

QUANTITATIVE GENETICS OF MULTIVARIATE MORPHOMETRIC VARIATION IN THE NEOTROPICAL STINGLESS BEE, *Scaptotrigona postica* (HYMENOPTERA: MELIPONINAE)

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ABSTRACT

A quantitative genetic analysis was performed to evaluate phenotypic and genotypic multivariate patterns of morphometric variation in *Scaptotrigona postica*. One hundred and fifty bees, collected from 15 man-made hives, were analyzed by measuring fifteen characters. Heritabilities found ranged from 0.799 to 0.201. Genetic and phenotypic correlations matrices were highly associated, considering their matrix correlation ($r_s = 0.809$; $P < 0.01$ by Mantel test) and the correspondence of varimax rotated eigenvectors of principal component analysis applied to both matrices. Also, phenotypic patterns of correlation were more associated with functional or developmental systems than genetic ones. There was, however, more overall integration in genotypic than in phenotypic components of morphometric variation. This is expected considering that most parts of the body of holometabolous insects are derived from imaginal discs, in such a way that final patterns of morphometric variation are not determined until relatively late in development. General results indicate that phenotypic patterns alone can be used to test evolutionary hypotheses in *S.postica*.

INTRODUCTION

During the last twenty years, patterns of morphometric variation in stingless bees have been used to propose systematic relationships among species (Kerr and Cunha, 1976; Pisani *et al.*, 1977; Cunha, 1973, 1991) and test hypotheses about their mechanisms of sex determination and caste differentiation (Kerr *et al.*, 1978; Bonetti and Kerr, 1985; Kerr, 1987; Kerr and Cunha, 1990; Hartfelder and Engels, 1992). These studies are usually based on phenotypic patterns of variation and covariation among characters, measured in individual bees. However, in some situations, hypothesis about correlated response to selection or correlated patterns in morphogenesis imply specific relationships about genetic parameters which may not be accurately described by phenotypic statistics alone (Atchley *et al.*, 1981; Atchley, 1983; Barton and Turelli, 1989).

Morphometric variation in an organism reflect a polygenically controlled integration of metric characters, whose phenotypic expression changes during ontogeny and evolution, and can be analyzed using standard quantitative genetics techniques (Falconer, 1975; Atchley *et al.*, 1981). In social bees, haploid-diploid organisms living in colonial aggregations and showing caste as well as sex differences, some modifications in the theoretical basis of quantitative genetics are necessary (Rinderer, 1977; Collins, 1986). The difficulties are related to changes in the coefficients of relatedness within colonies (caused by multiple insemination of the queens in some species), to the castes (that are distinct phenotypic expressions of the same combination of genetic material), and to the social organization, that can introduce confusion concerning the relative importance of additive genetic variation and environmental variation, since offspring in the sib groups share the same colonial conditions. Also, several characteristics of economic interest are expressed by colonies as a whole, not by individual bees. In *Apis mellifera* L., breeding programs, based on instrumental insemination, can be used to overcome these problems (Collins, 1986). In stingless bees it is usually very difficult to control the mates and establish a breeding program.

However, Oldroyd and Moran (1983) proposed a different system to obtain genetic parameters for characters measured in worker bees. The method does not require instrumental insemination and can be used to analyse natural populations of social Hymenoptera if average relatedness within colonies can be estimated.

In this paper, we used Oldroyd and Moran's (1983) method to evaluate the morphometric variation in a local population of a neotropical stingless bee, the trigonine *Scaptotrigona postica* Latreille, 1807. In spite of some possible upward bias in the genetic parameters, we expected to answer to three basic questions: 1) to what degree are the multivariate patterns of phenotypic variation in *S.postica* heritable? ; 2) are there phenotypic and genotypic complexes of characters or do they express a single system?; 3) are phenotypic and genotypic patterns of correlations among characters concordant?

MATERIAL AND METHODS

Fifteen morphometric characters were measured in 10 worker bees, randomly collected in fifteen hives of *S.postica* maintained in the animal-rearing building of the Instituto de Biociências of UNESP, Campus of Rio Claro, São Paulo, Brazil. The characters measured were: maximum head width (HDW), inferior interorbital distance (IND), glossa length (GLS), prementum length (PRM), maxillary palpus length (PLL), forewing length (FWL), forewing width (FWW), length of vein M in forewing (VML), hindwing length (HWL), hindwing width (HWW), number of hamuli (HAM), extension of hamuli on hindwing (EXH), mesonotum width (MSW), hind femur length (FEL) and hind tibia length (TBL). Characters were measured under a stereo microscope equipped with an ocular scale and converted to millimeters prior to the analysis.

Heritabilities (h^2) measure the proportion of additive genetic variance in relation to the total variance, and were estimated using Oldroyd and Moran's (1983) method. This method consists in estimating intraclass correlation (t) from the mean-squares derived from a single classification analysis of variance (ANOVA)(Sokal and Rohlf, 1981), using colonies as the source of variation. Heritability is then given by

$$h^2 = t / r$$

where r is the average relatedness of sib groups. Queens of *S.postica*, as in most stingless bees, mate only once (Kerr *et al.*, 1962; Contel and Kerr, 1976; Page, 1986), except in non-natural conditions (Engels and Engels, 1988; Campos and Melo, 1990). Relatedness within colonies is therefore equal to 0.75. Single male insemination, however, can increase the upward bias in h^2 due to dominance deviation

(Page and Kerr, 1991). In this case, the coefficient of dominance bias is equal to $0.666 V_D/V_P$, where V_D and V_P are dominance and phenotypic variances, respectively. So, serious upward bias in h^2 can be introduced if the characters studied are partially controlled by dominant effects. The Bartlett test (Sokal and Rohlf, 1981) was used to check the assumption of homocedasticity before ANOVA, to determine if a linear-additive model can be adjusted to morphometric differences among colonies. The other problem of Oldroyd and Moran's (1983) method is the upward bias in the h^2 estimates caused by the fact that sib groups are raised in a common environment (colony). For this analysis, we tried to minimize this effect, analysing only colonies established in man-made hives, with approximatedly the same size and kept near each other, with the same general environmental conditions.

To analyse the multivariate patterns of relationship among the characters measured, the phenotypic correlation matrix among them was partitioned into genetic and environmental correlation matrices, following Atchley *et al.* (1981). The genetic correlation coefficient (r_G), which contains only the additive part of the association between characters (indicating linkage or pleiotropic effects), say X and Y, is given as

$$r_G = \text{cov}_G / (\text{var}_{G_X} \text{var}_{G_Y})^{1/2}$$

where var_{G_X} and var_{G_Y} are the between colony variance components from ANOVA (used to estimate heritabilities of X and Y) and cov_G is the genetic covariance between X and Y, calculated by

$$\text{cov}_G = (\text{var}_{G(x+y)} - \text{var}_{G_X} - \text{var}_{G_Y}) / 2$$

Standard errors of genetic correlations were calculated using the procedures described in Falconer (1975). The matrices of phenotypic and genetic correlations among characters were compared using the Mantel test (Manly, 1986). Each matrix was also analyzed by extracting eigenvalues and eigenvectors, using Principal Component Analysis. Following Atchley *et al.*(1981), only principal components with eigenvalues greater than one were extracted and subsequently orthogonally rotated to a new varimax solution (Harris, 1975). The first unrotated principal component of each matrix, interpreted as a general size factor, was also analyzed. Heritabilities for the new phenotypic axes (rotated principal components) were also estimated. Environmental correlations are derived from phenotypic and genotypic ones, and were not analyzed here.

Variance components and Principal Component Analyses were performed using procedures of SAS-PC (SAS Institute Inc., 1987). Mantel tests were performed using NTSYS-Pc, version 1.5 (Rohlf, 1989).

RESULTS

Heritabilities ranged from about 0.2 for FEL to 0.8 for FWW (Table I). None of the F approximations for Bartlett tests for homocedasticity were statistically significant at the 5% level, indicating a high level of additivity in differences among colonies. So, upward bias due to dominance deviations should be small.

Table I - F approximation for Bartlett test, heritabilities and standard errors for fifteen morphometric characters of *S. postica*.

Character	F*	h^2 (\pm S.E.)
HDW	0.717	0.674 \pm 0.262
IND	0.541	0.571 \pm 0.229
GLS	0.828	0.754 \pm 0.287
PRM	1.120	0.719 \pm 0.276
PLL	0.682	0.619 \pm 0.244
FWL	0.382	0.716 \pm 0.275
FWW	1.201	0.799 \pm 0.301
VML	0.287	0.510 \pm 0.210
HWL	0.358	0.393 \pm 0.173
HWW	0.901	0.582 \pm 0.233
HAM	0.294	0.208 \pm 0.114
EXH	0.534	0.704 \pm 0.271
MSW	0.673	0.626 \pm 0.246
FEL	0.848	0.201 \pm 0.112
TBL	0.632	0.265 \pm 0.132

F value with 14 and 10251 degrees of freedom.

*All non significant at 5% level.

The phenotypic and genetic correlation matrices and their standard errors are available from the authors upon request.

Out of 105 phenotypic correlation coefficients, 89.5% were statistically different from zero at the 5% level. The average phenotypic r^2 was equal to 0.235. Four eigenvectors with eigenvalues greater than one were extracted and rotated, accounting for 76.7% of the phenotypic correlation patterns. The first unrotated principal component, which accounts for 50.5% of the original phenotypic correlation matrix, can be interpreted as a general size factor, with coefficients of the same signal and similar magnitude for all characters (except HAM). Heritability estimated for this composite axis was equal to 0.587 (\pm 0.234). Varimax orthogonal rotation breaks down this general size axis and reveals more detailed patterns of morphological integration. Eigenvalues and heritabilities for these new composite axes can be also seen in Table II. The first rotated principal component can still be interpreted as a size axis, with the greatest contributions from FWW, FWL, HDW, MSW, IND. The second rotated

principal component had the highest coefficients from TBL and FEL. The third rotated principal component had the highest coefficients for characters related to the bucal apparatus (GLS, PRM and PLL). The fourth rotated principal component had the greatest contributions from HAM, characters of forewing (HWL, HWW and EXH) and VML. These patterns reveal some correspondence between correlation structure among characters and some functional or developmental systems of the phenotype.

Table II - Coefficients of eigenvectors, eigenvalues and heritabilities of principal components extracted from phenotypic correlation matrix of fifteen morphometric characters of *S. postica*. UNR1 is the first unrotated principal component.

Character	UNR1	Rotated principal components			
		I	II	III	IV
HDW	0.847	0.868	0.167	0.226	0.033
IND	0.803	0.791	0.229	0.138	0.095
GLS	0.360	0.084	-0.071	0.865	0.183
PRM	0.743	0.487	0.440	0.441	0.149
PLL	0.631	0.307	0.388	0.724	0.051
FWL	0.848	0.861	0.153	0.005	0.274
FWW	0.846	0.899	0.030	0.190	0.144
VML	0.809	0.641	0.179	0.166	0.528
HWL	0.795	0.580	0.154	0.352	0.493
HWW	0.662	0.445	0.333	-0.113	0.642
HAM	0.274	-0.002	-0.010	0.200	0.680
EXH	0.771	0.716	0.263	-0.134	0.401
MSW	0.821	0.804	0.237	0.345	-0.082
FEL	0.613	0.239	0.862	0.260	0.037
TBL	0.499	0.147	0.908	-0.056	0.155
eigenvalue (%)	50.456	36.319	15.416	13.238	11.692
h^2 (S.E.)	0.587 \pm 0.234	0.882 \pm 0.328	0.815 \pm 0.307	0.413 \pm 0.179	0.469 \pm 0.196

Out of 105 genetic correlation coefficients, 44.7% were statistically different from zero at the 5% level. The smaller number of significant genetic correlations, in relation to phenotypic ones, may be due to a smaller number of degrees of freedom (D.F.=13). Fifty-two of the 105 genetic correlation coefficients were larger than the respective phenotypic ones. The average genetic r^2 was slightly larger than the phenotypic one and equal to 0.255 (although statistical tests for this difference are difficult to perform due to dependence among elements in the matrices).

Four principal axes with eigenvalues greater than one were also extracted from the genetic correlation matrix and rotated to a new varimax solution (Table III). These four components accounted for almost 90% of the genetic correlations.

Table III - Coefficients of eigenvectors and eigenvalues of principal components extracted from genetic correlation matrix of fifteen morphometric characters of *S.postica*. UNR1 is the first unrotated principal component.

character	UNR1	Rotated principal components			
		I	II	III	IV
HDW	0.875	0.895	-0.081	0.005	0.346
IND	0.805	0.823	0.163	0.315	-0.074
GLS	0.096	0.007	-0.801	0.518	-0.114
PRM	0.836	0.546	0.167	0.431	0.539
PLL	0.625	0.257	-0.262	0.368	0.803
FWL	0.855	0.880	0.156	0.225	0.032
FWW	0.834	0.948	-0.133	0.033	0.089
VML	0.901	0.763	0.157	0.474	0.162
HWL	0.773	0.506	-0.235	0.662	0.304
HWW	0.524	0.298	0.690	0.454	0.100
HAM	0.487	0.104	0.079	0.905	0.116
EXH	0.862	0.750	0.434	0.162	0.357
MSW	0.815	0.869	-0.294	0.026	0.268
FEL	0.414	0.095	0.365	-0.034	0.881
TBL	0.020	-0.116	0.990	0.069	0.016
eigenvalue (%)	49.588	38.629	18.424	16.409	14.672

The first unrotated principal component of genetic correlation had large unidirectional coefficients on all characters, except TBL, and accounted for 49.6% of the genetic correlations. Following Atchley *et al.* (1981), this vector can be interpreted as a "general pleiotropic" or "genetic size" function.

Patterns of morphological integration in genetic correlations, obtained by varimax rotation, are not so clear as phenotypic ones. First rotated principal component possess highest coefficients for characters related to size (FWL, FWW, HDW and MSW) as in the first varimax rotated phenotypic principal component. Second rotated genetic principal component has highest inverse contributions from TBL and GLS. Third rotated principal component has highest contributions from characters of hindwing (HAM, HWL and HWW)(except EXH). Fourth rotated principal component has highest contributions from FEL and PLL. Eigenvalues for each rotated genetic principal components are given in Table III.

The correspondence between phenotypic and genetic correlations was initially evaluated by a matrix correlation between them. The Spearman rank correlation between these two matrices was equal to 0.809, different from zero at 0.1% level by Mantel test, using 5000 random permutations. The scatterplot between these matrices (Figure 1) shows a nonlinear relationship between them. Deleting characters with low heritabilities (HAM, CTB and FEL), correlation grows up to 0.917. There is a high degree of correspondence, explained by the high heritabilities found for most part of characters and by the pattern of phenotypic integration in functional or developmental sets of characters.

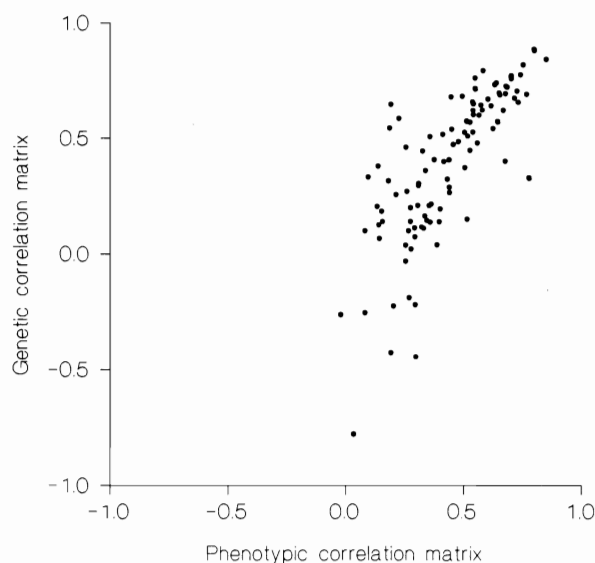


Figure 1 - Relationship between genetic and phenotypic correlation matrices of 15 morphometric characters of *Scaptotrigona postica*.

Correspondence between phenotypic and genetic varimax rotated principal components was evaluated by vector correlations. First rotated phenotypic and genetic eigenvectors were highly correlated ($r = 0.967$; $P < 0.01$). The fourth phenotypic eigenvector corresponds to the third genetic one ($r = 0.729$; $P < 0.01$). Four phenotypic components accounted for 76.7% of the correlations among characters, contrasting with 88.1% of the four genetic components. Average genetic r^2 is also slight larger than phenotypic one. These patterns (especially the relative importance of the first eigenvalue) indicate that overall genetic integration among morphometric characters is greater than overall phenotypic integration.

DISCUSSION

Heritabilities found for characters of *S.postica* are in the range usually found for morphological characters in

bees (Moritz and Klepsch, 1985; Oldroyd *et al.*, 1991). However, heritabilities for morphometric characters of *A. mellifera* decrease when offspring are not reared in the maternal environment, indicating effects of nursing or the within-colony environment confounded with additive genetic variance (Oldroyd *et al.*, 1991). These effects in stingless bees must be smaller than in honey bees, since they provision their brood cells before oviposition, and all steps of larval development take place in a sealed cell, without interference from adult bees (Kerr *et al.*, 1962; Nogueira-Neto, 1970; Wille, 1985).

The distribution of coefficients of characters on phenotypic varimax rotated principal components revealed an interesting pattern of morphological integration, with correlated characters integrated into functional or developmental systems (Cheverud, 1982). These systems seem to correspond to the imaginal discs that are responsible for the final size and shape of the adult bee (Hartfelder and Engels, 1992). All of these integrated axes are highly heritable, but phenotypic patterns of morphological integration may reflect linkage among genes, pleiotropic effects and similar developmental patterns, all acting in the same developmental unity (imaginal discs) (Cowley and Atchley, 1988).

The genetic patterns of morphological integration are not as clear as phenotypic ones, indicating that both genetic and environmental (including hormonal) effects, during ontogeny, contribute to this integration. As pointed out by Hartfelder and Engels (1992), large portions of the adult body of holometabolous insects, and in particular appendages, are derived from imaginal discs, in such a way that the final patterns of size and shape are not determined until relatively late in development. This way, a higher overall level of integration of the genotype, in relation to overall phenotypic integration, is expected. This differentiation, defining the morphometric patterns of workers, queens or males, may be the result of a shift in the balance of maleness and femaleness genes caused by juvenile hormone deficiency (Kerr, 1974; Campos *et al.*, 1979) or of a stepwise programming and reprogramming of a few growth parameters acting on imaginal discs during ontogeny (Wheeler, 1986; Hartfelder and Engels, 1992).

We conclude that genotype is highly integrated in *S. postica*, with fifteen characters being reduced to four new axes, all highly heritable. There is a reasonable correspondence between phenotypic and genetic patterns of correlation, in such a way that some microevolutionary inferences using evolutionary quantitative genetic models (Lande, 1976; 1979; Lande and Arnold, 1983; Barton and Turelli, 1989), based on phenotypic patterns, seem to be reasonable (Cheverud, 1989). Although matrices of genetic correlation among characters can be obtained easily by generalizing Oldroyd and Moran's (1983) method, large samples (more than 100 individual bees in

several colonies) must be obtained. This can be prohibitive in geographic variation or systematic studies, in which many populations or species should be analyzed. In geographic variation studies, for example, random samples of individual bees in each locality could be used to estimate pooled within-group covariance or correlation matrix. Following Manly (1985), genetic covariance matrix (CovG) could then be approximated by $CovG = \bar{h}^2 CovP$, where $CovP$ is the pooled within-group phenotypic covariance matrix and \bar{h}^2 is an average heritability value. For our data with *S. postica*, this approximation can be done by multiplying the phenotypic covariance matrix by 0.556. The approximated genetic covariance matrix obtained was then compared with the genetic covariance matrix obtained using Oldroyd and Moran's (1983) method, and the result can be seen in Figure 2. Matrix correlation between them was equal to 0.881, significant at 0.1% level by the Mantel test.

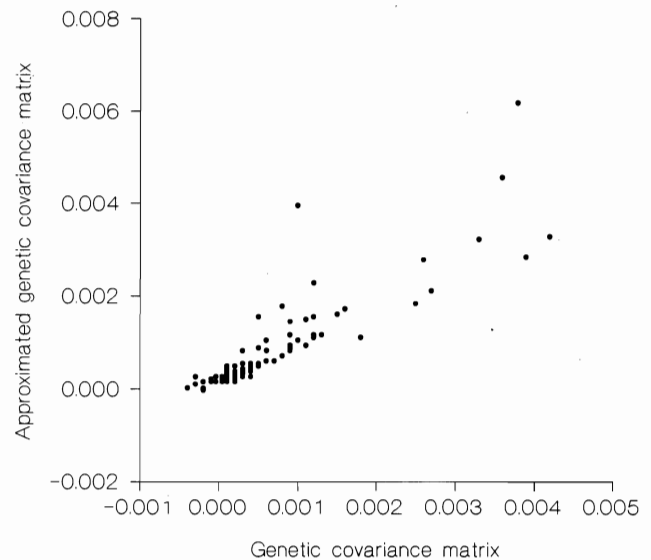


Figure 2 - Relationship between genetic covariance matrix and approximated genetic covariance matrix. This approximation was performed as $0.556 (COV_P)$, where COV_P is the phenotypic covariance matrix and 0.556 is the average heritability for the 15 characters.

Oldroyd and Moran's (1983) method permits a simple evaluation of genetic parameters in natural populations of social insects, if average relatedness within colonies is available and if dominance or microenvironmental effects can be ruled out. With this method it is possible to overcome most of the critiques to the contemporary morphometric studies properly pointed out by Atchley *et al.* (1981) and Atchley (1983) and increase the application of morphometric data to test evolutionary hypotheses in social insects.

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RESUMO

Neste trabalho, foram determinados os componentes fenotípicos e genotípicos da variação morfométrica multidimensional em uma população local de *Scaptotrigona postica*. Foram analisados quinze caracteres em cento e cinquenta abelhas, provenientes de quinze colônias. As herdabilidades, estimadas através do método de Oldroyd and Moran (*Aust. J. Biol. Sci.* 36: 323-332, 1983), variaram entre 0.799 e 0.201. Matrizes de correlações fenotípicas e genotípicas estão bastante associadas, considerando sua correlação matricial ($r_S = 0,809$; $P < 0,01$ através do teste de Mantel com 5000 permutações) e a correspondência de seus autovetores rotacionados através do critério Varimax. As correlações fenotípicas estão mais associadas a complexos funcionais do corpo da abelha do que as correlações genotípicas, embora haja maior integração total no genótipo (quatro autovalores explicando 88,13% da variabilidade total) do que no fenótipo (quatro autovalores explicando 76,66% da variabilidade total). Isso pode ser explicado uma vez que a maior parte do corpo dos insetos holometábolos é derivada de discos imaginais, de modo que os padrões finais de variação morfométrica só se definem relativamente tarde no desenvolvimento ontogenético. Os resultados indicam que inferências microevolutivas utilizando os modelos de genética evolutiva (Lande, *Evolution* 30: 314-334, 1976; Lande, *Evolution* 33: 402-416, 1979; Lande and Arnold, *Evolution* 37: 1210-1226, 1983) podem ser baseadas nos padrões fenotípicos multivariados.

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