

GEOMETRIC ESTIMATES OF HERITABILITY IN BIOLOGICAL SHAPE

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Abstract.—The recently developed geometric morphometrics methods represent an important contribution of statistics and geometry to the study of biological shapes. We propose simple protocols using shape distances that incorporate geometric techniques into linear quantitative genetic models that should provide insights into the contribution of genetics to shape variation in organisms. The geometric approaches use Procrustes distances in a curved shape space and distances in tangent spaces within and among families to estimate shape heritability. We illustrate the protocols with an example of wing shape variation in the honeybee, *Apis mellifera*. The heritability of overall shape variation was small, but some localized components depicting shape changes on distal wing regions showed medium to large heritabilities. The genetic variance-covariance matrix of the geometric shape variables was significantly correlated with the phenotypic shape variance-covariance matrix. A comparison of the results of geometric methods with the traditional multivariate analysis of interlandmark distances indicated that even with a larger dimensionality, the interlandmark distances were not as rich in shape information as the landmark coordinates. Quantitative genetics studies of shape should greatly benefit from the application of geometric methods.

Key words.—*Apis*, geometric morphometrics, heritability, Procrustes distances, quantitative genetics, wing shape.

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The shape of organisms and biological structures has been of scientific interest for centuries. This is understandable because biological shape is one of the most conspicuous aspects of an organism's phenotype and provides a link between the genotype and the environment (Ricklefs and Miles 1994). This interaction between the phenotypic and environmental spaces also may lead to processes of selection if there are alterations in the genotypic space (Atchley and Hall 1991) or to phenotypic plasticity if only the phenotypic space is altered (Travis 1994). Because measurement and variation of biological shape lies at the core of organismal biology, much effort has been spent on understanding the quantitative genetics of variation in biological shape (Cowley and Atchley 1992; Atchley et al. 1994), and some interesting models have been proposed (Atchley and Hall 1991).

Historically, the study of shape variation has been concentrated on using distances between landmarks in biological structures, as well as angles and distance ratios. These variables were generally combined and analyzed through an array of canonical and cluster analyses called "traditional morphometrics" (Marcus 1990). This approach has been gradually replaced by the modern geometric morphometric methods (Rohlf and Marcus 1993). One important contribution from geometry is the clear mathematical definition of shape (the geometric information of an object that is invariant to translation, rotation, and rescaling) and size (any positive real valued function from a landmark configuration X that satisfies the condition $g[aX] = ag[X]$ for any positive scalar a ; Dryden and Mardia 1998). These definitions clearly separate these two components of biological form and have allowed the

development of geometric methods to measure separately shape and size differences among organisms (Small 1996; Dryden and Mardia 1998). The field of statistical shape analysis is based on a positively curved shape space called Kendall's shape space (Kendall 1984; Dryden and Mardia 1998; Rohlf 1999). The study of shape variation in such nonlinear manifolds required the development of special statistical techniques (Goodall 1991; Dryden and Mardia 1998). An alternative is to project the shapes to tangent linear spaces, where shape variation can be studied by linear multivariate methods (Rohlf 1996, 1999).

A considerable amount of research both in shape analysis theory and morphometric applications in the last decade has provided both theoretical and empirical reasons for trading the traditional morphometrics for the geometric methods. Most of these reasons arise from the amount of information present in the different types of data (shape variables derived from rectangular coordinates vs. interlandmark distances) used. Let us first examine the theoretical reasons for using geometric morphometrics. The interlandmark distances used by the traditional approach are limited to providing information regarding differences between specimens that are aligned with its axis (Zelditch et al. 1995), whereas the rectangular coordinates associated with landmarks capture variation in all possible directions of figure space. For interlandmark distances to have the same amount of information concerning shape and size variation present in landmark coordinates, one has to include all possible interlandmark directions in the analysis. This means generating a problem of much higher dimensionality with distances than with coor-

dinates. For example, in a configuration of 10 planar landmarks, there are 20 dimensions in figure space, $2p - 4 = 16$ shape space dimensions, and one size dimension (Rohlf 1996). A set of corresponding interlandmark distances would need 45 dimensions to describe the same form (size + shape), a much higher dimensionality than for the coordinate data. As we increase the number of landmarks in the configuration, the discrepancy increases further. Each new landmark adds two new coordinate dimensions in figure space (in our two-dimensional example), but adds p (the previous number of landmarks) new interlandmark distances to the form matrix. If we consider that dataset dimensionality is important in regulating minimum sample sizes required (usually the minimum number of observations is three times the number of dimensions), one can predict that landmark coordinates will require smaller sample size than interlandmark distances (leading to a more powerful analysis). In recent simulation studies, Rohlf (2000a,b) showed that the coordinate based geometric morphometric methods are indeed the most powerful methods available for the statistical analysis of shape.

A second criticism of traditional methods is that it is a very difficult task to extract the shape information from the matrix of interlandmark distances. Some attempts in this direction may be found in the literature (Humphries et al. 1981; Rohlf and Bookstein 1987), but the methods used could not satisfactorily analyze size-independent shape because there was no explicit definition of size and shape or of what was an appropriate shape or size variable (Bookstein 1993). As stated by the linearity condition of its geometric definition, size can be approximated by different variables. The traditional studies use a linear combination of the interlandmark distances (usually the first principal component), but several other size measures can be used such as volumes, areas, and the preferred geometric size measure: centroid size (the square root of summed squared distances from each landmark to the configuration centroid). Bookstein (1986) showed that centroid size is the only size measure uncorrelated with shape in the absence of allometry. In defining and capturing size more effectively, the geometric methods are able to remove it from the landmark coordinates in a more efficient way than any of the protocols for size removal available for traditional data. Recent advances in evolutionary developmental biology have shown that the shape of complex morphological structures such as vertebrate skulls or insect wings results from the interfacing of and complex mappings between morphogenetic rules, ecological phenomena and deterministic and stochastic evolutionary forces (Murray 1990; Atchley and Hall 1991). Under this dynamic framework it is expected that the description of variability in shape will be conditional to the chosen level of organizational complexity and scale (Levin 1992; Bar-Yam 1997) and that incorporating spatial scale with geometric shape information should lead to insightful interpretations on the mechanisms that generate shape divergence (e.g., Guralnick and Lindberg 1999; Duarte et al. 2000).

In conjunction with the theoretical reasons presented above, there is also a growing body of empirical evidence showing the capacity of geometric methods in providing new insights into biological shape variation patterns that could not be assessed by traditional methods. Adams and Funk

(1997) showed that geometric morphometric methods were able to discriminate sibling species of a leaf beetle, whereas traditional methods failed to discover discriminating characters. Monteiro and Abe (1999) discovered patterns of shape variation in the scapula of xenarthran mammals that were overlooked by previous traditional analyses. Monteiro-Filho et al. (2002) successfully discriminated oceanic and river populations of the bottlenose dolphin *Sotalia fluviatilis* based on geometric shape variables, whereas previous morphometric analyses using traditional methods of the same biological problem could only find size differences between populations. Reis et al. (2002) found a clear geographic pattern for skull shape variation (using geometric morphometrics) in populations of the rodent *Thrichomys apereoides*. The same samples analyzed by traditional methods with distance measurements showed only size differences among populations. These are just a few examples from a growing number of studies that may be found in the literature.

Given the richness of information extracted from the data by geometric methods, and the relatively easy construction of linear models for such shape variables (Monteiro 1999), a natural extension would involve the assessment of the heritable component of shape as defined by geometric descriptors. Such effort would require the integration of geometric procedures of shape description into the framework of quantitative genetics. One of the major goals of quantitative genetics is the partitioning of phenotypic variance into genetic and environmental components (Lynch and Walsh 1998). A number of methods are available for the decomposition of variance into genetic and environmental components, all of them based on the principle of resemblance in the phenotypic traits among relatives. The contribution of the genetic component to total phenotypic variation is referred to as "heritability" and is a critical parameter that controls the ability of a phenotype to respond to selection (Falconer and Mackay 1996; Lynch and Walsh 1998).

Historically, the heritability of size and shape in biological structures has been estimated using size and shape variables derived from angles and ratios (Venable and Burquez 1989; Cassidy et al. 1998; Bitner-Mathé and Klaczko 1999a) or traditional multivariate morphometric methods such as principal components from covariance matrices of distance measurements (Atchley et al. 1981; Cheverud 1982; Diniz-Filho and Pignata 1994; Roff and Bradford 1998; Bitner-Mathé and Klaczko 1999b). Recently, there have been attempts to include information from contour shapes in heritability estimates using principal components of Fourier descriptors (Currie et al. 2000; Iwata et al. 2000; Zeng et al. 2000). The geometric methods have been successfully applied to quantitative trait loci mapping (Klingenberg et al. 2001), as a neoclassical approach to quantitative genetics (Walsh 2001), but the contribution of geometric morphometrics to a classical (Fisherian) quantitative genetics has not been fully explored beyond the multivariate extension of the response to selection (Klingenberg and Leamy 2001).

The reasons for reducing complex multivariate shapes to single variables (with a sensible variance maximizing criterion) is that the methods available for estimating heritability can only handle univariate quantitative characters (Falconer and Mackay 1996). The development of statistical theory for

shape data has allowed the study of shape variation by general linear models that explore the multivariate nature of shape configurations but can be handled as univariate models, using the appropriate degrees of freedom (Goodall 1991; Monteiro 1999).

In this paper, we propose a multivariate generalization of heritability estimates (while retaining the univariate simplicity) based on shape distances and an expansion of geometric morphometric methods to encompass linear models for quantitative genetics and shape heritability estimation. The reason for estimating univariate heritabilities for complex multidimensional shapes is that, in addition to the interest in predicting the outcome of selection, the heritability of a trait is a population parameter that varies in space and time (Coyne and Beecham 1987; Young et al. 1994; Bitner-Mathé and Klaczko 1999b) and univariate shape heritability can be easily compared within (temporal) and between (spatial) populations as a single variable. Another reason is that low heritabilities are also indicative of strong developmental constraints in shape evolution (Maynard Smith et al. 1985) and the calculation of univariate shape heritabilities should provide a test between selection versus developmental constraints in the explanation of nonexisting shapes. We illustrate the protocols with a simple example of wing shape variation in honeybee (*Apis mellifera* L.) colonies.

MATERIALS AND METHODS

Theoretical Background

Geometric morphometric techniques are based on a metric known as Procrustes distance, which measures the amount of shape difference. Although several distance measurements have been grouped under the name "Procrustes distance" (Dryden and Mardia 1998; Rohlf 1999), the term is now restricted to a great circle distance within a hemispheric shape space generated by the superimposition of shapes, where the centroid size of the configurations (square root of the summed squared distances from each landmark to the configuration centroid) is equal to unity, the centroid coordinates are (0,0), and the landmark configurations have been optimally rotated to match a reference configuration, which is usually the sample mean (Rohlf 1999). The chord (Euclidean) distance within this space (square root of squared differences between corresponding landmark coordinates in two configurations) is the partial Procrustes distance, and the full Procrustes distance is obtained after scaling the configurations to a size equal to $\cos(\rho)$, where ρ is the Procrustes distance from each configuration to the sample mean (Rohlf 1999). The scale changes generate a spherical shape space also known as Kendall's shape space (Kendall 1984; Dryden and Mardia 1998). The differences between these shape distances are negligible for real datasets, because they are usually so concentrated (occupying a small region) that the effects due to space curvature are negligible. This is true even for datasets with considerable shape variation such as mammal skulls from different orders (Marcus et al. 2000).

The approaches based on Procrustes distances explained here depend heavily on the fact that squared Procrustes chord distances (or squared partial Procrustes distances) (Dryden and Mardia 1998; Rohlf 1999) are sums of squares and can

be transformed to mean squares using appropriate degrees of freedom (Goodall 1991). The partial Procrustes distance between two shapes (X_1 and X_2) is simply the square root of summed squared distances between corresponding landmarks in the two configurations after translation to a common position (centroid at the origin), scaling to a common centroid size and rotating one of them by an angle determined by the least squares criterion. It is calculated as

$$d_P(X_1, X_2) = \sqrt{2} \left(1 - \sum_{i=1}^m \lambda_i \right)^{1/2}, \quad (1)$$

where λ_i are the m singular values from the singular value decomposition

$$Z_2^T Z_1 = V \Lambda U^T, \quad (2)$$

where Z_1 and Z_2 are the preshapes of configurations X_1 and X_2 and $\Lambda = \text{diag}(\lambda_1, \lambda_2, \dots, \lambda_m)$. The preshapes correspond to the landmark configurations after translating so that the centroids (mean configuration point) are located at position (0,0) and scaling such that the centroid size of the configurations (the square root of the summed squared distances from each landmark to the centroid) is equal to one (Goodall 1991; Rohlf 1996; Dryden and Mardia 1998). The rotation operation is embedded in the singular value decomposition of the pre-shape cross-products. If more than two objects are being compared, generalized Procrustes analysis estimates a mean shape from the sample and aligns the specimens to this mean. The mean shape can be estimated iteratively by a series of superimpositions (Slice 1996). The average configuration is the point in shape space that has the minimum sum of Procrustes distances from each point (specimen) in the sample.

For the estimation of heritabilities from the analysis of sibships, a combination of the model of multiple group analysis of variance from Goodall (1991) and the intraclass correlation coefficient (Sokal and Rohlf 1995) can be used. According to Goodall's model, the F -statistic for the analysis of variance can be calculated as

$$F = \frac{L(L-1)M \sum_{i=1}^M d_P^2(\bar{X}_j, \bar{\bar{X}})}{(M-1) \sum_{j=1}^{ML} d_P^2(X_{ij}, \bar{X}_j)} = \frac{MS_{\text{among}}}{MS_{\text{pooledwithin}}}, \quad (3)$$

where L is the number of individuals in each family (provided the experiment has yielded a balanced design) and M is the number of families, with the summation being carried over the squared Procrustes chord distances between each family average shape \bar{X}_j and the grand mean shape $\bar{\bar{X}}$ (numerator) and between each individual shape X_{ij} and its family average shape \bar{X}_j . The degrees of freedom for this F -value can be calculated as $df_1 = (M-1)m$ and $df_2 = M(L-1)m$, where m is the dimensionality of shape space $m = pk - k - 1 - [k(k-1)]/2$, where p is the number of landmarks and k is the number of coordinate dimensions (two for flat structures or images and three for tridimensional shapes). The F -test proposed by Goodall (1991) assumes homogeneous scatter at every landmark and independence of the scatter at different landmarks. To avoid these assumptions, which are seldom found in real datasets, a permutation test can be performed (Klingenberg and McIntyre 1998).

From these geometric estimates of among-family mean squares and pooled within-family mean square, the variance components among families can be estimated as

$$s_a^2 = \frac{MS_{among} - MS_{pooledwithin}}{L}. \quad (4)$$

The error variance component (s_e^2) is equal to the error mean square ($MS_{pooledwithin}$), and the among-family shape intraclass correlation coefficient (t) can be expressed as a proportion of the total shape variation

$$t = \frac{s_a^2}{s_a^2 + s_e^2}. \quad (5)$$

The shape heritability estimate, based on the method for heritability of univariate characters (Falconer and Mackay 1996) can be calculated dividing t by the degree of relatedness within sibling groups, $h^2 = t/r$, where r is the degree of relatedness among siblings. The value of r is usually 0.5 for full-siblings and 0.25 for half-siblings (but for a discussion about the bias and precision of estimates using different degrees of relatedness among siblings, see Falconer and Mackay 1996).

To estimate heritability from a parent-offspring regression, the approach should be somewhat different. In this case, one can use partial warp scores (Rohlf 1996) as shape variables in a multivariate regression (Monteiro 1999). Partial warps are projections from the spherical shape space to a tangent space based on the eigenvectors of a between-landmark square bending energy matrix that is a function of the distances between landmarks in a reference specimen (usually the average of the sample; Rohlf 1996). These shape variables are described in more detail below. The regression model can be expressed as $\hat{\mathbf{W}}_F = \mathbf{B}(\mathbf{W}_P)$, where $\hat{\mathbf{W}}_F$ is an estimated matrix of partial warp scores for the offspring, \mathbf{W}_P is the matrix of partial warp scores for the parents, and $\mathbf{B} = (\mathbf{W}_P^T \mathbf{W}_P)^{-1} \mathbf{W}_P^T \mathbf{W}_F$ is the matrix of regression coefficients of partial warp scores for offspring (\mathbf{W}_F) on parental partial warp scores (\mathbf{W}_P). The estimated partial warp scores from the parent-offspring relationship can be readily translated into estimated offspring shapes (\hat{X}_{io}) in the figure space (Monteiro 1999). From this point, we can return to Procrustes distances for the calculation of shape heritabilities. A multivariate shape R^2 (coefficient of determination) can be calculated as

$$R^2 = \frac{\sum_{i=1}^M d_p^2(\hat{X}_{io}, \bar{X}_o)}{\sum_{i=1}^M d_p^2(X_{io}, \bar{X}_o)}, \quad (6)$$

where the numerator is the sum of squared partial Procrustes distances from each estimated offspring specimen to the sample mean (explained sums of squares) and the denominator is the sum of squared partial Procrustes distances from each observed offspring specimen to the sample mean (total sums of squares). The square root of this coefficient of determination is a multivariate shape correlation coefficient for the parent offspring pairs. Correlation coefficients can be transformed to a multivariate regression coefficient by the relationship

$$B_{O.P} = R \frac{S_O}{S_P}, \quad (7)$$

where

$$S_O = \left(\frac{\sum_{i=1}^M d_p^2(X_{io}, \bar{X}_o)}{(n-1)(2p-4)} \right)^{1/2} \quad (8)$$

is the standard deviation of offspring shape around the mean, based on squared partial Procrustes distances, and

$$S_P = \left(\frac{\sum_{i=1}^M d_p^2(X_{ip}, \bar{X}_P)}{(n-1)(2p-4)} \right)^{1/2} \quad (9)$$

is the standard deviation of parent shape (the subscript P denotes parent).

Provided we are using parental average shape and offspring average shapes, the multivariate regression coefficient directly estimates multivariate shape heritability (h^2). However, if a single parent is used, the heritability is obtained by multiplying the regression coefficient by two (for details on the most efficient designs in each case, see Falconer and Mackay 1996).

Another possibility in geometric methods that may prove highly informative is to analyze the major axes of shape variation in the tangent space generated by partial warps and uniform components. This corresponds to relative warps analysis (Rohlf 1993, 1996, 1999). The partial warp scores are projections from Kendall's curved shape space into a tangent space spanned by principal warps, which are eigenvectors of a between point bending energy matrix. Partial warps and relative warps are described and explained in greater detail elsewhere (Bookstein 1989; Rohlf 1993, 1996). The bending energy matrix is a function of the distances among the landmarks in the reference configuration (usually the sample average configuration), and the principal warps describe shape changes in different scales, ranging from uniform global changes (the uniform component) to changes in decreasing spatial scales (Bookstein 1989). The partial warp scores are obtained by projecting least squares aligned shapes on principal warps. These shape variables span an informative shape space, but bear no biological significance if analyzed as univariate characters (Rohlf 1998). Dimensionality in the tangent space of partial warps can be reduced using a sensible criterion, such as the variance maximization of principal components. The principal components of a partial warp scores matrix are called relative warps, and the potential of this analysis was discussed by Rohlf (1993). The first relative warps can be analyzed as a single variable (the major axis of shape variation) using standard methods for heritability estimation for both sibling and parent-offspring analyses (Falconer and Mackay 1996). A genetic variance-covariance matrix can be calculated directly from partial warp scores and uniform components using among-family variance and covariance components (Lynch and Walsh 1998). The principal components (relative warps) of this matrix will express the genetic contributions to the major directions of within-sample shape variation. In this paper, we calculated partial warps with $\alpha = 0$ and added the uniform components to the

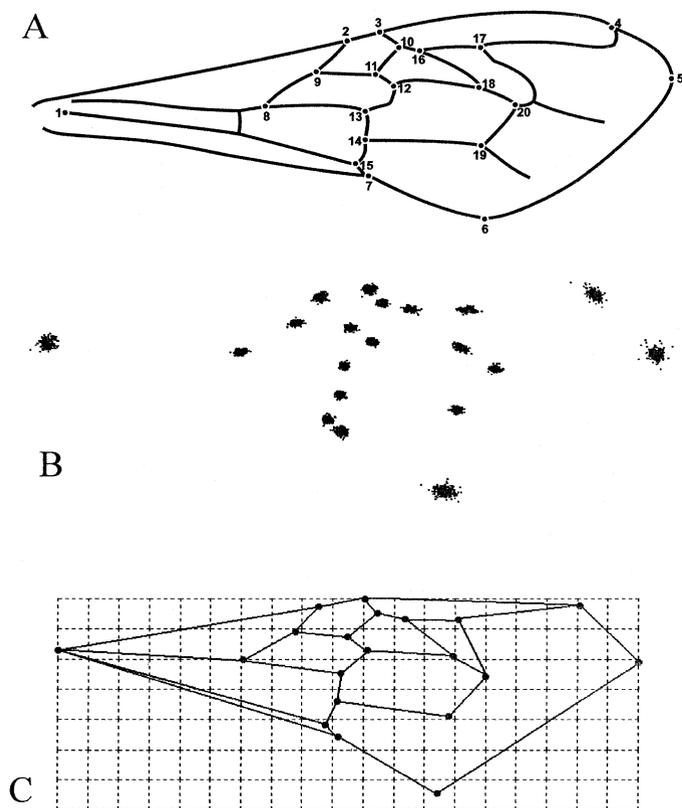


FIG. 1. (A) Wing of an *Apis mellifera* worker showing the landmarks used. (B) Least squares superimposition of the 210 specimens, showing the observed scatters (total variation) around each landmark. (C) Average wing shape configuration among the 21 colonies, used as the reference or tangent configuration.

partial warp scores matrix for the relative warps analysis, as advised by Rohlf (1993).

Size heritability can be studied using centroid size (described above) or any other size variable, such as baseline size, complex hull areas, volumes (Dryden and Mardia 1998), or a general size factor extracted from principal components analysis (Marcus 1990). The size measure most commonly used in geometric studies is centroid size, which is the measure we use here. Because size is a single dimension, standard methods for estimating its heritability can be used.

Example Dataset: Wing Shape Heritability in *Apis mellifera*

As an example of the application of the shape heritability methods described above, we collected 20 landmark coordinates (Fig. 1A) from the right forewings of workers from 21 colonies of Africanized honeybees (*Apis mellifera*) reared in the apiary at Universidade Estadual Paulista (Rio Claro, São Paulo, Brazil). The wings were dissected, mounted, and photographed with a Zeiss (Göttingen, Germany) stereomicroscope along with a scale for size calibration. The pictures were digitized using a flatbed scanner. The landmark coordinate values were obtained using the TpsDig software (Rohlf 1998). Each colony was represented by 10 workers, yielding a total sample size of 210 specimens.

Heritability estimates for worker characters in honeybees have commonly been estimated using the method of Oldroyd

and Moran (1983). The idea is simply to obtain among- and within-colony variance components (each colony can be considered a single family), as described before, and divide the intraclass correlation coefficient by the relatedness (r) among workers from the same colony. Because colonies in social bees are families combining supersisters ($r = 0.75$, due to a haplo-diploid sex determination system) and half-sisters ($r = 0.25$), the average relatedness is a function of the degree of polyandry in the species or population (Page 1986; Page and Kerr 1991). Despite some upward bias resulting from the common environment shared by workers in the same colonies and possible dominance effects in monoandric species (Collins 1986; Diniz-Filho & Pignata 1994; Diniz-Filho et al. 1994), the method furnishes a reasonable approximation of heritability values, especially for large-scale comparisons, using different characters and populations (Oldroyd et al. 1991). When estimating shape heritability using geometric methods, the main advantage of this simple experimental design is that it allows an easy understanding of the protocols proposed.

In the original method of Oldroyd and Moran (1983), heritability was estimated by dividing the intraclass correlation obtained with a one-way analysis of variance (ANOVA; t) by the average relatedness within colonies (r), which in turn was a function of the degree of polyandry. Assuming that drones who inseminate the queen are unrelated, r is given as

$$r = \frac{1}{2D} + 0.25, \quad (10)$$

where D is the number of drones. Traditionally, a D -value of 17 has been cited for Africanized honeybees (Adams et al. 1977; Lobo & Krieger 1992), yielding $r = 0.279$. Oldroyd and Moran (1983) and Oldroyd et al. (1991) used $D = 13$ drones, with an equivalent $r = 0.29$. Based on molecular data, Haberl and Moritz (1994) suggested that this D -value may be overestimated and found that average relatedness was closer to 0.33 (equivalent to around six matings by the queen). In this paper, we used an intermediate value of $r = 0.30$, which should provide a more conservative estimate of the heritabilities (Palmer and Oldroyd 2000). In any case, because of the functional relationship between r and D , differences in h^2 are small when D is large (>10). These small variations will not qualitatively affect the conclusions presented below.

To compare the geometric with the traditional methods for estimation of size and shape heritability, we generated a data matrix of interlandmark distances, containing all possible distances between the 20 landmarks shown in Figure 1A. The set was composed of 190 interlandmark distances. This space of interlandmark distances contains the same amount of information present in the 36 dimensions of shape space plus one size dimension from used in geometric morphometrics. The space of interlandmark distances was rotated to its major axes of variation by a principal components analysis. In this context, the first principal component is usually interpreted as size (provided all the eigenvector coefficients have the same sign) and from the second principal component on, the axes are interpreted as shape vectors (Marcus 1990). Heritabilities were calculated from the first and second principal

TABLE 1. Procrustes estimates of overall shape and size variance components, heritability (h^2) and its standard error (SE h^2) for *Apis mellifera* worker wings. Bartlett's chi-square approximation for wing shape homoscedasticity was $B = 1.404$, $P = 1.000$, and for wing size heteroscedasticity $B = 42.481$, $P = 0.0024$.

Source	df	MS	Variance component	Intraclass correlation	h^2	SE h^2
Wing Shape						
Colony	720	8.60706	0.54986	0.046934	0.1564	0.0245
Error	6804	3.10842	3.10842			
Wing Size						
Colony	20	0.091062	0.006230	0.178025	0.5934	0.1270
Error	189	0.028765	0.028765			

component scores to estimate the heritability of size and shape as depicted by traditional methods. This approach is commonly found in the literature (Leamy and Atchley 1984; Diniz-Filho and Pignata 1994; Bitner-Mathé and Klaczko 1999a,b).

RESULTS

The wings were superimposed by Procrustes analysis. The limited scatter of landmarks (Fig. 1B) indicated that there was little variation within each landmark. The largest scatters were those related to landmarks 5 and 6, which are the less-well-defined landmarks (maxima of curvature). The mean configuration used as a reference or point of tangency between the curved and tangent spaces is shown in Figure 1C.

The Procrustes estimate of heritability was significant for wing shape and wing centroid size ($P < 0.001$, based on 1000 permutations). However, the magnitude of shape heritability was smaller than for centroid size (Table 1). The Bartlett test for variance homogeneity (homoscedasticity) was not significant for wing shape, but highly significant for wing size, which indicates that the estimate of wing size heritability may be biased upward because of dominance deviations. The Procrustes estimates accounted for total shape variation, that is, global and localized shape changes. Because

developmental processes of wing veins may generate small-scale shape differences in restricted areas of the wing, further examination of shape heritability with relative warps is desirable at this point.

The first relative warp explained 16.5% of total shape variation and presented a larger heritability than total shape ($h^2 = 0.2935$; standard error $h^2 = 0.0971$; Bartlett test for homoscedasticity $B = 12.99$; $P = 0.8778$). The first relative warp (Fig. 2) depicts a large-scale shape change localized on the distal part of the wing. Specimens with positive scores present wings with larger extremities than specimens with negative scores.

A genetic variance-covariance matrix was calculated from the partial warp scores. The genetic matrix was compared to the respective phenotypic variance-covariance matrix by a Mantel test (using 10,000 random permutations to test for significance), yielding a large matrix correlation coefficient ($r_M = 0.57181$; $P = 0.0004$). Thus, the phenotypic variance-covariance matrix approximates the genetic variance-covariance matrix satisfactorily. As expected from the high correlation between the genetic and phenotypic covariance matrices, the first eigenvector of the genetic variance-covariance of partial warp scores and uniform components is very similar (vector correlation coefficient = 0.566) to the first eigenvector of the phenotypic variance-covariance matrix.

The first principal component from the analysis of 190 interlandmark distances explained 50.07% of the variance, and presented 190 positive coefficients, being interpreted as a size function. The second principal component presented positive and negative coefficients and explained 9.13% of total variation, being interpreted as a shape function. The heritability of the first component from interlandmark distances ($h^2 = 0.613$; standard error $h^2 = 0.235$; Bartlett test for homoscedasticity $B = 42.81$; $P = 0.0022$) was similar to the heritability estimated for centroid size (including the significant homoscedasticity test). The second component (shape) presented heritabilities similar to the magnitude of the values estimated for shape by Procrustes distances ($h^2 = 0.1637$; standard error $h^2 = 0.1487$; Bartlett test for homoscedasticity $B = 9.99$; $P = 0.9682$). However, the very large standard error indicates that the heritability found is not significantly different from zero. The interpretation of the results from interlandmark distances analysis would lead us to believe that the amount of shape variability in this sample is so small (and close to measurement error) that it is not inheritable.

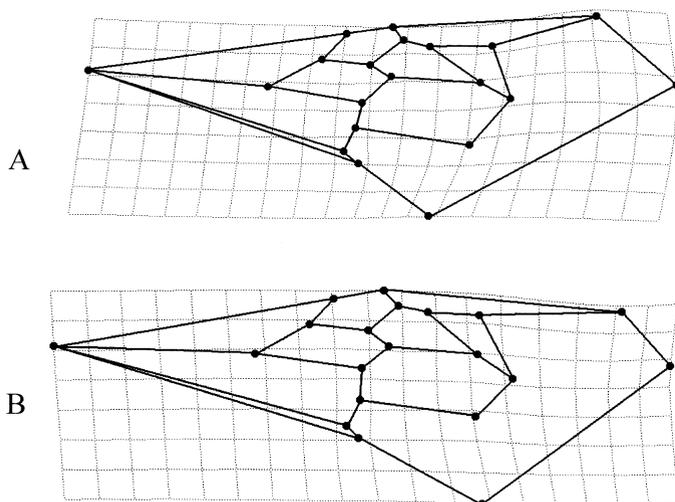


FIG. 2. Shape changes depicted by the first relative warp. (A) Deformation relative to the mean shape toward the positive direction. (B) Deformation toward the negative direction.

DISCUSSION

Our estimates of shape heritability were similar in magnitude to other estimates of the heritability of morphological structures in bees (Rinderer 1977; Milne 1985; Oldroyd et al. 1991; Diniz-Filho and Pignata 1994; Diniz-Filho et al. 1994). However, the shape variables used here are statistically more powerful and better behaved than the variables traditionally used in shape studies (Monteiro et al. 2000). Relative warps can be highly informative about specific shape differences occurring along major axes of variation in shape space. Because there was no major effect on total wing shape, the Procrustes heritability estimate was rather low, indicating that most overall wing shape variation in the sample was probably due to random noise.

Relatively small amounts of heritability have been reported for *Apis* wing shape variables such as venation angles (for a comparison of subspecific patterns worldwide, see Oldroyd et al. 1991). However, further analyses of wing shape at localized spatial scales performed here detected localized shape differences with higher amounts of heritability. These shape differences usually cannot be detected by traditional variables such as angles and distance ratios because these only provide information on overall shape (Monteiro and Abe 1999). Apart from its conceptual interest, this localized heritable shape variation in honeybee wings may provide new insights on the morphological evolution and subspecific divergence in honeybees (Ruttner 1988; Wagner 1990; Diniz-Filho et al. 1999).

The first relative warp explained a small amount of total shape variance but with larger heritability than total shape (measured by Procrustes analysis). This can be easily explained by the fact that the single trend of major variation should present less noise variation than the full shape space. It can be noted from Figure 1B that the scatter around the wing landmarks are rather small and circular. One should expect a large proportion of shape variation to arise due to measurement error.

The homoscedasticity of all shape variables examined indicates that wing shape genetic variation in *Apis* is generated by a large number of loci of roughly equal and small additive effects (Lynch and Walsh 1998), as would be expected for a multidimensional feature such as shape and as determined beforehand by studies on insect wing development (García-Bellido and de Cellis 1992; Sturtevant and Bier 1995; Biehs et al. 1998). On the other hand, size variation showed a significant heteroscedasticity, which indicates that wing size may be determined by a few major genes and that the heritability estimate might be biased upward because of dominance deviations or unique environmental effects within a few colonies (Diniz-Filho and Pignata 1994).

The procedures proposed here (shape heritability estimates from Procrustes distances and relative warps) are not directly comparable because they are designed to yield different, yet complementary results. The heritability estimates based on Procrustes distances were obtained from the full shape space (uniform and localized components), without the restriction of alignment with major axes of phenotypic variation. However, the relative warps are univariate directions of major intrapopulation variation in shape space that accounted for

a fraction of the total shape variance. The introduction of the criterion of maximum variance generates shape variables (relative warps) that depict maximum shape differences among individuals, but that do not span the entire range of variation in shape space. Based on this difference between estimators, the relative warps would be expected to show greater heritabilities than the Procrustes distances because they focus on highly variable directions within shape space.

The estimate of shape heritability using Procrustes distances allows us to rewrite the traditional expression for the rate of evolution or response to selection of a trait ($R = h^2S$; Falconer and Mackay 1996; Lynch and Walsh 1998). In these terms, the response to selection (R) that we can regard as the Procrustes distance between two generation means (parental and F_1) is equal to the heritability (h^2) of shape multiplied by the selection differential (S), which is the Procrustes distance between the mean shape of selected parents and the population mean shape. Procrustes distances (like other distances) measure resemblance between shapes, but provide no information about the direction of shape differences. The visualization of shape changes as a response to selection in shape space should come from a comparison of generation means. This can be obtained from the superimposition of landmark configurations or deformed grids (Bookstein 1996). Apart from the use of heritability to directly estimate the response to selection in a given trait, it has also been regarded as a measure of the importance of the genetic component of variation that can vary in time and space (Coyne and Beecham 1987; Young et al. 1994; Bitner-Mathé and Klaczko 1999a). In this context, it might prove interesting to be able to estimate the heritability of a complex feature such as the shape of a biological structure (or of parts of it) as a scalar that can be easily compared among populations from different geographic locations or in a time series. A third use of the shape heritability proposed here is a possible test of developmental constraints versus natural selection (as discussed by Maynard Smith et al. 1985) as a major evolutionary force explaining the gaps between species clusters in shape space (as proposed before for gaps between species in other theoretical morphospaces). Whenever the trait presents a significant heritability estimate, developmental constraints should be discarded as an explanation for the gaps observed in shape space. A fourth use of the shape heritability estimates presented here is as a parameter of divergence rate tests for the inference of evolutionary processes (Turelli et al. 1988; Spicer 1993). For the reasons presented above, the estimates of shape heritability presented and discussed here (particularly the scalar estimates based on Procrustes distances) have an enormous potential of application in the study of morphological evolution.

Comparing the geometric estimates of shape and size heritability with the estimates from the analysis of interlandmark distances, we can draw a series of conclusions that reinforce the theoretical expectations. First, to approximate the same amount of information present in the 36 shape dimensions derived from landmark coordinates, we needed about five times more dimensions (190) using interlandmark distances. An important point is that any dataset of interlandmark distances with less than 190 dimensions would be less informative than the landmark coordinates and therefore not de-

sirable. The increased number of dimensions needed also requires larger sample sizes to maintain the same statistical power. Second, size variation is usually so dominant in interlandmark distance datasets that it is sometimes difficult to study shape variation. Our example illustrates well this point. The traditional methods do not go much further than stating that shape variation in this sample is so small that it is not inheritable. On the other hand, the geometric protocols proposed here showed that there was a major axis in shape space that presented a significant heritability. The information extracted by geometric methods from landmark coordinates is richer than the information extracted from interlandmark distances by the traditional morphometrics. We believe that the choice of methods for a morphometric study should be driven by the information content that a method is capable to extract from a dataset.

As shown above, the techniques of geometric morphometrics can be highly informative in studies of shape heritability. Methods of shape analysis that use landmark coordinates and are based on Kendall's shape space and on the tangent space have recently proven to have an adequate topology for multivariate within-group variance maximizing techniques such as principal components analysis (Monteiro et al. 2000; Rohlf 2000a). They are also more powerful in detecting shape differences among groups than are methods that use distance ratios and angles (Rohlf 2000b). The estimation of heritability and other quantitative genetic parameters should benefit from these desirable characteristics of geometric morphometric methods and should provide a methodological framework for modeling the phenotypic evolution of complex morphological structures.

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