105:81–91.
Villano, J. L., and F. N. Katz. 1995. *four-jointed* is required for intermediate growth in the proximal-distal axis in *Drosophila.* Development 121:2767–2777.
von Dassow, G., and E. Munro. 1999. Modularity in animal development and evolution: elements of a conceptual framework for EvoDevo. J. Exp. Zool. (Mol. Dev. Evol.) 285:307–325.
Waddington, C. H. 1940. The genetic control of wing development in *Drosophila.* J. Genet. 41:75–139.
Wagner, G. P. 1984. On the eigenvalue distribution of genetic and phenotypic dispersion matrices: evidence for a nonrandom organization of quantitative character variation. J. Math. Biol. 21: 77–95.
———. 1996. Homologues, natural kinds and the evolution of modularity. Am. Zool. 36:36–43.
Wagner, G. P., and L. Altenberg. 1996. Complex adaptations and the evolution of evolvability. Evolution 50:967–976.
Weber, K. E. 1990. Selection on wing allometry in *Drosophila melanogaster*. Genetics 126:975–989.

———. 1992. How small are the smallest selectable domains of form? Genetics 130:345–353.
Weigmann, K., S. M. Cohen, and C. F. Lehner. 1997. Cell cycle progression, growth and patterning in imaginal discs despite inhibition of cell division after inactivation of *Drosophila* Cdc2 kinase. Development 124:3555–3563.
Yu, K., M. A. Sturtevant, B. Biehs, V. François, R. W. Padgett, R. K. Blackman, and E. Bier. 1996. The *Drosophila decapentaplegic* and *short gastrulation* genes function antagonistically during adult wing vein development. Development 122:4033–4044.
Zelditch, M. L. 1987. Evaluating models of developmental integration in the laboratory rat using confirmatory factor analysis. Syst. Zool. 36:368–380.

Corresponding Editor: M. Zelditch

Appendix

Algebra of Partial Least Squares

The PLS method is designed to analyze the patterns of covariation between two sets of variables, such as the coordinates of landmarks in the anterior versus posterior wing compartments.

Let $\mathbf{S}$ be the covariance matrix including both subsets of variables,

$$\mathbf{S} = \begin{bmatrix} \mathbf{S}_{11} & \mathbf{S}_{12} \\ \mathbf{S}_{21} & \mathbf{S}_{22} \end{bmatrix}, \tag{A1}$$

where $\mathbf{S}_{11}$ and $\mathbf{S}_{22}$ are the covariance matrices of the first and second variable sets, that is the matrices of within-set variances and covariances. $\mathbf{S}_{12}$ ($\mathbf{S}_{12} = \mathbf{S}_{21}'$) is the matrix of cross-covariances between the two sets of variables. If the first and second set consist of p and q variables, respectively, then $\mathbf{S}_{12}$ is of format $p \times q$. In the following paragraph, it is assumed that the set with more variables is considered the first, so that $p \geq q$ will hold.

The core of the PLS method is the singular value decomposition (e.g., Jolliffe 1986, p. 37 f.; Marcus 1993) of the matrix of cross-covariances:

$$\mathbf{S}_{12} = \mathbf{U}\mathbf{L}_{12}\mathbf{V}', \tag{A2}$$

where $\mathbf{U}$ is an orthonormal $p \times q$ matrix containing the left singular vectors ($\mathbf{u}_1, \mathbf{u}_2, \ldots, \mathbf{u}_q$), and $\mathbf{V}$ is an orthonormal $q \times q$ matrix with the right singular vectors ($\mathbf{v}_1, \mathbf{v}_2, \ldots, \mathbf{v}_q$). By convention, the singular vectors are scaled to unit length, so that $\mathbf{u}_i'\mathbf{u}_i = \mathbf{v}_i'\mathbf{v}_i = 1$ ($i = 1, \ldots, q$). The left and right singular vectors contain the coefficients for the pairs of linear combinations (PLS axes) of the two variable sets that have maximum covariances. $\mathbf{L}_{12}$ is a diagonal $q \times q$ matrix of singular values, which are the covariances between sets that are associated with each pair of PLS axes. The pairs of PLS axes are ranked by these covariances. The first $n < q$ pairs of PLS axes provide the best least-squares approximation of rank n to the matrix of cross-covariances.

Relationships of the Partial Least-Squares Method to Principal Component Analysis

The analogies of the PLS technique to PCA stem from their algebraic similarities. The singular value decomposition of a rectangular matrix used in PLS has properties that correspond in some ways to the spectral decomposition of a (symmetric) covariance matrix in PCA. The spectral decomposition of the covariance matrix of the total set of variables is

$$\mathbf{S} = \mathbf{B}\mathbf{L}\mathbf{B}', \tag{A3}$$

where $\mathbf{B}$ is the matrix of eigenvectors (PCs; $\mathbf{b}_1, \mathbf{b}_2, \ldots, \mathbf{b}_{p+q}$), and $\mathbf{L}$ is a diagonal matrix of eigenvalues (the variances associated with the corresponding PCs).

Statistical Comparison of Partial Least-Squares Analysis and Principal Component Analysis

The following statistical comparison is motivated by the empirical finding that the results of PLS and PCA were remarkably similar (see Results). Here we first explore the limiting condition under which the results of PLS and PCA are the same. This requires a model of complete covariation, where the correlations between the pairs of PLS axes are all 1.0, and the first q eigenvalues of $\mathbf{S}$ equal the singular values, that is, $\mathbf{L}_q = \mathbf{L}_{12}$ (where $\mathbf{L}_q$ is the top-left $q \times q$ portion of $\mathbf{L}$). The model further implies that the first q PCs of the overall covariance matrix can be obtained as

$$\mathbf{B}_q = \begin{bmatrix} \sqrt{\frac{p}{p+q}}\mathbf{U} \\ \sqrt{\frac{q}{p+q}}\mathbf{V} \end{bmatrix}, \tag{A4}$$

that is, by stacking the matrices of PLS axes and rescaling (the weighting by the expressions under the square roots serves only to give every variable equal weight when $p \neq q$). In empirical studies, this condition can never be met, but it is possible to examine to what extent the data approach the limit given in this model by comparing the first few PCs with the corresponding combined PLS axes and by comparing the eigenvalues with the corresponding singular values.

Parametric Bootstrap Test for Covariation

To test the similarity between PCs and PLS axes statistically, we used a parametric bootstrap test (Efron and Tibshirani 1993), that is, a computer simulation of random samples according to a parametric distribution (the multivariate normal) using the parameter values estimated from the original sample. For this purpose, we used a model that was slightly less extreme than the one outlined above, and did not require that the correlations between pairs of PLS axes in the two compartments were unity. This model still assumed that the PLS axes could account for all variation and covariation within and between compartments, but it allowed that each PLS axis also had some additional within-compartment variation. The $\mathbf{U}$, $\mathbf{V}$, and $\mathbf{L}_{12}$ matrices were estimated from the observed $\mathbf{S}_{12}$ by singular value decomposition, as above. In order ensure that the correlations of PLS axes between compartments were the same as in the original data, we set the within-compartment variation equal to the variance for which the $\mathbf{u}_i$ or $\mathbf{v}_i$ accounted in that compartment (i.e., $\mathbf{u}_i'\mathbf{S}_{11}\mathbf{u}_i$ or $\mathbf{v}_i'\mathbf{S}_{22}\mathbf{v}_i$, $i = 1, \ldots, q$), whenever this was greater than the respective value in $\mathbf{L}_{12}$. Let $\mathbf{E}_{11}$ and $\mathbf{E}_{22}$ be diagonal $q \times q$ matrices containing these values. We used these with the $\mathbf{U}$ and $\mathbf{V}$ matrices to model not only the cross-covariances between compartments, but also the within-compartment variation. Altogether, therefore, the covariance matrix used in the parametric bootstrap test was

$$\mathbf{S}_{\mathrm{BT}} = \begin{bmatrix} \mathbf{U}\mathbf{E}_{11}\mathbf{U}' & \mathbf{U}\mathbf{L}_{12}\mathbf{V}' \\ \mathbf{V}\mathbf{L}_{12}\mathbf{U}' & \mathbf{V}\mathbf{E}_{22}\mathbf{V}' \end{bmatrix}. \tag{A5}$$

Notice that there are only $2q$ dimensions with nonzero variance, not $p + q$.

We carried out the parametric bootstrap test (Efron and Tibshirani 1993) using a random number generator to produce 10,000 bootstrap samples equal in size to the original sample. To generate the bootstrap samples, we used a multivariate normal distribution with means of zero for all variables (this is possible because the analyses performed here do not concern the means) and the covariance matrix $\mathbf{S}_{\mathrm{BT}}$. For each of these simulated samples, the covariance matrix was computed, from which we extracted the first three PCs and PLS axes and calculated the angles between corresponding pairs. The achieved significance levels for these angles were the proportions of angles in the simulations that equaled or exceeded the observed angles.